AOP ID and Title:

AOP 478: Deposition of energy leading to occurrence of cataracts
Short Title: Deposition of energy leading to cataracts

Graphical Representation

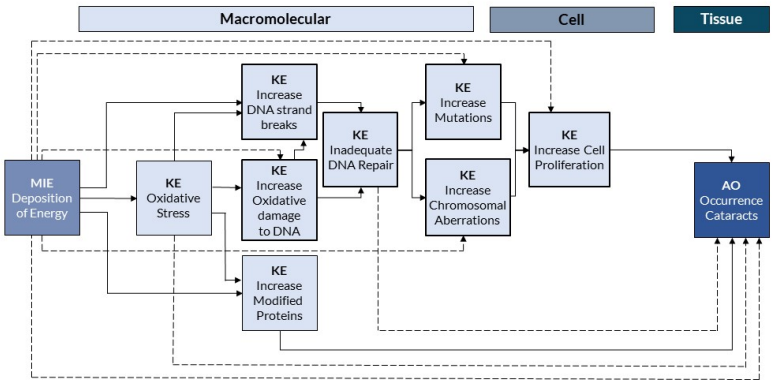

Figure 1. Visual representation depicting the AOP leading from the deposition of energy to cataracts. Both adjacent KERs and non-adjacent KERs are included in this pathway. Adjacent KERs demonstrate the causal relationship between two KEs. Non-adjacent KERs can be used to support the weight of evidence (WOE) of the whole AOP by bypassing KEs with less evidence.

Authors

Emma Carrothers[1], Meghan Appleby[1], Vita Lai[1], Tatiana Kozbenko[1], Dalya Alomar[1], Benjamin Smith[1], Robyn Hocking[3], Carole Yauk[2], Ruth Wilkins[1], Vinita Chauhan[1]

(1) Consumer and Clinical Radiation Protection Bureau, Environmental and Radiation Health Sciences Directorate, Health Canada, Ottawa, Ontario

(2) Department of Biology, University of Ottawa, Ottawa, Ontario

(3) Learning and Knowledge and Library Services, Health Canada, Ottawa, Ontario, Canada

Consultants

Nobuyuki Hamada[1], Patricia Hinton[2], Elizabeth A. Ainsbury[3]

(1) Biology and Environmental Chemistry Division, Sustainable System Research Laboratory, Central Research Institute of Electric Power Industry (CRIEPI), Komae, Tokyo, Japan

(2) Canadian Forces Environmental Medicine Establishment, Toronto, Ontario, Canada

(3) Radiation, Chemical and Environmental Hazards Division, UK Health Security Agency, United Kingdom.

Environmental Research Group within the School of Public Health, Faculty of Medicine at Imperial College of Science, Technology and Medicine, London, UK.

Status

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Abstract

An AOP was developed describing a simplified path from “deposition of energy” (MIE;KE#1686) to cataracts (AO; KE#2083). The AOP is initiated by deposition of energy resulting in oxidative stress (KE#1392) within cells from

increased free radical generation. If this exceeds antioxidant defence mechanisms, the oxidative stress, in turn, can damage molecules, the most well-studied include DNA and proteins. Within the lens of the eye modified proteins (KE#2081) can aggregate, such as crystalline, and if not eliminated, can accumulate resulting in lens opacity. Concurrently, unmanaged oxidative stress can increase oxidative DNA damage (KE#1634) leading to DNA strand breaks (KE#1635). If these lesions are inadequately repaired (KE#155), an increase mutation frequency (KE#185) in critical genes and chromosomal aberrations (KE#1636) can occur. Mutations in genes associated with cell cycling can lead to uncontrolled cell proliferation (KE#870) of lens epithelial cells and the eventual AO, cataracts. The overall assessment of this AOP indicates high biological plausibility of the KERs as they are well established and understood; moderate levels of evidence support the essentiality and the Bradford-Hill empirical evidence criteria; low weight of evidence was identified for quantitative understanding across adjacent relationships, with some uncertainties and inconsistencies in mechanisms. Broadly, the information presented in this AOP can be used to support the review of radiation effects classification and broadly the system of radiological protection.

Background

Cataracts, one of the leading causes of blindness, are a progressive condition in which the lens of the eye develops opacities and becomes cloudy, resulting in blurred vision as well as glare and haloes around lights (National Eye Institute, 2022). For this AOP, a cataract is defined when over 5% of the lens is opacified. Cataracts typically occur after the age of 50 in humans, as an age-related disease (Liu et al., 2017); however, progression of this disease can be initiated or accelerated after exposure to a variety of agents, one of which is radiation.

For radiation induced cataracts, most research shows that the anatomical location is within the posterior sub capsular region of the eye with limited occurrence in the cortical and nuclear region. Available epidemiological evidence confirms a positive statistically significant association between radiation exposure and cataracts (Nakashima et al., 2006; Worgul et al., 2007; Chylack et al., 2012; Little et al., 2018). The data comes from Chernobyl workers, radiologic technologists, and patients exposed to radiation through medical procedures, with the most compelling evidence derived from atomic bomb survivors. Although there is concern for the role of long duration space flight missions in cataract formation, there is limited data from astronauts.

In 2012, the International Commission on Radiological Protection (ICRP) recommended lowering the occupational eye lens dose limit from 150 mSv per year to an average of 20 mSv, with no single year exceeding 50 mSv. This revision was based on new evidence from both radiobiological studies and relevant epidemiological data. Assessment of the literature indicated a threshold dose for radiation induced cataracts of about 0.5 Gy (ICRP, 2012). This change in exposure limit has led to a need to further understand radiation-induced effects at lower doses and dose-rates. It is believed the progression of cataracts at high doses and s higher dose-rates generally induce more damage than lower dose-rates (Brooks et al., 2016).

This AOP provides a summary of the relevant studies and endpoints that can inform future research designed to understand the role of radiation in causing cataracts. Assays and study designs spanning biological levels of organization across relevant models were identified, with the end goal to improve testing strategies and understanding in risk from low dose low dose-rate exposures.

Summary of the AOP

Events

Molecular Initiating Events (MIE), Key Events (KE), Adverse Outcomes (AO)

Sequence	Type	Event ID	Title	Short name
	MIE	1686	Deposition of Energy	Energy Deposition
	KE	2081	Increased Modified Proteins	Modified Proteins
	KE	1634	Increase, Oxidative DNA damage	Increase, Oxidative DNA damage
	KE	1635	Increase, DNA strand breaks	Increase, DNA strand breaks
	KE	155	Inadequate DNA repair	Inadequate DNA repair
	KE	185	Increase, Mutations	Increase, Mutations
	KE	1636	Increase, Chromosomal aberrations	Increase, Chromosomal aberrations
	KE	870	Increase, Cell Proliferation	Increase, Cell Proliferation
	KE	1392	Oxidative Stress	Oxidative Stress
	AO	2083	Occurrence of Cataracts	Cataracts

Key Event Relationships

Upstream Event	Relationship Type	Downstream Event	Evidence	Quantitative Understanding
Deposition of Energy	adjacent	Increase, DNA strand breaks	High	High
Deposition of Energy	adjacent	Oxidative Stress	High	High
Deposition of Energy	adjacent	Increased Modified Proteins	Moderate	Moderate
Oxidative Stress	adjacent	Increase, Oxidative DNA damage	Moderate	Low
Oxidative Stress	adjacent	Increase, DNA strand breaks	Moderate	Low
Oxidative Stress	adjacent	Increased Modified Proteins	Moderate	Low
Increase, Oxidative DNA damage	adjacent	Inadequate DNA repair	Moderate	Low
Increase, DNA strand breaks	adjacent	Inadequate DNA repair	Moderate	Moderate
Inadequate DNA repair	adjacent	Increase, Mutations	High	Low
Inadequate DNA repair	adjacent	Increase, Chromosomal aberrations	Low	Low
Increase, Mutations	adjacent	Increase, Cell Proliferation	Moderate	Low
Increase, Chromosomal aberrations	adjacent	Increase, Cell Proliferation	Moderate	Low
Increased Modified Proteins	adjacent	Occurrence of Cataracts	Moderate	Low
Increase, Cell Proliferation	adjacent	Occurrence of Cataracts	Moderate	Low
Increase, Oxidative DNA damage	adjacent	Increase, DNA strand breaks	Low	Low
Deposition of Energy	non-adjacent	Increase, Oxidative DNA damage	Moderate	Moderate
Deposition of Energy	non-adjacent	Increase, Mutations	High	High
Deposition of Energy	non-adjacent	Increase, Chromosomal aberrations	High	High
Deposition of Energy	non-adjacent	Increase, Cell Proliferation	Moderate	Moderate
Deposition of Energy	non-adjacent	Occurrence of Cataracts	High	High
Inadequate DNA repair	non-adjacent	Occurrence of Cataracts	Low	Low
Oxidative Stress	non-adjacent	Occurrence of Cataracts	Moderate	Low

Stressors

Name	Evidence
Ionizing Radiation	

Overall Assessment of the AOP

Summary of evidence (KE & KER relationships and evidence)

This assessment provides an overview of the pathway. Further details and references can be found in the individual KEs and KERs and within the AOP report.

Biological Plausibility

This AOP begins with an MIE (deposition of energy) and then branches to cataract formation either from modified proteins or through DNA damage processes. Ionization events from deposition of energy interact directly or indirectly with the DNA. Indirect damage can also occur when water molecules dissociate producing radicals such as reactive oxygen species (ROS) that induce DNA breaks (Ahmadi et al., 2022). Moreover, a cascade of ionization events can cause the formation of clustered damage (Joiner and van der Kogel, 2009). Many studies use ultraviolet radiation as a stressor, and it is important to note that ionizing and non-ionizing radiation interact through different mechanisms when inducing cataracts. Ionizing radiation can remove tightly bound electrons from atoms to create charged particles, but also excite molecules without ionization. The absorption of non-ionizing radiation results in heat

generation from molecular vibrations (Alcócer et al., 2020).

Deposition of energy can also lead to high levels of ROS and reactive nitrogen species (RNS) (collectively RONS) (Tangvarasittichai & Tangvarasittichai, 2019). There are several pathways leading to ROS, but radiolysis is the most prominent. Free radicals can combine to produce hydrogen peroxide, hydroxide, superoxide, and hydroxyl (Tian et al., 2017; Venkatesulu et al., 2018). Interactions with NO can also lead to RNS (Wang et al., 2019). Additionally, deposited energy can directly upregulate enzymes involved in reactive RONS production (de Jager et al., 2017). Activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX) within mitochondria can generate more ROS (Soloviev & Kizub, 2019). Energy absorption by an unstable molecule, such as the chromophore NADPH (Jurja et al., 2014), is another route for radical production. Overwhelming amounts of free radicals can decrease antioxidant levels, causing oxidative stress (Wang et al., 2019).

Protein damage leading to cataracts

Alongside DNA as a target to energy deposition, other macromolecules can be damaged. In terms of cataracts, there is much evidence to show that protein modifications such as phosphorylation, deamidation, oxidation, disulfide bonds (Hanson et al., 2000), increased cross-linking, altered water-solubility, and increased protein aggregation are critical to disease progression (Fochler & Durchschlag, 1997; Reisz et al., 2014; Wang et al. 2020; Chandrasekher et al., 2004). ROS can also cause many alterations including conformational changes, protein cross-link formation, oxidation of amino acid side chains (Uwineza et al., 2019), and protein aggregation (Moreau et al., 2012). For example, alpha crystallin aggregation can be induced by free radicals oxidizing the thiol groups (Cabrera & Chihuailaf, 2011; Moreau & King 2012; Stohs, 1995). Finally, these modified proteins can lead directly to cataracts, the AO. Aggregated proteins cause improper lens epithelial cell (LEC) organization and increases light scattering, therefore resulting in lens opacity (Hamada et al., 2014). Additionally, modifications to connexin protein can lead to improper LEC layering, which has been linked to cataracts in humans (NCRP, 2016).

DNA damage leading to cataracts

Oxidative stress is also directly connected to increased DNA strand breaks. Cells under oxidative stress have excessive levels of ROS, molecules that can oxidize and remove nitrogenous bases, producing nicks in the DNA strand known as single strand breaks (SSB). Under circumstances when multiple SSBs are in close proximity, they may combine to form double strand breaks (DSB). Furthermore, these strand breaks and a combination of various DNA abnormalities occurring in close proximity can create complex lesions that are more difficult to repair (Nickoloff et al., 2020). The formation of SSBs induces base excision repair (BER), a DNA repair mechanism; however, cells are often unable to support multiple sites of repair in one area, leading to residual unrepaired SSBs that will increase the number of DSBs. It has been shown that radiation-generated ROS are more likely to produce clustered damage (Cannan & Pederson, 2016). Increased oxidative stress can also lead to increased oxidative DNA damage. In this case, ROS can induce DNA lesions, such as oxidized nucleotides or DNA breaks (Collins, 2014).

DNA strand breaks can lead to inadequate DNA repair. DSBs, the most detrimental form of this damage (Iliakis et al., 2015), are often formed in the G1 phase of the cell cycle. Cells utilize various systems to repair DNA damage, the most error-prone pathway being non-homologous end-joining (NHEJ). Since NHEJ is an active pathway for DNA DSB repair in the G1 phase of the cell cycle, DSBs are repaired using this pathway, leading to decreased repair accuracy (Jeggo et al., 1998). Although NHEJ is predominantly the preferred repair mechanism throughout the cell cycle, homologous recombination (HR) and single-stranded annealing (SSA) are favored during the S and G2 phases in scenarios where the NHEJ repair pathway is inhibited. The absence of HR leading to an increase in SSA activity is still a matter to debate (Ceccaldi et al., 2016). Furthermore, clustered damage generated by high linear energy transfer radiation (Nikitaki et al., 2016), overwhelms the repair systems, leading to increased probability of inadequate repair (Tsao, 2007).

Similarly, increased oxidative damage to DNA can also lead to inadequate repair. Repair systems are unable to deal with increased levels of lesions within a small area, resulting in decreased repair ability and therefore, inadequate repair (Georgakilas et al., 2013). Moreover, unrepaired oxidative lesions may be incorrectly bypassed during DNA replication, leading to the insertion of incorrect bases opposite unrepaired lesions (Shah et al., 2018). Imbalances between the level of oxidative DNA lesions and cellular repair capacity can also lead to inadequate repair (Brenerman et al., 2014). Non-DSB oxidative DNA damage can alter nuclease or glycosylase activity, resulting in decreased local DNA repair ability (Georgakilas et al., 2013).

One of the possible outcomes of inadequate repair is increased mutations. DNA repair mechanisms, such as NHEJ (Sishc & Davis, 2017), break-induced replication (BIR), and microhomology-mediated break-induced replication (MMBIR) can be error-prone, leading to increased mutagenesis and genomic instability (Kramara et al., 2018).

Inadequate repair can also lead to increased chromosomal aberrations (CA). The best-known model for this KER holds that unrepaired DSBs eventually lead to CAs (Schipler & Iliakis, 2013). Alternate models suggests that CAs occur when the enzymes responsible for binding DNA strands during the repair of enzyme-induced DNA breaks dysfunctions. Failure of different binding enzymes would lead to different forms of CAs (Bignold, 2009).

Increased mutations and increased CAs are both linked to increased cell proliferation; however, as no lens-specific data was found, the existing relationships in the Wiki (KER: 1978 and 1979) have not been altered. This presents a possible focus for future research.

Finally, increased cell proliferation of the metabolically active LECs can lead to cataracts. The lens is composed of several zones, with the germinative zone (GZ) being the only one that is mitotically active. In healthy lenses, cells in the GZ replicate and differentiate into lens fiber cells (LFCs). The LFCs are organelle-free, allowing light to pass

through the lens. However, in cases of increased proliferation, cells are forced out of the GZ before forming fully differentiated LFCs. These improperly differentiated cells have not lost all of their organelles, resulting in reduced lens transparency (Ainsbury et al., 2016; Hamada, 2017; McCarron et al., 2022). As the lens is a closed system with little turnover, these cells are not removed, and their accumulation contributes to the cataractogenic load, a gradual lens opacification throughout life, which can eventually lead to cataracts (Ainsbury et al., 2016; Uwineza et al., 2019).

Time- and dose- and incidence-concordance

Overall evidence of time- and dose-concordance is moderate to low throughout the AOP. Certain relationships, particularly those directly connected with deposition of energy, are well supported with measurable changes in expression in a temporal and dose concordant manner. However, KEs at the cellular and organ level are generally supported by a weaker WOE with inconsistencies. The use of different models, time-points and radiation types across studies may be the reason for inconsistencies.

Evidence of time concordance is demonstrated by the occurrence of upstream KEs at earlier timepoints than the downstream KEs. Time concordance involving deposition of energy is well supported by studies showing the deposition of energy followed by downstream changes later in a time course. Studies using in vitro and in vivo models have found downstream effects occurring within minutes to years of the MIE. For example, oxidative stress in human LECs can occur within an hour following 0.25 Gy γ-rays exposure (Ahmadi et al., 2022), while it may take months to years for cataract development under radiotherapy or space radiation exposure (Gragoudas et al., 1995; Cucinotta et al., 2001). However, cellular and organ level events are not well-studied to demonstrate consistent time concordance.

Studies in the AOP provide evidence of upstream KEs observed at the same doses or lower doses as the downstream KEs. The KERs directly involving the deposition of energy contain the most evidence for dose concordance. Downstream KERs have limited support for dose concordance in a lens model but are supported with evidence from other cell types.

A few studies demonstrate greater changes produced by the upstream KE than the downstream KE following a stressor (incidence concordance). One KER showing incidence concordance is oxidative stress to DNA strand breaks. For example, there was a 5x increase of DNA strand breaks, while only a ~1.4-fold increase in oxidative stress marker in human LECs (Liu et al., 2013a).

Domain of Applicability

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	High	NCBI
mouse	Mus musculus	High	NCBI
rat	Rattus norvegicus	High	NCBI
rhesus monkeys	Macaca mulatta	Moderate	NCBI
rabbit	Oryctolagus cuniculus	Moderate	NCBI
guinea pig	Cavia porcellus	Moderate	NCBI

Sex Applicability

Sex	Evidence
Male	High
Female	High

Overall, this AOP is applicable to all organisms with DNA that require a clear lens for vision. Of these, Homo sapiens (humans), Mus musculus (mice), and Rattus norvegicus (rats) had a moderate level of support, and Oryctolagus cuniculus (rabbits) had a low level of support throughout most of the pathway. However, portions of the pathway were also supported in Bos taurus (bovine), Sus scrofa (pigs), Cavia porcellus (guinea pigs), Sciurus linnaeus (squirrels), Macaca mulatta (monkeys) and Anura (frogs).

This AOP is also applicable to all life stages, with a moderate level of support. However, it should also be noted that cataracts are primarily an age-related disease, generally occurring in humans after the age of 50 (Liu et al., 2017). As such, older organisms are at a higher risk of radiation-induced cataracts, as a gradual opacification of the lens may have already begun.

Essentiality of the Key Events

The present AOP encompasses several notable uncertainties.

1. There is no objective, universally acknowledged, definition for cataracts. A large variety of cataract scoring systems are used, with the major ones being the Lens Opacities Classification System I, II, and III (LOC I, II, and III), and the Merriam-Focht Cataract Scoring System. However, they are all subjective, relying partly on the examiner’s judgement.

2. Many studies do not directly measure cataracts, instead measuring indirect indicators, such as minor opacities, that do not always progress into cataracts.

3. Observation periods used in many studies may be too short to account for cataract development, leading to an apparent decrease in cataract prevalence.

4. Certain KERs, such as increased oxidative stress to increased oxidative DNA damage, increased oxidative stress to increased DNA strand breaks, increased oxidative stress to modified proteins, modified proteins to cataracts, inadequate DNA repair to cataracts, increased oxidative stress to cataracts, and deposition of energy to increased cell proliferation are only weakly supported by empirical evidence.

5. KERs describing increased oxidative DNA damage to inadequate DNA repair, inadequate DNA repair to increased mutations, inadequate DNA repair to increased chromosomal aberrations, and increased oxidative DNA damage to increased DNA strand breaks, while supported by non-lens evidence, are not supported by lens-based studies.

6. The use of different assays to assess KEs can result in diverse quantitative interpretations of data.

Essentiality of the Key Events

Essentiality of the Deposition of Energy (MIE#1686)

- Radiation exposure increases levels of DNA strand breaks (Reddy et al., 1998; Barnard et al., 2019; Barnard et al., 2021), modified proteins (Zigman et al., 1975; Abdelkawi et al., 2008; Anbaraki et al., 2016), oxidative stress (Zigman et al., 1995; Zigman et al., 2000; Kubo et al., 2010; Ahmadi et al., 2022), oxidative DNA damage (Pendergrass et al., 2010; Bahia et al., 2018), chromosomal aberrations (Dalke et al., 2018; Bains et al., 2019; Udroiu et al., 2020), cell proliferation (Pirie & Drance, 1959; Markiewicz et al., 2015; Bahia et al., 2018), and cataracts (Worgul et al., 1993; Jones et al., 2007; Kocer et al., 2007) above background levels. Removing/reducing the amount of radiation decreases the amount of damage to macromolecules found within the cell.

Essentiality of Increased Oxidative Damage to DNA (KE#1634)

- Depletion of antioxidant removing enzymes reduces oxidative DNA damage and initiate adequate repair mechanisms (Mesa & Bassnett, 2013) and increases DNA breaks (Domijan et al., 2006).

Essentiality of Increased DNA Strand Breaks (KE#1635)

- It is difficult to demonstrate the essentiality of increased DNA strand breaks as there are no modulators that can alter the KE and show effects to inadequate repair. However, it has been indirectly demonstrated that knock-out of mechanism related to repair processes can lead to increased strand breaks.

Essentiality of Inadequate DNA Repair (KE#155)

- The essentiality of inadequate DNA repair can be assessed through knock-out studies examining the effect of altering important repair genes on downstream KEs. In this way, inadequate DNA repair has been found to be essential in increasing mutations (Perera et al., 2016), chromosomal aberrations (Wilhelm et al., 2014), and cataracts (Kleiman et al., 2007) above background levels. For example, cataracts are up to 90% more common in ATM mutant mice, which have decreased DNA repair, compared to wild type mice (Worgul et al., 2002).

Essentiality of Increased Mutations (KE#185)

- The essentiality of this KE has been assessed as part of other AOPs, as described in the overall assessment of AOP #272. However, there was no available data from eye lens models to demonstrate the essentiality of this KE and show the effects on downstream KEs.

Essentiality of Increased Chromosomal Aberrations (KE#1636)

- The essentiality of this KE has been assessed as part of other AOPs, as described in the overall assessment of AOP #272. However, there was no available data from eye lens models to demonstrate the essentiality of this KE and show the effects on downstream KEs.

Essentiality of Increased Cell Proliferation (KE#870)

- There is a moderate level of evidence supporting the essentiality of increased cell proliferation. Mice with decreased cell proliferation (Ptch1) have lower lens opacity compared to wild-type mice (McCarron et al., 2021) and vice versa (De Stefano et al., 2021).

Essentiality of Oxidative Stress (KE#1392)

- Oxidative stress causes an increase in levels of DNA strand breaks (Li et al., 1998; Liu et al., 2013b; Cencer et al., 2018; Ahmadi et al., 2022) and cataract indicators (Karslioğlu et al., 2005; Varma et al., 2011; Liu et al., 2013b; Qin et al., 2019) above background levels. Additionally, inhibition of oxidative stress reduces DNA strand breaks (Spector et al., 1997; Liu et al., 2013b) and cataract risk (Van Kuijk, 1991; Spector, 1995; Smith et al., 2016; Qin et al., 2019).

Essentiality of Modified Proteins (KE#2081)

- There is a low level of evidence supporting the essentiality of radiation in promoting modified proteins above a normal level. One study found that return of the lens protein solubility ratio to near control levels resulted in decreased lens opacity (Menard et al., 1986).

Weight of Evidence Summary

	Defining Question	**High**	**Moderate**	**Low**
1. Support for Biological Plausibility	Is there a mechanistic relationship between KEup and KEdown consistent with established biological knowledge?	Extensive understanding of the KER based on extensive previous documentation and broad acceptance.	KER is plausible based on analogy to accepted biological relationships, but scientific understanding is incomplete	Empirical support for association between KEs, but the structural or functional relationship between them is not understood.
MIE#1686→ KE#1635: Deposition of energy → Increase DNA strand breaks	**High** It is well established that deposition of energy can cause various types of DNA damage including SSBs and DSBs. Structural damage from the deposited energy can induce chemical modifications in the form of breaks to the phosphodiester backbone of both strands of the DNA. DSBs are also often formed by indirect interactions with radiation through water radiolysis and subsequent reactive oxygen species generation that can then damage the DNA.			
MIE#1686→ KE#2081: Deposition of Energy → Modified Proteins	**High** It is well established that the deposition of energy leads to protein modifications. Energy deposited into cells, results in proteins undergoing post-translational modifications. These modifications culminate into larger protein changes such as high molecular weight aggregates and water-insolubility.			
MIE#1686→ KE#1392: Deposition of Energy → Oxidative Stress	**High** When deposited energy reaches a cell it reacts with water and organic materials to produce free radicals such as ROS. If the ROS cannot be eliminated quickly and efficiently enough by the cell's defense system, oxidative stress ensues.			
KE#1392→ KE#1634: Oxidative Stress →Increase Oxidative DNA Damage	**High** There is a large amount of evidence supporting the mechanistic relationship between increased oxidative stress and increased oxidative DNA damage. ROS react with DNA, causing changes such as DNA-protein cross-links, inter and intra-strand links, tandem base lesions, single and double strand breaks, abasic sites, and oxidized bases. The most common and best-studied lesion is 8-oxodG.			
KE#1392→ KE#1635: Oxidative Stress → Increase, DNA Strand Breaks	**High** There is a strong understanding of the mechanistic relationship between increased oxidative stress leading to increased DNA strand breaks. ROS oxidize bases on the DNA strand, triggering base excision repair, which removes the altered bases. These altered bases are usually adenine and guanine, as they have the lowest oxidation potentials. When multiple bases in close proximity are removed, the repair efforts cause strain which can lead to strand breaks. Increased levels of ROS have also been linked to DNA strand fragmentation. Furthermore, decreased antioxidant levels have also been linked to increased DNA strand damage.			

KE#1392→ KE#2081: Oxidative Stress → Modified Proteins	**High** There is high evidence to support increased oxidative stress leading to modified proteins. Studies show that following increases in ROS, proteins undergo cross-linking, thiol group oxidation, increased disulfide bonds, and amino acid oxidation and carbonylation. The increased amount of inter-protein linkages leads to aggregation, insolubility, and reduced chaperone action.
KE#1634→ KE#155: Increase, Oxidative DNA Damage → Inadequate DNA Repair	**High** There is a risk of increased genomic instability and mutation potential associated with repairing the lesions. The high-risk area can become resistant to repair when non-DSB oxidative DNA damage results in altered nuclease or glycosylase activity. There are limited data from eye lens models to support this relationship. However, this KER has been assessed as part of other AOPs, as described in the overall assessment of AOP #296.
KE#1635→ KE#155: Increase DNA strand breaks → Inadequate DNA repair	**High** It is well recognized that almost all types of DNA lesions will result in recruitment of repair enzymes and factors to the site of damage, and the pathway involved in the repair of DSBs has been well-documented in a number of reviews, many of which also discuss the error-prone nature of DNA repair. Error-prone repair processes are particularly important when DSBs are biologically induced and repaired during V(D)J recombination of developing lymphocytes and during meiotic divisions to generate gametes.
KE#155→ KE#185: Inadequate DNA Repair → Increase, Mutations	**High** This KER has been assessed as part of other AOPs, as described in the overall assessment of AOP #272. However, there was no available data from eye lens models to support this relationship.
KE#155→ KE#1636: Inadequate DNA Repair → Increase, Chromosomal Aberrations	**High** There is low support for the biological plausibility of this relationship in lens cells; however, the relationship is well supported in other cell types. One of the repair mechanisms most commonly used for DSBs is NHEJ, which is error-prone and can lead to CAs.
MIE#1686→ KE#1634: Deposition of energy → Increase oxidative DNA damage	**High** A large body of evidence supports the biological plausibility of this KER. The deposition of energy produces ROS, which then overwhelms the cell's defense mechanisms and induces a state of oxidative stress, leading to increases in oxidative DNA damage. For energy such as ultraviolet (UV), a form of electromagnetic radiation, this process occurs through the MAPK pathway.
MIE#1686→ KE#1636: Deposition of energy → Increase chromosomal aberrations	**High** Extensive and diverse data from human, animal and*in vitro*-based studies show ionizing radiation induces a rich variety of chromosomal aberrations. The mechanism leading from deposition of energy to chromosomal aberrations has been described in several reviews. Other evidence is derived from studies examining the mechanism of copy number variant formation and induction of radiation-induced chromothripsis.
MIE#1686→ KE#870: Deposition of Energy → Increase, Cell Proliferation	**Moderate** There is moderate available information to support the mechanistic relationship between energy deposition to increase cell proliferation. Energy deposited onto cells causes increased cell proliferation via the combined efforts of oncogene activation, tumor suppressor deactivation, and upregulated signaling pathways.
MIE#1686→ KE#2083: Deposition of Energy → Cataracts	**High** It is well understood that the deposition of radiation energy leads to cataract development. It has been clearly shown that radiation affects lenses structurally. These structural changes can be characterized by the measurement of lens opacification. Opacification may be the result of uncontrolled cell proliferation due to overwhelming DNA damages and conformational alteration in lens crystallin proteins. However, the effect of radiation on the functionality of lenses is uncertain, since adverse effects of opacification on vision are largely dependent on the proportion and location of the opacification. Whether minor opacification progress into vision-impairing cataracts is also uncertain.
KE#2081→ KE#2083: Modified Proteins → Cataracts	**High** It is well understood that the alteration of proteins leads to the development of cataracts/increased lens opacity. Changes in protein confirmation leads to aggregation, altering the ability of light to pass to the lens and leading to opaque regions within the eye. Protein alterations also result in the loss of protein functionality, which prevents repair and causes structural disorganization of lens proteins and loss of transparency and eventual cataracts.

KE#155→ KE#2083: Inadequate DNA Repair → Cataracts	**Moderate** There is moderate evidence to support inadequate DNA repair leading to the development of cataracts. Poor DNA repair leads to aberrant lens fiber cell differentiation, contributing to light scattering and cataracts.
KE#1392→ KE#2083: Oxidative stress → Cataracts	**High** There is a large amount of evidence for the biological plausibility of increases in oxidative stress leading to cataracts. This includes various different pathways such as protein oxidation, lipid peroxidation, increased calcium levels, DNA damage, apoptosis, and gap junction damage. The best-studied pathway, through increased protein oxidation, results in increased protein cross-linking, leading to decreased protein solubility, increased protein aggregation, increased light scattering, and therefore increased lens opacity and cataract occurrence.
KE#870→ KE#2083: Increase, Cell Proliferation → Cataracts	**Moderate** There is biological plausibility support for the relation between increased cell proliferation and cataracts. Since the lens is a closed system with little turnover, the increased proliferation of the metabolically active LECs can result in cataracts. Gradual lens opacification and eventual cataract development can result from the improperly differentiated and proliferating cells that are not removed from the system.
KE#1634→ KE#1635: Increase, Oxidative DNA Damage → Increase, DNA Strand Breaks	**Moderate** There is moderate support for the biological plausibility of this relationship, the mechanism is generally understood. Findings include guanine and adenine being the most likely bases to be damaged, and clustered oxidized bases raise the risk of strand breaks.

2. Support for Essentiality of KEs	**Defining Question**	**High**	**Moderate**	**Low**
	Are downstream KEs and/or the AO prevented if an upstream KE is blocked?	Direct evidence from specifically designed experimental studies illustrating essentiality for at least one of the important KEs	Indirect evidence that sufficient modification of an expected modulating factor attenuates or augments a KE	No or contradictory experimental evidence of the essentiality of any of the KEs.

MIE#1686: Deposition of energy	**High** Radiation exposure has been found to increase levels of DNA strand breaks, modified proteins, oxidative stress, oxidative DNA damage, chromosomal aberrations, cell proliferation, and cataracts above background levels. Removing the amount of radiation decreases the amount of damage to macromolecules found within the cell.
KE#1634: Increase, oxidative damage to DNA	**Moderate** Depletion of antioxidant removing enzymes can reduce oxidative DNA damage and initiate adequate repair mechanisms and increased DNA breaks.
KE#1635: Increase, DNA strand breaks	**Low** The essentiality of increased DNA strand breaks is difficult to demonstrate as there are no modulators that can alter the KE and show effects to inadequate repair. However, indirectly, it has been shown that knock-out of mechanism related to repair processes can lead to increased strand breaks.
KE#155: Inadequate DNA repair	**High** The essentiality of inadequate DNA repair can be assessed through knock-out studies examining the effect of altering important repair genes on downstream KEs. In this way, inadequate DNA repair has been found to be essential in increasing mutations, chromosomal aberrations, and cataracts above background levels.
KE#870: Increase, cell proliferation	**Moderate** There is a moderate level of evidence supporting the essentiality of increased cell proliferation leading to cataracts. Under homeostatic conditions, cells duplicate at a rate set by the speed of the cell cycle. Any disruption in regulators of the cell cycle can result in cellular transformation. Cell proliferation rates can be altered via deposited energy-induced genetic alterations, signaling pathway activation, and increased production of growth factors.
KE#1392: Oxidative stress	**Moderate** Oxidative stress increases levels of DNA strand breaks above background levels. Inhibition of oxidative stress through the use of antioxidants reduces DNA strand breaks.

KE#2081: Modified proteins	**Low** There is a low level of evidence supporting the essentiality of radiation in promoting modified proteins above a normal level.

	Defining Question	**High**	**Moderate**	**Low**
3. Empirical Support for KERs	Does empirical evidence support that a change in KEup leads to an appropriate change in KEdown? Does KEup occur at lower doses and earlier time points than KE down and is the incidence of KEup> than that for KEdown? Inconsistencies?	Multiple studies showing dependent change in both events following exposure to a wide range of specific stressors. No or few critical data gaps or conflicting data	Demonstrated dependent change in both events following exposure to a small number of stressors. Some inconsistencies with expected pattern that can be explained by various factors.	Limited or no studies reporting dependent change in both events following exposure to a specific stressor; and/or significant inconsistencies in empirical support across taxa and species that don't align with hypothesized AOP

MIE#1686→ KE#1635: Deposition of energy → Increase DNA strand breaks	**High** There is a high level of empirical evidence to support the relationship between energy deposition and increased DNA strand breaks. The evidence collected to support this relationship was gathered from various *in vitro* and *in vivo* studies. Various stressors were applied, including X-rays, gamma rays, protons and photons. The studies supported a dose and time concordance between the deposition of energy and DNA strand breaks.
MIE#1686→ KE#2081: Deposition of Energy → Modified Proteins	**Low** There are a number of studies to support a dose response between energy deposition and protein modification, but no time response data. Evidence suggests that *in vitro* and *in vivo* model exposure to higher (>2 Gy) doses and long UV exposures can initiate protein modification.
MIE#1686→ KE#1392: Deposition of Energy → Oxidative Stress	**High** There is a large body of evidence supporting a time and dose relationship from the deposition of energy to oxidative stress. Various studies using *in vitro* and *in vivo* rat, mice, rabbit, squirrel, bovine and human models provided evidence for this KER. A wide range of stressors were applied, including UV light (UV-B and UV-A) and ionizing radiation (gamma rays, X-rays, protons, photons, neutrons, and heavy ions). A dose-dependent increase in oxidative stress was observed in studies that examined a range of ionizing radiation doses (0-10 Gy).
KE#1392→ KE#1634: Oxidative Stress →Increase Oxidative DNA Damage	**Low** There is very limited evidence supporting time and dose concordance for this KER.*In vivo* rodent studies informed a dose concordance following O_2 exposure as a stressor and a time concordance following 11 Gy X-rays.
KE#1392→ KE#1635: Oxidative Stress → Increase, DNA Strand Breaks	**Moderate** There is evidence supporting the dose and incidence concordance of this relationship. There is limited evidence to support a time concordance. A limited variety of stressors are used as evidence supporting this KER. Most studies informing this relationship come from *in vitro* human models.

KE#1392→ KE#2081: Oxidative Stress → Modified Proteins	**Low** Limited evidence supports dose and incidence concordance for this relationship. No evidence found supporting time concordance. Stressor types are limited to UVA radiation or H_2O_2 exposure.
KE#1634→ KE#155: Increase, Oxidative DNA Damage → Inadequate DNA Repair	**Moderate** There is limited available data from eye lens models to support this relationship This KER has been assessed as part of other AOPs, as described in the overall assessment of AOP #296.
KE#1635→ KE#155: Increase DNA strand breaks → Inadequate DNA repair	**Moderate** There is limited available data from eye lens models to support this relationship. This KER has been assessed as part of other AOPs, as described in the overall assessment of AOP #272.
KE#155→ KE#185: Inadequate DNA Repair → Increase, Mutations	**Moderate** This KER has been assessed as part of other AOPs, as described in the overall assessment of AOP #272. However, there was no available data from eye lens models to support this relationship.
KE#155→ KE#1636: Inadequate DNA Repair → Increase, Chromosomal Aberrations	**Moderate** This KER has been assessed as part of other AOPs, as described in the overall assessment of AOP #296. However, there was no available data from eye lens models to support this relationship.
MIE#1686→ KE#1634: Deposition of energy → Increase oxidative DNA damage	**Low** There is limited evidence to support this KER. No incidence concordance evidence is available. Low variety of stressors (X-rays and UVB) inform this relationship. *In vitro* human lens epithelial cells exposed to X-rays or UVB lead to a dose concordant increase in oxidative DNA damage. *In vivo* mice models exposed to X-rays showed a time concordant increase in oxidative DNA damage.
MIE#1686→ KE#1636: Deposition of energy → Increase chromosomal aberrations	**High** There is a high level of empirical evidence to support this KER. Various studies demonstrate dose and time concordance between the deposition of energy and increased frequency of chromosomal aberrations. The evidence collected to support this relationship was gathered from various *in vitro* and *in vivo* studies. Various stressors were applied, including X-rays, gamma rays, and heavy ions. Chromosomal aberrations were detected as early as 30 minutes post-irradiation.
MIE#1686→ KE#870: Deposition of Energy → Increase, Cell Proliferation	**High** There is high evidence to support dose and time concordance between energy deposition and cell proliferation, but no evidence for incidence concordance. Various *in vivo* and *in vitro* studies on rodents, rabbits, or human models inform this relationship. A variety of stressor types such as gamma rays, UV, or X-rays were used as evidence for this KER.
MIE#1686→ KE#2083: Deposition of Energy → Cataracts	**High** There is high evidence to support dose and time concordance between energy deposition and cataract development. Various *in vivo* and *in vitro* studies and a variety of stressor types inform this relationship.
KE#2081→ KE#2083: Modified Proteins → Cataracts	**Low** There is a small pool of evidence to support the time and incidence concordance between modified proteins and cataracts. Limited *in vivo* rodent studies and stressor types (gamma rays, X-rays) provide support for incidence and time concordance.
KE#155→ KE#2083: Inadequate DNA Repair → Cataracts	**Low** There is a low amount of empirical evidence to support the relationship. The only available studies involve mice genetically predisposed towards inadequate DNA repair. Time concordance is supported by the *in vivo* studies, but no evidence is available for dose and incidence concordance. Mice irradiated with X-rays show cataract development 1-3 weeks sooner than wild type animals.

KE#1392→ KE#2083: Oxidative stress → Cataracts	**Low** There is a limited amount of empirical support for this KER. No available studies support incidence concordance. Dose and time concordance are supported by a low amount of empirical evidence from *in vitro* and *in vivo* studies exposed to gamma rays or H_2O_2.
KE#870→ KE#2083: Cell Proliferation → Cataracts	**Low** There is no confident empirical evidence to accurately demonstrate a dependant relationship between the two events. Limited studies support time and dose concordance using relevant stressors and models.
KE#1634→ KE#1635: Increase, Oxidative DNA Damage → Increase, DNA Strand Breaks	**Moderate** This KER has been assessed as part of other AOPs, as described in the overall assessment of AOP #296. However, there was no available data from eye lens models to support this relationship.
KE#1636 → KE#870: Increase, chromosomal aberrations → Increase, Cell Proliferation	**Moderate** This KER has been assessed as part of other AOPs, as described in the overall assessment of AOP #272. However, there was no available data from eye lens models to support this relationship.
KE#185 → KE#870: Increase, Mutations → Increase, Cell Proliferation	**Moderate** This KER has been assessed as part of other AOPs, as described in the overall assessment of AOP #272. However, there was no available data from eye lens models to support this relationship.
MIE#1686→ KE#185: Deposition of energy → Increase, Mutations	**High** This KER has been assessed as part of other AOPs, as described in the overall assessment of AOP #272. However, there was no available data from eye lens models to support this relationship.

Quantitative Consideration

Quantitative understanding of the KERs in this AOP was rated as low. While certain KERs, such as MIE to AO, are well understood quantitatively with the literature, the understanding of other KERs is limited. For example, the quantitative understanding regarding the amount of DNA strand breaks and oxidative DNA lesions that would exceed cellular repair capacities to predict downstream effects require further investigation. Furthermore, studies often examined different endpoints at various time-points, using different stressors, doses, dose-rates, and models within each KER, causing difficulty in accurately comparing studies and deriving a quantitative understanding of the relationship, including precisely predicting the downstream KEs from the upstream KEs. As such, the areas with low quantitative understanding could be the focus of future experimental work using a more co-ordinated approach to experimental design, data collection and analysis. This would allow for more informative quantitative data that could be combined to understand the quantitative concordance of direct relationships and better support risk modeling and understanding of minimal risk dose estimates.

	Defining Question	High	Moderate	Low
Review of the				

Quantitative Understanding for each KER	To what extent can a change in KEdownstream be predicted from KEupstream? With what precision can the uncertainty in the prediction of KEdownstream be quantified? To what extent are the known modulating factors of feedback mechanisms accounted for? To what extent can the relationships described be reliably generalized across the applicability domain of the KER?	Change in KEdownstream can be precisely predicted based on a relevant measure of KEupstream; uncertainty in the quantitative prediction can be precisely estimated from the variability in the relevant KEupstream measure. Known modulating factors and feedback/feedforward mechanisms are accounted for in the quantitative description. Evidence that the quantitative relationship between the KEs generalizes across the relevant applicability domain of the KER.	Change in KEdownstream can be precisely predicted based on a relevant measure of KEupstream; uncertainty in the quantitative prediction is influenced by factors other than the variability in the relevant KEupstream measure. Quantitative description does not account for all known modulating factors and/or known feedback/feedforward mechanisms. The quantitative relationship has only been demonstrated for a subset of the overall applicability domain of the KER.	Only a qualitative or semi-quantitative prediction of the change in KEdown can be determined from a measure of KEup. Known modulating factors and feedback/feedforward mechanisms are not accounted for. Quantitative relationship has only been demonstrated for a narrow subset of the overall applicability domain of the KER.
MIE#1686→ KE#1635: Deposition of energy → Increase DNA strand breaks	**High** The vast majority of studies examining energy deposition and incidence of DSBs suggest a positive, linear relationship between these two events. Predicting the exact number of DSBs from the deposition of energy, however, appears to be highly dependent on the biological model, the type of radiation and the radiation dose range, as evidenced by the differing calculated DSB rates across studies.			
MIE#1686→ KE#2081: Deposition of Energy → Modified Proteins	**Moderate** There is a large amount of quantitative evidence supporting an increased amount of modified proteins following the deposition of energy; however, no trend emerged that could reliably predict the changes. There is a large variety of protein alterations that are possible and measurable. This makes finding connections between studies difficult, especially due to the wide range of doses used with inconsistencies as to the minimum dose needed to see effect.			
MIE#1686→ KE#1392: Deposition of Energy → Oxidative Stress	**High** There is a large body of evidence supporting a quantitative understanding of the change in the deposition of energy needed to produce a change in the level of elements of oxidative stress. Oxidative stress can be represented by several different endpoints, including catalase, glutathione (GSH), superoxide dismutase, glutathione peroxidase (GSH-Px), malondialdehyde (MDA), and ROS levels. Measurements have also been made over a large range of doses and dose rates			
KE#1392→ KE#1634: Oxidative Stress →Increase Oxidative DNA Damage	**Low** There are a small number of studies that provide quantitative evidence for this KER.			
KE#1392→ KE#1635: Oxidative Stress → Increase, DNA Strand Breaks	**Low** There is a considerable amount of evidence supporting dose concordance for an increased amount of DNA strand breaks following exposure to increased oxidative stress, however no trend has emerged that could reliably predict the changes. Measurements of oxidative stress are quite varied across studies. There is a clear association between the two events, positive changes in oxidative stress indicators increase DSB.			
KE#1392→ KE#2081: Oxidative Stress → Modified Proteins	**Low** There is a moderate amount of quantitative evidence supporting an increased the number of modified proteins following exposure to increased oxidative stress; however, no trend has emerged that could reliably predict the changes.			
KE#1634→ KE#155: Increase, Oxidative DNA Damage → Inadequate DNA Repair	**Low** This KER has been assessed as part of other AOPs, as described in the overall assessment of AOP #296. However, there was no available data from eye lens models to support this relationship.			

KE#1635→ KE#155: Increase DNA strand breaks → Inadequate DNA repair	**Moderate** According to studies examining DSBs and DNA repair after exposure to radiation, a positive linear relationship between DSBs and radiation dose has been observed, and a linear-quadratic relationship between the number of misrejoined DSBs and radiation dose which varied according to LET and dose-rate of the radiation. Overall, 1 Gy of radiation may induce between 35 and 70 DSBs, with 10 - 15% being misrepaired at 10 Gy and 50 - 60% being misrepaired at 80 Gy. This KER has been assessed as part of other AOPs, as described in the overall assessment of AOP #272.
KE#155→ KE#185: Inadequate DNA Repair → Increase, Mutations	**Moderate** This KER has been assessed as part of other AOPs, as described in the overall assessment of AOP #272. However, there was no available data from eye lens models to support this relationship.
KE#155→ KE#1636: Inadequate DNA Repair → Increase, Chromosomal Aberrations	**Low** This KER has been assessed as part of other AOPs, as described in the overall assessment of AOP #296. However, there was no available data from eye lens models to support this relationship.
MIE#1686→ KE#1634: Deposition of energy → Increase oxidative DNA damage	**Moderate** There is a moderate amount of quantitative understanding for this KER. The majority of the data investigates different indicators of oxidative DNA damage, namely 8-OH-DG, 8-OH G, cyclobutane pyrimidine dimers, and multiple chromophores such as NADH.
MIE#1686→ KE#1636: Deposition of energy → Increase chromosomal aberrations	**High** Most studies indicate a positive, linear-quadratic relationship between the deposition of energy by ionizing radiation and the frequency of chromosomal aberrations. Equations describing this relationship were provided in a number of studies. In terms of time scale predictions, this may still be difficult owing to the often-lengthy cell cultures required to assess chromosomal aberrations post-irradiation, as well as the potential inapplicability of long-term cultures in predicting events *in vivo*.
MIE#1686→ KE#870: Deposition of Energy → Increase, Cell Proliferation	**Moderate** There is a large amount of quantitative evidence supporting an increased amount of cell proliferation following the deposition of energy, however no trend can reliably predict the changes.
MIE#1686→ KE#2083: Deposition of Energy → Cataracts	**High** The levels of cataract prevalence and severity generally can be predicted quantitatively from the level of radiation exposure. Many studies show that cataract development is dose dependent. The prediction of cataract development can be made more reliably with higher-dose exposures than with lower-dose exposures. Low-dose exposures typically show long lag periods for the onset of cataractogenesis, that coupled with the short observation periods frequently used make the prediction of cataract severity or prevalence less reliable. There are many known modulating factors that influence cataract development such as quality and dose of the radiation, gender, age at exposure, and genetic predispositions. These factors all affect the onset timing, prevalence, and severity of cataract development. Radiation-induced cataracts have been observed consistently across several mammalian species.
KE#2081→ KE#2083: Modified Proteins → Cataracts	**Low** There is limited quantitative understanding of increased lens opacity/cataracts from protein alteration. Age is a known modulator of this relationship; protein aggregation increases naturally as the individual ages.
KE#155→ KE#2083: Inadequate DNA Repair → Cataracts	**Low** There is limited quantitative evidence supporting the development of cataracts following inadequate DNA repair, and as such, there is not enough information to observe a trend that could reliably predict the changes.
KE#1392→ KE#2083: Oxidative stress → Cataracts	**Low** There is limited quantitative understanding for this KER. Most of the data has been obtained using H_2O_2 to induce oxidative stress, and cataracts are assessed indirectly.

KE#870→ KE#2083: Cell Proliferation → Cataracts	**Low** The quantitative understanding of this KER is weak. There is no confident empirical evidence to accurately demonstrate a dependant relationship between the two events.
KE#1634→ KE#1635: Increase, Oxidative DNA Damage → Increase, DNA Strand Breaks	**Low** This KER has been assessed as part of other AOPs, as described in the overall assessment of AOP #296. However, there was no available data from eye lens models to support this relationship.
KE#1636 → KE#870: Increase, chromosomal aberrations → Increase, Cell Proliferation	**Low** This KER has been assessed as part of other AOPs, as described in the overall assessment of AOP #272. However, there was no available data from eye lens models to support this relationship.
KE#185 → KE#870: Increase, Mutations → Increase, Cell Proliferation	**Low** This KER has been assessed as part of other AOPs, as described in the overall assessment of AOP #272. However, there was no available data from eye lens models to support this relationship.
MIE#1686→ KE#185: Deposition of energy → Increase, Mutations	**High** This KER has been assessed as part of other AOPs, as described in the overall assessment of AOP #272. However, there was no available data from eye lens models to support this relationship.

Considerations for Potential Applications of the AOP (optional)

As the International Commission on Radiological Protection works to review literature on health effects from radiation exposure, the collected knowledge presented in this AOP will provide a structured approach to guide future recommendations. With better designed experiments that cross biological levels of organization, more informative quantitative data will be generated that can then inform risk assessment strategies. A stronger evidence base can provide better justification to support guidelines and standards for future space missions and settings related to occupational, environmental, and medical exposures, where cataracts are of concern.

References

Abdelkawi, S., M. Abo-Elmagd and H. Soliman. (2008), “Development of cataract and corneal opacity in mice due to radon exposure”, Radiation Effects and Defects in Solids, Vol.163/7, Taylor & Francis, Oxfordshire, https://doi.org/10.1080/10420150701249603.

Ahmadi, M. et al. (2022), “Early responses to low-dose ionizing radiation in cellular lens epithelial models”, Radiation Research, Vol. 197/1, Radiation Research Society, United States, https://doi.org/10.1667/RADE-20-00284.1

Ainsbury, E. A. et al. (2016), “Ionizing radiation induced cataracts: recent biological and mechanistic developments and perspectives for future research”, Mutation research. Reviews in mutation research, Vol. 770, Elsevier B.V., https://doi.org/10.1016/j.mrrev.2016.07.010

Anbaraki, A. et al. (2016), “Preventive role of lens antioxidant defense mechanism against riboflavin-mediated sunlight damaging of lens crystallin”, International Journal of Biological Macromolecules, Vol.91, Elsevier, Amsterdam, https://doi.org/10.1016/j.ijbiomac.2016.06.047.

Bahia, S. et al. (2018), “Oxidative and nitrative stress-related changes in human lens epithelial cells following exposure to X-rays”, International journal of radiation biology, Vol. 94/4, England, https://doi.org/10.1080/09553002.2018.1439194

Bains, S. K. et al. (2019), “Effects of ionizing radiation on telomere length and telomerase activity in cultured human lens epithelium cells”, International journal of radiation biology, Vol. 95/1, Taylor & Francis, Abingdon, https://doi.org/10.1080/09553002.2018.1466066

Barnard, S. et al. (2021), “Lens epithelial cell proliferation in response to ionizing radiation”, Radiation Research, Vol. 197/1, Radiation Research Society, https://doi.org/10.1667/RADE-20-00294.1

Barnard, S. G. R. et al. (2019), “Inverse dose-rate effect of ionising radiation on residual 53BP1 foci in the eye lens”, Scientific Reports, Vol. 9/1, Nature Publishing Group, England, https://doi.org/10.1038/s41598-019-46893-3

Bignold, L.P. (2009), "Mechanisms of clastogen-induced chromosomal aberrations : A critical review and description of a model based on failures of tethering of DNA strand ends to strand-breaking enzymes.", Mutation Research, Vol. 681/2-3, Elsevier, Amsterdam https://doi.org/10.1016/j.mrrev.2008.11.004.

Brenerman, B., J. Illuzzi, and D. Wilson III (2014), “Base excision repair capacity in informing healthspan”, Carcinogenesis, Vol. 35/12, Oxford Academic, https://doi.org/10.1093/carcin/bgu225.

Brooks, A. L., Hoel, D. G., Preston, R. J. (2016), “The role of dose rate in radiation cancer risk: evaluating the effect of dose rate at the molecular, cellular and tissue levels using key events in critical pathways following exposure to low LET radiation.” International journal of radiation biology, Vol. 92/8, Informa, London, https://doi.org/10.1080/09553002.2016.118630

Cabrera, M. and R. Chihuailaf. (2011), “Antioxidants and the integrity of ocular tissues”, Veterinary Medicine International, Vol. 2011, Hindawi Limited, London, https://doi.org/10.4061/2011/905153.

Cannan, W. and D. Pederson. (2016), “Mechanisms and consequences of double-strand DNA break formation in chromatin”, Journal of Cell Physiology, Vol.231/1, Wiley, Hoboken, https://doi.org/10.1002/jcp.25048.

Ceccaldi, R., Rondinelli B., D’Andrea A. D. (2016), “Repair Pathway Choices and Consequences at the Double-Strand Break.” Trends in cell biology, Vol. 26/1, Elsevier, Amsterdam, https://doi.org/10.1016/j.tcb.2015.07.009

Cencer, C. et al. (2018), “PARP-1/PAR Activity in Cultured Human Lens Epithelial Cells Exposed to Two Levels of UVB Light”, Photochemistry and Photobiology, Vol.94/1, Wiley-Blackwell, Hoboken, https://doi.org/10.1111/php.12814.

Chandrasekher, G., & Sailaja, D. (2004),. Alterations in lens protein tyrosine phosphorylation and phosphatidylinositol 3-kinase signaling during selenite cataract formation,. Current eye research, Vol.28(2),. https://doi.org/10.1076/ceyr.28.2.135.26232 https://doi.org/10.1076/ceyr.28.2.135.26232

Chylack, L.T. Jr et al. (2012), “NASCA Report 2: Longitudinal Study of Relationship of Exposure to Space Radiation and Risk of Lens Opacity”, Radiation Research, Vol.178/1, Radiation Research Society, Indianapolis, https://doi.org/10.1667/RR2876.1.

Collins, A. R. (2014), “Measuring oxidative damage to DNA and its repair with the comet assay”, Biochimica et biophysica acta. General subjects, Vol. 1840/2, Elsevier, Amsterdam, https://doi.org/10.1016/j.bbagen.2013.04.022

Cucinotta, F.A. et al. (2001), “Space Radiation and Cataracts in Astronauts”, Radiation Research, Vol.156/5 Pt 1, Radiation Research Society, Indianapolis, https://doi.org/10.1667/0033-7587(2001)156[0460:SRACIA]2.0.CO;2.

Dalke, C. et al. (2018), “Lifetime study in mice after acute low-dose ionizing radiation: a multifactorial study with special focus on cataract risk”, Radiation and environmental biophysics, Vol. 57/2, Springer Berlin Heidelberg, Berling/Heidelberg, https://doi.org/10.1007/s00411-017-0728-z.

de Jager, T. L., A. E. Cockrell, S. S. Du Plessis (2017), “Ultraviolet Light Induced Generation of Reactive Oxygen Species”, in: Ultraviolet Light in Human Health, Diseases and Environment, vol 996. Springer Cham, https://doi.org/10.1007/978-3-319-56017-5_2.

De Stefano, I. et al. (2021), “Contribution of genetic background to the radiation risk for cancer and non-cancer diseases in Ptch1+/- mice”, Radiation Research, Vol. 197/1, Radiation Research Society, https://doi.org/10.1667/RADE-20-00247.1

Domijan, A., Zeljezic, D., Kopjar, D., Peraica, M. (2006), “Standard and Fpg-modified comet assay in kidney cells of ochratoxin A- and fumonisin B(1)-treated rats”, Toxciology, Vol. 222/1-2, Elsevier, Amsterdam, https://doi.org/10.1016/j.tox.2006.01.024.

Fochler, C. and H. Durchschlag. (1997), “Investigation of irradiated eye-lens proteins by analytical ultracentrifugation and other techniques”, Progress in Colloid and Polymer Science, Vol.107, Springer, Berlin, https://doi.org/10.1007/BFb0118020.

Georgakilas, A. G et al. (2013), “Induction and repair of clustered DNA lesions: what do we know so far?”, Radiation Research, Vol. 180/1, The Radiation Research Society, United States, https://doi.org/10.1667/RR3041.1.

Gragoudas, E.S. et al. (1995), “Lens Changes after Proton Beam Irradiation for Uveal Melanoma”, American Journal of Ophthalmology, Vol.119/2, Elsevier, Amsterdam, https://doi.org/10.1016/S0002-9394(14)73868-1.

Hamada, N. (2014), “What Are the Intracellular Targets and Intratissue Target Cells for Radiation Effects?”, Radiation Research, Vol.181/1, Radiation Research Society, Indianapolis, https://doi.org/10.1667/RR13505.1.

Hamada, N. (2017), “Ionizing radiation sensitivity of the ocular lens and its dose rate dependence”, International journal of radiation biology, Vol. 93/10, Taylor & Francis, England, https://doi.org/10.1080/09553002.2016.1266407

Hanson, S. et al. (2000), “The major in vivo modifications of the human water-insoluble lens crystallins are disulfide bonds, deamidation, methionine oxidation and backbone cleavage”, Experimental Eye Research, Vol.71/2, Academic Press Inc, Cambridge, https://doi.org/10.1006/EXER.2000.0868.

ICRP (2012), “ICRP Publication #118: ICRP Statement on Tissue Reactions and Early and Late Effects of Radiation in Normal Tissues and Organs - Threshold Doses for Tissue Reactions in a Radiation Protection Context”, Annals of the

ICRP, Vol.41/1-2, Elsevier, Amsterdam, https://doi.org/10.1016/j.icrp.2012.02.001.

Iliakis, G., T. Murmann & A. Soni (2015), "Alternative end-joining repair pathways are the ultimate backup for abrogated classical non-homologous end-joining and homologous recombination repair: Implications for the formation of chromosome translocations", Mutation research. Genetic toxicology and environmental mutagenesis, Vol. 793, https://doi.org/10.1016/j.mrgentox.2015.07.001.

Jeggo, P.A. (1998) “DNA breakage and repair”, Advances in genetics, Vol. 38, Elsevier, Amsterdam, https://doi.org/10.1016/s0065-2660(08)60144-3

Joiner, M. and A. van der Kogel (2009), "Basic Clinical Radiobiology", 4th ed., CRC Press, https://doi.org/10.1201/b15450.

Jones, J.A. et al. (2007), “Cataract Formation Mechanisms and Risk in Aviation and Space Crews”, Aviation, Space and Environmental Medicine, Vol.78/4 Suppl, Aerospace Medical Association, A56-66.

Jurja, S. et al. (2014), “Ocular cells and light: harmony or conflict?”, Romanian Journal of Morphology & Embryology, Vol. 55/2, Romanian Academy Publishing House, Bucharest, pp. 257–261.

Karslioğlu, I. et al. (2005), “Radioprotective effects of melatonin on radiation-induced cataract”, Journal of radiation research, Vol. 46/2, The Japan Radiation Research Society, England, https://doi.org/10.1269/jrr.46.277

Kleiman, N. J. et al. (2007), “Mrad9 and Atm haplinsufficiency enhance spontaneous and X-ray-induced cataractogenesis in mice”, Radiation research, Vol. 168/5, Radiation Research Society, United States, https://doi.org/10.1667/rr1122.1

Kocer, I. et al. (2007), “The effect of L-carnitine in the prevention of ionizing radiation-induced cataracts: A rat model”, Graefe’s Archive for Clinical and Experimental Ophthalmology, Vol. 245/4, Springer, Germany, https://doi.org/10.1007/s00417-005-0097-1

Kozbenko, T. et al. (2022), “Deploying elements of scoping review methods for adverse outcome pathway development: a space travel case example”, International Journal of Radiation Biology, Vol. 98/12, Taylor & Francis, London, https://doi.org/10.1080/09553002.2022.2110306

Kramara, J., B. Osia, and A. Malkova (2018), "Break-Induced Replication: The Where, The Why, and The How", Trends Genet, Vol. 34/7, Elsevier, Amsterdam, https://doi.org/10.1016/j.tig.2018.04.002.

Kubo, E. et al. (2010), “Protein expression profiling of lens epithelial cells from Prdx6-depleted mice and their vulnerability to UV radiation exposure”, American Journal of Physiology, Vol. 298/2, American Physiological Society, Rockville, https://doi.org/10.1152/ajpcell.00336.2009.

Li, Y. et al. (1998), “Response of lens epithelial cells to hydrogen peroxide stress and the protective effect of caloric restriction”, Experimental Cell Research, Vol.239/2, Elsevier, Amsterdam, https://doi.org/10.1006/excr.1997.3870.

Little, M. P. et al. (2018), “Occupational radiation exposure and risk of cataract incidence in a cohort of US radiologic technologists”, European Journal of Epidemiology, Vol. 33/12, Springer, https://doi.org/10.1007/s10654-018-0435-3.

Liu, H. et al. (2013a), “Sulforaphane can protect lens cells against oxidative stress: Implications for cataract prevention”, Investigative Ophthalmology and Visual Science, Vol.54/8, Association for Research in Vision and Ophthalmology, Rockville, https://doi.org/10.1167/iovs.13-11664.

Liu B. et al. (2013b). “Computational methods for detecting copy number variations in cancer genome using next generation sequencing: principles and challenges”, Oncotarget. 4(11):1868-81. Doi: 10.18632/oncotarget.1537.

Liu, Y. et al. (2017), “Cataracts”, The Lancet (British edition), Vol.390/10094, Elsevier Ltd, England, https://doi.org/10.1016/S0140-6736(17)30544-5

Markiewicz, E. et al. (2015), “Nonlinear ionizing radiation-induced changes in eye lens cell proliferation, cyclin D1 expression and lens shape”, Open Biology, Vol.5/4, Royal Society, London, https://doi.org/10.1098/rsob.150011.

McCarron, R. et al. (2022), “Radiation-Induced Lens Opacity and Cataractogenesis: A Lifetime Study Using Mice of Varying Genetic Backgrounds”, Radiation Research, Vol.196, Radiation Research Society, Indianapolis, https://doi.org/10.1667/RADE-20-00266.1.

Menard, T. et al. (1986), “Radioprotection against cataract formation by WR-77913 in gamma-irradiated rats”, International Journal of Radiation Oncology Biology Physics, Vol.12, Elsevier, Amsterdam, https://doi.org/10.1016/0360-3016(86)90199-9.

Mesa, R. and S. Bassnett (2013), “UV-B induced DNA damage and repair in the mouse lens”, Investigative ophthalmology & visual science, Vol. 54/10, the Association for Research in Vision and Ophthalmology, United States, https://doi.org/10.1167/iovs.13-12644

Moreau, K. and J. King. (2012), “Protein misfolding and aggregation in cataract disease and prospects for prevention”, Trends in Molecular Medicine, Vol.18/5, Elsevier Ltd, London, https://doi.org/10.1016/j.molmed.2012.03.005.

Nakashima, E., K. Neriishi and A. Minamoto. (2006), “A Reanalysis of Atomic-Bomb Cataract Data, 2000-2002: A

Threshold Analysis", Health Physics, Vol.90/2, Lippincott Williams & Wilkins, Philadelphia, https://doi.org/10.1097/01.HP.0000175442.03596.63.

National Eye Institute (2022), Cataracts, https://www.nei.nih.gov/learn-about-eye-health/eye-conditions-and-diseases/cataracts (accessed November 29, 2022).

NCRP (2016), "Guidance on radiation dose limits for the lens of the eye", NCRP Commentary, Vol.26, National Council on Radiation Protection and Measurements Publications, Bethesda.

Nickoloff, J. A., Sharma N., and Taylor L. (2020) "Clustered DNA Double-Strand Breaks: Biological Effects and Relevance to Cancer Radiotherapy." Genes, Vol. 11/1, MDPI, Basel, https://doi.org/10.3390/genes11010099

Nikitaki, Z. et al. (2016), "Measurement of complex DNA damage induction and repair in human cellular systems after exposure to ionizing radiations of varying linear energy transfer (LET)", Free Radiacal Research, Vol. 50/sup1, Taylor & Francis, London, https://doi.org/10.1080/10715762.2016.1232484.

Pendergrass, W. et al. (2010), "X-ray induced cataract is preceded by LEC loss, and coincident with accumulation of cortical DNA, and ROS; similarities with age-related cataracts", Molecular Vision, Vol. 16, Emory University, Atlanta, pp. 1496-513.

Perera, D. et al. (2016), "Differential DNA repair underlies mutation hotspots at active promoters in cancer genomes", Nature, Vol. 532/7598, Springer Nature, https://doi.org/10.1038/nature17437.

Pirie, A. and S. M. Drance (1959), "Modification of X-ray damage to the lens by partial shielding", International Journal of Radiation Biology and Related Studies in Physics, Chemistry and Medicine, Vol. 1/3, Taylor & Francis, London, https://doi.org/10.1080/09553005914550391.

Qin, Z. et al. (2019), "Opacification of lentoid bodies derived from human induced pluripotent stem cells is accelerated by hydrogen peroxide and involves protein aggregation", Journal of cellular physiology, Vol. 234/12, Wiley Subscriptions, United States, https://doi.org/10.1002/jcp.28943.

Reddy, V. N. et al. (1998), "The effect of aqueous humor ascorbate on ultraviolet-B-induced DNA damage in lens epithelium", Investigative ophthalmology & visual science, Vol. 39/2, Arvo, pp. 344-350.

Reisz, J. et al. (2014), "Effects of ionizing radiation on biological molecules - mechanisms of damage and emerging methods of detection", Antioxidants and Redox Signaling, Vol.21(2), Mary Ann Liebert Inc, Larchmont, https://doi.org/10.1089/ars.2013.5489.

Schipler, A. & G. Iliakis (2013), "DNA double-strand – break complexity levels and their possible contributions to the probability for error-prone processing and repair pathway choice.", Nucleic Acids Research, Vol. 41/165, http://doi.org/10.1093/nar/gkt556.

Shah, A. et al. (2018), "Defective Base Excision Repair of Oxidative DNA Damage in Vascular Smooth Muscle Cells Promotes Atherosclerosis", Circulation, Vol. 138/14, https://doi.org/10.1161/CIRCULATIONAHA.117.033249.

Sishc, B.J. & A.J. Davis (2017), "The Role of the Core Non-Homologous End Joining Factors in Carcinogenesis and Cancer.", Cancers, Vol. 9/7, Basel, http://doi.org/10.3390/cancers9070081.

Smith, A. J. O. et al. (2016), "PARP-1 inhibition influences the oxidative stress response of the human lens", Redox biology, Vol. 8/C, Elsevier, Netherlands, https://doi.org/10.1016/j.redox.2016.03.003.

Soloviev, A. I. and I.V. Kizub (2019), "Mechanisms of vascular dysfunction evoked by ionizing radiation and possible targets for its pharmacological correction", Biochemical pharmacology, Vol. 159, Elsevier, Amsterdam, https://doi.org/10.1016/j.bcp.2018.11.019.

Spector, A. et al. (1997), "Microperoxidases catalytically degrade reactive oxygen species and may be anti-cataract agents", Experimental Eye Research, Vol.65/4, Academic Press Inc, Cambridge, https://doi.org/10.1006/exer.1997.0336.

Spector, A. (1995), "Oxidative stress-induced cataract: mechanism of action", The FASEB Journal, Vol.9/12, Federation of American Societies for Experimental Biology, Bethesda, https://doi.org/10.1096/fasebj.9.12.7672510.

Stohs, S. (1995), "The role of free radicals in toxicity and disease", Journal of Basic and Clinical Physiology and Pharmacology, Vol. 6/3-4, Walter de Gruyter GmbH, Berlin, pp. 205-228.

Tangvarasittichai, O and S. Tangvarasittichai (2019), "Oxidative stress, ocular disease, and diabetes retinopathy", Current Pharmaceutical Design, Vol. 24/40, Bentham Science Publishers, https://doi.org/10.2174/1381612825666190115121531.

Tian, Y. et al. (2017), "The Impact of Oxidative Stress on the Bone System in Response to the Space Special Environment", International Journal of Molecular Sciences, Vol. 18/10, Multidisciplinary Digital Publishing Institute, Basel, https://doi.org/10.3390/ijms18102132.

Tsao, D. et al. (2007), "Induction and processing of oxidative clustered DNA lesions in 56Fe-ion-irradiated human monocytes", Radiation Research, Vol.168/1, United States, https://doi.org/10.1667/RR0865.1

Tuieng, R. J., Cartmell, S. H., Kirwan, C. C., & Sherratt, M. J. (2021). The Effects of Ionising and Non-Ionising Electromagnetic Radiation on Extracellular Matrix Proteins, Cells, Vol. 10(11), MDPI, Basel, https://doi.org/10.3390/cells10113041

Udroiu, I. et al. (2020), “DNA damage in lens epithelial cells exposed to occupationally-relevant X-ray doses and role in cataract formation”, Scientific reports, Vol. 10/1, Nature Research, Berlin, https://doi.org/10.1038/s41598-020-78383-2

Uwineza, A. et al. (2019), “Cataractogenic load – A concept to study the contribution of ionizing radiation to accelerated aging in the eye lens”, Mutation Research - Reviews in Mutation Research, Vol.779, Elsevier, Amsterdam, https://doi.org/10.1016/j.mrrev.2019.02.004.

van Kuijk, F. J. (1991), “Effects of ultraviolet light on the eye: role of protective glasses”, Environmental health perspectives, Vol. 96, National Institute of Environmental Health Sciences, Unites States, https://doi.org/10.1289/ehp.9196177.

Varma, S. D., S. Kovtun and K. R. Hegde (2011), “Role of ultraviolet irradiation and oxidative stress in cataract formation – medical prevention by nutritional antioxidants and metabolic agonists”, Eye & contact lens, Vol. 37/4, Lippincott Willians & Wilkins Inc, United States, https://doi.org/10.1097/ICL.0b013e31821ec4f2.

Venkatesulu, B. P. et al. (2018), “Radiation-Induced Endothelial Vascular Injury: A Review of Possible Mechanisms”, JACC: Basic to Translational Science, Vol. 3/4, Elsevier, Amsterdam, https://doi.org/10.1016/j.jacbts.2018.01.014.

Wang, H. et al. (2019), “Radiation-induced heart disease: a review of classification, mechanism and prevention”, International Journal of Biological Sciences, Vol. 15/10, Ivyspring International Publisher, Sydney, https://doi.org/10.7150/ijbs.35460.

Wang, Z. et al. (2020). MDM2 phosphorylation mediates H2O2-induced lens epithelial cells apoptosis and age-related cataract,. Biochemical and biophysical research communications, Vol. (1), . https://doi.org/10.1016/j.bbrc.2020.05.060

Wilhelm, T. et al. (2014), "Spontaneous slow replication fork progression elicits mitosis alterations in homologous recombination-deficient mammalian cells.", Proceedings of the National Academy of Sciences of the United States of America, Vol. 111/2, https://doi.org/10.1073/pnas.1311520111.

Worgul, B. V et al. (2002), “Atm heterozygous mice are more sensitive to radiation-induced cataracts than are their wild-type counterparts,” Proceedings of the National Academy of Sciences, Vol. 99/15, National Academy of Sciences, https://doi.org/10.1073/pnas.162349699.

Worgul, B. V. et al. (1993), “Accelerated heavy particles and the lens VII: The cataractogenic potential of 450 MeV/amu iron ions”, Investigative Ophthalmology and Visual Science, Vol. 34/1, pp. 184–193.

Worgul, B. V. et al. (2007), “Cataracts among Chernobyl Clean-up Workers: Implications Regarding Permissible Eye Exposures”, Radiation Research, Vol.167/2, Radiation Research Society, Indianapolis, https://doi.org/10.1667/RR0298.1.

Zigman, S. et al. (1975), “The response of mouse ocular tissues to continuous near-UV light exposure”, Investigative Ophthalmology, Vol.14/September, Association for Research in Vision and Ophthalmology, Rockville, pp.710-713.

Zigman, S. et al. (1995), “Damage to cultured lens epithelial cells of squirrels and rabbits by UV-A (99.9%) plus UV-B (0.1%) radiation and alpha tocopherol protection”, Molecular and cellular biochemistry, Vol. 143, Springer, London, https://doi.org/10.1007/BF00925924

Zigman, S. et al. (2000), “Effects of intermittent UVA exposure on cultured lens epithelial cells”, Current Eye Research, Vol. 20/2, Informa UK Limited, London, https://doi.org/10.1076/0271-3683(200002)2021-DFT095

Appendix 1

List of MIEs in this AOP

Event: 1686: Deposition of Energy

Short Name: Energy Deposition

Key Event Component

Process	Object	Action
energy deposition event		increased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:272 - Deposition of energy leading to lung cancer	MolecularInitiatingEvent
Aop:432 - Deposition of Energy by Ionizing Radiation leading to Acute Myeloid Leukemia	MolecularInitiatingEvent
Aop:386 - Deposition of ionizing energy leading to population decline via inhibition of photosynthesis	MolecularInitiatingEvent
Aop:387 - Deposition of ionising energy leading to population decline via mitochondrial dysfunction	MolecularInitiatingEvent
Aop:388 - Deposition of ionising energy leading to population decline via programmed cell death	MolecularInitiatingEvent
Aop:435 - Deposition of ionising energy leads to population decline via pollen abnormal	MolecularInitiatingEvent
Aop:216 - Deposition of energy leading to population decline via DNA strand breaks and follicular atresia	MolecularInitiatingEvent
Aop:238 - Deposition of energy leading to population decline via DNA strand breaks and oocyte apoptosis	MolecularInitiatingEvent
Aop:311 - Deposition of energy leading to population decline via DNA oxidation and oocyte apoptosis	MolecularInitiatingEvent
Aop:299 - Deposition of energy leading to population decline via DNA oxidation and follicular atresia	MolecularInitiatingEvent
Aop:441 - Ionizing radiation-induced DNA damage leads to microcephaly via apoptosis and premature cell differentiation	MolecularInitiatingEvent
Aop:444 - Ionizing radiation leads to reduced reproduction in Eisenia fetida via reduced spermatogenesis and cocoon hatchability	MolecularInitiatingEvent
Aop:470 - Deposition of energy leads to abnormal vascular remodeling	MolecularInitiatingEvent
Aop:473 - Energy deposition from internalized Ra-226 decay lower oxygen binding capacity of hemocyanin	MolecularInitiatingEvent
Aop:478 - Deposition of energy leading to occurrence of cataracts	MolecularInitiatingEvent
Aop:482 - Deposition of energy leading to occurrence of bone loss	MolecularInitiatingEvent
Aop:483 - Deposition of Energy Leading to Learning and Memory Impairment	MolecularInitiatingEvent

Stressors

Name
Ionizing Radiation

Biological Context

Level of Biological Organization

Molecular

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	Moderate	NCBI
rat	Rattus norvegicus	Moderate	NCBI
mouse	Mus musculus	Moderate	NCBI
nematode	Caenorhabditis elegans	High	NCBI
zebrafish	Danio rerio	High	NCBI
thale-cress	Arabidopsis thaliana	High	NCBI
Scotch pine	Pinus sylvestris	Moderate	NCBI

Term	Scientific Term	Evidence	Links
Daphnia magna	Daphnia magna	High	NCBI
Chlamydomonas reinhardtii	Chlamydomonas reinhardtii	Moderate	NCBI
common brandling worm	eisenia fetida	Moderate	NCBI
Lemna minor	Lemna minor	High	NCBI
Salmo salar	Salmo salar	Low	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Unspecific	Low

Energy can be deposited into any substrate, both living and non-living; it is independent of age, taxa, sex, or life-stage.

Taxonomic applicability: This MIE is not taxonomically specific.

Life stage applicability: This MIE is not life stage specific.

Sex applicability: This MIE is not sex specific.

Key Event Description

Deposition of energy refers to events where energetic subatomic particles, nuclei, or electromagnetic radiation deposit energy in the media through which they transverse. The energy may either be sufficient (e.g. ionizing radiation) or insufficient (e.g. non-ionizing radiation) to ionize atoms or molecules (Beir et al.,1999).

Ionizing radiation can cause the ejection of electrons from atoms and molecules, thereby resulting in their ionization and the breakage of chemical bonds. The excitation of molecules can also occur without ionization. These events are stochastic and unpredictable. The energy of these subatomic particles or electromagnetic waves ranges from 124 keV to 5.4 MeV and is dependent on the source and type of radiation (Zyla et al., 2020). Not all electromagnetic radiation is ionizing; as the incident radiation must have sufficient energy to free electrons from the electron orbitals of the atom or molecule. The energy deposited can induce direct and indirect ionization events and can result from internal (injections, inhalation, ingestion) or external exposure.

Direct ionization is the principal path where charged particles interact with biological structures such as DNA, proteins or membranes to cause biological damage. Photons, which are electromagnetic waves can also deposit energy to cause direct which themselves can indirectly damage critical targets such as DNA (Beir et al., 1999; Balagamwala et al., 2013) or alter cellular processes. Given the fundamental nature of energy deposition by radioactive/unstable nuclei, nucleons or elementary particles in material, this process is universal to all biological contexts.

The spatial structure of ionizing energy deposition along the resulting particle track is represented as linear energy transfer (LET) (Hall and Giaccia, 2018 UNSCEAR, 2020). High LET refers to energy mostly above 10 keV µm-1 which produces more complex, dense structural damage than low LET radiation (below 10 keV µm-1). Low-LET particles produce sparse ionization events such as photons (X- and gamma rays), as well as high-energy protons. Low LET radiation travels farther into tissue but deposits smaller amounts of energy, whereas high LET radiation, which includes heavy ions, alpha particles and high-energy neutrons, does not travel as far but deposits larger amounts of energy into tissue at the same absorbed dose. The biological effect of the deposition of energy can be modulated by varying dose and dose rate of exposure, such as acute, chronic, or fractionated exposures (Hall and Giaccia, 2018).

Non-ionizing radiation is electromagnetic waves that does not have enough energy to break bonds and induce ion formation but it can cause molecules to excite and vibrate faster resulting in biological effects. Examples of non-ionizing radiation include radio waves (wavelength: 100 km-1m), microwaves (wavelength: 1m-1mm), infrared radiation (wavelength: 1mm- 1 um), visible light (wavelengths: 400-700 nm), and ultraviolet radiation of longer wavelengths such as UVB (wavelengths: 315-400nm) and UVA (wavelengths: 280-315 nm).

How it is Measured or Detected

Radiation type	Assay Name	References	Description	OECD Approved Assay

Ionizing radiation	Monte Carlo Simulations (eg. Geant4)	Douglass et al., 2013; Douglass et al., 2012; Zyla et al., 2020	Monte Carlo simulations are based on a computational algorithm that mathematically models the deposition of energy into materials.	No
Ionizing radiation	Fluorescent Nuclear Track Detector (FNTD)	Sawakuchi, 2016; Niklas, 2013; Kodaira & Konishi, 2015	FNTDs are biocompatible chips with crystals of aluminum oxide doped with carbon and magnesium; used in conjunction with fluorescent microscopy, these FNTDs allow for the visualization and the linear energy transfer (LET) quantification of tracks produced by the deposition of energy into a material.	No
Ionizing radiation	Tissue equivalent proportional counter (TEPC)	Straume et al, 2015	Measure the LET spectrum and calculate the equivalent dose	No
Ionizing radiation	alanine dosimeters/NanoDots	Lind et al. 2019 Xie et al., 2022	Alanine dosimeters use the amino acid alanine to detect radiation-induced changes, and nanodots leverage nano-scale technology to provide high precision and sensitivity in radiation dose measurements	No
Non-ionizing radiation	UV meters or radiometers	Xie et al., 2020	UVA/UVB (irradiance intensity), UV dosimeters (accumulated irradiance over time), Spectrophotometer (absorption of UV by a substance or material)	No

References

Balagamwala, E. H. et al. (2013), “Introduction to radiotherapy and standard teletherapy techniques”, Dev Ophthalmol, Vol. 52, Karger, Basel, https://doi.org/10.1159/000351045

Beir, V. et al. (1999), “The Mechanistic Basis of Radon-Induced Lung Cancer”, in Health Risks of Exposure to Radon: BEIR VI, National Academy Press, Washington, D.C., https://doi.org/10.17226/5499

Douglass, M. et al. (2013), “Monte Carlo investigation of the increased radiation deposition due to gold nanoparticles using kilovoltage and megavoltage photons in a 3D randomized cell model”, Medical Physics, Vol. 40/7, American Institute of Physics, College Park, https://doi.org/10.1118/1.4808150

Douglass, M. et al. (2012), “Development of a randomized 3D cell model for Monte Carlo microdosimetry simulations.”, Medical Physics, Vol. 39/6, American Institute of Physics, College Park, https://doi.org/10.1118/1.4719963

Hall, E. J. and Giaccia, A.J. (2018), Radiobiology for the Radiologist, 8th edition, Wolters Kluwer, Philadelphia.

Kodaira, S. and Konishi, T. (2015), “Co-visualization of DNA damage and ion traversals in live mammalian cells using a fluorescent nuclear track detector.”, Journal of Radiation Research, Vol. 56/2, Oxford University Press, Oxford, https://doi.org/10.1093/jrr/rru091

Lind, O.C., D.H. Oughton and Salbu B. (2019), "The NMBU FIGARO low dose irradiation facility", International Journal of Radiation Biology, Vol. 95/1, Taylor & Francis, London, https://doi.org/10.1080/09553002.2018.1516906.

Sawakuchi, G.O. and Akselrod, M.S. (2016), “Nanoscale measurements of proton tracks using fluorescent nuclear track detectors.”, Medical Physics, Vol. 43/5, American Institute of Physics, College Park, https://doi.org/10.1118/1.4947128

Straume, T. et al. (2015), “Compact Tissue-equivalent Proportional Counter for Deep Space Human Missions.”, Health physics, Vol. 109/4, Lippincott Williams & Wilkins, Philadelphia, https://doi.org/10.1097/HP.0000000000000334

Niklas, M. et al. (2013), “Engineering cell-fluorescent ion track hybrid detectors.”, Radiation Oncology, Vol. 8/104, BioMed Central, London, https://doi.org/10.1186/1748- 717X-8-141

UNSCEAR (2020), Sources, effects and risks of ionizing radiation, United Nations.

Xie, Li. et al. (2022), "Ultraviolet B Modulates Gamma Radiation-Induced Stress Responses in Lemna Minor at Multiple Levels of Biological Organisation", SSRN, Elsevier, Amsterdam, http://dx.doi.org/10.2139/ssrn.4081705 .

Zyla, P.A. et al. (2020), Review of particle physics: Progress of Theoretical and Experimental Physics, 2020 Edition, Oxford University Press, Oxford.

List of Key Events in the AOP

Event: 2081: Increased Modified Proteins

Short Name: Modified Proteins

Key Event Component

Process	Object	Action
protein modification process		increased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:478 - Deposition of energy leading to occurrence of cataracts	KeyEvent

Biological Context

Level of Biological Organization

Molecular

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	Moderate	NCBI
mouse	Mus musculus	Low	NCBI
rat	Rattus norvegicus	Moderate	NCBI
bovine	Bos taurus	Moderate	NCBI

Life Stage Applicability

Life Stage	Evidence
Juvenile	Moderate
Adult	Moderate

Sex Applicability

Sex	Evidence
Male	Moderate
Female	Low
Unspecific	High

Taxonomic applicability: Modified proteins are applicable to all animals as proteins exist in some form in the cells of all animals (Cray, 2012).

Life stage applicability: This key event is not life stage specific as individuals in all life stages have proteins that can be modified (Dalle-Donne et al., 2006; Krisko & Radman, 2019). However, older individuals have naturally higher baseline levels of modified proteins, and those levels can even be used to determine an individual's age (Krisko & Radman, 2019).

Sex applicability: This key event is not sex specific as both sexes have proteins that can be modified. Evidence shows that males have a slightly higher level of protein carbonylation than their age-matched female counterparts (Barreiro et al., 2006).

Evidence for perturbation by a stressor: There is evidence to demonstrate that protein modification can occur as a result of multiple stressor types including oxidizing agents and ionizing & non-ionizing radiation (Hightower, 1995, Hamada et al., 2014; Lipman et al., 1988; Reisz et al., 2014).

Key Event Description

Proteins are considered to be modified following any change in structural components, as well as protein levels. Modifications to proteins can occur at any one of the structural levels of proteins, primary structure (amino acid or polypeptide sequence), the secondary structure (alpha helix or beta sheet structures), or the tertiary structure (globular protein forms) (Alberts et al., 2002). Protein modifications can include post-translational

modifications such as deamidation, oxidation, phosphorylation and carbonylation. Protein structure specificity can be crucial to their ability to execute their functional duties within a cell. Protein modifications can in turn affect protein-protein interactions, potentially hindering the ability to perform those functions (Dalle-Donne et al., 2006; Krisko & Radman, 2019). These affected protein interactions can result in unfolding, aggregation, insolubility, and increased molecular weight (Toyoma et al., 2013; Young, 1994). This can lead to the development of various age-related diseases, such as cataracts. As an example, modification of the tertiary structure of lens crystallin proteins can cause protein aggregation, increased lens opacity, and eventually cataracts (Moreau & King, 2012).

Modified proteins also refers to changes in protein levels which can result from changes in how proteins are synthesized (through transcription and translation), modified, and regulated in cells (Krisko & Radman, 2019). These processes are governed spatially and temporally by transcriptional and translational regulators as well as other signaling moieties and are tightly linked to the functional needs of cells, which can change depending on the presence of stressors or other external signaling factors (Reisz et al., 2014. Misregulation of protein expression can trigger a cascade of changes in downstream intracellular activities, which can then cause abnormal cellular dynamics. This misregulation can include abnormally high or low levels of particular proteins or even abnormalities in their breakdown (Hamada et al., 2014.

How it is Measured or Detected

Listed below are common methods for detecting the KE, however there may be other comparable methods that are not listed.

Method of Measurement	References	Description	OECD-Approved Assay
Mass Spectrometry	(Noble & Bailey, 2009)	Technique involves measuring the mass-to-charge ratio of ions to identify and quantify molecules and architectural changes such as the post-translational modifications of proteins	No
Proximity Ligation Assay	(Noble & Bailey, 2009)	An immunohistochemical tool that can help perform in situ detection of endogenous proteins, protein modifications, and protein interactions with high specificity and sensitivity	No
Western Blot	(Noble & Bailey, 2009)	Immunoblotting technique using antibody to detect its antigen and can be used for measuring protein levels.	No
Bicinchoninic Acid Assay (BCA)	(Noble & Bailey, 2009)	Can assist in quantification of total protein in a sample with colorimetric changes propagated through proteins mediated reduction of Cu+2 to Cu+1.	No
A280(Spectroscopy)	(Noble & Bailey, 2009)	Direct assay method for protein concentration determination in solution through measuring absorbance at 280 nm.	No
Lowry Assay	(Noble & Bailey, 2009)	Binding of administered agents to proteins causes measurable spectral shift to the blue form of the dye which can be used to quantify protein-levels.	No
Protein Mass Spectrometry	(Noble & Bailey, 2009)	Proteins initially digested various recombinant proteases, most often trypsin and are then subsequently observed at the tandem mass spectrometer (MS1) as a series of peaks, each with a different mass-to-charge ratio.	No
ELISA	(Alomari et al. 2018)	Carbonyl content on proteins detected using a plate reader following chromogenic reaction	No

References

Alberts B. et al. (2002), “The Shape and Structure of Proteins”, Molecular Biology of the Cell, 4, New York: Garland Science.

Alomari, E. et al. (2018), “Protein carbonylation detection methods: A comparison”, Data in Brief, Vol.19, Elsevier, Amsterdam, https://doi.org/10.1016/j.dib.2018.06.088.

Barreiro, E. et al. (2006), “Aging, sex differences, and oxidative stress in human respiratory and limb muscles”, Free Radical Biology & Medicine, Vol.41/5, Elsevier, Amsterdam, https://doi.org/10.1016/j.freeradbiomed.2006.05.027.

Cray, C. (2012), “Chapter 5 - Acute Phase Proteins in Animals”, Progress in Molecular Biology and Translational Science, Vol.105, Elsevier, Amsterdam, https://doi.org/10.1016/B978-0-12-394596-9.00005-6.

Dalle-Donne, I. et al. (2006), “Protein carbonylation, cellular dysfunction, and disease progression”, Journal of Cellular and Molecular Medicine, Vol.10/2, Wiley-Blackwell, Hoboken, https://doi.org/10.1111/j.1582-4934.2006.tb00407.x.

Hamada, N. et al. (2014), “Emerging issues in radiogenic cataracts and cardiovascular disease”, Journal of Radiation Research, Vol.55/5, Oxford University Press, Oxford, https://doi.org/10.1093/jrr/rru036.

Hightower, K. (1995), “The role of the Lens epithelium in development of UV cataract”, Current Eye Research, Vol.14/1, Taylor & Francis, Oxfordshire, https://doi.org/10.3109/02713689508999916.

Krisko, A. and M. Radman. (2019), “Protein damage, ageing and age-related diseases”, Open Biology, 9/3, The Royal Society, London, https://doi.org/10.1098/rsob.180249.

Lipman, R., B. Tripathi and R. Tripathi. (1988), “Cataracts induced by microwave and ionizing radiation”, Survey of Ophthalmology, Vol.33/3, Elsevier, Amsterdam, https://doi.org/10.1016/0039-6257(88)90088-4.

Moreau, K. L. and J. A. King (2012), “Protein misfolding and aggregation in cataract disease and prospects for prevention”, Trends in Molecular Medicine, Vol. 18/5, Elsevier Ltd, England, https://doi.org/10.1016/j.molmed.2012.03.005

Reisz, J. et al. (2014), “Effects of ionizing radiation on biological molecules - mechanisms of damage and emerging methods of detection”, Antioxidants and Redox Signaling, Vol.21(2), Mary Ann Liebert Inc, Larchmont, https://doi.org/10.1089/ars.2013.5489.

Noble, J. E., & Bailey, M. J. A. (2009). Chapter 8 Quantitation of Protein. In Methods in Enzymology (Vol. 463, Issue C, pp. 73–95). Academic Press Inc.

Toyama, B., & Hetzer, M. (2013). Protein homeostasis: Live long, won't prosper. Nature Reviews Molecular Cell Biology, 14(1), pp.55-61.

Young, R. (1994). The family of sunlight-related eye diseases. Optometry and Vision Science, 71(2), pp.125-144.

Event: 1634: Increase, Oxidative DNA damage

Short Name: Increase, Oxidative DNA damage

Key Event Component

Process	Object	Action
regulation of response to reactive oxygen species	reactive oxygen species	occurrence

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:296 - Oxidative DNA damage leading to chromosomal aberrations and mutations	MolecularInitiatingEvent
Aop:299 - Deposition of energy leading to population decline via DNA oxidation and follicular atresia	KeyEvent
Aop:311 - Deposition of energy leading to population decline via DNA oxidation and oocyte apoptosis	KeyEvent
Aop:478 - Deposition of energy leading to occurrence of cataracts	KeyEvent
Aop:330 - Excessive reactive oxygen species production leading to mortality (4)	KeyEvent
Aop:324 - Excessive reactive oxygen species leading to growth inhibition via oxidative DNA damage and cell death	KeyEvent
Aop:331 - Excessive reactive oxygen species leading to growth inhibition via oxidative DNA damage and reduced cell proliferation	KeyEvent

Stressors

Name
Hydrogen peroxide
Potassium bromate
Ionizing Radiation
Sodium arsenite
Reactive oxygen species

Biological Context

Level of Biological Organization
Molecular

Cell term

Cell term
eukaryotic cell

Organ term

Organ term
organ

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human and other cells in culture	human and other cells in culture	Moderate	NCBI
yeast	Saccharomyces cerevisiae	Low	NCBI
mouse	Mus musculus	High	NCBI
rat	Rattus norvegicus	Low	NCBI
bovine	Bos taurus	Low	NCBI
human	Homo sapiens	High	NCBI
rabbit	Oryctolagus cuniculus	Low	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Unspecific	Moderate

Taxonomic applicability: Theoretically, DNA oxidation can occur in any cell type, in any organism. Oxidative DNA lesions have been measured in mammalian cells (human, mouse, calf, rat) in vitro and in vivo, and in prokaryotes.

Life stage applicability: This key event is not life stage specific (Mesa & Bassnett, 2013; Suman et al., 2019).

Sex applicability: This key event is not sex specific (Mesa & Bassnett, 2013).

Evidence for Perturbation by Prototypic Stressor: H_2O_2 and $KBrO_3$ – A concentration-dependent increase in oxidative lesions was observed in both Fpg- and hOGG1-modified comet assays of TK6 cells treated with increasing concentrations of glucose oxidase (an enzyme that generates H_2O_2) and potassium bromate for 4 h (Platel et al., 2011).

Evidence indicates that oxidative DNA damage is also induced by X-rays (Bahia et al., 2018), ^{60}Co γ-rays, ^{12}C ions, α particles, electrons (Georgakilas, 2013), UVB (Mesa and Bassnett, 2013), γ-rays, ^{56}Fe ions (Datta et al., 2012), and protons (Suman et al., 2019).

Key Event Description

The nitrogenous bases of DNA are susceptible to oxidation in the presence of oxidizing agents. Oxidative adducts form mainly on C5 and to a lesser degree on C6 of thymine and cytosine, and on C8 of guanine and adenine. Guanine is most prone to oxidation due to its low oxidation potential (Jovanovic and Simic, 1986). Indeed, 8-oxo-2’-deoxyguanosine (8-oxodG)/8-hydroxy-2’-deoxyguanosine (8-OHdG) is the most abundant and well-studied oxidative DNA lesion in the cell (Swenberg et al., 2011). It causes an A(anti):8-oxo-G(syn) mispair instead of the normal C(anti):8-oxo-G(syn) pair. This pairing does not cause large structural changes to the DNA backbone, and therefore remains undetected by the polymerase’s proofreading mechanism. Consequently, one of the daughter strands will have an AT pair instead of the correct GC pair after replication (Markkanen, 2017).

Formamidopyrimidine lesions on guanine and adenine (FaPyG and FaPyA), 8-hydroxy-2'-deoxyadenine (8-oxodA), and thymidine glycol (Tg) are other common oxidative lesions. We refer the reader to reviews on this topic to see the full set of potential oxidative DNA lesions (Whitaker et al., 2017). Oxidative DNA lesions are present in the cell at a steady state due to endogenous redox processes (Swenberg et al., 2010). Under normal conditions, cells are able to withstand the baseline level of oxidized bases through efficient repair and regulation of free radicals in the cell. However, direct chemical insult from specific compounds, exposure to various forms of radiation, or induction of reactive oxygen species (ROS) from the reduction of endogenous molecules, as well as through the release of inflammatory cell-derived oxidants, can lead to increased DNA oxidation, a state known as oxidative stress (Turner et al., 2002; Schoenfeld et al., 2012; Tangvarasittichai and Tangvarasittichai, 2019). It is worth noting that ROS must be generated near the DNA to cause damage, otherwise, if ROS was produced more distantly, then it can be removed by the cell (Nilsson & Liu, 2020). Furthermore, although cells do possess repair mechanisms to deal with oxidative DNA damage, sometimes the repair intermediates can interfere with genome function or decrease stability of the genome. This creates a balancing act between when it is best to repair damage and when it is best to leave it (Poetsch, 2020a).

This KE describes an increase in oxidative lesions of a broad spectrum (ie. superoxide radical (O2•−), hydroxyl radical (OH), peroxyl radical (RO22), single oxygen (1O2) in the nuclear DNA above the steady-state level. Oxidative DNA

damage can occur in any cell type with nuclear DNA under oxidative stress.

How it is Measured or Detected

Relative Quantification of Oxidative DNA Lesions

- Comet assay (single cell gel electrophoresis) with Fpg and hOGG1 modifications (Smith et al., 2006; Platel et al., 2011)
  - Oxoguanine glycosylase (hOGG1) and formamidopyrimidine-DNA glycosylase (Fpg) are base excision repair (BER) enzymes in eukaryotic and prokaryotic cells, respectively
  - Both enzymes are bi-functional; the glycosylase function cleaves the glycosidic bond between the ribose and the oxidized base, giving rise to an abasic site, and the apurinic/apymidinic (AP) site lyase function cleaves the phosphodiester bond via β-elimination reaction and creates a single strand break
  - Treatment of DNA with either enzyme prior to performing the electrophoresis step of the comet assay allows detection of oxidative lesions by measuring the increase in comet tail length when compared against untreated samples.
- Enzyme-linked immunosorbant assay (ELISA) (Dizdaroglu et al., 2002; Breton et al., 2003; Xu et al., 2008; Zhao et al. 2017)
  - 8-oxodG can be detected using immunoassays, such as ELISA, that use antibodies against 8-oxodG lesions. It has been noted that immunodetection of 8-oxodG can be interfered by certain compounds in biological samples.

Absolute Quantification of Oxidative DNA Lesions

- Quantification of 8-oxodG using HPLC-EC (Breton et al., 2003; Chepelev et al., 2015)
  - 8-oxodG can be separated from digested DNA and precisely quantified using high performance liquid chromatography (HPLC) with electrochemical detection
- Mass spectrometry LC-MRM/MS (Mangal et al., 2009)
  - Liquid chromatography can also be coupled with multiple reaction monitoring/ mass spectrometry to detect and quantify oxidative lesions. Correlation between lesions measured by hOGG1-modified comet assay and LC-MS has been reported

Gas chromatography-mass spectrometry (GC-MS)

- DNA is hydrolyzed to release either free bases or nucleosides and then undergoes derivatization in order to increase their volatility. Finally, samples run through a gas chromatograph and then a mass spectrometer. The mass spectrometer results are used to determine oxidative DNA damage by identifying modified bases or nucleosides (Dizdaroglu, 1994).

Sequencing assays

- Various markers are used to detect and highlight sites of DNA damage; the result is then processed and sequenced. This category encompasses a wide range of assays such as snAP-seq, OGG1-AP-seq, oxiDIP-seq, OG-seq, and click-code-seq (Yun et al., 2017; Wu et al., 2018; Amente et al., 2019; Poetsch, 2020b).
- We note that other types of oxidative lesions can be quantified using the methods described above.

References

Amente, S. et al. (2019), “Genome-wide mapping of 8-oxo-7,8-dihydro-2’-deoxyguanosine reveals accumulation of oxidatively-generated damage at DNA replication origins within transcribed long genes of mammalian cells”, *Nucleic Acids Research 2019*, Vol. 47/1, Oxford University Press, England, https://doi.org/10.1093/nar/gky1152

Bahia, S. et al. (2018), “Oxidative and nitrative stress-related changes in human lens epithelial cells following exposure to X-rays”, *International journal of radiation biology*, Vol. 94/4, England, https://doi.org/10.1080/09553002.2018.1439194

Breton J, Sichel F, Bainchini F, Prevost V. (2003). Measurement of 8-Hydroxy-2′-Deoxyguanosine by a Commercially Available ELISA Test: Comparison with HPLC/Electrochemical Detection in Calf Thymus DNA and Determination in Human Serum. Anal Lett 36:123-134.

Cabrera, M. P., R. Chihuailaf and F. Wittwer Menge (2011), “Antioxidants and the integrity of ocular tissues”, *Veterinary medicine international*, Vol. 2011, SAGE-Hindawi Access to Research, United States, https://doi.org/10.4061/2011/905153

Cadet, J. et al. (2012), “Oxidatively generated complex DNA damage: tandem and clustered lesions”,*Cancer letters*, Vol. 327/1, Elsevier Ireland Ltd, Ireland. https://doi.org/10.1016/j.canlet.2012.04.005

Chepelev N, Kennedy D, Gagne R, White T, Long A, Yauk C, White P. (2015). HPLC Measurement of the DNA Oxidation Biomarker, 8-oxo-7,8-dihydro-2'-deoxyguanosine, in Cultured Cells and Animal Tissues. Journal of Visualized Experiments 102:e52697.

Collins, A. R. (2014), “Measuring oxidative damage to DNA and its repair with the comet assay”,*Biochimica et biophysica acta. General subjects*, Vol. 1840/2, Elsevier B.V., https://doi.org/10.1016/j.bbagen.2013.04.022

Datta, K. et al. (2012), “Exposure to heavy ion radiation induces persistent oxidative stress in mouse intestive”,*PloS One*, Vol. 7/8, Public Library of Science, United States, https://doi.org/10.1371/journal.pone.0042224

Dizdaroglu, M. (1994), “Chemical determination of oxidative DNA damage by gas chromatography-mass spectrometry”, *Methods in Enzymology*, Vol. 234, Elsevier Science & Technology, United States, https://doi.org/10.1016/0076-6879(94)34072-2

Dizdaroglu, M. et al. (2002), “Free radical-induced damage to DNA : mechanisms and measurement”,*Free radical biology & medicine*, Vol. 32/11, United States, pp. 1102-1115

Eaton, J. W. (1995), “UV-mediated cataractogenesis: a radical perspective”, *Documenta ophthalmologica*, Vol. 88/3-4, Springer, Dordrecht, https://doi.org/10.1007/BF01203677

Fletcher, A. E. (2010), “Free radicals, antioxidants and eye diseases: evidence from epidemiological studies on cataract and age-related macular degeneration”, *Ophthalmic Research*, Vol. 44/3, Karger international, Basel, https://doi.org/10.1159/000316476

Georgakilas, A. G et al. (2013), “Induction and repair of clustered DNA lesions: what do we know so far?”, Radiation Research, Vol. 180/1, *The Radiation Research Society*, United States, https://doi.org/10.1667/RR3041.1

Jose, D. et al. (2009). “Spectroscopic studies of position-specific DNA “breathing” fluctuations at replication forks and primer-template junctions”, *Proceedings of the National Academy of Sciences of the United States of America*,Vol. 106/11, https://doi.org/10.1073/pnas.0900803106

Jovanovic S, Simic M. (1986). One-electron redox potential of purines and pyrimidines. J Phys Chem 90:974-978.

Kruk, J., K. Kubasik-Kladna and H. Y. Aboul-Enein (2015), “The role oxidative stress in the pathogenesis of eye diseases: current status and a dual role of physical activity”, *Mini-reviews in medicinal chemistry*, Vol. 16/3, Bentham Science Publishers Ltd, Netherlands, https://doi.org/10.2174/1389557516666151120114605

Lee, J. et al. (2004), “Reactive oxygen species, aging, and antioxidative nutraceuticals”,*Comprehensive reviews in food science and food safety*, Vol. 3/1, Blackwell Publishing Ltd, Oxford, https://doi.org/10.1111/j.1541-4337.2004.tb00058.x

Mangal D, Vudathala D, Park J, Lee S, Penning T, Blair I. (2009). Analysis of 7,8-Dihydro-8-oxo-2′-deoxyguanosine in Cellular DNA during Oxidative Stress. Chem Res Toxicol 22:788-797.

Markkanen, E. (2017), “Not breathing is not an option: How to deal with oxidative DNA damage”,*DNA repair*, Vol. 59, Elsevier B.V., Netherlands, https://doi.org/10.1016/j.dnarep.2017.09.007

Mesa, R. and S. Bassnett (2013), “UV-B induced DNA damage and repair in the mouse lens”,*Investigative ophthalmology & visual science*, Vol. 54/10, the Association for Research in Vision and Ophthalmology, United States, https://doi.org/10.1167/iovs.13-12644

Nilsson R. and Liu N. (2020), “Nuclear DNA damages generated by reactive oxygen molecules (ROS) under oxidative stress and their relevance to human cancers, including ionizing radiation-induced neoplasia part I: Physical, chemical and molecular biology aspects”, Radiation Medicine and Protection, Vol. 1/3(3), https://doi.org/10.1016/j.radmp.2020.09.002

Pendergrass, W. et al. (2010), “X-ray induced cataract is preceded by LEC loss, and coincident with accumulation of cortical DNA, and ROS; similarities with age-related cataracts”, *Molecular vision*, Vol. 16, Molecular Vision, United States, pp. 1496-1513

Platel A, Nesslany F, Gervais V, Claude N, Marzin D. (2011). Study of oxidative DNA damage in TK6 human lymphoblastoid cells by use of the thymidine kinase gene-mutation assay and the *in vitro* modified comet assay: Determination of No-Observed-Genotoxic-Effect-Levels. Mutat Res 726:151-159.

Poetsch, Anna R. (2020a), “The genomics of oxidative DNA damage, repair, and resulting mutagenesis”, *Computational and structural biotechnology journal 2020*, Vol. 18, Elsevier B.V., Netherlands https://doi.org/10.1016/j.csbj.2019.12.013

Poetsch, A. R. (2020b), “AP-Seq: A method to measure apurinic sites and small base adducts genome-wide”, The Nucleus, Springer US, New York, Sacca, S. C. et al. (2009), “Gene-environment interactions in ocular diseases”, *Mutation research – fundamental and molecular mechanisms of mutagenesis*, Vol. 667/1-2, Elsevier, Amsterdam, https://doi.org/10.1016/j.mrfmmm.2008.11.002

Schoenfeld, M. P. et al. (2012), “A hypothesis on biological protection from space radiation through the use of new therapeutic gases as medical counter measures”, *Medical gas research*, Vol. 2/1, BioMed Central Ltd, India, https://doi.org/10.1186/2045-9912-2-8

Smith C, O'Donovan M, Martin E. (2006). hOGG1 recognizes oxidative damage using the comet assay with greater specificity than FPG or ENDOIII. Mutagenesis 21:185-190.

Stohs, S. J. (1995), “The role of free radicals in toxicity and disease”,*Journal of Basic and Clinical Physiology and Pharmacology*, Vol. 6/3-4, Freund Publishing House Ltd, https://doi.org/10.1515/JBCPP.1995.6.3-4.205

Suman, S. et al. (2019), “Fractionated and acute proton radiation show differential intestinal tumorigenesis and DNA damage and repair pathway response in ApcMin/+ mice”, *International Journal of Radiation Oncology*, Biology, Physics, Vol. 105/3, Elsevier Inc, https://doi.org/10.1016/j.ijrobp.2019.06.2532

Swenberg J. et al. (2011). "Endogenous versus Exogenous DNA Adducts: Their Role in Carcinogenesis, Epidemiology, and Risk Assessment." Toxicol Sci 120:S130-S145.

Tangvarasittichai, O and S. Tangvarasittichai (2018), “Oxidative stress, ocular disease, and diabetes retinopathy”, *Current Pharmaceutical Design*, Vol. 24/40, Bentham Science Publishers, https://doi.org/10.2174/1381612825666190115121531

Turner, N. D. et al. (2002), “Opportunities for nutritional amelioration of radiation-induced cellular damage”,*Nutrition*, Vol. 18/10, Elsevier Inc, New York, https://doi.org/10.1016/S0899-9007(02)00945-0

Whitaker A, Schaich M, Smith MS, Flynn T, Freudenthal B. (2017). Base excision repair of oxidative DNA damage: from mechanism to disease. Front Biosci 22:1493-1522.

Wu, J. (2018), “Nucleotide-resolution genome-wide mapping of oxidative DNA damage by click-code-seq”,*Journal of the American Chemical Society 2018*, American Chemical Society, United States https://doi-org.proxy.bib.uottawa.ca/10.1021/jacs.8b03715

Xu, X. et al. (2008). “Fluorescence recovery assay for the detection of protein-DNA binding”,*Analytical Chemistry*, Vol. 80/14, https://doi.org/10.1021/ac8007016

Zhao M, Howard E, Guo Z, Parris A, Yang X. (2017). p53 pathway determines the cellular response to alcohol-induced DNA damage in MCF-7 breast cancer cells. PLoS One 12:e0175121.

Event: 1635: Increase, DNA strand breaks

Short Name: Increase, DNA strand breaks

Key Event Component

Process	Object	Action
DNA Strand Break	Deoxyribonucleic acid	increased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:296 - Oxidative DNA damage leading to chromosomal aberrations and mutations	KeyEvent
Aop:272 - Deposition of energy leading to lung cancer	KeyEvent
Aop:322 - Alkylation of DNA leading to reduced sperm count	KeyEvent
Aop:216 - Deposition of energy leading to population decline via DNA strand breaks and follicular atresia	KeyEvent
Aop:238 - Deposition of energy leading to population decline via DNA strand breaks and oocyte apoptosis	KeyEvent
Aop:478 - Deposition of energy leading to occurrence of cataracts	KeyEvent
Aop:483 - Deposition of Energy Leading to Learning and Memory Impairment	KeyEvent
Aop:470 - Deposition of energy leads to abnormal vascular remodeling	KeyEvent

Stressors

Name
Ionizing Radiation
Topoisomerase inhibitors
Radiomimetic compounds

Biological Context

Level of Biological Organization

Molecular

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human and other cells in culture	human and other cells in culture		NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Unspecific	High

Taxonomic applicability: DNA strand breaks are relevant to all species, including vertebrates such as humans, that contain DNA (Cannan & Pederson, 2016).

Life stage applicability: This key event is not life stage specific as all life stages display strand breaks. However, there is an increase in baseline levels of DNA strand breaks seen in older individuals though it is unknown whether this change due to increased break induction or a greater retention of breaks due to poor repair (White & Vijg, 2016).

Sex applicability: This key event is not sex specific as both sexes display evidence of strand breaks. In some cell types, such as peripheral blood mononuclear cells, males show higher levels of single strand breaks than females (Garm et al., 2012).

Evidence for perturbation by a stressor: There are studies demonstrating that increased DNA strand breaks can result from exposure to multiple stressor types including ionizing & non-ionizing radiation, chemical agents, and oxidizing agents (EPRI, 2014; Hamada, 2014; Cencer et al., 2018; Cannan & Pederson, 2016; Yang et al., 1998).

Key Event Description

DNA strand breaks are a type of damage resulting from the hydrolysis of phosphodiester groups in the backbone of DNA molecules (Gates, 2009) and can occur on a single strand (single strand breaks; SSBs) or both strands (double strand breaks; DSBs). SSBs arise when the sugar phosphate backbones connecting adjacent nucleotides in DNA are simultaneously hydrolyzed such that the hydrogen bonds between complementary bases are not able to hold the two strands together. DSBs are generated when both strands are simultaneously broken at sites that are sufficiently close to one another that base-pairing and chromatin structure are insufficient to keep the two DNA ends juxtaposed. As a consequence, the two DNA ends generated by a DSB can physically dissociate from one another, becoming difficult to repair and increasing the chance of inappropriate recombination with other sites in the genome (Jackson, 2002). SSB can turn into DSB if the replication fork stalls at the lesion leading to fork collapse. Strand breaks are intermediates in various biological events, including DNA repair (e.g., excision repair), as well as other normal cellular processes where DSBs act as genetic shufflers to generate genetic diversity for V(D)J recombination in lymphoid cells, and chromatin remodeling in both somatic cells and germ cells, and meiotic recombination in gametes.

Strand breaks are intermediates in various biological events, including DNA repair (e.g., excision repair), V(D)J recombination in developing lymphoid cells and chromatin remodeling in both somatic cells and germ cells. The spectrum of damage can be complex, particularily if the stressor is from large amounts of deposited energy which can result in complex lesions and clustered damage defined as two or more oxidized bases, abasic sites or starnd breaks on opposing DNA strands within a few helical turns. These lesions are more difficult to repair and have been studied in many types of models (Barbieri et al., 2019 and Asaithamby et al., 2011). DSBs and complex lesions are of particular concern, as they are considered the most lethal and deleterious type of DNA lesion. If misrepaired or left unrepaired, DSBs may drive the cell towards genomic instability, apoptosis or tumorigenesis (Beir, 1999).

How it is Measured or Detected

Please refer to the table below for details regarding these and other methodologies for detecting DNA DSBs.

Method of Measurement	References	Description	OECD Approved Method?

Comet Assay (Single Cell Gel Eletrophoresis - Alkaline)	Collins, 2004; Olive and Banath, 2006; Platel et al., 2011; Nikolova et al., 2017	To detect SSBs or DSBs, single cells are encapsulated in agarose on a slide, lysed, and subjected to gel electrophoresis at an alkaline pH (pH >13); DNA fragments are forced to move, forming a "comet"-like appearance	Yes
γ-H2AX Foci Quantification - Flow Cytometry	Rothkamm and Horn, 2009; Bryce et al., 2016	Measurement of γ-H2AX immunostaining in cells by flow cytometry, normalized to total levels of H2AX	No
γ-H2AX Foci Quantification - Western Blot	Burma et al., 2001; Revet et al., 2011	Measurement of γ-H2AX immunostaining in cells by Western blotting, normalized to total levels of H2AX	No
γ-H2AX Foci Quantification - Microscopy	Redon et al., 2010; Mah et al., 2010; Garcia-Canton et al., 2013	Quantification of γ-H2AX immunostaining by counting γ-H2AX foci visualized with a microscope	No
γ-H2AX Foci Quantification - ELISA	Ji et al., 2017	Measurement of γ-H2AX in cells by ELISA, normalized to total levels of H2AX	No
Pulsed Field Gel Electrophoresis (PFGE)	Ager et al., 1990; Gardiner et al., 1985; Herschleb et al., 2007; Kawashima et al., 2017	To detect DSBs, cells are embedded and lysed in agarose, and the released DNA undergoes gel electrophoresis in which the direction of the voltage is periodically alternated; Large DNA fragments are thus able to be separated by size	No
The TUNEL (Terminal Deoxynucleotidyl Transferase dUTP Nick End Labeling) Assay	Loo, 2011	To detect strand breaks, dUTPs added to the 3’OH end of a strand break by the DNA polymerase terminal deoxynucleotidyl transferase (TdT) are tagged with a fluorescent dye or a reporter enzyme to allow visualization	No
In Vitro DNA Cleavage Assays using Topoisomerase	Nitiss, 2012	Cleavage of DNA can be achieved using purified topoisomerase; DNA strand breaks can then be separated and quantified using gel electrophoresis	No
PCR assay	Figueroa-González & Pérez-Plasencia, 2017	Assay of strand breaks through the observation of DNA amplification prevention. Breaks block Taq polymerase, reducing the number of DNA templates, preventing amplification	No
Sucrose density gradient centrifuge	Raschke et al. 2009	Division of DNA pieces by density, increased fractionation leads to lower density pieces, with the use of a sucrose cushion	No
Alkaline Elution Assay	Kohn, 1991	Cells lysed with detergent-solution, filtered through membrane to remove all but intact DNA	No
Unwinding Assay	Nacci et al. 1992	DNA is stored in alkaline solutions with DNA-specific dye and allowed to unwind following removal from tissue, increased strand damage associated with increased unwinding	Yes
STRIDE assay	Zilio and Ulrich, 2021	STRIDE (SensiTive Recognition of Individual DNA Ends) combines in situ nick translation with the proximity ligation assay (PLA) to detect single-strand breaks (sSTRIDE) or double-strand breaks (dSTRIDE). In this process, lesions labeled through nick translation with biotinylated nucleotides are identified by a PLA signal, which arises from the interaction of two anti-biotin antibodies from different species.	No
sBLISS	Bouwmann et al. 2020	sBLISS (in-suspension breaks labeling in situ and sequencing) labels double-strand breaks (DSBs) in cells immobilized on glass coverslips, using double-stranded oligonucleotide adaptors that facilitate selective linear amplification through T7-mediated in vitro transcription (IVT), followed by next-generation sequencing (NGS) library preparation	No

References

Ager, D. D., et al. (1990). Measurement of radiation-induced DNA double-strand breaks by pulsed-field gel

electrophoresis. Radiation research, 122/(2), 181–187.

Anderson, D. & Laubenthal J. (2013), “Analysis of DNA Damage via Single-Cell Electrophoresis. In: Makovets S, editor. DNA Electrophoresis. Totowa.”, NJ: Humana Press. p 209-218.

Asaithamby, A., B. Hu and D.J. Chen. (2011) “Unrepaired clustered DNA lesions induce chromosome breakage in human cells.” Proc Natl Acad Sci U S A 108(20): 8293-8298 .

Barbieri, S., G. Babini, J. Morini et a l (2019). . Predicting DNA damage foci and their experimental readout with 2D microscopy: a unified approach applied to photon and neutron exposures. Scientific Reports 9(1): 14019

Bouwman, B. et al. (2020), “Genome-wide detection of DNA double-strand breaks by in-suspension BLISS”, Nature protocols,.15/12, Springer Nature, London, https://doi.org/10.1038/s41596-020-0397-2

Bryce, S. et al. (2016), “Genotoxic mode of action predictions from a multiplexed flow cytometric assay and a machine learning approach.”, Environ Mol Mutagen. 57:171-189. Doi: 10.1002/em.21996.

Burma, S. et al. (2001), “ATM phosphorylates histone H2AX in response to DNA double-strand breaks.”, J Biol Chem, 276(45): 42462-42467. doi:10.1074/jbc.C100466200

Cannan, W.J. and D.S. Pederson (2016), "Mechanisms and Consequences of Double-Strand DNA Break Formation in Chromatin.", Journal of Cellular Physiology, Vol.231(/1), Wiley, New York, https://doi.org/10.1002/jcp.25048.

Cencer, C. et al. (2018), “PARP-1/PAR Activity in Cultured Human Lens Epithelial Cells Exposed to Two Levels of UVB Light”, Photochemistry and Photobiology, Vol.(94/1), Wiley-Blackwell, Hoboken, https://doi.org/10.1111/php.12814.

Charlton, E. D. et al. (1989), “Calculation of Initial Yields of Single and Double Stranded Breaks in Cell Nuclei from Electrons, Protons, and Alpha Particles.”, Int. J. Radiat. Biol. 56(1): 1-19. doi: 10.1080/09553008914551141.

Collins, R. A. (2004), “The Comet Assay for DNA Damage and Repair. Molecular Biotechnology.”, Mol Biotechnol. 26(3): 249-61. doi:10.1385/MB:26:3:249

EPRI (2014), Epidemiology and mechanistic effects of radiation on the lens of the eye: Review and scientific appraisal of the literature, EPRI, California.

Figueroa-González, G. and C. Pérez-Plasencia. (2017), “Strategies for the evaluation of DNA damage and repair mechanisms in cancer”, Oncology Letters, Vol.133(/6), Spandidos Publications, Athens, https://doi.org/10.3892/ol.2017.6002.

Garcia-Canton, C. et al. (2013), “Assessment of the in vitro p-H2AX assay by High Content Screening asa novel genotoxicity test.”, Mutat Res. 757:158-166. Doi: 10.1016/j.mrgentox.2013.08.002

Gardiner, K. et al. (1986), “Fractionation of Large Mammalian DNA Restriction Fragments Using Vertical Pulsed-Field Gradient Gel Electrophoresis.”, Somatic Cell and Molecular Genetics. 12(2): 185-95.Doi: 10.1007/bf01560665.

Garm, C. et al. (2012), “Age and gender effects on DNA strand break repair in peripheral blood mononuclear cells”, Aging Cell, Vol.12/1, Blackwell Publishing Ltd, Oxford, https://doi.org/10.1111/acel.12019.

Hamada, N. (2014), “What are the intracellular targets and intratissue target cells for radiation effects?”, Radiation research, Vol. 181/1, The Radiation Research Society, Indianapolis, https://doi.org/10.1667/RR13505.1.

Herschleb, J. et al. (2007), “Pulsed-field gel electrophoresis.”, Nat Protoc. 2(3): 677-684. doi:10.1038/nprot.2007.94

Iliakis, G. et al. (2015), “Alternative End-Joining Repair Pathways Are the Ultimate Backup for Abrogated Classical Non-Homologous End-Joining and Homologous Recombination Repair: Implications for the Formation of Chromosome Translocations.”, Mutation Research/Genetic Toxicology and Environmental Mutagenesis. 2(3): 677-84. doi: 10.1038/nprot.2007.94

Jackson, S. (2002). “Sensing and repairing DNA double-strand breaks.”, Carcinogenesis. 23:687-696. Doi:10.1093/carcin/23.5.687.

Ji, J. et al. (2017), “Phosphorylated fraction of H2AX as a measurement for DNA damage in cancer cells and potential applications of a novel assay.”, PLoS One. 12(2): e0171582. doi:10.1371/journal.pone.0171582

Kawashima, Y.(2017), “Detection of DNA double-strand breaks by pulsed-field gel electrophoresis.”, Genes Cells 22:84-93. Doi: 10.1111/gtc.12457.

Khoury, L. et al. (2013), “Validation of high-throughput genotoxicity assay screening using cH2AX in-cell Western assay on HepG2 cells.”, Environ Mol Mutagen, 54:737-746. Doi: 10.1002/em.21817.

Khoury, L. et al. (2016), “Evaluation of four human cell lines with distinct biotransformation properties for genotoxic screening.”, Mutagenesis, 31:83-96. Doi: 10.1093/mutage/gev058.

Kohn, K.W. (1991), “Principles and practice of DNA filter elution”, Pharmacology & Therapeutics, Vol.49(/1), Elsevier, Amsterdam, https://doi.org/10.1016/0163-7258(91)90022-E.

Loo, DT. (2011), “In Situ Detection of Apoptosis by the TUNEL Assay: An Overview of Techniques. In: Didenko V, editor.

DNA Damage Detection In Situ, Ex Vivo, and In Vivo. Totowa.", NJ: Humana Press. p 3-13.doi: 10.1007/978-1-60327-409-8_1.

Mah, L. J. et al. (2010), "Quantification of gammaH2AX foci in response to ionising radiation.", J Vis Exp(38). doi:10.3791/1957.

Nacci, D. et al. (1992), "Application of the DNA alkaline unwinding assay to detect DNA strand breaks in marine bivalves", Marine Environmental Research, Vol.33(/2), Elsevier BV, Amsterdam, https://doi.org/10.1016/0141-1136(92)90134-8.

Nikolova, T., F. et al. (2017), "Genotoxicity testing: Comparison of the γH2AX focus assay with the alkaline and neutral comet assays.", Mutat Res 822:10-18. Doi: 10.1016/j.mrgentox.2017.07.004.

Nitiss, J. L. et al. (2012), "Topoisomerase assays. ", Curr Protoc Pharmacol. Chapter 3: Unit 3 3.

OECD. (2014). Test No. 489: "In vivo mammalian alkaline comet assay." OECD Guideline for the Testing of Chemicals, Section 4 .

Olive, P. L., & Banáth, J. P. (2006), "The comet assay: a method to measure DNA damage in individual cells.", Nature Protocols. 1(1): 23-29. doi:10.1038/nprot.2006.5.

Platel A. et al. (2011), "Study of oxidative DNA damage in TK6 human lymphoblastoid cells by use of the thymidine kinase gene-mutation assay and the in vitro modified comet assay: Determination of No-Observed-Genotoxic-Effect-Levels.", Mutat Res 726:151-159. Doi: 10.1016/j.mrgentox.2011.09.003.

Raschke, S., J. Guan and G. Iliakis. (2009), "Application of alkaline sucrose gradient centrifugation in the analysis of DNA replication after DNA damage", Methods in Molecular Biology, Vol.521, Humana Press, Totowa, https://doi.org/10.1007/978-1-60327-815-7_18.

Redon, C. et al. (2010), "The use of gamma-H2AX as a biodosimeter for total-body radiation exposure in non-human primates.", PLoS One. 5(11): e15544. doi:10.1371/journal.pone.0015544

Revet, I. et al. (2011), "Functional relevance of the histone γH2Ax in the response to DNA damaging agents." Proc Natl Acad Sci USA.108:8663-8667. Doi: 10.1073/pnas.1105866108

Rogakou, E.P. et al. (1998), "DNA Double-stranded Breaks Induce Histone H2AX Phosphorylation on Serine 139." , J Biol Chem, 273:5858-5868. Doi: 10.1074/jbc.273.10.5858

Rothkamm, K. & Horn, S. (2009), "γ-H2AX as protein biomarker for radiation exposure.", Ann Ist Super Sanità, 45(3): 265-71.

White, R.R. and J. Vijg. (2016), "Do DNA Double-Strand Breaks Drive Aging?", Molecular Cell, Vol.63, Elsevier, Amsterdam, http://doi.org/10.1016/j.molcel.2016.08.004.

Yang, Y. et al. (1998), "The effect of catalase amplification on immortal lens epithelial cell lines", Experimental Eye Research, Vol.67(/6), Academic Press Inc, Cambridge, https://doi.org/10.1006/exer.1998.0560.

Zilio, N. and H. D. Ulrich (2021), "Exploring the SSBreakome: genome-wide mapping of DNA single-strand breaks by next-generation sequencing", The FEBS journal, 288(13), Wiley, Hoboken, https://doi.org/10.1111/febs.15568

Event: 155: Inadequate DNA repair

Short Name: Inadequate DNA repair

Key Event Component

Process	Object	Action
DNA repair	deoxyribonucleic acid	abnormal

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:15 - Alkylation of DNA in male pre-meiotic germ cells leading to heritable mutations	KeyEvent
Aop:141 - Alkylation of DNA leading to cancer 2	KeyEvent

AOP ID and Name	Event Type
Aop:139 - Alkylation of DNA leading to cancer 1	KeyEvent
Aop:296 - Oxidative DNA damage leading to chromosomal aberrations and mutations	KeyEvent
Aop:272 - Deposition of energy leading to lung cancer	KeyEvent
Aop:322 - Alkylation of DNA leading to reduced sperm count	KeyEvent
Aop:397 - Bulky DNA adducts leading to mutations	KeyEvent
Aop:432 - Deposition of Energy by Ionizing Radiation leading to Acute Myeloid Leukemia	KeyEvent
Aop:443 - DNA damage and mutations leading to Metastatic Breast Cancer	KeyEvent
Aop:478 - Deposition of energy leading to occurrence of cataracts	KeyEvent

Stressors

Name
Ionizing Radiation

Biological Context

Level of Biological Organization
Cellular

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
mouse	Mus musculus	High	NCBI
rat	Rattus norvegicus	Moderate	NCBI
Syrian golden hamster	Mesocricetus auratus	Moderate	NCBI
Homo sapiens	Homo sapiens	High	NCBI
cow	Bos taurus	Low	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Unspecific	High

The retention of adducts has been directly measured in many different types of eukaryotic somatic cells (in vitro and in vivo). In male germ cells, work has been done on hamsters, rats and mice. The accumulation of mutation and changes in mutation spectrum has been measured in mice and human cells in culture. Theoretically, saturation of DNA repair occurs in every species (prokaryotic and eukaryotic). The principles of this work were established in prokaryotic models. Nagel et al. (2014) have produced an assay that directly measures DNA repair in human cells in culture.

NHEJ is primarily used by vertebrate multicellular eukaryotes, but it also been observed in plants. Furthermore, it has recently been discovered that some bacteria (Matthews et al., 2014) and yeast (Emerson et al., 2016) also use NHEJ. In terms of invertebrates, most lack the core DNA-PK_{cs} and Artemis proteins; they accomplish end joining by using the RA50:MRE11:NBS1 complex (Chen et al., 2001). HR occurs naturally in eukaryotes, bacteria, and some viruses (Bhatti et al., 2016).

Taxonomic applicability: Inadequate DNA repair is applicable to all species, as they all contain DNA (White & Vijg, 2016).

Life stage applicability: This key event is not life stage specific as any life stage can have poor repair, though as individuals age their repair process become less effective (Gorbunova & Seluanov, 2016).

Sex applicability: There is no evidence of sex-specificity for this key event, with initial rate of DNA repair not significantly different between sexes (Trzeciak et al., 2008).

Evidence for perturbation by a stressor: Multiple studies demonstrate that inadequate DNA repair can occur as a result of stressors such as ionizing and non-ionizing radiation, as well as chemical agents (Kuhne et al., 2005; Rydberg et al., 2005; Dahle et al., 2008; Seager et al., 2012; Wilhelm, 2014; O'Brien et al., 2015).

Key Event Description

DNA lesions may result from the formation of DNA adducts (i.e., covalent modification of DNA by chemicals), or by the action of agents such as radiation that may produce strand breaks or modified nucleotides within the DNA molecule. These DNA lesions are repaired through several mechanistically distinct pathways that can be categorized as follows:

1. **Damage reversal** acts to reverse the damage without breaking any bonds within the sugar phosphate backbone of the DNA. The most prominent enzymes associated with damage reversal are photolyases (Sancar, 2003) that can repair UV dimers in some organisms, and O6-alkylguanine-DNA alkyltransferase (AGT) (Pegg 2011) and oxidative demethylases (Sundheim et al., 2008), which can repair some types of alkylated bases.
2. **Excision repair** involves the removal of a damaged nucleotide(s) through cleavage of the sugar phosphate backbone followed by re-synthesis of DNA within the resultant gap. Excision repair of DNA lesions can be mechanistically divided into:

    a) Base excision repair (BER) (Dianov and Hübscher, 2013), in which the damaged base is removed by a damage-specific glycosylase prior to incision of the phosphodiester backbone at the resulting abasic site. This leads to an intermediate that contains a DNA strand break, whereby DNA ligase is then recruited to seal the ends of the DNA.

    b) Nucleotide excision repair (NER) (Schärer, 2013), in which the DNA strand containing the damaged nucleotide is incised at sites several nucleotides 5' and 3' to the site of damage, and a polynucleotide containing the damaged nucleotide is removed prior to DNA resynthesis within the resultant gap and sealing of the ends by DNA ligase.

    c) Mismatch repair (MMR) (Li et al., 2016) which does not act on DNA lesions but does recognize mispaired bases resulting from replication errors. In MMR the strand containing the misincorporated base is removed prior to DNA resynthesis.

    The major pathway that removes oxidative DNA damage is base excision repair (BER), which can be either monofunctional or bifunctional; in mammals, a specific DNA glycosylase (OGG1: 8-Oxoguanine glycosylase) is responsible for excision of 8-oxoguanine (8-oxoG) and other oxidative lesions (Hu et al., 2005; Scott et al., 2014; Whitaker et al., 2017). We note that long-patch BER is used for the repair of clustered oxidative lesions, which uses several enzymes from DNA replication pathways (Klungland and Lindahl, 1997). These pathways are described in detail in various reviews e.g., (Whitaker et al., 2017).

3. **Single strand break repair (SSBR)** involves different proteins and enzymes depending on the origin of the SSB (e.g., produced as an intermediate in excision repair or due to direct chemical insult) but the same general steps of repair are taken for all SSBs: detection, DNA end processing, synthesis, and ligation (Caldecott, 2014). Poly-ADP-ribose polymerase1 (PARP1) detects and binds unscheduled SSBs (i.e., not deliberately induced during excision repair) and synthesizes PAR as a signal to the downstream factors in repair. PARP1 is not required to initiate SSBR of BER intermediates. The XRCC1 protein complex is then recruited to the site of damage where a common DNA intermediate as BER was generated, and acts as a scaffold for proteins and enzymes required for repair. Depending on the nature of the damaged termini of the DNA strand, different enzymes are required for end processing to generate the substrates that DNA polymerase β (Polβ; short patch repair) or Pol δ/ε (long patch repair) can bind to synthesize over the gap, although end processing is generally done by polynucleotide kinase. Synthesis in long-patch repair displaces a single stranded flap which is excised by flap endonuclease 1 (FEN1). In short-patch repair, the XRCC1/Lig3α complex joins the two ends after synthesis. In long-patch repair, the PCNA/Lig1 complex ligates the ends. (Caldecott, 2014).
4. **Double strand break repair (DSBR)** is necessary to preserve genomic integrity when breaks occur in both strands of a DNA molecule. There are two major pathways for DSBR: homologous recombination (HR), which operates primarily during the S phase of dividing cell types, and nonhomologous end joining (NHEJ), which can function in both dividing and non-dividing cell types. No repair occurs in the M phase (Teruaki Iyama and David M. Wilson III, 2013). DNA repair in mitosis is controversial (Mladenov et al., 2023).

    Complex lesions can be created by a single mutagen and can be more difficult to repair, as the mechanism behind how different repair pathways cooperate to address this is still unclear (Aleksandrov et al., 2018). In higher eukaryotes such as mammals, NHEJ is usually the preferred pathway for DNA DSBR. Its use, however, is dependent on the cell type, the gene locus, and the nuclease platform (Miyaoka et al., 2016). The use of NHEJ is also dependent on the cell cycle; NHEJ is generally not the pathway of choice when the cell is in the late S or G2 phase of the cell cycle, or in mitotic cells when the sister chromatid is directly adjacent to the double-strand break (DSB) (Lieber et al., 2003). In these cases, the HR pathway is commonly used for repair of DSBs. Despite this, NHEJ is still used more commonly than HR in human cells. Classical NHEJ (C-NHEJ) is the most common NHEJ repair mechanism, but alternative NHEJ (alt-NHEJ) can also occur, especially in the absence of C-NHEJ and HR.

The process of C-NHEJ in humans requires at least seven core proteins: Ku70, Ku86, DNA-dependent protein kinase complex (DNA-PK_{CS}), Artemis, X-ray cross-complementing protein 4 (XRCC4), XRCC4-like factor (XLF), and DNA ligase IV (Boboila et al., 2012). When DSBs occur, the Ku proteins, which have a high affinity for DNA ends, will bind to the break site and form a heterodimer. This protects the DNA from exonucleolytic attack and acts to recruit DNA-PK_{CS}, the catalytic subunit, thus forming a trimeric complex on the ends of the DNA strands. Alternative NHEJ, or alt NHEJ, uses small similar sequences in two broken DNA ends to join them together. Unlike the usual repair method (cNHEJ), aNHEJ doesn't need specific proteins like LIG4 and KU. Instead, it relies on the MRN complex to process the breaks. However, alt NHEJ tends to cause mutations by adding or removing bits of DNA during the repair (Chaudhuri and Nussenzweig, 2017). The kinase activity of DNA-PK_{CS} is then triggered, causing DNA-PK_{CS} to auto-phosphorylate and thereby lose its kinase activity; the now phosphorylated DNA-PK_{CS} dissociates from the DNA-bound Ku proteins. The free DNA-PK_{CS} phosphorylates Artemis, an enzyme that possesses 5’-3’ exonuclease and endonuclease activity in the presence of DNA-PK_{CS} and ATP. Artemis is responsible for ‘cleaning up’ the ends of the DNA. For 5’ overhangs, Artemis nicks the overhang, generally leaving a blunt duplex end. For 3’ overhangs, Artemis will often leave a four- or five-nucleotide single stranded overhang (Pardo et al., 2009; Fattah et al., 2010; Lieber et al., 2010). Next, the XLF and XRCC4 proteins form a complex which makes a channel to bind DNA and aligns the ends for efficient ligation via DNA ligase IV (Hammel et al., 2011).

The process of alt-NHEJ is less well understood than C-NHEJ and is a lower fidelity mechanism. Alt-NHEJ is known to involve slightly different core proteins than C-NHEJ and required microhomology repeats, but the steps of the pathway are essentially the same between the two processes (reviewed in Chiruvella et al., 2013). It is established, however, that alt-NHEJ is more error-prone in nature than C-NHEJ, which contributes to incorrect DNA repair. Alt-NHEJ is thus considered primarily to be a backup repair mechanism (reviewed in Chiruvella et al., 2013).

In contrast to NHEJ, HR takes advantage of similar or identical DNA sequences to repair DSBs and is not error-prone (Sung and Klein, 2006). The initiating step of HR is the creation of a 3’ single strand DNA (ss-DNA) overhang. Combinases such as RecA and Rad51 then bind to the ss-DNA overhang, and other accessory factors, including Rad54, help recognize and invade the homologous region on another DNA strand. From there, DNA polymerases are able to elongate the 3’ invading single strand and resynthesize the broken DNA strand using the corresponding sequence on the homologous strand.

Fidelity of DNA Repair

Most DNA repair pathways are extremely efficient. However, in principal, all DNA repair pathways can be overwhelmed when the DNA lesion burden exceeds the capacity of a given DNA repair pathway to recognize and remove the lesion. Exceeded repair capacity may lead to toxicity or mutagenesis following DNA damage. Apart from extremely high DNA lesion burden, inadequate repair may arise through several different specific mechanisms. For example, during repair of DNA containing O6-alkylguanine adducts, AGT irreversibly binds a single O6-alkylguanine lesion and as a result is inactivated (this is termed suicide inactivation, as its own action causes it to become inactivated). Thus, the capacity of AGT to carry out alkylation repair can become rapidly saturated when the DNA repair rate exceeds the de novo synthesis of AGT (Pegg, 2011).

A second mechanism relates to cell specific differences in the cellular levels or activity of some DNA repair proteins. For example, XPA is an essential component of the NER complex. The level of XPA that is active in NER is low in the testes, which may reduce the efficiency of NER in testes as compared to other tissues (Köberle et al., 1999). Likewise, both NER and BER have been reported to be deficient in cells lacking functional p53 (Adimoolam and Ford, 2003; Hanawalt et al., 2003; Seo and Jung, 2004). A third mechanism relates to the importance of the DNA sequence context of a lesion in its recognition by DNA repair enzymes. For example, 8-oxoguanine (8-oxoG) is repaired primarily by BER; the lesion is initially acted upon by a bifunctional glycosylase, OGG1, which carries out the initial damage recognition and excision steps of 8-oxoG repair. However, the rate of excision of 8-oxoG is modulated strongly by both chromatin components (Menoni et al., 2012) and DNA sequence context (Allgayer et al., 2013) leading to significant differences in the repair of lesions situated in different chromosomal locations.

DNA repair is also remarkably error-free. However, misrepair can arise during repair under some circumstances. DSBR is notably error prone, particularly when breaks are processed through NHEJ, during which partial loss of genome information is common at the site of the double strand break (Iyama and Wilson, 2013). This is because NHEJ rejoins broken DNA ends without the use of extensive homology; instead, it uses the microhomology present between the two ends of the DNA strand break to ligate the strand back into one. When the overhangs are not compatible, however, indels (insertion or deletion events), duplications, translocations, and inversions in the DNA can occur. These changes in the DNA may lead to significant issues within the cell, including alterations in the gene determinants for cellular fatality (Moore et al., 1996).

Activation of mutagenic DNA repair pathways to withstand cellular or replication stress either from endogenous or exogenous sources can promote cellular viability, albeit at a cost of increased genome instability and mutagenesis (Fitzgerald et al., 2017). These salvage DNA repair pathways including, Break-induced Replication (BIR) and Microhomology-mediated Break-induced Replication (MMBIR). BIR repairs one-ended DSBs and has been extensively studied in yeast as well as in mammalian systems. BIR and MMBIR are linked with heightened levels of mutagenesis, chromosomal rearrangements and ensuing genome instability (Deem et al., 2011; Sakofsky et al., 2015; Saini et al., 2017; Kramara et al., 2018). In mammalian genomes BIR-like synthesis has been proposed to be involved in late stage

Mitotic DNA Synthesis (MiDAS) that predominantly occurs at so-called Common Fragile Sites (CFSs) and maintains telomere length under s conditions of replication stress that serve to promote cell viability (Minocherhomji et al., 2015; Bhowmick et al., 2016; Dilley et al., 2016).

Misrepair may also occur through other repair pathways. Excision repair pathways require the resynthesis of DNA and rare DNA polymerase errors during gap resynthesis will result in mutations (Brown et al., 2011). Errors may also arise during gap resynthesis when the strand that is being used as a template for DNA synthesis contains DNA lesions (Kozmin and Jinks-Robertson, 2013). In addition, it has been shown that sequences that contain tandemly repeated sequences, such as CAG triplet repeats, are subject to expansion during gap resynthesis that occurs during BER of 8-oxoG damage (Liu et al., 2009).

How it is Measured or Detected

There is no test guideline for this event. The event is usually inferred from measuring the retention of DNA adducts or the creation of mutations as a measure of lack of repair or incorrect repair. These ‘indirect’ measures of its occurrence are crucial to determining the mechanisms of genotoxic chemicals and for regulatory applications (i.e., determining the best approach for deriving a point of departure). More recently, a fluorescence-based multiplex flow-cytometric host cell reactivation assay (FM-HCR) has been developed to directly measure the ability of human cells to repair plasmid reporters (Nagel et al., 2014).

Indirect Measurement

In somatic and spermatogenic cells, measurement of DNA repair is usually inferred by measuring DNA adduct formation/removal. Insufficient repair is inferred from the retention of adducts and from increasing adduct formation with dose. Insufficient DNA repair is also measured by the formation of increased numbers of mutations and alterations in mutation spectrum. The methods will be specific to the type of DNA adduct that is under study.

Some EXAMPLES are given below for alkylated DNA.

DOSE-RESPONSE CURVE FOR ALKYL ADDUCTS/MUTATIONS: It is important to consider that some adducts are not mutagenic at all because they are very effectively repaired. Others are effectively repaired, but if these repair processes become overwhelmed mutations begin to occur. The relationship (shape of dose-response curve) between exposure to mutagenic agents and mutations provide an indication of whether the removal of adducts occurs, and whether it is more efficient at low doses. Sub-linear dose-response curves (hockey stick or j-shape curves) for mutation induction indicates that adducts are not converted to mutations at low doses. This suggests the effective repair of adducts at low doses, followed by saturation of repair at higher doses (Clewell et al., 2019). Thus, measurement of a clear point of inflection in the dose-response curve for mutations suggests that repair does occur, at least to some extent, at low dosees but that reduced repair efficiency arises above the inflection point. A lack of increase in mutation frequencies (i.e., flat line for dose-response) for a compound showing a dose-dependent increase in adducts would imply that the adducts formed are either not mutagenic or are effectively repaired.

RETENTION OF ALKYL ADDUCTS: Alkylated DNA can be found in cells long after exposure has occurred. This indicates that repair has not effectively removed the adducts. For example, DNA adducts have been measured in hamster and rat spermatogonia several days following exposure to alkylating agents, indicating lack of repair (Seiler et al., 1997; Scherer et al., 1987).

MUTATION SPECTRUM: Shifts in mutation spectrum (i.e., the specific changes in the DNA sequence) following a chemical exposure (relative to non-exposed mutation spectrum) indicates that repair was not operating effectively to remove specific types of lesions. The shift in mutation spectrum is indicative of the types of DNA lesions (target nucleotides and DNA sequence context) that were not repaired. For example, if a greater proportion of mutations occur at guanine nucleotides in exposed cells, it can be assumed that the chemical causes DNA adducts on guanine that are not effectively repaired.

Direct Measurement

Nagel et al. (2014) we developed a fluorescence-based multiplex flow-cytometric host cell reactivation assay (FM-HCR) to measures the ability of human cells to repair plasmid reporters. These reporters contain different types and amounts of DNA damage and can be used to measure repair through by NER, MMR, BER, NHEJ, HR and MGMT.

Please refer to the table below for additional details and methodologies for detecting DNA damage and repair.

Assay Name	References	Description	DNA Damage/Repair Being Measured	OECD Approved Assay

Dose-Response Curve for Alkyl Adducts/ Mutations	Lutz 1991 Clewell 2016	Creation of a curve plotting the stressor dose and the abundance of adducts/mutations; Characteristics of the resulting curve can provide information on the efficiency of DNA repair	Alkylation, oxidative damage, or DSBs	N/A
Retention of Alkyl Adducts	Seiler 1997 Scherer 1987	Examination of DNA for alkylation after exposure to an alkylating agent; Presence of alkylation suggests a lack of repair	Alkylation	N/A
Mutation Spectrum	Wyrick 2015	Shifts in the mutation spectrum after exposure to a chemical/mutagen relative to an unexposed subject can provide an indication of DNA repair efficiency, and can inform as to the type of DNA lesions present	Alkylation, oxidative damage, or DSBs	N/A
DSB Repair Assay (Reporter constructs)	Mao et al., 2011	Transfection of a GFP reporter construct (and DsRed control) where the GFP signal is only detected if the DSB is repaired; GFP signal is quantified using fluorescence microscopy or flow cytometry	DSBs	N/A
Primary Rat Hepatocyte DNA Repair Assay	Jeffrey and Williams, 2000 – Butterworth et al., 1987	Rat primary hepatocytes are cultured with a ^{3}H-thymidine solution in order to measure DNA synthesis in response to a stressor in non-replicating cells; Autoradiography is used to measure the amount of ^{3}H incorporated in the DNA post-repair	Unscheduled DNA synthesis in response to DNA damage	N/A
Repair synthesis measurement by ^{3}H-thymine incorporation	Iyama and Wilson, 2013	Measure DNA synthesis in non-dividing cells as indication of gap filling during excision repair	Excision repair	N/A
Comet Assay with Time-Course	Olive et al., 1990 – Trucco et al., 1998 - Dunkenberger et al., 2022	Comet assay is performed with a time-course under alkaline conditions to detect SSBs and DSBs. Quantity of DNA in the tail should decrease as DNA repair progresses	DSBs	Yes (No. 489)

Flow Cytometry	Corneo et al., 2007	The alt-NHEJ flow cytometer method involves utilizing an extrachromosomal substrate. Green fluorescent protein (GFP) expression is indicative of successful alt-NHEJ activity, contingent on the removal of 10 nucleotides from each end of the DNA and subsequent rejoining within a 9-nucleotide microhomology region. This approach provides a quantitative and visual means to measure the efficiency of alternative non-homologous end joining in cellular processes.	Alt NHEJ	No
Pulsed Field Gel Electro-phoresis (PFGE) with Time-Course	Biedermann_et al., 1991	PFGE assay with a time-course; Quantity of small DNA fragments should decrease as DNA repair progresses	DSBs	N/A
Fluorescence -Based Multiplex Flow-Cytometric Host Reactivation Assay (FM-HCR)	Nagel et al., 2014	Measures the ability of human cells to repair plasma reporters, which contain different types and amounts of DNA damage; Used to measure repair processes including HR, NHEJ, BER, NER, MMR, and MGMT	HR, NHEJ, BER, NER, MMR, or MGMT	N/A
Alkaline Unwinding Assay with Time Course	Nacci et al. 1991	DNA is stored in alkaline solutions with DNA-specific dye and allowed to unwind following removal from tissue, increased strand damage associated with increased unwinding. Samples analyzed at different time points to compare remaining damage following repair opportunities	DSBs	Yes (No. 489)
Sucrose Density Gradient Centrifugation with Time Course	Larsen et al. 1982	Strand breaks alter the molecular weight of the DNA piece. DNA in alkaline solution centrifuged into sugar density gradient, repeated set time apart. The less DNA breaks identified in the assay repeats, the more repair occurred	SSBs	N/A

y-H2AX Foci Staining with Time Course	Mariotti et al. 2013 Penninckx et al. 2021	Histone H2AX is phosphorylated in the presence of DNA strand breaks, the rate of its disappearance over time is used as a measure of DNA repair	DSBs	N/A
Alkaline Elution Assay with Time Course	Larsen et al. 1982	DNA with strand breaks elute faster than DNA without, plotted against time intervals to determine the rate at which strand breaks repair	SSBs	N/A
53BP1 foci Detection with Time Course	Penninckx et al. 2021	53BP1 is recruited to the site of DNA damage, the rate at which its level decreases over time is used to measure DNA repair	DSBs	N/A

References

Adimoolam, S. & J.M. Ford (2003), "p53 and regulation of DNA damage recognition during nucleotide excision repair" *DNA Repair* (Amst), 2(9): 947-54.

Aleksandrov, Radoslav et al. (2018), “Protein Dynamics in Complex DNA Lesions.” Molecular cell,69(6): 1046-1061.e5. doi:10.1016/j.molcel.2018.02.016

Allgayer, J. et al. (2013), "Modulation of base excision repair of 8-oxoguanine by the nucleotide sequence", *Nucleic Acids Res*, 41(18): 8559-8571. Doi: 10.1093/nar/gkt620.

Beranek, D.T. (1990), "Distribution of methyl and ethyl adducts following alkylation with monofunctional alkylating agents", *Mutation Research*, 231(1): 11-30. Doi: 10.1016/0027-5107(90)90173-2.

Bhatti, A. et al., (2016), “Homologous Recombination Biology.”, *Encyclopedia Britannica*.

Bhowmick, R., S. et al. (2016), "RAD52 Facilitates Mitotic DNA Synthesis Following Replication Stress", *Mol Cell*, 64:1117-1126. Doi: 10.1016/j.molcel.2016.10.037.

Biedermann, A. K. et al. (1991), “SCID mutation in mice confers hypersensitivity to ionizing radiation and a deficiency in DNA double-strand break repair”, *Cell Biology*, 88(4): 1394-7. Doi: 10.1073/pnas.88.4.1394.

Boboila, C., F. W. Alt & B. Schwer. (2012), “Classical and alternative end-joining pathways for repair of lymphocyte-specific and general DNA double-strand breaks.” *Adv Immunol*, 116, 1-49. doi:10.1016/B978-0-12-394300-2.00001-6

Bronstein, S.M. et al. (1991), "Toxicity, mutagenicity, and mutational spectra of N-ethyl-N-nitrosourea in human cell lines with different DNA repair phenotypes", *Cancer Research*, 51(19): 5188-5197.

Bronstein, S.M. et al. (1992), "Efficient repair of O6-ethylguanine, but not O4-ethylthymine or O2-ethylthymine, is dependent upon O6-alkylguanine-DNA alkyltransferase and nucleotide excision repair activities in human cells", *Cancer Research*, 52(7): 2008-2011.

Brown, J.A. et al. (2011), "Efficiency and fidelity of human DNA polymerases λ and β during gap-filling DNA synthesis", *DNA Repair (Amst).*, 10(1):24-33.

Butterworth, E. B. et al., (1987), A protocol and guide for the in vitro rat hepatocyte DNA-repair assay. *Mutation Research*. 189, 113-21. Doi: 10.1016/0165-1218(87)90017-6.

Caldecott, K. W. (2014), "DNA single-strand break repair", Exp Cell Res, 329(1): 2-8.

Chaudhuri, R.A. and Nussenzweig, A. (2017), “The multifaceted roles of PARP1 in DNA repair and chromatin remodelling”. Nat Rev Mol Cell Biol 18, 610–621. https://doi.org/10.1038/nrm.2017.53

Chen, L. et al., (2001), Promotion of DNA ligase IV-catalyzed DNA end-joining by the Rad50/Mre11/Xrs2 and Hdf1/Hdf2 complexes. *Mol Cell*. 8(5), 1105-15.

Chiruvella, K. K., Z. Liang & T. E. Wilson, (2013), Repair of Double-Strand Breaks by End Joining. *Cold Spring Harbor Perspectives in Biology*, 5(5):127-57. Doi: 10.1101/cshperspect.a012757.

Clewell, R. A. et al. (2019). “Dose-dependence of chemical carcinogenicity: Biological mechanisms for thresholds and implications for risk assessment”. Chem Biol Interact. 2019 Mar 1;301:112-127. doi: 10.1016/j.cbi.2019.01.025.

Corneo, B. et al., 2007, "Rag mutations reveal robust alternative end joining". Nature 449, 483–486 (2007). https://doi.org/10.1038/nature06168

Dahle, J., et al. (2008), "Overexpression of human OGG1 in mammalian cells decreases ultraviolet A induced mutagenesis", Cancer Letters, Vol.267, Elsevier, Amsterdam, https://doi.org/10.1016/j.canlet.2008.03.002.

Deem, A. et al. (2011), "Break-Induced Replication Is Highly Inaccurate.", *PLoS Biol*. 9:e1000594. Doi: 10.1371/journal.pbio.1000594.

Dianov, G.L. & U. Hübscher (2013), "Mammalian base excision repair: the forgotten archangel", *Nucleic Acids Res*., 41(6):3483-90. Doi: 10.1093/nar/gkt076.

Dilley, R.L. et al. Greenberg (2016), "Break-induced telomere synthesis underlies alternative telomere maintenance", *Nature*, 539:54-58. Doi: 10.1038/nature20099.

Douglas, G.R. et al. (1995), "Temporal and molecular characteristics of mutations induced by ethylnitrosourea in germ cells isolated from seminiferous tubules and in spermatozoa of lacZ transgenic mice", *Proceedings of the National Academy of Sciences of the United States of America*, 92(16):7485-7489. Doi: 10.1073/pnas.92.16.7485.

Dunkenberger, Logan et al. (2022), "Comet Assay for the Detection of Single and Double-Strand DNA Breaks." Methods in molecular biology (Clifton, N.J.), 2422: 263-269. doi:10.1007/978-1-0716-1948-3_18

Fattah, F. et al., (2010), Ku regulates the non-homologous end joining pathway choice of DNA double-strand break repair in human somatic cells. PLoS Genet, 6(2), doi:10.1371/journal.pgen.1000855

Fitzgerald, D.M., P.J. Hastings, and S.M. Rosenberg (2017), "Stress-Induced Mutagenesis: Implications in Cancer and Drug Resistance", Ann Rev Cancer Biol, 1:119-140. Doi: 10.1146/annurev-cancerbio-050216-121919.

Gorbunova, V. and A. Seluanov. (2016), "DNA double strand break repair, aging and the chromatin connection", Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, Vol.788/1-2, Elsevier, Amsterdam, http://dx.doi.org/10.1016/j.mrfmmm.2016.02.004.

Hammel, M. et al., (2011), XRCC4 protein interactions with XRCC4-like factor (XLF) create an extended grooved scaffold for DNA ligation and double strand break repair. J Biol Chem, 286(37), 32638-32650. doi:10.1074/jbc.M111.272641.

Hanawalt, P.C., J.M. Ford and D.R. Lloyd (2003), "Functional characterization of global genomic DNA repair and its implications for cancer", *Mutation Research*, 544(2-3): 107–114.

Harbach, P. R. et al., (1989), "The in vitro unscheduled DNA synthesis (UDS) assay in rat primary hepatocytes", *Mutation Research*, 216(2):101-10. Doi:10.1016/0165-1161(89)90010-1.

Iyama, T. and D.M. Wilson III (2013), "DNA repair mechanisms in dividing and non-dividing cells", *DNA Repair*, 12(8): 620– 636.

Jeffrey, M. A.& M. G. Williams, (2000), "Lack of DNA-damaging Activity of Five Non-nutritive Sweeteners in the Rat Hepatocyte/DNA Repair Assay", *Food and Chemical Toxicology*, 38: 335-338. Doi: 10.1016/S0278-6915(99)00163-5.

Köberle, B. et al. (1999), "Defective repair of cisplatin-induced DNA damage caused by reduced XPA protein in testicular germ cell tumours", *Curr. Biol*., 9(5):273-6. Doi: 10.1016/s0960-9822(99)80118-3.

Kozmin, S.G. & S. Jinks-Robertson S. (2013), "The mechanism of nucleotide excision repair-mediated UV-induced mutagenesis in nonproliferating cells", *Genetics*, 193(3): 803-17. Doi: 10.1534/genetics.112.147421.

Kramara, J., B. Osia, and A. Malkova (2018), "Break-Induced Replication: The Where, The Why, and The How", *Trends Genet*, 34:518-531. Doi: 10.1016/j.tig.2018.04.002.

Kuhne, M., G. Urban and M. Lo (2005), "DNA Double-Strand Break Misrejoining after Exposure of Primary Human Fibroblasts to CK Characteristic X Rays, 29 kVp X Rays and 60Co γ Rays", Radiation. Research, Vol.164/5, Radiation Research Society, Indianapolis, https://doi.org/10.1667/RR3461.1.

Larsen, K.H. et al. (1982), "DNA repair assays as tests for environmental mutagens: A report of the U.S. EPA gene-tox program", Mutation Research, Vol.98/3, Elsevier, Amsterdam, https://doi.org/10.1016/0165-1110(82)90037-9.

Li Z, A. H. Pearlman, and P. Hsieh (2016), "DNA mismatch repair and the DNA damage response", *DNA Repair (Amst)*, 38:94-101.

Lieber, M. R., (2010), "The mechanism of double-strand DNA break repair by the nonhomologous DNA end-joining pathway." *Annu Rev Biochem*. 79:181-211. doi:10.1146/annurev.biochem.052308.093131.

Lieber, M. R. et al., (2003), "Mechanism and regulation of human non-homologous DNA end-joining", *Nat Rev Mol Cell Biol*. 4(9):712-720. doi:10.1038/nrm1202.

Liu, Y. et al. (2009), "Coordination between polymerase beta and FEN1 can modulate CAG repeat expansion", J. Biol. Chem., 284(41): 28352-28366. Doi: 10.1074/jbc.M109.050286.

Mao, Z. et al., (2011), "SIRT6 promotes DNA repair under stress by activating PARP1", *Science*. 332(6036): 1443-1446. doi:10.1126/science.1202723.

Mariotti, L.G. et al. (2013), “Use of the γ-H2AX Assay to Investigate DNA Repair Dynamics Following Multiple Radiation Exposures”, PLoS ONE, Vol.8/11, PLoS, San Francisco, https://doi.org/10.1371/journal.pone.0079541.

Matthews, L. A., & L. A. Simmons, (2014), “Bacterial nonhomologous end joining requires teamwork”,*J Bacteriol*. 196(19): 3363-3365. doi:10.1128/JB.02042-14.

Menoni, H. et al. (2012), "Base excision repair of 8-oxoG in dinucleosomes", *Nucleic Acids Res.* ,40(2): 692-700. Doi: 10.1093/nar/gkr761.

Minocherhomji, S. et al. (2015), "Replication stress activates DNA repair synthesis in mitosis", *Nature*, 528:286-290. Doi: 10.1038/nature16139.

Miyaoka, Y. et al., (2016), “Systematic quantification of HDR and NHEJ reveals effects of locus, nuclease, and cell type on genome-editing”, *Sci Rep*, 6, 23549. doi:10.1038/srep23549/.

Mladenov. et al. (2023), . “New Facets of DNA Double Strand Break Repair: Radiation Dose as Key Determinant of HR versus c-NHEJ Engagement”. International journal of molecular sciences, 24(19), 14956. https://doi.org/10.3390/ijms241914956

Moore, J. K., & J. E. Haber, (1996), “Cell cycle and genetic requirements of two pathways of nonhomologous end-joining repair of double-strand breaks in Saccharomyces cerevisiae”, *Molecular and Cellular Biology*, 16(5), 2164–73. Doi: 10.1128/MCB.16.5.2164.

Nacci, D. et al. (1992), “Application of the DNA alkaline unwinding assay to detect DNA strand breaks in marine bivalves”, Marine Environmental Research, Vol.33/2, Elsevier BV, Amsterdam, https://doi.org/10.1016/0141-1136(92)90134-8.

Nagel, Z.D. et al. (2014), "Multiplexed DNA repair assays for multiple lesions and multiple doses via transcription inhibition and transcriptional mutagenesis", *Proc. Natl. Acad. Sci. USA*, 111(18):E1823-32. Doi: 10.1073/pnas.1401182111.

O’Brien, J.M. et al. (2015), "Sublinear response in lacZ mutant frequency of Muta™ Mouse spermatogonial stem cells after low dose subchronic exposure to N-ethyl-N-nitrosourea", *Environ. Mol. Mutagen.*, 56(4): 347-55. Doi: 10.1002/em.21932.

Olive, L. P., J. P. Bnath & E. R. Durand, (1990), “Heterogeneity in Radiation-Induced DNA Damage and Repairing Tumor and Normal Cells Measured Using the "Comet" Assay”, *Radiation Research*. 122: 86-94. Doi: 10.1667/rrav04.1.

Pardo, B., B. Gomez-Gonzalez & A. Aguilera, (2009), “DNA repair in mammalian cells: DNA double-strand break repair: how to fix a broken relationship*”, Cell Mol Life Sci*, 66(6), 1039-1056. doi:10.1007/s00018-009-8740-3.

Pegg, A.E. (2011), "Multifaceted roles of alkyltransferase and related proteins in DNA repair, DNA damage, resistance to chemotherapy, and research tools", *Chem. Res. Toxicol.*, 4(5): 618-39. Doi: 10.1021/tx200031q.

Penninckx, S. et al. (2021), “Quantification of radiation-induced DNA double strand break repair foci to evaluate and predict biological responses to ionizing radiation”, NAR Cancer, Vol.3/4, Oxford University Press, Oxford, https://doi.org/10.1093/narcan/zcab046.

Rydberg, B. et al. (2005), "Dose-Dependent Misrejoining of Radiation-Induced DNA Double-Strand Breaks in Human Fibroblasts: Experimental and Theoretical Study for High- and Low-LET Radiation", Radiation Research, Vol.163/5, Radiation Research Society, Indianapolis, https://doi.org/10.1667/RR3346.

Sancar, A. (2003), "Structure and function of DNA photolyase and cryptochrome blue-light photoreceptors", *Chem Rev.*, 103(6): 2203-37. Doi: 10.1021/cr0204348.

Saini, N. et al. (2017), "Migrating bubble during break-induced replication drives conservative DNA synthesis", *Nature*, 502:389-392. Doi: 10.1038/nature12584.

Sakofsky, C.J. et al. (2015), "Translesion Polymerases Drive Microhomology-Mediated Break-Induced Replication Leading to Complex Chromosomal Rearrangements", *Mol Cell*, 60:860-872. Doi: 10.1016/j.molcel.2015.10.041.

Schärer, O.D. (2013), "Nucleotide excision repair in eukaryotes", *Cold Spring Harb. Perspect. Biol.*, 5(10): a012609. Doi: 10.1101/cshperspect.a012609.

Scherer, E., A.A. Jenner and L. den Engelse (1987), "Immunocytochemical studies on the formation and repair of O6-alkylguanine in rat tissues", *IARC Sci Publ.*, 84: 55-8.

Seiler, F., K. Kamino, M. Emura, U. Mohr and J. Thomale (1997), "Formation and persistence of the miscoding DNA alkylation product O6-ethylguanine in male germ cells of the hamster", *Mutat Res.*, 385(3): 205-211. Doi: 10.1016/s0921-8777(97)00043-8.

Shelby, M.D. and K.R. Tindall (1997), "Mammalian germ cell mutagenicity of ENU, IPMS and MMS, chemicals selected for a transgenic mouse collaborative study", *Mutation Research*, 388(2-3): 99-109. Doi: 10.1016/s1383-5718(96)00106-4.

Seo, Y.R. and H.J. Jung (2004), "The potential roles of p53 tumor suppressor in nucleotide excision repair (NER) and base excision repair (BER)", *Exp. Mol. Med.*, 36(6): 505-509. Doi: 10.1038/emm.2004.64.

Sundheim, O. et al. (2008), "AlkB demethylases flip out in different ways", *DNA Repair (Amst).*, 7(11): 1916-1923. Doi: 10.1016/j.dnarep.2008.07.015.

Sung, P., & H. Klein, (2006), “Mechanism of homologous recombination: mediators and helicases take on regulatory functions”, *Nat Rev Mol Cell Biol*, 7(10), 739-750. Doi:10. 1038/nrm2008.

Trucco, C., et al., (1998), “DNA repair defect i poly(ADP-ribose) polymerase-deficient cell lines”, Nucleic Acids Research. 26(11): 2644–2649. Doi: 10.1093/nar/26.11.2644.

Trzeciak, A.R. et al. (2008), “Age, sex, and race influence single-strand break repair capacity in a human population”, Free Radical Biology & Medicine, Vol. 45, Elsevier, Amsterdam, https://doi.org/10.1016/j.freeradbiomed.2008.08.031.

White, R.R. and J. Vijg. (2016), “Do DNA Double-Strand Breaks Drive Aging?”, Molecular Cell, Vol.63, Elsevier, Amsterdam, http://doi.org/10.1016/j.molcel.2016.08.004.

Wyrick, J.J. & S. A. Roberts, (2015), “Genomic approaches to DNA repair and mutagenesis”, DNA Repair (Amst). 36:146-155. doi: 10.1016/j.dnarep.2015.09.018.

van Zeeland, A.A., A. de Groot and A. Neuhäuser-Klaus (1990), "DNA adduct formation in mouse testis by ethylating agents: a comparison with germ-cell mutagenesis", *Mutat. Res.*, 231(1): 55-62.

Event: 185: Increase, Mutations

Short Name: Increase, Mutations

Key Event Component

Process	Object	Action
mutation	deoxyribonucleic acid	increased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:15 - Alkylation of DNA in male pre-meiotic germ cells leading to heritable mutations	KeyEvent
Aop:141 - Alkylation of DNA leading to cancer 2	KeyEvent
Aop:139 - Alkylation of DNA leading to cancer 1	KeyEvent
Aop:294 - Increased reactive oxygen and nitrogen species (RONS) leading to increased risk of breast cancer	AdverseOutcome
Aop:293 - Increased DNA damage leading to increased risk of breast cancer	AdverseOutcome
Aop:296 - Oxidative DNA damage leading to chromosomal aberrations and mutations	AdverseOutcome
Aop:272 - Deposition of energy leading to lung cancer	KeyEvent
Aop:397 - Bulky DNA adducts leading to mutations	AdverseOutcome
Aop:443 - DNA damage and mutations leading to Metastatic Breast Cancer	KeyEvent
Aop:478 - Deposition of energy leading to occurrence of cataracts	KeyEvent

Stressors

Name
Ionizing Radiation

Biological Context

Level of Biological Organization

Molecular

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Mus musculus	Mus musculus	High	NCBI
medaka	Oryzias latipes	Moderate	NCBI
rat	Rattus norvegicus	High	NCBI
Homo sapiens	Homo sapiens	Moderate	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Unspecific	High

Taxonomic applicability: Mutations can occur in any organism and in any cell type, and are the fundamental material of evolution. The test guidelines described above range from analysis from prokaryotes, to rodents, to human cells in vitro. Mutations have been measured in virtually every human tissue sampled in vivo.

Life stage applicability: This key event is not life stage specific as all stages of life have DNA that can be mutated; however, baseline levels of mutations are seen to increase with age (Slebos et al., 2004; Kirkwood, 1989).

Sex applicability: This key event is not sex specific as both sexes undergo mutations. Males have a higher mutation rate than females (Hedrick, 2007).

Evidence for perturbation by a stressor: Many studies demonstrate that increased mutations can occur as a result of ionizing radiation (Sankaranarayanan & Nikjoo, 2015; Russell et al., 1957; Winegar et al., 1994; Gossen et al., 1995).

Key Event Description

A mutation is a change in DNA sequence. Mutations can thus alter the coding sequence of genes, potentially leading to malformed or truncated proteins. Mutations can also occur in promoter regions, splice junctions, non-coding RNA, DNA segments, and other functional locations in the genome. These mutations can lead to various downstream consequences, including alterations in gene expression. There are several different types of mutations including missense, nonsense, insertion, deletion, duplication, and frameshift mutations, all of which can impact the genome and its expression in unique ways.

Missense mutations are the substitution of one base in the codon with another. This change is significant because the three bases in a codon code for a specific amino acid and the new combination may signal for a different amino acid to be formed. Nonsense mutations also result from changes to the codon bases, but in this case, they cause the generation of a stop codon in the DNA strand where there previously was not one. This stop codon takes the place of a normal coding triplet, preventing its translation into an amino acid. This will cause the translation of the strand to prematurely stop. Both missense and nonsense mutations can result from substitutions, insertions, or deletions of bases (Chakarov et al. 2014).

Insertion and deletion mutations are the addition and removal of bases from the strand, respectively. These often accompany a frameshift mutation, as the alteration in the number of bases in the strand causes the frame of the base reader to shift by the added or reduced number, altering the amino acids that are produced if that number is not devisable by three. Codons come in specific orders, sectioned into groups of three. When the boundaries of which three bases are included in one group are changed, this can change the whole transcriptional output of the strand (Chakaroy et al. 2014).

Mutations can be propagated to daughter cells upon cellular replication. Mutations in stem cells (versus terminally differentiated non-replicating cells) are the most concerning, as these will persist in the organism. The consequence of the mutation, and thus the fate of the cell, depends on the location (e.g., coding versus non-coding) and the type (e.g., nonsense versus silent) of mutation.

Mutations can occur in somatic cells or germ cells (sperm or egg).

How it is Measured or Detected

Mutations can be measured using a variety of both OECD and non-OECD mutagenicity tests. Listed below are common methods for detecting the KE, however there may be other comparable methods that are not listed.

Somatic cells: The Salmonella mutagenicity test (Ames Test) is generally used as part of a first tier screen to determine if a chemical can cause gene mutations. This well-established test has an OECD test guideline (OECD TG 471, 2020). A variety of bacterial strains are used, in the presence and absence of a metabolic activation system

(e.g., rat liver microsomal S9 fraction), to determine the mutagenic potency of chemicals by dose-response analysis. A full description is found in Test No. 471: Bacterial Reverse Mutation Test (OECD, 2016).

A variety of in vitro mammalian cell gene mutation tests are described in OECD's Test Guidelines 476 (2016) and 490 (2015). TG 476 (2016) is used to identify substances that induce gene mutations at the hprt (hypoxanthine-guanine phosphoribosyl transferase) gene, or the transgenic xprt (xanthine-guanine phosphoribosyl transferase) reporter locus. The most commonly used cells for the HPRT test include the CHO, CHL and V79 lines of Chinese hamster cells, L5178Y mouse lymphoma cells, and TK6 human lymphoblastoid cells. The only cells suitable for the XPRT test are AS52 cells containing the bacterial xprt (or gpt) transgene (from which the hprt gene was deleted).

The new OECD TG 490 (2015) describes two distinct in vitro mammalian gene mutation assays using the thymidine kinase (tk) locus and requiring two specific tk heterozygous cells lines: L5178Y tk+/-3.7.2C cells for the mouse lymphoma assay (MLA) and TK6 tk+/- cells for the TK6 assay. The autosomal and heterozygous nature of the thymidine kinase gene in the two cell lines enables the detection of cells deficient in the enzyme thymidine kinase following mutation from tk+/- to tk-/-.

It is important to consider that different mutation spectra are detected by the different mutation endpoints assessed. The non-autosomal location of the hprt gene (X-chromosome) means that the types of mutations detected in this assay are point mutations, including base pair substitutions and frameshift mutations resulting from small insertions and deletions. Whereas, the autosomal location of the transgenic xprt, tk, or gpt locus allows the detection of large deletions not readily detected at the hemizygous hprt locus on X-chromosomes. Genetic events detected using the tk locus include both gene mutations (point mutations, frameshift mutations, small deletions) and large deletions.

The transgenic rodent mutation assay (OECD TG 488, 2020) is the only assay capable of measuring gene mutation in virtually all tissues in vivo. Specific details on the rodent transgenic mutation reporter assays are reviewed in Lambert et al. (2005, 2009). The transgenic reporter genes are used for detection of gene mutations and/or chromosomal deletions and rearrangements resulting in DNA size changes (the latter specifically in the lacZ plasmid and Spi- test models) induced in vivo by test substances (OECD, 2009, OECD, 2011; Lambert et al., 2005). Briefly, transgenic rodents (mouse or rat) are exposed to the chemical agent sub-chronically. Following a manifestation period, genomic DNA is extracted from tissues, transgenes are rescued from genomic DNA, and transfected into bacteria where the mutant frequency is measured using specific selection systems.

The Pig-a (phosphatidylinositol glycan, Class A) gene on the X chromosome codes for a catalytic subunit of the N-acetylglucosamine transferase complex that is involved in glycosylphosphatidyl inositol (GPI) cell surface anchor synthesis. Cells lacking GPI anchors, or GPI-anchored cell surface proteins are predominantly due to mutations in the Pig-a gene. Thus, flow cytometry of red blood cells expressing or not expressing the Pig-a gene has been developed for mutation analysis in blood cells from humans, rats, mice, and monkeys. The assay is described in detail in Dobrovolsky et al. (2010). Development of an OECD guideline for the Pig-a assay is underway. In addition, experiments determining precisely what proportion of cells expressing the Pig-a mutant phenotype have mutations in the Pig-a gene are in progress (e.g., Nicklas et al., 2015, Drobovolsky et al., 2015). A recent paper indicates that the majority of CD48 deficient cells from 7,12-dimethylbenz[a]anthracene-treated rats (78%) are indeed due to mutation in Pig-a (Drobovolsky et al., 2015).

Germ cells: Tandem repeat mutations can be measured in bone marrow, sperm, and other tissues using single-molecule PCR. This approach has been applied most frequently to measure repeat mutations occurring in sperm DNA. Isolation of sperm DNA is as described above for the transgenic rodent mutation assay, and analysis of tandem repeats is done using electrophoresis for size analysis of allele length using single-molecule PCR. For expanded simple tandem repeat this involved agarose gel electrophoresis and Southern blotting, whereas for microsatellites sizing is done by capillary electrophoresis. Detailed methodologies for this approach are found in Yauk et al. (2002) and Beal et al. (2015).

Mutations in rodent sperm can also be measured using the transgenic reporter model (OECD TG 488, 2020). A description of the approach is found within this published TG. Further modifications to this protocol have been made as of 2022 for the analysis of germ cells. Detailed methodology for detecting mutant frequency arising in spermatogonia is described in Douglas et al. (1995), O'Brien et al. (2013); and O'Brien et al. (2014). Briefly, male mice are exposed to the mutagen and killed at varying times post-exposure to evaluate effects on different phases of spermatogenesis. Sperm are collected from the vas deferens or caudal epididymis (the latter preferred). Modified protocols have been developed for extraction of DNA from sperm.

A similar transgenic assay can be used in transgenic medaka (Norris and Winn, 2010).

Please note, gene mutations that occur in somatic cells in vivo (OECD Test. No. 488, 2020) or in vitro (OECD Test No. 476: In vitro Mammalian Cell Gene Mutation Test, 2016), or in bacterial cells (i.e., OECD Test No. 471, 2020) can be used as an indicator that mutations in male pre-meiotic germ cells may occur for a particular agent (sensitivity and specificity of other assays for male germ cell effects is given in Waters et al., 1994). However, given the very unique biological features of spermatogenesis relative to other cell types, known exceptions to this rule, and the small database on which this is based, inferring results from somatic cell or bacterial tests to male pre-meiotic germ cells must be done with caution. That mutational assays in somatic cells may predict mutations in germ cells has not been rigorously tested empirically (Singer and Yauk, 2010). The IWGT working group on germ cells specifically addressed this gap in knowledge in their report (Yauk et al., 2015) and recommended that additional research address this issue. Mutations can be directly measured in humans (and other species) through the application of next-generation sequencing. Although single-molecule approaches are growing in prevalence, the most robust approach to measure

mutation using next-generation sequencing today requires clonal expansion of the mutation to a sizable proportion (e.g., sequencing tumours; Shen et al., 2015), or analysis of families to identify germline derived mutations (reviewed in Campbell and Eichler, 2013; Adewoye et al., 2015).

Please refer to the table below for additional details and methodologies for measuring mutations.

Assay Name	**References**	**Description**	**OECD Approved Assay**
Assorted Gene Loci Mutation Assays	Tindall et al., 1989; Kruger et al., 2015	After exposure to a chemical/mutagen, mutations can be measured by the ability of exposed cells to form colonies in the presence of specific compounds that would normally inhibit colony growth; Usually only cells -/- for the gene of interest are able to form colonies	N/A
TK Mutation Assay	Yamamoto et al., 2017; Liber et al., 1982; Lloyd and Kidd, 2012	After exposure to a chemical/mutagen, mutations are detected at the thymidine kinase (TK) loci of L5178Y wild-type mouse lymphoma TK (+/-) cells by measuring resistance to lethaltriflurothymidine (TFT); Only TK-/- cells are able to form colonies	Yes (No. 490)
HPRT Mutation Assay	Ayres et al., 2006; Parry and Parry, 2012	Similar to TK Mutation Assay above, X-linked HPRT mutations produced in response to chemical/mutagen exposure can be measured through colony formation in the presence of 6-TG or 8-azoguanine; Only HPRT-/- cells are able to form colonies	Yes (No. 476)
Salmonella Mutagenicity Test (Ames Test)	OECD, 1997	After exposure to a chemical/mutagen, point mutations are detected by analyzing the growth capacity of different bacterial strains in the presence and absence of various metabolic activation systems	Yes (No. 471)
PIG-A / PIG-O Assay	Kruger et al., 2015; Nakamura, 2012; Chikura, 2019	After exposure to a chemical/mutagen, mutations in PIG-A or PIG-O (which decrease the biosynthesis of the glycosylphosphatidylinositol (GPI) anchor protein) are assessed by the colony-forming capabilities of cells after *in vitro* exposure, or by flow cytometry of blood samples after *in vivo* exposure	N/A

Single Molecule PCR	Kraytsberg & Khrapko, 2005; Yauk, 2002	This PCR technique uses a single DNA template, and is often employed for detection of mutations in microsatellites, recombination studies, and generation of polonies	N/A
ACB-PCR	Myers et al., 2014 (Textbook, pg 345-363); Banda et al., 2013; Banda et al., 2015; Parsons et al., 2017	Using this PCR technique, single base pair substitution mutations within oncogenes or tumour suppressor genes can be detected by selectively amplifying specific point mutations within an allele and selectively blocking amplification of the wild-type allele	N/A
Transgenic Rodent Mutation Assay	OECD 2013; Lambert 2005; Lambert 2009	This *in vivo* test detects gene mutations using transgenic rodents that possess transgenes and reporter genes; After *in vivo* exposure to a chemical/mutagen, the transgenes are analyzed by transfecting bacteria with the reporter gene and examining the resulting phenotype	Yes (No. 488)
Conditionally inducible transgenic mouse models	Parsons 2018 (Review)	Inducible mutations linked to fluorescent tags are introduced into transgenic mice; Upon exposure of the transgenic mice to an inducing agent, the presence and functional assessment of the mutations can be easily ascertained due to expression of the linked fluorescent tags	N/A
Error-Corrected Next Generation Sequencing (NGS)	Salk 2018 (Review)	This technique detects rare subclonal mutations within a pool of heterogeneous DNA samples through the application of new error-correction strategies to NGS; At present, few laboratories in the world are capable of doing this, but commercial services are becoming available (e.g., Duplex sequencing at TwinStrand BioSciences)	N/A

References

Adewoye, A.B. et al. (2015), "The genome-wide effects of ionizing radiation on mutation induction in the mammalian germline", *Nat. Commu.*, 6:6684. Doi: 10.1038/ncomms7684.

Ayres, M. F. et al. (2006), "Low doses of gamma ionizing radiation increase hprt mutant frequencies of TK6 cells without triggering the mutator phenotype pathway", *Genetics and Molecular Biology.* 2(3): 558-561. Doi:10.1590/S1415-47572006000300002.

Banda M, Recio L, and Parsons BL. (2013), "ACB-PCR measurement of spontaneous and furan-induced H-ras codon 61

CAA to CTA and CAA to AAA mutation in B6C3F1 mouse liver", *Environ Mol Mutagen*. 54(8):659-67. Doi:10.1002/em.21808.

Banda, M. et al. (2015), "Quantification of Kras mutant fraction in the lung DNA of mice exposed to aerosolized particulate vanadium pentoxide by inhalation", *Mutat Res Genet Toxicol Environ Mutagen* 789-790:53-60. Doi: 10.1016/j.mrgentox.2015.07.003

Campbell, C.D. & E.E. Eichler (2013), "Properties and rates of germline mutations in humans",*Trends Genet.*, 29(10): 575-84. Doi: 10.1016/j.tig.2013.04.005

Chakarov, S. et al. (2014), "DNA damage and mutation. Types of DNA damage", BioDiscovery, Vol.11, Pensoft Publishers, Sofia, https://doi.org/10.7750/BIODISCOVERY.2014.11.1.

Chikura, S. et al. (2019), "Standard protocol for the total red blood cell Pig-a assay used in the interlaboratory trial organized by the Mammalian Mutagenicity Study Group of the Japanese Environmental Mutagen Society", *Genes Environ*. 27:41-5. Doi: 10.1186/s41021-019-0121-z.

Dobrovolsky, V.N. et al. (2015), "CD48-deficient T-lymphocytes from DMBA-treated rats have de novo mutations in the endogenous Pig-a gene. CD48-Deficient T-Lymphocytes from DMBA-Treated Rats Have De Novo Mutations in the Endogenous Pig-a Gene", Environ. Mol. Mutagen., (6): 674-683. Doi: 10.1002/em.21959.

Douglas, G.R. et al. (1995), "Temporal and molecular characteristics of mutations induced by ethylnitrosourea in germ cells isolated from seminiferous tubules and in spermatozoa of lacZ transgenic mice", *Proceedings of the National Academy of Sciences of the United States of America*, 92(16): 7485-7489. Doi: 10.1073/pnas.92.16.7485.

Gossen, J.A. et al. (1995), "Spontaneous and X-ray-induced deletion mutations in a LacZ plasmid-based transgenic mouse model", Mutation Research, 331/1, Elsevier, Amsterdam, https://doi.org/10.1016/0027-5107(95)00055-N.

Hedrick, P.W. (2007), "Sex: Differences In Mutation, Recombination, Selection, Gene Flow, And Genetic Drift", Evolution, Vol.61/12, Wiley, Hoboken, https://doi.org/10.1111/j.1558-5646.2007.00250.x.

Kirkwood, T.B.L. (1989), "DNA, mutations and aging", Mutation Research, Vol.219/1, Elsevier B.V., Amsterdam, https://doi.org/10.1016/0921-8734(89)90035-0

Kraytsberg,Y. & Khrapko, K. (2005), "Single-molecule PCR: an artifact-free PCR approach for the analysis of somatic mutations", *Expert Rev Mol Diagn*. 5(5):809-15. Doi: 10.1586/14737159.5.5.809.

Krüger, T. C., Hofmann, M., & Hartwig, A. (2015), "The in vitro PIG-A gene mutation assay: mutagenicity testing via flow cytometry based on the glycosylphosphatidylinositol (GPI) status of TK6 cells", *Arch Toxicol*. 89(12), 2429-43. Doi: 10.1007/s00204-014-1413-5.

Lambert, I.B. et al. (2005), "Detailed review of transgenic rodent mutation assays",*Mutat Res.*, 590(1-3):1-280. Doi: 10.1016/j.mrrev.2005.04.002.

Liber, L. H., & Thilly, G. W. (1982), "Mutation assay at the thymidine kinase locus in diploid human lymphoblasts", *Mutation Research*. 94: 467-485. Doi:10.1016/0027-5107(82)90308-6.

Lloyd, M., & Kidd, D. (2012), "The Mouse Lymphoma Assay. In: Parry J., Parry E. (eds) Genetic Toxicology, Methods in Molecular Biology (Methods and Protocols), 817. Springer, New York, NY.

Myers, M. B. et al., (2014), "ACB-PCR Quantification of Somatic Oncomutation", *Molecular Toxicology Protocols, Methods in Molecular Biology*. DOI: 10.1007/978-1-62703-739-6_27

Nakamura, J. et al., (2012), "Detection of PIGO-deficient cells using proaerolysin: a valuable tool to investigate mechanisms of mutagenesis in the DT40 cell system", *PLoS One*.7(3): e33563. Doi:10.1371/journal.pone.0033563.

Nicklas, J.A., E.W. Carter and R.J. Albertini (2015), "Both PIGA and PIGL mutations cause GPI-a deficient isolates in the Tk6 cell line", Environ. Mol. Mutagen., 6(8):663-73. Doi: 10.1002/em.21953.

Norris, M.B. and R.N. Winn (2010), "Isolated spermatozoa as indicators of mutations transmitted to progeny", Mutat Res., 688(1-2): 36–40. Doi: 10.1016/j.mrfmmm.2010.02.008.

O'Brien, J.M. et al.(2013), "No evidence for transgenerational genomic instability in the F1 or F2 descendants of Muta™Mouse males exposed to N-ethyl-N-nitrosourea", *Mutat. Res.*, 741-742:11-7. Doi: 10.1016/j.mrfmmm.2013.02.004.

O'Brien, J.M. et al. (2014), "Transgenic rodent assay for quanitifying male germ cell mutation frequency",*Journal of Visual Experimentation*, Aug 6;(90). Doi: 10.3791/51576.

O'Brien, J.M. et al. (2015), "Sublinear response in lacZ mutant frequency of Muta™ Mouse spermatogonial stem cells after low dose subchronic exposure to N-ethyl-N-nitrosourea", *Environ. Mol. Mutagen.*, 6(4): 347-355. Doi: 10.1002/em.21932.

OECD (2020), Test No. 471: Bacterial Reverse Mutation Test, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris.

OECD (2016), Test No. 476: In vitro Mammalian Cell Gene Mutation Test, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris.

OECD (2009), Detailed Review Paper on Transgenic Rodent Mutation Assays, Series on Testing and Assessment, N° 103, ENV/JM/MONO 7, OECD, Paris.

OECD (2020), Test No. 488: Transgenic Rodent Somatic and Germ Cell Gene Mutation Assays, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris.

OECD (2016), Test. No. 490: In vitro mammalian cell gene mutation mutation tests using the thymidine kinase gene, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris.

OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris.

Parry MJ, & Parry ME. 2012. Genetic Toxicology Principles and Methods. Humana Press. Springer Protocols.

Parsons BL, McKim KL, Myers MB. 2017. Variation in organ-specific PIK3CA and KRAS mutant levels in normal human tissues correlates with mutation prevalence in corresponding carcinomas. Environ Mol Mutagen. 58(7):466-476. Doi: 10.1002/em.22110.

Parsons BL. Multiclonal tumor origin: Evidence and implications. *Mutat Res*. 2018. 777:1-18. doi: 10.1016/j.mrrev.2018.05.001.

Russell, W.L. et al. (1957), "Radiation Dose Rate and Mutation Frequency.", Science, Vol.128/3338, American Association for the Advancement of Science, Washington, https://doi.org/10.1126/science.128.3338.1546.

Salk JJ, Schmitt MW, &Loeb LA. (2018), “Enhancing the accuracy of next-generation sequencing for detecting rare and subclonal mutations”, *Nat Rev Genet*. 19(5):269-285. Doi: 10.1038/nrg.2017.117.

Sankaranarayanan, K. & H. Nikjoo (2015), "Genome-based, mechanism-driven computational modeling of risks of ionizing radiation: The next frontier in genetic risk estimation?", Mutation Research, Vol.764, Elsevier, Amsterdam, https://doi.org/10.1016/j.mrrev.2014.12.003.

Shen, T., S.H. Pajaro-Van de Stadt, N.C. Yeat and J.C. Lin (2015), "Clinical applications of next generation sequencing in cancer: from panels, to exomes, to genomes" *Front. Genet.*, 6: 215. Doi: 10.3389/fgene.2015.00215.

Singer, T.M. and C.L. Yauk CL (2010), "Germ cell mutagens: risk assessment challenges in the 21st century",*Environ. Mol. Mutagen.*, 51(8-9): 919-928. Doi: 10.1002/em.20613.

Slebos, R.J.C. et al. (2004), “Mini-and microsatellite mutations in children from Chernobyl accident cleanup workers”, Mutation Research/Genetic Toxicology and Environmental Mutagenesis, Vol.559/1-2, Elsevier, Amsterdam, https://doi.org/10.1016/j.mrgentox.2004.01.003.

Tindall, R. K., & Stankowski Jr., F. L. (1989), “Molecular analysis of spontaneous mutations at the GPT locus in Chinese hamster ovary (AS52) cells”, *Mutation Research*, 220, 241-53. Doi: 10.1016/0165-1110(89)90028-6.

Waters, M.D. et al. (1994), "The performance of short-term tests in identifying potential germ cell mutagens: a qualitative and quantitative analysis", *Mutat. Res.*, 341(2): 109-31. Doi: 10.1016/0165-1218(94)90093-0.

Winegar, R.A. et al. (1994), "Radiation-induced point mutations, deletions and micronuclei in lacI transgenic mice", Mutation Research, Vol.307/2, Elsevier, Amsterdam, https://doi.org/10.1016/0027-5107(94)90258-5.

Yamamoto, A. et al. (2017), “Radioprotective activity of blackcurrant extract evaluated by in vitro micronucleus and gene mutation assays in TK6 human lymphoblastoid cells”, *Genes and Environment.* 39: 22. Doi: 10.1186/s41021-017-0082-z.

Yauk, C.L. et al. (2002), "A novel single molecule analysis of spontaneous and radiation-induced mutation at a mouse tandem repeat locus", Mutat. Res., 500(1-2): 147-56. Doi: 10.1016/s0027-5107(02)00005-2.

Yauk, C.L. et al. (2015), "Approaches for Identifying Germ Cell Mutagens: Report of the 2013 IWGT Workshop on Germ Cell Assays", *Mutat. Res. Genet. Toxicol. Environ. Mutagen.*, 783: 36-54. Doi: 10.1016/j.mrgentox.2015.01.008.

Yeat and J.C. Lin. 2015. Clinical applications of next generation sequencing in cancer: from panels, to exomes, to genomes. *Front. Genet.*, 6: 215. Doi: 10.3389/fgene.2015.00215.

Event: 1636: Increase, Chromosomal aberrations

Short Name: Increase, Chromosomal aberrations

Key Event Component

Process	Object	Action

Process	Object	Action
	chromosome	increased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:296 - Oxidative DNA damage leading to chromosomal aberrations and mutations	AdverseOutcome
Aop:272 - Deposition of energy leading to lung cancer	KeyEvent
Aop:478 - Deposition of energy leading to occurrence of cataracts	KeyEvent

Stressors

Name
Ionizing Radiation

Biological Context

Level of Biological Organization
Cellular

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	High	NCBI
rat	Rattus norvegicus	High	NCBI
mouse	Mus musculus	High	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Unspecific	High

Taxonomic applicability: CAs are possible in nucleated cells of any species (Ferguson-Smith, 2015).

Life stage applicability: This key event is not life stage specific as subjects of all ages have chromosomes that can be improperly structured. However, older individuals have naturally higher baseline levels of CAs (Vick et al., 2017). Individuals born with stable type aberrations will retain them throughout their lifetime (Gardner et al., 2011).

Sex applicability: This key event is not sex specific, with both sexes experiencing CAs at comparable rates (Kašuba et al., 1995).

Evidence for perturbation by a stressor: Many studies have provided evidence to support increased CAs occurring as a result of exposure to ionizing radiation (Franken et al., 2012; Cornforth et al., 2002; Loucas et al., 2013).

Key Event Description

Structural chromosomal aberrations describe the damage to chromosomes that results from breaks along the DNA and may lead to deletion, addition, or rearrangement of sections in the chromosome. Chromosomal aberrations can be divided in two major categories: chromatid-type or chromosome-type depending on whether one or both chromatids are involved, respectively. They can be further classified as rejoined or non-rejoined aberrations. Rejoined aberrations include translocations, insertions, dicentrics and rings, while unrejoined aberrations include acentric fragments and breaks (Savage, 1976). Some of these aberrations are stable (i.e., reciprocal translocations) and can

persist for many years (Tucker and Preston, 1996). Others are unstable (i.e., dicentrics, acentric fragments) and decline at each cell division because of clonogenic inactivation (Boei et al., 1996). These events may be detectable after cell division and such damage to DNA is irreversible. Chromosomal aberrations are associated with clonogenic inactivation and carcinogenicity (Mitelman, 1982).

Chromosomal aberrations (CA) refer to a missing, extra or irregular portion of chromosomal DNA. These DNA changes in the chromosome structure may be produced by different double strand break (DSB) repair mechanisms (Obe et al., 2002).

There are 4 main types of CAs: deletions, duplications, translocations, and inversions. Deletions happen when a portion of the genetic material from a chromosome is lost. Terminal deletions occur when an end piece of the chromosome is cleaved. Interstitial deletions arise when a chromosome breaks in two separate locations and rejoins incorrectly, with the center piece being omitted. Duplications transpire when there is any addition or rearrangement of excess genetic material; types of duplications include transpositions, tandem duplications, reverse duplications, and displaced duplications (Griffiths et al., 2000). Translocations result from a section of one chromosome being transferred to a non-homologous chromosome (Bunting and Nussenzweig, 2013). When there is an exchange of segments on two non-homologous chromosomes, it is called a reciprocal translocation. Inversions occur in a single chromosome and involve both of the ends breaking and being ligated on the opposite ends, effectively inverting the DNA sequence.

A fifth type of CA that can occur in the genome is the copy number variant (CNV). CNVs, which may comprise greater than 10% of the human genome (Shlien et al., 2009; Zhang et al., 2016; Hastings et al., 2009), are deletions or duplications that can vary in size from 50 base pairs (Arlt et al., 2012; Arlt et al., 2014; Liu et al., 2013) up into the megabase pair range (Arlt et al., 2012; Wilson et al., 2015; Arlt et al., 2014; Zhang et al., 2016). CNV regions are especially enriched in large genes and large active transcription units (Wilson et al., 2015), and are of particular concern when they cause deletions in tumour suppressor genes or duplications in oncogenes (Liu et al., 2013; Curtis et al., 2012). There are two types of CNVs: recurrent and non-recurrent. Recurrent CNVs are thought to be produced through a recombination process during meiosis known as non-allelic homologous recombination (NAHR) (Arlt et al., 2012; Hastings et al., 2009). These recurrent CNVs, also called germline CNVs, could be inherited and are thus common across different individuals (Shlien et al., 2009; Liu et al., 2013). Non-recurrent CNVs are believed to be produced in mitotic cells during the process of replication. Although the mechanism is not well studied, it has been suggested that stress during replication, in particular stalling replication forks, prompt microhomology-mediated mechanisms to overcome the replication stall, which often results in duplications or deletions. Two models that have been proposed to explain this mechanism include the Fork Stalling and Template Switching (FoSTeS) model, and the Microhomology-Mediated Break-Induced Replication (MMBIR) model (Arlt et al., 2012; Wilson et al., 2015; Lee et al., 2007; Hastings et al., 2009).

CAs can be classified according to whether the chromosome or chromatid is affected by the aberration. Chromosome-type aberrations (CSAs) include chromosome-type breaks, ring chromosomes, marker chromosomes, and dicentric chromosomes; chromatid-type aberrations (CTAs) refer to chromatid breaks and chromatid exchanges (Bonassi et al., 2008; Hagmar et al., 2004). When cells are blocked at the cytokinesis step, When cells are blocked at the cytokinesis step, micronuclei (MN; small nucleus-like structures that contain a chromosome or a piece of a chromosome that was lost during mitosis) can appear in the cytoplasm of binucleated cells. These micronuclei are an indication of CAs and are often related to dicentric chromosomes. Dicentric chromosomes can also cause nucleoplasmic bridges (NPBs; physical connections that exist between the two nuclei) (El-Zein et al., 2014). Other CAs can be assessed by examining the DNA sequence, as is the case when detecting copy number variants (CNVs) (Liu et al., 2013).

OECD defines clastogens as ‘any substance that causes structural chromosomal aberrations in populations of cells or organisms’.

How it is Measured or Detected

CAs can be detected before and after cell division. Widely used assays are described in the table below, however there may be other comparable methods that are not listed.

Assay	References	Description	OECD-approved assay
Premature Chromosome Condensation (PCC)	Prasanna et al., 2000; Okayasu et al., 2019	Cells are exposed to mitosis-promoting factors (MPF) following cell fusion, causing the chromosomes to condense prematurely. In another approach, cells are exposed to protein phosphatase inhibitors, such as type 1 and 2A protein phosphatases, also causing premature chromosome condensation.	N/A

Chromosomal G-banding	Schwatz, 1990	Use of Giesma dye to stain chromosomal bands, abnormalities determined by the presence of altered morphology	N/A
Fluorescent In Situ Hybridization (FISH)	Beaton et al., 2013; Pathak et al., 2017	Fluorescent assay of metaphase chromosomes that can detect CAs through chromosome painting and microscopic analysis	N/A
Micronuclei (MN) Assay via Microscopy *in vitro*	OECD, 2016a	Micronuclei are scored in vitro using microscopy	Yes (No. 487)
Cytokinesis Block Micronucleus (CBMN) Assay with Microscopy in vitro	Fenech, 2000; OECD, 2016a	Cells are cultured with cytokinesis blocking agent, fixed to slides, and undergo MN quantification using microscopy.	Yes (No.487)
Micronucleus (MN) Assay by Microscopy in vivo	OECD, 2016b	Cells are fixed on slides and MN are scored using microscopy. Red blood cells can also be scored for MN using flow cytometry (see below)	Yes (No. 474)
CBMN with Imaging Flow Cytometry	Rodrigues et al., 2015	Cells are cultured with cytokinesis blocking agent, fixed in solution, and imaged with flow cytometry to quantify MN	N/A
Flow cytometry detection of MN	Dertinger et al., 2004; Bryce et al., 2007; OECD 2016a, 2016b	In vivo and in vitro flow cytometry-based, automated micronuclei measurements are also done without cytokinesis block. MN analysis in vivo is performed in peripheral blood cells to detect MN in erythrocytes and reticulocytes.	Yes (No.487; No. 474)
High-throughput biomarker assays (indirect measures to confirm clastogenicity)	Bryce et al. 2014, 2016, 2018 Khoury et al., 2013, Khoury et al., 2016) Hendriks et al., 2012, 2016; Wink et al., 2014	Multiplexed biomarkers can be measured by flow cytometry are used to discern clastogenic and aneugenic mechanisms for MN induction. Flow cytometry-based quantification of γH2AX foci and p53 protein expression (Bryce et al., 2016). Prediscreen Assay– In-Cell Western - based quantification of γH2AX Green fluorescent protein reporter assay to detect the activation of stress signaling pathways, including DNA damage signaling including a reporter porter that is associated with DNA double strand breaks.	N/A
Dicentric Chromosome Assay (DCA)	Abe et al., 2018	Cells are fixed on microscope slides, chromosomes are stained, and the number of dicentric chromosomes are quantified	N/A

High content imaging	Shahane et al., 2016	DNA can be stained using fluorescent dyes and micronuclei can be scored high-throughput microscopy image analysis.	N/A
Chromosomal aberration test	OECD, 2016c; 2016d; 20l16e	In vitro, the cell cycle is arrested at metaphase after 1.5 cell cycle following 3-6 hour exposure In vivo, the test chemical is administered as a single treatment and bone marrow is collected 18-24 hrs later (TG 475), while testis is collected 24-48 hrs later (TG 483). The cell cycle is arrested with a metaphase-arresting chemical (e.g., colchicine) 2-5 hours before cell collection. Once cells are fixed and stained on microscope slides, chromosomal aberrations are scored	Yes. In vitro (No. 473) In vivo (No. 475 and No. 483)
Array Comparative Genomic Hybridization (aCGH) or SNP Microarray	Adewoye et al., 2015; Wilson et al., 2015; Arlt et al., 2014; Redon et al., 2006; Keren, 2014; Mukherjee, 2017	CNVs are most commonly detected using global DNA microarray technologies; This method, however, is unable to detect balanced CAs, such as inversions	N/A
Next Generation Sequencing (NGS): Whole Genome Sequencing (WGS) or Whole Exome Sequencing (WES)	Liu, 2013; Shen, 2016; Mukherjee, 2017	CNVs are detected by fragmenting the genome and using NGS to sequence either the entire genome (WGS), or only the exome (WES); Challenges with this methodology include only being able to detect CNVs in exon-rich areas if using WES, the computational investment required for the storage and analysis of these large datasets, and the lack of computational algorithms available for effectively detecting somatic CNVs	N/A

References

Abe, Y et al. (2018), “Dose-response curves for analyzing of dicentric chromosomes and chromosome translocations following doses of 1000 mGy or less, based on irradiated peripheral blood samples from five healthy individuals”, *J Radiat Res*. 59(1), 35-42. doi:10.1093/jrr/rrx052

Adewoye, A.B.et al. (2015), “The genome-wide effects of ionizing radiation on mutation induction in the mammalian germline”, *Nat. Commun*. 6:66-84. doi: 10.1038/ncomms7684.

Arlt MF, Wilson TE, Glover TW. (2012), “Replication stress and mechanisms of CNV formation”, *Curr Opin Genet Dev*. 22(3):204-10. doi: 10.1016/j.gde.2012.01.009.

Arlt, MF. Et al. (2014), “Copy number variants are produced in response to low-dose ionizing radiation in cultured cells”, *Environ Mol Mutagen*. 55(2):103-13. doi: 10.1002/em.21840.

Beaton, L. A. et al. (2013), “Investigating chromosome damage using fluorescent in situ hybridization to identify biomarkers of radiosensitivity in prostate cancer patients”, Int J Radiat Biol. 89(12): 1087-1093. doi:10.3109/09553002.2013.825060

Boei, J.J., Vermeulen, S., Natarajan, A.T. (1996), “Detection of chromosomal aberrations by fluorescence in situ hybridization in the first three postirradiation divisions of human lymphocytes”, Mutat Res, 349:127-135. Doi: 10.1016/0027-5107(95)00171-9.

Bonassi, S. (2008),”Chromosomal aberration frequency in lymphocytes predicts the risk of cancer: results from a pooled cohort study of 22 358 subjects in 11 countries”, *Carcinogenesis.* 29(6):1178-83. doi: 10.1093/carcin/bgn075.

Bryce SM, Bemis JC, Avlasevich SL, Dertinger SD. In vitro micronucleus assay scored by flow cytometry provides a comprehensive evaluation of cytogenetic damage and cytotoxicity. Mutat Res. 2007 Jun 15;630(1-2):78-91. doi:

10.1016/j.mrgentox.2007.03.002. Epub 2007 Mar 19. PMID: 17434794; PMCID: PMC1950716.

Bryce, S. et al. (2014), “Interpreting In VitroMicronucleus Positive Results: Simple Biomarker Matrix Discriminates Clastogens, Aneugens, and Misleading Positive Agents”, Environ Mol Mutagen, 55:542-555. Doi:10.1002/em.21868.

Bryce, S. et al.(2016), “Genotoxic mode of action predictions from a multiplexed flow cytometric assay and a machine learning approach”, Environ Mol Mutagen, 57:171-189. Doi: 10.1002/em.21996.

Bryce SM, Bernacki DT, Smith-Roe SL, Witt KL, Bemis JC, Dertinger SD. Investigating the Generalizability of the MultiFlow ® DNA Damage Assay and Several Companion Machine Learning Models With a Set of 103 Diverse Test Chemicals. Toxicol Sci. 2018 Mar 1;162(1):146-166. doi: 10.1093/toxsci/kfx235. PMID: 29106658; PMCID: PMC6059150.

Bunting, S. F., & Nussenzweig, A. (2013), “End-joining, translocations and cancer”, Nature Reviews Cancer.13 (7): 443-454. doi:10.1038/nrc3537

Cornforth, M.N., S.M. Bailey, and E.H. Goodwin. (2002), “Dose Responses for Chromosome Aberrations Produced in Noncycling Primary Human Fibroblasts by Alpha Particles, and by Gamma Rays Delivered at Sublimating Low Dose Rates”, Radiation Research, Vol.158, Radiation Research Society, Indianapolis, https://doi.org/10.1667/0033-7587(2002)158[0043:DRFCAP]2.0.CO;2.

Curtis, C. et al. (2012), “The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups”, Nature. 486(7403):346-52. doi: 10.1038/nature10983.

Dertinger, S.D. et al.(2004), “Three-color labeling method for flow cytometric measurement of cytogenetic damage in rodent and human blood”, Environ Mol Mutagen, 44:427-435. Doi: 10.1002/em.20075.

El-Zein, RA. Et al. (2014), “The cytokinesis-blocked micronucleus assay as a strong predictor of lung cancer: extension of a lung cancer risk prediction model”, Cancer Epidemiol Biomarkers Prev. 23(11):2462-70. doi: 10.1158/1055-9965.EPI-14-0462.

Fenech, M. (2000), “The in vitro micronucleus technique”, Mutation Research. 455(1-2), 81-95. Doi: 10.1016/s0027-5107(00)00065-8

Ferguson-Smith, M.A. (2015), “History and evolution of cytogenetics”, Molecular Cytogenetics, Vol.8/19, Biomed Central, London, https://doi.org/10.1186/s13039-015-0125-8.

Franken, N.A.P. et al. (2012), “Relative biological effectiveness of high linear energy transfer alpha-particles for the induction of DNA-double-strand breaks, chromosome aberrations and reproductive cell death in SW-1573 lung tumour cells”, Oncology Reports, Vol.27, Spandidos Publications, Athens, https://doi.org/10.3892/or.2011.1604.

Gardner, R.M., G.R. Sutherland, and L.G. Shaffer. (2011), “Chapter 1: Elements in Medical Cytogenetics” in Chromosome abnormalities and genetic counseling (No. 61), Oxford University Press, USA, pp.7-15.

Griffiths, A. J. F., Miller, J. H., & Suzuki, D. T. (2000), “An Introduction to Genetic Analysis”, 7th edition. New York: W. H. Freeman. Available from: https://www.ncbi.nlm.nih.gov/books/NBK21766/

Hagmar, L. et al. (2004), “Impact of types of lymphocyte chromosomal aberrations on human cancer risk: results from Nordic and Italian cohorts”, Cancer Res. 64(6):2258-63.

Hastings PJ, Ira G & Lupski JR. (2009), “A microhomology-mediated break-induced replication model for the origin of human copy number variation”. PLoS Genet. 2009 Jan;5(1): e1000327. doi: 10.1371/journal.pgen.1000327.

Hendriks, G. et al. (2012), “The ToxTracker assay: novel GFP reporter systems that provide mechanistic insight into the genotoxic properties of chemicals”, Toxicol Sci, 125:285-298. Doi: 10.1093/toxsci/kfr281.

Hendriks, G. et al. (2016), “The Extended ToxTracker Assay Discriminates Between Induction of DNA Damage, Oxidative Stress, and Protein Misfolding”, Toxicol Sci, 150:190-203. Doi: 10.1093/toxsci/kfv323.

Kašuba, V., et al. (1995), “Chromosome aberrations in peripheral blood lymphocytes from control individuals”, Mutation Research Letters, Vol.346/4, Elsevier, Amsterdam, https://doi.org/10.1016/0165-7992(95)90034-9.

Keren, B. (2014),”The advantages of SNP arrays over CGH arrays”, Molecular Cytogenetics.7(1):I31. Doi: 10.1186/1755-8166-7-S1-I31.

Khoury, L., Zalko, D., Audebert, M. (2016), “Evaluation of four human cell lines with distinct biotransformation properties for genotoxic screening”, Mutagenesis. 31:83-96. Doi: 10.1093/mutage/gev058.

Khoury, L., Zalko, D., Audebert, M. (2013), “Validation of high-throughput genotoxicity assay screening using cH2AX in-cell Western assay on HepG2 cells”, Environ Mol Mutagen, 54:737-746. Doi: 10.1002/em.21817.

Lee JA, Carvalho CM, Lupski JR. (2007). “Replication mechanism for generating nonrecurrent rearrangements associated with genomic disorders”, Cell. 131(7):1235-47. Doi: 10.1016/j.cell.2007.11.037.

Liu B. et al. (2013). “Computational methods for detecting copy number variations in cancer genome using next generation sequencing: principles and challenges”, Oncotarget. 4(11):1868-81. Doi: 10.18632/oncotarget.1537.

Loucas, B.D., et al. (2013), “Chromosome Damage in Human Cells by Gamma Rays, Alpha Particles and Heavy Ions: Track Interactions in Basic Dose-Response Relationships”, Radiation Research, Vol.179/1, Radiation Research Society, Indianapolis, https://doi.org/10.1667/RR3089.1.

Mitelman, F. (1982), “Application of cytogenetic methods to analysis of etiologic factors in carcinogenesis”, IARC Sci Publ, 39:481-496.

Mukherjee. S. et al. (2017), “Addition of chromosomal microarray and next generation sequencing to FISH and classical cytogenetics enhances genomic profiling of myeloid malignancies, Cancer Genet. 216-217:128-141. doi: 10.1016/j.cancergen.2017.07.010.

Obe, G. et al. (2002), “Chromosomal Aberrations: formation, Identification, and Distribution”, Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis. 504(1-2), 17-36. Doi: 10.1016/s0027-5107(02)00076-3.

Savage, J.R. (1976), “Classification and relationships of induced chromosomal structual changes”, J Med Genet, 13:103-122. Doi: 10.1136/jmg.13.2.103.

Shahane SA, Nishihara K, Xia M. High-Throughput and High-Content Micronucleus Assay in CHO-K1 Cells. Methods Mol Biol. 2016;1473:77-85. doi: 10.1007/978-1-4939-6346-1_9. PMID: 27518626.

OECD (2016a), *Test No. 487: In Vitro Mammalian Cell Micronucleus Test*, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, https://doi.org/10.1787/9789264264861-en.

OECD (2016a), Test No. 487: In Vitro Mammalian Cell Micronucleus Test, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, https://doi.org/10.1787/9789264264861-en.

OECD. (2016b), “Test No. 474: Mammalian Erythrocyte Micronucleus Test. OECD Guideline for the Testing of Chemicals, Section 4.”Paris: OECD Publishing.

OECD. (2016c), “In Vitro Mammalian Chromosomal Aberration Test 473.”

OECD. (2016d). Test No. 475: Mammalian Bone Marrow Chromosomal Aberration Test. OECD Guideline for the Testing of Chemicals, Section 4. Paris: OECD Publishing.

OECD. (2016e). Test No. 483: Mammalian Spermatogonial Chromosomal Aberration Test. Paris: OECD Publishing.

Okayasu, R. and C. Liu. (2019), “G1 premature chromosome condensation (PCC) assay”, Methods in molecular biology, Humana Press, Totowa, https://doi.org/10.1007/978-1-4939-9432-8_4.

Pathak, R., Koturbash, I., & Hauer-Jensen, M. (2017), “Detection of Inter-chromosomal Stable Aberrations by Multiple Fluorescence In Situ Hybridization (mFISH) and Spectral Karyotyping (SKY) in Irradiated Mice”, J Vis Exp(119). doi:10.3791/55162.

Prasanna, P. G. S., N. D. Escalada and W. F. Blakely (2000), “Induction of premature chromosome condensation by a phosphatase inhibitor and a protein kinase in unstimulated human peripheral blood lymphocytes: a simple and rapid technique to study chromosome aberrations using specific whole-chromosome DNA hybridization probes for biological dosimetry”, Mutation Research, Vol. 466/2, Elsevier B.V., Amsterdam, https://doi/org/10.1016/S1383-5718(00)00011-5

Redon, R. et al. (2006), “Global variation in copy number in the human genome”, Nature. 444(7118):444-54. 10.1038/nature05329.

Rodrigues, M. A., Beaton-Green, L. A., & Wilkins, R. C. (2016), “Validation of the Cytokinesis-block Micronucleus Assay Using Imaging Flow Cytometry for High Throughput Radiation Biodosimetry”, Health Phys. 110(1): 29-36. doi:10.1097/HP.0000000000000371

Schwartz, G. G. (1990), “Chromosome aberrations. Biological Markers in Epidemiology (BS Hulka, TC Wlwosky, and JD Griffith, Eds.)”, Oxford University Press, Oxford, pp.147-172.

Shahane S, Nishihara K, Xia M. (2016), “High-Throughput and High-Content Micronucleus Assay in CHO-K1 Cells”, In: Zhu H, Xia M, editors. High-Throughput Screening Assays in Toxicology. New York, NY: Humana Press. p 77-85.

Shen.TW, (2016),”Concurrent detection of targeted copy number variants and mutations using a myeloid malignancy next generation sequencing panel allows comprehensive genetic analysis using a single testing strategy”, Br J Haematol. 173(1):49-58. doi: 10.1111/bjh.13921.

Shlien A, Malkin D. (2009), “Copy number variations and cancer”, Genome Med. 1(6):62. doi: 10.1186/gm62.

Tucker, J.D., Preston, R.J. (1996), “Chromosome aberrations, micronuclei, aneuploidy, sister chromatid exchanges, and cancer risk assessment”, Mutat Res, 365:147-159.

Vick, E. et al. (2017), Age-related chromosomal aberrations in patients with diffuse large B-cell lymphoma, American Society of Hematology, https://doi.org/10.1182/blood.V130.Suppl_1.1571.1571

Wilson, TE. et al. (2015), “Large transcription units unify copy number variants and common fragile sites arising under replication stress”, Genome Res. 25(2):189-200. doi: 10.1101/gr.177121.114.

Wink, S. et al. (2014), “Quantitative high content imaging of cellular adaptive stress response pathways in toxicity for chemical safety assessment”, Chem Res Toxicol, 27:338-355.

Zhang N, Wang M, Zhang P, Huang T. 2016. Classification of cancers based on copy number variation landscapes. Biochim Biophys Acta. 1860(11 Pt B):2750-5. doi: 10.1016/j.bbagen.2016.06.003.

Event: 870: Increase, Cell Proliferation

Short Name: Increase, Cell Proliferation

Key Event Component

Process	Object	Action
cell proliferation	epithelial cell	increased
cell proliferation	mesothelial cell	increased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:136 - Intracellular Acidification Induced Olfactory Epithelial Injury Leading to Site of Contact Nasal Tumors	KeyEvent
Aop:303 - Frustrated phagocytosis-induced lung cancer	KeyEvent
Aop:272 - Deposition of energy leading to lung cancer	KeyEvent
Aop:409 - Frustrated phagocytosis leads to malignant mesothelioma	KeyEvent
Aop:420 - Aryl hydrocarbon receptor activation leading to lung cancer through sustained NRF2 toxicity pathway	KeyEvent
Aop:432 - Deposition of Energy by Ionizing Radiation leading to Acute Myeloid Leukemia	KeyEvent
Aop:451 - Interaction with lung resident cell membrane components leads to lung cancer	KeyEvent
Aop:478 - Deposition of energy leading to occurrence of cataracts	KeyEvent

Stressors

Name
Ionizing Radiation

Biological Context

Level of Biological Organization
Cellular

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
rat	Rattus norvegicus	High	NCBI
mouse	Mus musculus	High	NCBI
human	Homo sapiens	High	NCBI

Life Stage Applicability

Life Stage	Evidence

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Unspecific	High

Cell proliferation is a central process supporting development, tissue homeostasis and carcinogenesis, each of which occur in all vertebrates. This key event has been observed nasal tissues of rats exposed to the chemical initiator vinyl acetate. In general, cell proliferation is necessary in the biological development and reproduction of most organisms. This KE is thus relevant and applicable to all multicellular cell types, tissue types, and taxa.

Life stage applicability: This key event is not life stage specific (Fujimichi and Hamada, 2014; Barnard et al., 2022).

Sex applicability: This key event is not sex specific (Markiewicz et al., 2015).

Evidence for perturbation by a stressor: There is a large body of evidence supporting the effectiveness of ionizing radiation, UV, and mechanical wounding as stressors for increased cell proliferation. These stressors can be subdivided into X-rays (van Sallmann, 1951; Ramsell and Berry, 1966; Richards, 1966; Riley et al., 1988; Riley et al., 1989; Kleiman et al., 2007; Pendergrass et al., 2010; Fujimichi and Hamada, 2014, Markiewicz et al., 2015; Bahia et al., 2018), 60Co γ-rays (Hanna and O'Brien, 1963; Barnard et al., 2022; McCarron et al., 2021), 137Cs γ-rays (Andley and Spector, 2005), neutrons (Richards, 1966; Riley et al., 1988; Riley et al., 1989), 40Ar (Worgul et al., 1986), 56Fe (Riley et al., 1989), UVB (Söderberg et al., 1986; Andley et al., 1994; Cheng et al., 2019), UVC (Trenton and Courtois, 1981), and mechanical wounding (Riley et al., 1989).

Key Event Description

Throughout their life, cells replicate their organelles and genetic information before dividing to form two new daughter cells, in a process known as cellular proliferation. This replicative process is known as the cell cycle and is subdivided into various stages notably, G1, S, G2, and M in mammals. G1 and G2 are gap phases, separating mitosis and DNA synthesis. Differentiated cells typically remain in G1; however, quiescent cells reside in an optional phase just before G1, known as G0.

Progression through the cycle is dependent on sufficient nutrient availability to provide optimal nucleic acid, protein, and lipid levels, as well as sufficient cell mass. To this end, the cell cycle is mediated by three major checkpoints: the restriction (R) point, or G1/S checkpoint, controlling entry into S phase, the G2/M checkpoint, controlling entry into mitosis, and one more controlling entry into cytokinesis. If conditions are ideal for division, cells will pass the restriction point (G1/S) and begin the activation and expression of genes used for duplicating centrosomes and DNA, eventually leading to proliferation (Cuyàs et al., 2014).

Various protein complexes, known as cyclins, cyclin-dependent kinases (CDKs), and cyclin-dependent kinase inhibitors (CKIs) regulate passage through each phase by activating and inhibiting specific processes (Lovicu et al., 2014). The CDKs are responsible for controlling progression through the cell cycle. They promote DNA synthesis and mitosis, and therefore cell division (Barnum & O'Connell, 2014). Furthermore, growth factors are required to stimulate cell division, but after passing through the restriction point at G1 they are no longer necessary (Lovicu et al., 2014).

In the context of cancer, one hallmark is the sustained and uncontrolled cell proliferation (Hanahan et al., 2011, Portt et al., 2011). When cells obtain a growth advantage due to mutations in critical genes that regulate cell cycle progression, they may begin to proliferate excessively, resulting in hyperplasia and potentially leading to the development of a tumor. This is often achieved through oncogene activation and inactivation of tumor suppressor genes (Hanahan et al., 2011). Cell inactivation and the replacement of these cells can initiate clonal expansion (Heidenreich adn Paretzke et al., 2008).

Sustained atrophy/degeneration olfactory epithelium under the influence of a cytotoxic agent leads to adaptive tissue remodeling. Cell types unique to olfactory epithelium, e.g. olfactory neurons, sustentacular cells and Bowmans glands, are replaced by cell types comprising respiratory epithelium or squamous epithelium.

How it is Measured or Detected

Two common methods of measuring cell proliferation in vivo are the use of Bromodeoxyuridine (5-bromo-2'-deoxyuridine, BrdU) labeling (Pera, 1977), and Ki67 immunostaining (Grogan, 1988). BrdU is a synthetic analogue of the nucleoside Thymidine. BrDu is incorporated into DNA synthesized during the S1 phase of cell replication and is stable for long periods. Labeling of dividing cells by BrdU is accomplished by infusion, bolus injection, or implantation of osmotic pumps containing BrdU for a period of time sufficient to generate measureable numbers of labeled cells. Tissue sections are stained immunhistochemically with antibodies for BrdU and labeled cells are counted as dividing cells. Similarly, 5-iodo-2'-deoxyuridine (IdU) is another analogue of thymidine used to measure cell proliferation as it is also incorporated into DNA during its synthesis (Devine & Behbehani, 2021). Ki67 is a cellular marker of replication not found in quiescent cells (Roche, 2015). Direct immunohistochemical staining of cells for protein Ki67 using antibodies is an alternative to the use of BrdU, with the benefit of not requiring a separate treatment (injection for

pulse-labeling). Cells positive for Ki67 are counted as replicating cells. Replicating cell number is reported per unit tissue area or per cell nuclei (Bogdanffy, 1997). Listed below are common methods for detecting the KE, however there may be other comparable methods that are not listed.

Assay Name	References	Description	OECD Approved Assay
CyQuant Cell Proliferation Assay	Jones et al., 2001	DNA-binding dye is added to cell cultures, and the dye signal is measured directly to provide a cell count and thus an indication of cellular proliferation	N/A
Nucleotide Analog Incorporation Assays (e.g. BrdU, EdU)	Romar et al., 2016, Roche; 2013	Nucleoside analogs are added to cells in culture or injected into animals and become incorporated into the DNA at different rates, depending on the level of cellular proliferation; Antibodies conjugated to a peroxidase or fluorescent tag are used for quantification of the incorporated nucleoside analogs using techniques such as ELISA, flow cytometry, or microscopy	Yes (No. 442B)
Cytoplasmic Proliferation Dye Assays	Quah & Parish, 2012	Cells are incubated with a cytoplasmic dye of a certain fluorescent intensity; Cell divisions decrease the intensity in such a way that the number of divisions can be calculated using flow cytometry measurements	N/A
Colourimetric Dye Assays	Vega-Avila & Pugsley, 2011; American Type Culture Collection	Cells are incubated with a dye that changes colour following metabolism; Colour change can be measured and extrapolated to cell number and thus provide an indication of cellular proliferation rates	N/A
BrdU, Ki67, IdU Quantification - Flow Cytometry	Ligasová et al., 2017; Devine & Behehani, 2021; Kim & Sederstrom, 2015	Measurement of cell proliferation biomarkers by flow cytometry, normalized to total levels of BrdU, Ki67 or IdU.	No

References

Andley, U. P. et al. (1994), “Modulation of lens epithelial cell proliferation by enhanced prostaglandin synthesis after UVB exposure”, Investigative Ophthalmology & Visual Science, Vol. 35/2, Rockville, pp. 374-381

Andley, U. and A. Spector (2005), “Peroxide resistance in human and mouse lens epithelial cell lines is related to long-term changes in cell biology and architecture”, Free Radical Biology & Medicine, Vol. 39/6, Elsevier B.V, United States, https://doi.org/10.1016/j.freeradbiomed.2005.04.028

Bahia, S. et al. (2018), “Oxidative and nitrative stress-related changes in human lens epithelial cells following exposure to X-rays”, International journal of radiation biology, Vol. 94/4, England, https://doi.org/10.1080/09553002.2018.1439194

Barnard, S. et al. (2022), “Lens Epithelial Cell Proliferation in Response to Ionizing Radiation.”, Radiation Research, Vol. 197/1, Radiation Research Society, United States, https://doi.org/10.1667/RADE-20-00294.1

Barnum, K. and M. O’Connell (2014), “Cell cycle regulation by checkpoints”, in Cell cycle control, Springer, New York, http://doi.org/ 10.1007/978-1-4939-0888-2

Bogdanffy. et al. (1997). “FOUR-WEEK INHALATION CELL PROLIFERATION STUDY OF THE EFFECTS OF VINYL ACETATE ON RAT NASAL EPITHELIUM”, Inhalation Toxicology, Taylor & Francis. 9: 331-350.

Cheng, T. et al. (2019), “lncRNA H19 contributes to oxidative damage repair in the early age-related cataract by regulating miR-29a/TDG axis”, Journal of cellular and molecular medicine, Vol. 23/9, Wiley Subscription Services, Inc. England, https://doi.org/10.1111/jcmm.14489

Cuyàs, E. et al. (2014), “Cell cycle regulation by the nutrient-sensing mammalian target of rapamycin (mTOR) pathway”, in Cell cycle control, Springer, New York, http://dx.doi.org/ 10.1007/978-1-4939-0888-2

Devine,R. D, and G. K. Behbehani (2021), “Use of the Pyrimidine Analog, 5-Iodo-2'-Deoxyuridine (IdU) with Cell Cycle Markers to establish Cell Cycle Phases in a Mass Cytometry Platform”, Journal of visualized experiments. (176). doi:10.3791/60556

Fujimichi, Y. and N. Hamada (2014), “Ionizing irradiation not only inactivates clonogenic potential in primary normal human diploid lens epithelial cells but also stimulates cell proliferation in a subset of this population”, PloS one, Vol. 9/5, e98154, Public Library of Science, United States, https://doi.org/10.1371/journal.pone.0098154

Grogan. et al. (1988). “Independent prognostic significance of a nuclear proliferation antigen in diffuse large cell

lymphomas as determined by the monoclonal antibody Ki-67", Blood. 71: 1157-1160.

Hanna, C. and J. E. O'Brien (1963), "Lens epithelial cell proliferation and migration in radiation cataracts", Radiation research, Academic Press, Inc, United States, https://doi.org/10.2307/3571405

Hanahan, D. & R. A. Weinberg, (2011)," Hallmarks of cancer: the next generation", Cell. 144(5):646-74. doi: 10.1016/j.cell.2011.02.013.

Heidenreich WF, Paretzke HG. (2008) Promotion of initiated cells by radiation-induced cell inactivation. Radiat Res. Nov;170(5):613-7. doi: 10.1667/RR0957.1. PMID: 18959457.

Jones, J. L. et al. (2001), Sensitive determination of cell number using the CyQUANT cell proliferation assay. Journal of Immunological Methods. 254(1-2), 85-98. Doi:10.1016/s0022-1759(01)00404-5.

Kim, K. H. and Sederstrom J. M. (2015), "Assaying Cell Cycle Status Using Flow Cytometry." Current protocols in molecular biology, 111:28.6.1-28.6.11., doi:10.1002/0471142727.mb2806s111

Kleiman, N. J. et al. (2007), "Mrad9 and Atm haplinsufficiency enhance spontaneous and X-ray-induced cataractogenesis in mice", Radiation research, Vol. 168/5, Radiation Research Society, United States, https://doi.org/10.1667/rr1122.1

Ligasová, A. et al. (2017), "Cell cycle profiling by image and flow cytometry: The optimised protocol for the detection of replicational activity using 5-Bromo-2'-deoxyuridine, low concentration of hydrochloric acid and exonuclease III." PloS one, 12(4): e0175880, doi:10.1371/journal.pone.0175880

Lovicu, J. et al (2014), "Lens epithelial cell proliferation", in Lens epithelium and posterior capsular opacification, Springer, Tokyo, http://dx.doi.org/ 10.1007/978-4-431-54300-8_4

Markiewicz, E. et al. (2015), "Nonlinear ionizing radiation-induced changes in eye lens cell proliferation, cyclin K1 expression and lens shape", Open biology, Vol. 5/4, The Royal Society, England, https://doi.org/10.1098/rsob.150011

McCarron, R. A. et al. (2021), "Radiation-induced lens opacity and cataractogenesis: a lifetime study using mice of varying genetic backgrounds", Radiation research, Vol. 197/1, Radiation Research Society, United States, https://doi.org/10.1667/RADE-20-00266.1

Pendergrass, W. et al. (2010), "X-ray induced cataract is preceded by LEC loss, and coincident with accumulation of cortical DNA, and ROS; similarities with age-related cataracts", Molecular vision, Vol. 16, Molecular Vision, United States, pp. 1496-1513

Pera, Mattias and Detzer (1977). "Methods for determining the proliferation kinetics of cells by means of 5-bromodeoxyuridine", Cell Tissue Kinet.10: 255-264. Doi: 10.1111/j.1365-2184.1977.tb00293.x.

Portt, L. et al. (2011), "Anti-apoptosis and cell survival: a review", Biochim Biophys Acta. 21813(1):238-59. doi: 10.1016/j.bbamcr.2010.10.010.

Quah, J. C. B. & R. C. Parish (2012), "New and improved methods for measuring lymphocyte proliferation in vitro and in vivo using CFSE-like fluorescent dyes", Journal of Immunological Methods. 379(1-2), 1-14. doi: 10.1016/j.jim.2012.02.012.

Ramsell, T. G. and R. J. Berry (1966), "Recovery from X-ray damage to the lens. The effects of fractionated X-ray doses observed in rabbit lens epithelium irradiated in vivo", British Journal of Radiology, Vol. 39/467, England, pp. 853-858

Riley, E. F. et al. (1988), "Recovery of murine lens epithelial cells from single and fractionated doses of X rays and neutrons", Radiation Research, Vol. 114/3, Academic Press Inc, Oak Brook, https://doi.org/10.2307/3577127

Riley, E. F. et al. (1989), "Comparison of recovery from potential mitotic abnormality in mitotically quiescent lens cells after X, neutron, and 56Fe irradiations", Radiation Research, Vol. 119/2, United States, pp. 232-254

Richards, R. D. (1966), "Changes in lens epithelium after X-ray or neutron irradiation (mouse and rabbit)", Transactions of the American Ophthalmological Society, Vol. 64, United States, pp. 700-734

Roche Applied Science, (2013), "Cell Proliferation Elisa, BrdU (Colourmetric) ». Version 16

Romar, A. G., S. T. Kupper & J. S. Divito (2015), "Research Techniques Made Simple: Techniques to Assess Cell Proliferation", Journal of Investigative Dermatology. 136(1), e1-7. doi: 10.1016/j.jid.2015.11.020.

Söderberg, P. G. et al. (1986), "Unscheduled DNA synthesis in lens epithelium after in vivo exposure to UV radiation in the 300 nm wavelength region", Acta Ophthalmologica, Vol. 64/2, Blackwell Publishing Ltd, Oxford, UK, https://doi.org/10.1111/j.1755-3768.1986.tb06894.x

Trenton, J. A. and Y. Courtois (1981), "Evolution of the distribution, proliferation and ultraviolet repair capacity of rat lens epithelial cells as a function of maturation and aging", Mechanisms of Ageing and Development, Vol. 15/3, Elsevier, Ireland, https://doi.org/1016/0047-6374(81)90134-2

Vega-Avila, E. & K. M. Pugsley (2011), "An Overview of Colorimetric Assay Methods Used to Assess Survival or

Proliferation of Mammalian Cells", Proc. West. Pharmacol. Soc. 54, 10-4.

von Sallmann, L. (1951), "Experimental studies on early lens changes after x-ray irradiation III. Effect of X-radiation on mitotic activity and nuclear fragmentation of lens epithelium in normal and cysteine-treated rabbits", Transactions of the American Ophthalmological Society, Vol. 48, United States, pp. 228-242

Worgul, B. V. et al. (1986), "Accelerated heavy particles and the lens II. Cytopathological changes", Investigative Ophthalmology and Visual Science, Vol 27/1, pp. 108-114

Event: 1392: Oxidative Stress

Short Name: Oxidative Stress

Key Event Component

Process	Object	Action
oxidative stress		increased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:220 - Cyp2E1 Activation Leading to Liver Cancer	KeyEvent
Aop:17 - Binding of electrophilic chemicals to SH(thiol)-group of proteins and /or to seleno-proteins involved in protection against oxidative stress during brain development leads to impairment of learning and memory	KeyEvent
Aop:284 - Binding of electrophilic chemicals to SH(thiol)-group of proteins and /or to seleno-proteins involved in protection against oxidative stress leads to chronic kidney disease	KeyEvent
Aop:377 - Dysregulated prolonged Toll Like Receptor 9 (TLR9) activation leading to Multi Organ Failure involving Acute Respiratory Distress Syndrome (ARDS)	KeyEvent
Aop:411 - Oxidative stress Leading to Decreased Lung Function	MolecularInitiatingEvent
Aop:424 - Oxidative stress Leading to Decreased Lung Function via CFTR dysfunction	MolecularInitiatingEvent
Aop:425 - Oxidative Stress Leading to Decreased Lung Function via Decreased FOXJ1	MolecularInitiatingEvent
Aop:429 - A cholesterol/glucose dysmetabolism initiated Tau-driven AOP toward memory loss (AO) in sporadic Alzheimer's Disease with plausible MIE's plug-ins for environmental neurotoxicants	KeyEvent
Aop:452 - Adverse outcome pathway of PM-induced respiratory toxicity	KeyEvent
Aop:464 - Calcium overload in dopaminergic neurons of the substantia nigra leading to parkinsonian motor deficits	KeyEvent
Aop:470 - Deposition of energy leads to abnormal vascular remodeling	KeyEvent
Aop:478 - Deposition of energy leading to occurrence of cataracts	KeyEvent
Aop:479 - Mitochondrial complexes inhibition leading to left ventricular function decrease via increased myocardial oxidative stress	KeyEvent
Aop:481 - AOPs of amorphous silica nanoparticles: ROS-mediated oxidative stress increased respiratory dysfunction and diseases.	KeyEvent
Aop:482 - Deposition of energy leading to occurrence of bone loss	KeyEvent
Aop:483 - Deposition of Energy Leading to Learning and Memory Impairment	KeyEvent
Aop:505 - Reactive Oxygen Species (ROS) formation leads to cancer via inflammation pathway	KeyEvent
Aop:521 - Essential element imbalance leads to reproductive failure via oxidative stress	KeyEvent
Aop:26 - Calcium-mediated neuronal ROS production and energy imbalance	AdverseOutcome
Aop:488 - Increased reactive oxygen species production leading to decreased cognitive function	KeyEvent

AOP ID and Name	Event Type
Aop:396 - Deposition of ionizing energy leads to population decline via impaired meiosis	KeyEvent
Aop:437 - Inhibition of mitochondrial electron transport chain (ETC) complexes leading to kidney toxicity	KeyEvent
Aop:535 - Binding and activation of GPER leading to learning and memory impairments	KeyEvent
Aop:171 - Chronic cytotoxicity of the serous membrane leading to pleural/peritoneal mesotheliomas in the rat.	KeyEvent
Aop:138 - Organic anion transporter (OAT1) inhibition leading to renal failure and mortality	KeyEvent
Aop:177 - Cyclooxygenase 1 (COX1) inhibition leading to renal failure and mortality	KeyEvent
Aop:186 - unknown MIE leading to renal failure and mortality	KeyEvent
Aop:200 - Estrogen receptor activation leading to breast cancer	KeyEvent
Aop:444 - Ionizing radiation leads to reduced reproduction in Eisenia fetida via reduced spermatogenesis and cocoon hatchability	KeyEvent
Aop:447 - Kidney failure induced by inhibition of mitochondrial electron transfer chain through apoptosis, inflammation and oxidative stress pathways	KeyEvent
Aop:476 - Adverse Outcome Pathways diagram related to PBDEs associated male reproductive toxicity	KeyEvent
Aop:497 - ERa inactivation alters mitochondrial functions and insulin signalling in skeletal muscle and leads to insulin resistance and metabolic syndrome	KeyEvent
Aop:457 - Succinate dehydrogenase inhibition leading to increased insulin resistance through reduction in circulating thyroxine	KeyEvent
Aop:459 - AhR activation in the thyroid leading to Subsequent Adverse Neurodevelopmental Outcomes in Mammals	KeyEvent
Aop:507 - Nrf2 inhibition leading to vascular disrupting effects via inflammation pathway	KeyEvent
Aop:509 - Nrf2 inhibition leading to vascular disrupting effects through activating apoptosis signal pathway and mitochondrial dysfunction	KeyEvent
Aop:510 - Demethylation of PPAR promotor leading to vascular disrupting effects	KeyEvent
Aop:511 - The AOP framework on ROS-mediated oxidative stress induced vascular disrupting effects	KeyEvent
Aop:538 - Adverse outcome pathway of PFAS-induced vascular disrupting effects via activating oxidative stress related pathways	KeyEvent
Aop:260 - CYP2E1 activation and formation of protein adducts leading to neurodegeneration	KeyEvent
Aop:450 - Inhibition of AChE and activation of CYP2E1 leading to sensory axonal peripheral neuropathy and mortality	KeyEvent
Aop:501 - Excessive iron accumulation leading to neurological disorders	KeyEvent
Aop:540 - Oxidative Stress in the Fish Ovary Leads to Reproductive Impairment via Reduced Vitellogenin Production	KeyEvent
Aop:471 - Various neuronal effects induced by elavl3, sox10, and mbp	KeyEvent
Aop:31 - Oxidation of iron in hemoglobin leading to hematotoxicity	KeyEvent

Stressors

Name
Acetaminophen
Chloroform
furan
Platinum
Aluminum
Cadmium

Name
Mercury
Uranium
Arsenic
Silver
Manganese
Nickel
Zinc
nanoparticles

Biological Context

Level of Biological Organization
Molecular

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
rodents	rodents	High	NCBI
Homo sapiens	Homo sapiens	High	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Mixed	High

Taxonomic applicability: Occurrence of oxidative stress is not species specific.

Life stage applicability: Occurrence of oxidative stress is not life stage specific.

Sex applicability: Occurrence of oxidative stress is not sex specific.

Evidence for perturbation by prototypic stressor: There is evidence of the increase of oxidative stress following perturbation from a variety of stressors including exposure to ionizing radiation and altered gravity (Bai et al., 2020; Ungvari et al., 2013; Zhang et al., 2009).

Key Event Description

Oxidative stress is defined as an imbalance in the production of reactive oxygen species (ROS) and antioxidant defenses. High levels of oxidizing free radicals can be very damaging to cells and molecules within the cell. As a result, the cell has important defense mechanisms to protect itself from ROS. For example, Nrf2 is a transcription factor and master regulator of the oxidative stress response. During periods of oxidative stress, Nrf2-dependent changes in gene expression are important in regaining cellular homeostasis (Nguyen, et al., 2009) and can be used as indicators of the presence of oxidative stress in the cell.

In addition to the directly damaging actions of ROS, cellular oxidative stress also changes cellular activities on a molecular level. Redox sensitive proteins have altered physiology in the presence and absence of ROS, which is caused by the oxidation of sulfhydryls to disulfides on neighboring amino acids (Antelmann & Helmann 2011). Importantly Keap1, the negative regulator of Nrf2, is regulated in this manner (Itoh, et al. 2010).

ROS also undermine the mitochondrial defense system from oxidative damage. The antioxidant systems consist of superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase, as well as antioxidants such as α-tocopherol and ubiquinol, or antioxidant vitamins and minerals including vitamin E, C, carotene, lutein, zeaxanthin, selenium, and zinc (Fletcher, 2010). The enzymes, vitamins and minerals catalyze the conversion of ROS to non-toxic molecules such as water and O2. However, these antioxidant systems are not perfect and endogenous metabolic

processes and/or exogenous oxidative influences can trigger cumulative oxidative injuries to the mitochondria, causing a decline in their functionality and efficiency, which further promotes cellular oxidative stress (Balasubramanian, 2000; Ganea & Harding, 2006; Guo et al., 2013; Karimi et al., 2017).

However, an emerging viewpoint suggests that ROS-induced modifications may not be as detrimental as previously thought, but rather contribute to signaling processes (Foyer et al., 2017).

Sources of ROS Production

Direct Sources: Direct sources involve the deposition of energy onto water molecules, breaking them into active radical species. When ionizing radiation hits water, it breaks it into hydrogen (H*) and hydroxyl (OH*) radicals by destroying its bonds. The hydrogen will create hydroxyperoxyl free radicals (HO2*) if oxygen is available, which can then react with another of itself to form hydrogen peroxide (H2O2) and more O2 (Elgazzar and Kazem, 2015). Antioxidant mechanisms are also affected by radiation, with catalase (CAT) and peroxidase (POD) levels rising as a result of exposure (Seen et al. 2018; Ahmad et al. 2021).

Indirect Sources: An indirect source of ROS is the mitochondria, which is one of the primary producers in eukaryotic cells (Powers et al., 2008). As much as 2% of the electrons that should be going through the electron transport chain in the mitochondria escape, allowing them an opportunity to interact with surrounding structures. Electron-oxygen reactions result in free radical production, including the formation of hydrogen peroxide (H2O2) (Zhao et al., 2019). The electron transport chain, which also creates ROS, is activated by free adenosine diphosphate (ADP), O2, and inorganic phosphate (Pi) (Hargreaves et al. 2020; Raimondi et al. 2020; Vargas-Mendoza et al. 2021). The first and third complexes of the transport chain are the most relevant to mammalian ROS production (Raimondi et al., 2020). The mitochondria has its own set of DNA and it is a prime target of oxidative damage (Guo et al., 2013). ROS is also produced through nicotinamide adenine dinucleotide phosphate oxidase (Nox) stimulation, an event commenced by angiotensin II, a product/effector of the renin-angiotensin system (Nguyen Dinh Cat et al. 2013; Forrester et al. 2018). Other ROS producers include xanthine oxidase, immune cells (macrophage, neutrophils, monocytes, and eosinophils), phospholipase A2 (PLA2), monoamine oxidase (MAO), and carbon-based nanomaterials (Powers et al. 2008; Jacobsen et al. 2008; Vargas-Mendoza et al. 2021).

How it is Measured or Detected

Oxidative Stress: Direct measurement of ROS is difficult because ROS are unstable. The presence of ROS can be assayed indirectly by measurement of cellular antioxidants, or by ROS-dependent cellular damage. Listed below are common methods for detecting the KE, however there may be other comparable methods that are not listed

- Detection of ROS by chemiluminescence (https://www.sciencedirect.com/science/article/abs/pii/S0165993606001683)
- Detection of ROS by chemiluminescence is also described in OECD TG 495 to assess phototoxic potential.
- Glutathione (GSH) depletion. GSH can be measured by assaying the ratio of reduced to oxidized glutathione (GSH:GSSG) using a commercially available kit (e.g., http://www.abcam.com/gshgssg-ratio-detection-assay-kit-fluorometric-green- ab138881.html).
- TBARS. Oxidative damage to lipids can be measured by assaying for lipid peroxidation using TBARS (thiobarbituric acid reactive substances) using a commercially available kit.
- 8-oxo-dG. Oxidative damage to nucleic acids can be assayed by measuring 8-oxo-dG adducts (for which there are a number of ELISA based commercially available kits),or HPLC, described in Chepelev et al. (Chepelev, et al. 2015).

Molecular Biology: Nrf2. Nrf2’s transcriptional activity is controlled post-translationally by oxidation of Keap1. Assay for Nrf2 activity include:

- Immunohistochemistry for increases in Nrf2 protein levels and translocation into the nucleus Western blot for increased Nrf2 protein levels
- Western blot of cytoplasmic and nuclear fractions to observe translocation of Nrf2 protein from the cytoplasm to the nucleus qPCR of Nrf2 target genes (e.g., Nqo1, Hmox-1, Gcl, Gst, Prx, TrxR, Srxn), or by commercially available pathway-based qPCR array (e.g., oxidative stress array from SABiosciences)
- Whole transcriptome profiling by microarray or RNA-seq followed by pathway analysis (in IPA, DAVID, metacore, etc.) for enrichment of the Nrf2 oxidative stress response pathway (e.g., Jackson et al. 2014)
- OECD TG422D describes an ARE-Nrf2 Luciferase test method

In general, there are a variety of commercially available colorimetric or fluorescent kits for detecting Nrf2 activationOxidative Stress. Direct measurement of ROS is difficult because ROS are unstable. The presence of ROS can be assayed indirectly by measurement of cellular antioxidants, or by ROS-dependent cellular damage. Listed below are common methods for detecting the KE, however there may be other comparable methods that are not listed

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Assay Type & Measured Content	**Description**	**Dose Range Studied**	**Assay Characteristics (Length/Ease of use/Accuracy)**
ROS Formation in the Mitochondria assay (Shaki et al., 2012)	"The mitochondrial ROS measurement was performed flow cytometry using DCFH-DA. Briefly, isolated kidney mitochondria were incubated with UA (0, 50, 100 and 200 µM) in respiration buffer containing (0.32 mM sucrose, 10mM Tris, 20 mM Mops, 50 µM EGTA, 0.5 mM MgCl2, 0.1 mM KH2PO4 and 5 mM sodium succinate) [32]. In the interval times of 5, 30 and 60 min following the UA addition, a sample was taken and DCFH-DA was added (final concentration, 10 µM) to mitochondria and was then incubated for 10 min.Uranyl acetate-induced ROS generation in isolated kidney mitochondria were determined through the flow cytometry (Partec, Deutschland) equipped with a 488-nm argon ion laser and supplied with the Flomax software and the signals were obtained using a 530-nm bandpass filter (FL-1 channel). Each determination is based on the mean fluorescence intensity of 15,000 counts."	0, 50,100 and 200 µM of Uranyl Acetate	Long/ Easy High accuracy
Mitochondrial Antioxidant Content Assay Measuring GSH content (Shaki et al., 2012)	"GSH content was determined using DTNB as the indicator and spectrophotometer method for the isolated mitochondria. The mitochondrial fractions (0.5 mg protein/ml) were incubated with various concentrations of uranyl acetate for 1 h at 30 °C and then 0.1 ml of mitochondrial fractions was added into 0.1 mol/l of phosphate buffers and 0.04% DTNB in a total volume of 3.0 ml (pH 7.4). The developed yellow color was read at 412 nm on a spectrophotometer (UV-1601 PC, Shimadzu, Japan). GSH content was expressed as µg/mg protein."	0, 50, 100, or 200 µM Uranyl Acetate	
H2O2 Production Assay Measuring H2O2 Production in isolated mitochondria (Heyno et al., 2008)	"Effect of CdCl2 and antimycin A (AA) on H2O2 production in isolated mitochondria from potato. H2O2 production was measured as scopoletin oxidation. Mitochondria were incubated for 30 min in the measuring buffer (see the Materials and Methods) containing 0.5 mM succinate as an electron donor and 0.2 µM mesoxalonitrile 3-chlorophenylhydrazone (CCCP) as an uncoupler, 10 U horseradish peroxidase and 5 µM scopoletin."	0, 10, 30 µM Cd2+ 2 µM antimycin A	

Flow Cytometry ROS & Cell Viability (Kruiderig et al., 1997)	“For determination of ROS, samples taken at the indicated time points were directly transferred to FACScan tubes. Dih123 (10 mM, final concentration) was added and cells were incubated at 37°C in a humidified atmosphere (95% air/5% CO2) for 10 min. At t 5 9, propidium iodide (10 mM, final concentration) was added, and cells were analyzed by flow cytometry at 60 ml/min. Nonfluorescent Dih123 is cleaved by ROS to fluorescent R123 and detected by the FL1 detector as described above for Dc (Van de Water 1995)”“For determination of ROS, samples taken at the indicated time points were directly transferred to FACScan tubes. Dih123 (10 mM, final concentration) was added and cells were incubated at 37°C in a humidified atmosphere (95% air/5% CO2) for 10 min. At t 5 9, propidium iodide (10 mM, final concentration) was added, and cells were analyzed by flow cytometry at 60 ml/min. Nonfluorescent Dih123 is cleaved by ROS to fluorescent R123 and detected by the FL1 detector as described above for Dc (Van de Water 1995)”		Strong/easy medium
DCFH-DA Assay Detection of hydrogen peroxide production (Yuan et al., 2016)	Intracellular ROS production was measured using DCFH-DA as a probe. Hydrogen peroxide oxidizes DCFH to DCF. The probe is hydrolyzed intracellularly to DCFH carboxylate anion. No direct reaction with H2O2 to form fluorescent production.	0-400 µM	Long/ Easy High accuracy
H2-DCF-DAAssay Detection of superoxide production (Thiebault etal., 2007)	This dye is a stable nonpolar compound which diffuses readily into the cells and yields H2-DCF. Intracellular OH or ONOO- react with H2-DCF when cells contain peroxides, to form the highly fluorescent compound DCF, which effluxes the cell. Fluorescence intensity of DCF is measured using a fluorescence spectrophotometer.	0–600 µM	Long/ Easy High accuracy
CM-H2DCFDA Assay (Eruslanov & Kusmartsev, 2009)	The dye (CM-H2DCFDA) diffuses into the cell and is cleaved by esterases, the thiol reactive chlormethyl group reacts with intracellular glutathione which can be detected using flow cytometry.		Long/Easy/ High Accuracy

Method of Measurement	**References**	**Description**	**OECD-Approved Assay**
Chemiluminescence	(Lu, C. et al., 2006; Griendling, K. K., et al., 2016)	ROS can induce electron transitions in molecules, leading to electronically excited products. When the electrons transition back to ground state, chemiluminescence is emitted and can be measured. Reagents such as luminol and lucigenin are commonly used to amplify the signal.	No
Spectrophotometry	(Griendling, K. K., et al., 2016)	NO has a short half-life. However, if it has been reduced to nitrite (NO2-), stable azocompounds can be formed via the Griess Reaction, and further measured by spectrophotometry.	No
Direct or Spin Trapping-Based electron paramagnetic resonance (EPR) Spectroscopy	(Griendling, K. K., et al., 2016)	The unpaired electrons (free radicals) found in ROS can be detected with EPR and is known as electron paramagnetic resonance. A variety of spin traps can be used.	No
Nitroblue Tetrazolium Assay	(Griendling, K. K., et al., 2016)	The Nitroblue Tetrazolium assay is used to measure O2.− levels. O2.− reduces nitroblue tetrazolium (a yellow dye) to formazan (a blue dye), and can be measured at 620 nm.	No
Fluorescence analysis of dihydroethidium (DHE) or Hydrocyans	(Griendling, K. K., et al., 2016)	Fluorescence analysis of DHE is used to measure O2.− levels. O2.− is reduced to O2 as DHE is oxidized to 2-hydroxyethidium, and this reaction can be measured by fluorescence. Similarly, hydrocyans can be oxidized by any ROS, and measured via fluorescence.	No

Amplex Red Assay	(Griendling, K. K., et al., 2016)	Fluorescence analysis to measure extramitochondrial or extracellular H2O2 levels. In the presence of horseradish peroxidase and H2O2, Amplex Red is oxidized to resorufin, a fluorescent molecule measurable by plate reader.	No
Dichlorodihydrofluorescein Diacetate (DCFH-DA)	(Griendling, K. K., et al., 2016)	An indirect fluorescence analysis to measure intracellular H2O2 levels. H2O2 interacts with peroxidase or heme proteins, which further react with DCFH, oxidizing it to dichlorofluorescein (DCF), a fluorescent product.	No
HyPer Probe	(Griendling, K. K., et al., 2016)	Fluorescent measurement of intracellular H2O2 levels. HyPer is a genetically encoded fluorescent sensor that can be used for in vivo and in situ imaging.	No
Cytochrome c Reduction Assay	(Griendling, K. K., et al., 2016)	The cytochrome c reduction assay is used to measure O2.− levels. O O2.− is reduced to O2 as ferricytochrome c is oxidized to ferrocytochrome c, and this reaction can be measured by an absorbance increase at 550 nm.	No
Proton-electron double-resonance imaging (PEDRI)	(Griendling, K. K., et al., 2016)	The redox state of tissue is detected through nuclear magnetic resonance/magnetic resonance imaging, with the use of a nitroxide spin probe or biradical molecule.	No
Glutathione (GSH) depletion	(Biesemann, N. et al., 2018)	A downstream target of the Nrf2 pathway is involved in GSH synthesis. As an indication of oxidation status, GSH can be measured by assaying the ratio of reduced to oxidized glutathione (GSH:GSSG) using a commercially available kit (e.g., http://www.abcam.com/gshgssg-ratio-detection-assay-kit-fluorometric-green-ab138881.html).	No
Thiobarbituric acid reactive substances (TBARS)	(Griendling, K. K., et al., 2016)	Oxidative damage to lipids can be measured by assaying for lipid peroxidation with TBARS using a commercially available kit.	No
Protein oxidation (carbonylation)	(Azimzadeh et al., 2017; Azimzadeh et al., 2015; Ping et al., 2020)	Can be determined with ELISA or a commercial assay kit. Protein oxidation can indicate the level of oxidative stress.	No
Seahorse XFp Analyzer	Leung et al. 2018	The Seahorse XFp Analyzer provides information on mitochondrial function, oxidative stress, and metabolic dysfunction of viable cells by measuring respiration (oxygen consumption rate; OCR) and extracellular pH (extracellular acidification rate; ECAR).	No

Molecular Biology: Nrf2. Nrf2's transcriptional activity is controlled post-translationally by oxidation of Keap1. Assays for Nrf2 activity include:

Method of Measurement	References	Description	OECD-Approved Assay
Immunohistochemistry	(Amsen, D., de Visser, K. E., and Town, T., 2009)	Immunohistochemistry for increases in Nrf2 protein levels and translocation into the nucleus	No

qPCR	(Forlenza et al., 2012)	qPCR of Nrf2 target genes (e.g., Nqo1, Hmox-1, Gcl, Gst, Prx, TrxR, Srxn), or by commercially available pathway-based qPCR array (e.g., oxidative stress array from SABiosciences)	No
Whole transcriptome profiling via microarray or via RNA-seq followed by a pathway analysis	(Jackson, A. F. et al., 2014)	Whole transcriptome profiling by microarray or RNA-seq followed by pathway analysis (in IPA, DAVID, metacore, etc.) for enrichment of the Nrf2 oxidative stress response pathway	No

References

Ahmad, S. et al. (2021), “60Co-γ Radiation Alters Developmental Stages of Zeugodacus cucurbitae (Diptera: Tephritidae) Through Apoptosis Pathways Gene Expression”, Journal Insect Science, Vol. 21/5, Oxford University Press, Oxford, https://doi.org/10.1093/jisesa/ieab080

Antelmann, H. and J. D. Helmann (2011), “Thiol-based redox switches and gene regulation.”, Antioxidants & Redox Signaling, Vol. 14/6, Mary Ann Leibert Inc., Larchmont, https://doi.org/10.1089/ars.2010.3400

Amsen, D., de Visser, K. E., and Town, T. (2009), “Approaches to determine expression of inflammatory cytokines”, in Inflammation and Cancer, Humana Press, Totowa, https://doi.org/10.1007/978-1-59745-447-6_5

Azimzadeh, O. et al. (2015), “Integrative Proteomics and Targeted Transcriptomics Analyses in Cardiac Endothelial Cells Unravel Mechanisms of Long-Term Radiation-Induced Vascular Dysfunction”, Journal of Proteome Research, Vol. 14/2, American Chemical Society, Washington, https://doi.org/10.1021/pr501141b

Azimzadeh, O. et al. (2017), “Proteome analysis of irradiated endothelial cells reveals persistent alteration in protein degradation and the RhoGDI and NO signalling pathways”, International Journal of Radiation Biology, Vol. 93/9, Informa, London, https://doi.org/10.1080/09553002.2017.1339332

Azzam, E. I. et al. (2012), “Ionizing radiation-induced metabolic oxidative stress and prolonged cell injury”, Cancer Letters, Vol. 327/1-2, Elsevier, Ireland, https://doi.org/10.1016/j.canlet.2011.12.012

Bai, J. et al. (2020), “Irradiation-induced senescence of bone marrow mesenchymal stem cells aggravates osteogenic differentiation dysfunction via paracrine signaling”, American Journal of Physiology - Cell Physiology, Vol. 318/5, American Physiological Society, Rockville, https://doi.org/10.1152/ajpcell.00520.2019.

Balasubramanian, D (2000), “Ultraviolet radiation and cataract”, Journal of ocular pharmacology and therapeutics, Vol. 16/3, Mary Ann Liebert Inc., Larchmont, https://doi.org/10.1089/jop.2000.16.285.

Biesemann, N. et al., (2018), “High Throughput Screening of Mitochondrial Bioenergetics in Human Differentiated Myotubes Identifies Novel Enhancers of Muscle Performance in Aged Mice”, Scientific Reports, Vol. 8/1, Nature Portfolio, London, https://doi.org/10.1038/s41598-018-27614-8.

Elgazzar, A. and N. Kazem. (2015), “Chapter 23: Biological effects of ionizing radiation” in The Pathophysiologic Basis of Nuclear Medicine, Springer, New York, pp. 540-548

Eruslanov, E., & Kusmartsev, S. (2010). Identification of ROS using oxidized DCFDA and flow-cytometry. Methods in molecular biology ,N.J., Vol. 594, https://doi.org/10.1007/978-1-60761-411-1_4

Fletcher, A. E (2010), “Free radicals, antioxidants and eye diseases: evidence from epidemiological studies on cataract and age-related macular degeneration”, Ophthalmic Research, Vol. 44, Karger International, Basel, https://doi.org/10.1159/000316476.

Forlenza, M. et al. (2012), “The use of real-time quantitative PCR for the analysis of cytokine mRNA levels” in Cytokine Protocols, Springer, New York, https://doi.org/10.1007/978-1-61779-439-1_2

Forrester, S.J. et al. (2018), “Angiotensin II Signal Transduction: An Update on Mechanisms of Physiology and Pathophysiology”, Physiological Reviews, Vol. 98/3, American Physiological Society, Rockville, https://doi.org/10.1152/physrev.00038.201

Foyer, C. H., A. V. Ruban, and G. Noctor (2017), “Viewing oxidative stress through the lens of oxidative signalling rather than damage”, Biochemical Journal, Vol. 474/6, Portland Press, England, https://doi.org/10.1042/BCJ20160814

Ganea, E. and J. J. Harding (2006), “Glutathione-related enzymes and the eye”, Current eye research, Vol. 31/1, Informa, London, https://doi.org/10.1080/02713680500477347.

Griendling, K. K. et al. (2016), “Measurement of reactive oxygen species, reactive nitrogen species, and redox-dependent signaling in the cardiovascular system: a scientific statement from the American Heart Association”, Circulation research, Vol. 119/5, Lippincott Williams & Wilkins, Philadelphia, https://doi.org/10.1161/RES.0000000000000110

Guo, C. et al. (2013), “Oxidative stress, mitochondrial damage and neurodegenerative diseases”, Neural regeneration research, Vol. 8/21, Publishing House of Neural Regeneration Research, China, https://doi.org/10.3969/j.issn.1673-5374.2013.21.009

Hargreaves, M., and L. L. Spriet (2020), “Skeletal muscle energy metabolism during exercise.”, Nature Metabolism, Vol. 2, Nature Portfolio, London, https://doi.org/10.1038/s42255-020-0251-4

Hladik, D. and S. Tapio (2016), “Effects of ionizing radiation on the mammalian brain”, Mutation Research/Reviews in Mutation Research, Vol. 770, Elsevier, Amsterdam, https://doi.org/10.1016/j.mrrev.2016.08.003

Itoh, K., J. Mimura and M. Yamamoto (2010), “Discovery of the negative regulator of Nrf2, Keap1: a historical overview”, Antioxidants & Redox Signaling, Vol. 13/11, Mary Ann Leibert Inc., Larchmont, https://doi.org/10.1089/ars.2010.3222

Jackson, A.F. et al. (2014), “Case study on the utility of hepatic global gene expression profiling in the risk assessment of the carcinogen furan.”, Toxicology and Applied Pharmacology, Vol. 274/11, Elsevier, Amsterdam, https://doi.org/10.1016/j.taap.2013.10.019

Jacobsen, N.R. et al. (2008), “Genotoxicity, cytotoxicity, and reactive oxygen species induced by single-walled carbon nanotubes and C60 fullerenes in the FE1-MutaTM Mouse lung epithelial cells”, Environmental and Molecular Mutagenesis, Vol. 49/6, John Wiley & Sons, Inc., Hoboken, https://doi.org/10.1002/em.20406

Karimi, N. et al. (2017), “Radioprotective effect of hesperidin on reducing oxidative stress in the lens tissue of rats”, International Journal of Pharmaceutical Investigation, Vol. 7/3, Phcog Net, Bengaluru, https://doi.org/10.4103/jphi.JPHI_60_17.

Leung, D.T.H., and Chu, S. (2018), “Measurement of Oxidative Stress: Mitochondrial Function Using the Seahorse System” In: Murthi, P., Vaillancourt, C. (eds) Preeclampsia. Methods in Molecular Biology, vol 1710. Humana Press, New York, NY. https://doi.org/10.1007/978-1-4939-7498-6_22

Lu, C., G. Song, and J. Lin (2006), “Reactive oxygen species and their chemiluminescence-detection methods”, TrAC Trends in Analytical Chemistry, Vol. 25/10, Elsevier, Amsterdam, https://doi.org/10.1016/j.trac.2006.07.007

Nguyen Dinh Cat, A. et al. (2013), “Angiotensin II, NADPH oxidase, and redox signaling in the vasculature”, Antioxidants & redox signaling, Vol. 19/10, Mary Ann Liebert, Larchmont, https://doi.org/10.1089/ars.2012.4641

Ping, Z. et al. (2020), “Oxidative Stress in Radiation-Induced Cardiotoxicity”, Oxidative Medicine and Cellular Longevity, Vol. 2020, Hindawi, https://doi.org/10.1155/2020/3579143

Powers, S.K. and M.J. Jackson. (2008), “Exercise-Induced Oxidative Stress: Cellular Mechanisms and Impact on Muscle Force Production”, Physiological Reviews, Vol. 88/4, American Physiological Society, Rockville, https://doi.org/10.1152/physrev.00031.2007

Raimondi, V., F. Ciccarese and V. Ciminale. (2020), “Oncogenic pathways and the electron transport chain: a dangeROS liason”, British Journal of Cancer, Vol. 122/2, Nature Portfolio, London, https://doi.org/10.1038/s41416-019-0651-y

Seen, S. and L. Tong. (2018), “Dry eye disease and oxidative stress”, Acta Ophthalmologica, Vol. 96/4, John Wiley & Sons, Inc., Hoboken, https://doi.org/10.1111/aos.13526

Ungvari, Z. et al. (2013), “Ionizing Radiation Promotes the Acquisition of a Senescence-Associated Secretory Phenotype and Impairs Angiogenic Capacity in Cerebromicrovascular Endothelial Cells: Role of Increased DNA Damage and Decreased DNA Repair Capacity in Microvascular Radiosensitivity”, The Journals of Gerontology Series A: Biological Sciences and Medical Sciences, Vol. 68/12, Oxford University Press, Oxford, https://doi.org/10.1093/gerona/glt057.

Vargas-Mendoza, N. et al. (2021), “Oxidative Stress, Mitochondrial Function and Adaptation to Exercise: New Perspectives in Nutrition”, Life, Vol. 11/11, Multidisciplinary Digital Publishing Institute, Basel, https://doi.org/10.3390/life11111269

Wang, H. et al. (2019), “Radiation-induced heart disease: a review of classification, mechanism and prevention”, International Journal of Biological Sciences, Vol. 15/10, Ivyspring International Publisher, Sydney, https://doi.org/10.7150/ijbs.35460

Zhang, R. et al. (2009), “Blockade of AT1 receptor partially restores vasoreactivity, NOS expression, and superoxide levels in cerebral and carotid arteries of hindlimb unweighting rats”, Journal of applied physiology, Vol. 106/1, American Physiological Society, Rockville, https://doi.org/10.1152/japplphysiol.01278.2007.

Zhao, R. Z. et al. (2019), “Mitochondrial electron transport chain, ROS generation and uncoupling”, International journal of molecular medicine, Vol. 44/1, Spandidos Publishing Ltd., Athens, https://doi.org/10.3892/ijmm.2019.4188

List of Adverse Outcomes in this AOP

Event: 2083: Occurrence of Cataracts

Short Name: Cataracts

Key Event Component

Process	Object	Action
eye opacity	Lens	increased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:478 - Deposition of energy leading to occurrence of cataracts	AdverseOutcome

Biological Context

Level of Biological Organization
Organ

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	High	NCBI
rat	Rattus norvegicus	High	NCBI
mouse	Mus musculus	High	NCBI
rabbit	Oryctolagus cuniculus	High	NCBI
Monkey	Monkey	Moderate	NCBI
Pig	Pig	Moderate	NCBI
guinea pig	Cavia porcellus	Moderate	NCBI
rainbow trout	Oncorhynchus mykiss	Moderate	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Female	High
Male	High

Taxonomic applicability: This KE is relevant to any species requiring a clear lens for vision.

Life stage applicability: This key event can occur at any life stage; however, it is most common in older adults. Among humans, cataract changes usually begin after the age of 50 and become increasingly prevalent with age.

Sex applicability: The adverse outcome can develop in both sexes and is not sex-specific. Females, however, have a small increased background risk of cataracts (Ainsbury et al., 2016). They also have a higher risk for radiation-induced cataracts including PSC, cortical and nuclear cataracts (Choshi et al., 1983, Nakashima et al., 2006; Henderson et al., 2010; Dynlacht et al., 2011; Azizova et al. 2018; Little et al., 2018; Garrett et al., 2020).

Evidence for perturbation by prototypic stressors: A large body of evidence supports cataract induction via both ionizing and non-ionizing radiation. This includes X-rays, γ-rays, UV, neutrons, protons, β particles, and various heavy ions (^{56}Fe, ^{40}Ar, ^{12}C, ^{20}Ne, ^{224}Ra, and He). Of these, X-rays and γ-rays are the best supported (Yang &

Ainsworth, 1987; Chmelevsky, 1988l; Brenner et al., 1991; Fedorenko, 1995; Char et al., 1998; Nakashima et al., 2006; Worgul et al., 2007; Davis et al., 2010; Karatasakis et al., 2018; Garrett et al., 2020; Kang et al., 2020; McCarron et al., 2021).

Key Event Description

Cataracts are a progressive condition in which the lens of the eye develops opacities and becomes cloudy, resulting in blurred vision as well as glare and haloes around lights (National Eye Institute, 2022). For this AOP, a cataract is defined when over 5% of the lens is opacified. It is one of the leading causes of blindness around the world (Raj et al., 2009; Liu et al., 2017), and surgery is currently the only cure.

The lens is a transparent, biconvex tissue located at the front of the eye. It is responsible for focusing light onto the retina, producing a clear image. However, under certain conditions, sections of the lens may develop small opacities, losing their transparency and resulting in blurred vision (Hildreth et al., 2009). As the lens has low metabolic and mitotic activity, there is very little tissue turnover. Therefore, damaged proteins that are not removed can acucmulate over time contributing to opacities and formation of cataracts (Hamada, 2017).

A variety of factors are essential for maintaining the transparency of the lens, and therefore preventing cataracts. These include proper organization, development and balance of proteins such as crystallins (Hildreth et al., 2009; Ainsbury et al., 2016; Hamada, 2017; Wu et al., 2018), no organelles within the mature lens fiber cells (Pendergrass, 2010; Fujimichi et al., 2014; Hamada, 2017; Heitmancik & Shiels, 2015), and a low water content in the lens (Ainsbury et al., 2016). Genetic factors can also play a role, such as mutations in genes coding for molecular chaperones, growth factors, gap-junction proteins, intermediated filament proteins, membrane proteins, and RNA binding proteins (Hamada & Fujimichi, 2015; Lachke, 2022). When any of these factors are affected, it causes light scattering, which increases lens opacity, contributing to cataract formation and density.

In general, there are three main categories of cataract: pediatric, age-related and those secondary to other causes. Age-related cataracts are the most common and can be subdivided into nuclear, cortical, or posterior subcapsular cataracts (PSC) based on which portion of the lens becomes opaque. In nuclear cataracts the opacities are in the nucleus of the lens, in cortical cataracts they are in the cortex, and in posterior subcapsular cataracts they are located beneath the posterior capsule (Van Kuijk, 1991). Research has shown that posterior subcapsular (PSC) cataracts are a subtype of cataract that are most often found with ionizing radiation exposure. This may be due to radiation exposure causing the improperly differentiated lens epithelial cells (LECs) to leave the germinative zone (GZ) and migrate along the posterior capsule towards the center of the lens. As atypical lens fiber cells (LFCs), and atypical LECs accumulate in this area, they may cause the development of a PSC cataract (Loganovsky et al., 2020).

Cataracts can be diagnosed through several different methods and there is no universally accepted grading system. The most common grading systems are the Lens Opacities Classification System I, II, or III (LOC I, II, or III), the Modified Merriam-Focht Cataract Scoring System, and the slit lamp grading system. They classify cataracts on a scale of severity, which is often subjective, relying upon the examiner’s judgement. However, there are some methods such as Scheimpflug imaging which are less subjective as they measure lens density (Barraquer et al., 2017; Singh Grewal & Singh Grewal, 2012).

How it is Measured or Detected

Listed below are common methods for detecting the KE, however there may be other comparable methods that are not listed.

Assay	Reference	Description	OECD Approved Assay
Lens opacification grading systems	Barraquer et al., 2017	Systems used to classify the severity of cataracts. There are multiple types including: the Modified Merriam-Focht Cataract Scoring System, Lens Opacities Classification System III (LOC III), Word Health Organization Cataract Grading System, Lens Opacities Classification System I (LOCI), Lens Opacities Classification System II (LOC II), Wisconsin Clinical and Photographic Cataract Grading System, Wilmer Clinical and Photographic Grading System, Oxford Clinical Cataract Grading System, Age-Related Eye Disease Study, National Eye Institute Clinical Cataract Grading System, Japanese Cooperative Cataract Research Group Cataract Grading System	No
Slit Lamp Grading System	Barraquer et al., 2017; Robert & Alastair, 2017	Measures the light intensity reflected from opacities in nuclear cataracts. This also includes various techniques such as retroillumination.	No
Microscopy Examination	Stirling and Griffiths, 1991	Tests can help to examine interlocking processes and membrane architecture of lens.	No
Histological staining	Singh et al., 2003	Uses dyes such as trypan blue to differentiate different parts of the lens.	No

Optical coherence tomography (OCT)	Sharma, 2016	Optical signals are sent towards a tissue, where they either pass through or are reflected. These signals are then interpreted to build a spatial image of the tissue.	No
Scheimpflug imaging	Singh Grewal & Singh Grewal, 2012	This technique allows for the photography of obliquely tilted specimens without losing focus. Cataract grading systems that utilize this principle include the Oxford Scheimpflug System, the Nidek EAS-1000, and the Zeiss Schfeimpflug video camera.	No

References

Ainsbury, E. A. et al. (2016), “Ionizing radiation induced cataracts: recent biological and mechanistic developments and perspectives for future research”, Mutation research. Reviews in mutation research, Vol. 770, Elsevier B.V., https://doi.org/10.1016/j.mrrev.2016.07.010

Barraquer, R. I. et al. (2017), “Validation of the nuclear cataract grading system BCN 10”, Ophthalmic Research, Vol. 57/4, Karger, https://doi.org/10.1159/000456720

Brenner, D. J. et al. (1991), “Accelerated heavy particles and the lens: VI. RBE studies at low doses”, Radiation Research, Vol. 128/1, Academic Press, Inc, United States, https://doi.org/10.2307/3578069

Char, D. H., S. M. Kroll, and J. Castro (1998), “Ten-year follow-up of helium ion therapy for uveal melanoma”, American Journal of Ophthalmology, Vol. 125/1, Elsevier Inc, New York, https://doi.org/10.1016/S0002-9394(99)80238-4

Chmelevsky, D et al. (1988), “An epidemiological assessment of lens opacifications that impaired vision in patients injected with radium-224”, Radiation Research, Vol. 115/2, Academic Press, Oak Brook, https://doi.org/10.2307/3577161

Choshi, K et al. (1983), “Ophthalmologic changes related to radiation exposure and age in adult health study sample, Hiroshima and Nagasaki”, Radiation Research, Vol. 96/3, Academic Press, Oak Brook, https://doi.org/10.2307/3576122

Davis, J. G et al. (2010), “Dietary supplements reduce the cataractogenic potential of proton and HZE-particle radiation in mice”, Radiation Research, Vol. 173/3, The Radiation Research Society, United States, https://doi.org/10.1667/RR1398.1

Dynlacht, J. R et al. (2011), “Age and hormonal status as determinants of cataractogenesis induced by ionizing radiation. I. Densely ionizing (high-LET) radiation”, Radiation Research, Vol. 175/1, The Radiation Research Society, United States, https://doi.org/10.1667/RR2319.1

Fujimichi, Y., N. Hamada and M. Duncan (2014), “Ionizing irradiation not only inactivates clonogenic potential in primary normal human diploid lens epithelial cells but also stimulates cell proliferation in a subset of this population”, PloS one, Vol. 9/5, Public Library of Science, United States, https://doi.org/10.1371/journal.pone.0098154

Garrett, J et al. (2020), “The protective effect of estrogen against radiation cataractogenesis is dependent upon the type of radiation”, Radiation Research, Vol. 194/5, Radiation Research Society, United States, https://doi.org/10.1667/RADE-20-00015.1

Hamada, N. (2015), “Role of carcinogenesis related mechanisms in cataractogenesis and its implications for ionizing radiation cataractogenesis.” Cancer letters, Vol. 368,2 . https://doi.org/10.1016/j.canlet.2015.02.017

Hamada, N. (2017), “Ionizing radiation sensitivity of the ocular lens and its dose rate dependence”, International journal of radiation biology, Vol. 93/10, Taylor & Francis, England, https://doi.org/10.1080/09553002.2016.1266407

Hausmann, J. C. et al. (2020), “Ophthalmic examination findings and intraocular pressure measurements in six species of Anura”, Journal of Zoo and Wildlife Medicine, Vol. 50/4, American Association of Zoo Veterinarians, United States, https://doi.org/10.1638/2019-0115

Henderson, M. A. et al. (2010), “Effects of estrogen and gender on cataractogenesis induced by high-let radiation” Radiation Research, Vol. 173/2, The Radiation Research Society, United States, https://doi.org/10.1667/RR1917.1

Hildreth, C. J., A. E. Burke, and R. M. Glass (2009), “Cataracts”, JAMA: the journal of the American Medical Association, Vol. 301/19, https://doi.org/10.1001/jama.301.19.2060

Kang, L. H. et al. (2020), “Ganoderic acid A protects lens epithelial cells from UVB irradiation and delays lens opacity”, Chinese Journal of Natural Medicines, Vol. 18/12, Elsevier B. V., China, https://doi.org/10.1016/S1875-5364(20)60037-1

Karatasakis, A. et al. (2018), “Radiation-associated lens changes in the cardiac catheterization laboratory: Results from the IC-CATARACT (CATaracts Attributed to Radiation in the CaTh lab) study”, Catheterization and Cardiovascular Interventions, Vol. 91/4, Wiley Subscription Services, United States, https://doi.org/10.1002/ccd.27173

Lachke, S. A. (2022), “RNA-binding proteins and post-transcriptional regulation in lens biology and cataract: mediating spatiotemporal expression of key factors that control the cell cycle, transcription, cytoskeleton and transparency”, Experimental Eye Research, Vol. 214, Elsevier Ltd, England, https://doi.org/10.1016/j.exer.2021.108889

Little, M. P. et al. (2018), “Occupational radiation exposure and risk of cataract incidence in a cohort of US radiologic technologists”, European Journal of Epidemiology, Vol. 33/12, Springer, https://doi.org/10.1007/s10654-018-0435-3.

Liu, Y. et al. (2017), “Cataracts”, The Lancet (British edition), Vol. 390/10094, Elsevier Ltd, England, https://doi.org/10.1016/S0140-6736(17)30544-5

Loganovsky. et al (2020),. “Radiation-Induced Cerebro-Ophthalmic Effects in Humans”,. Life (Basel, Switzerland), Vol10(4). https://doi.org/10.3390/life10040041

Loganovsky. eEt al (2021),. “Radiation-associated brain-ophthalmic effects in long-duration space missions”,. Revista Brasileira de Oftalmologia. Vol. 80. 10.37039/1982.8551.20210058.

McCarron, R. A. et al. (2021), “Radiation-induced lens opacity and cataractogenesis: a lifetime study using mice of varying genetic backgrounds”, Radiation research, Vol. 197/1, Radiation Research Society, United States, https://doi.org/10.1667/RADE-20-00266.1

Nakashima, E. et al. (2006), “A reanalysis of atomic-bomb cataract data, 2000-2002: A threshold analysis”, Health Physics, Vol. 90/2, Health Physics Society, Philadelphia, https://doi.org/10.1097/01.HP.0000175442.03596.63

National Eye Institute (2022), Cataracts,https://www.nei.nih.gov/learn-about-eye-health/eye-conditions-and-diseases/cataractshttps://www.nei.nih.gov/learn-about-eye-health/eye-conditions-and-diseases/cataracts (accessed November 29, 2022)

Pendergrass, W. et al. (2010), “X-ray induced cataract is preceded by LEC loss, and coincident with accumulation of cortical DNA, and ROS; similarities with age-related cataracts”, Molecular vision, Vol. 16, Molecular Vision, United States, pp. 1496-1513

Raj et al. (2009), “Post-operative capsular opacification”, Nepalese journal of ophthalmology, Nepal, https://doi.org/10.3126/nepjoph.v1i1.3673

Singh, A. J. et al. (2003), “A histological analysis of lens capsules stained with trypan blue doe capsulorrhexis in phacoemulsification cataract surgery”, Eye, Vol. 17/5, Springer Nature, London, https://doi.org/10.1038/sj.eye.6700440

Sing Grewal, D. and S. P. Singh Grewal (2012), “Clinical applications of Scheimpflug imaging in cataract surgery”, Saudi Journal of Ophthalmology, Vol. 26, Elsevier, pp. 25-32

Stirling, R.J. and P. G. Griffiths (1991), “Scanning EM studies of normal human lens fibres and fibres from nuclear cataracts”, Eye, Vol. 5/1, Springer Nature, London, https://doi.org/10.1038/eye.1991.17

Worgul, B. V et al. (2007), “Cataracts among Chernobyl clean-up workers: Implications regarding permissible eye exposures”, Radiation Research, Vol. 167/2, Radiation Research Society, Lawrence, https://doi.org/10.1667/RR0298.1

Wu, Shu-Yu et al. (2018), “Transgenic zebrafish models reveal distinct molecular mechanisms for cataract-linked αA-crystallin mutants”, PloS One, Vol. 13/11, Public Library of Science, United States, https://doi.org/10.1371/journal.pone.0207540

Yang, V. C. and E. J. Ainsworth (1987), “A histological study on the cataractogenic effects of heavy charged particles”, Vol. 11/1, National Science Council of the Republic of China, pp. 18-28

Appendix 2

List of Key Event Relationships in the AOP

List of Adjacent Key Event Relationships

Relationship: 1977: Energy Deposition leads to Increase, DNA strand breaks

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Deposition of energy leading to lung cancer	adjacent	High	High

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Deposition of energy leading to population decline via DNA strand breaks and follicular atresia	adjacent	High	
Deposition of energy leading to population decline via DNA strand breaks and oocyte apoptosis	adjacent		
Deposition of energy leading to occurrence of cataracts	adjacent	High	High
Deposition of energy leads to abnormal vascular remodeling	adjacent	High	High
Deposition of Energy Leading to Learning and Memory Impairment	adjacent	High	High

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
mouse	Mus musculus	High	NCBI
human	Homo sapiens	High	NCBI
rat	Rattus norvegicus	High	NCBI
bovine	Bos taurus	Low	NCBI
rabbit	Oryctolagus cuniculus	Low	NCBI
Pig	Pig	Low	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Unspecific	High

This KER is plausible in all life stages, sexes, and organisms with DNA. The majority of the evidence is from in vivo adult mice and human in vitro models that do not specify the sex.

Key Event Relationship Description

Direct deposition of ionizing energy refers to imparted energy interacting directly with the DNA double helix and producing randomized damage. This can be in the form of double strand breaks (DSBs), single-strand breaks, base damage, or the crosslinking of DNA to other molecules (Smith et al., 2003; Joiner, 2009; Christensen, 2014; Sage & Shikazono, 2017). Among these, the most detrimental type of DNA damage to a cell is DSBs. They are caused by the breaking of the sugar-phosphate backbone on both strands of the DNA double helix molecule, either directly across from each other or several nucleotides apart (Ward, 1988; Iliakis et al., 2015). This occurs when high-energy subatomic particles interact with the orbital electrons of the DNA causing ionization (where electrons are ejected from atoms) and excitation (where electrons are raised to higher energy levels) (Joiner, 2009). The number of DSBs produced and the complexity of the breaks is highly dependent on the amount of energy deposited on and absorbed by the cell. This can vary as a function of the dose-rate (Brooks et al., 2016) and the radiation quality which is a function of its linear energy transfer (LET) (Sutherland et al., 2000; Nikjoo et al., 2001; Jorge et al., 2012). LET describes the amount of energy that an ionizing particle transfers to media per unit distance (Smith et al., 2003; Okayasu, 2012a; Christensen et al., 2014). High LET radiation, such as alpha particles, heavy ion particles, and neutrons can deposit larger quantities of energy within a single track than low LET radiation, such as γ-rays, X-rays, electrons, and protons (Kadhim et al., 2006; Franken et al., 2012; Frankenberg et al., 1999; Rydberg et al., 2002; Belli et al., 2000; Antonelli et al., 2015). As such, radiation with higher LETs tends to produce more complex, dense structural damage, particularly in the form of clustered damage, in comparison to lower LET radiation (Nikjoo et al., 2001; Terato and Ide, 2005; Hada and Georgakilas, 2008; Okayasu, 2012a; Lorat et al., 2015; Nikitaki et al., 2016). Thus, the complexity and yield of clustered DNA damage increases with ionizing density (Ward, 1988; Goodhead, 2006). However, clustered damage can also be induced even by a single radiation track through a cell.

While the amount of DSBs produced depends on the radiation dose (see dose concordance), it also depends on several other factors. As the LET increases, the complexity of DNA damage increases, decreasing the repair rate, and increasing toxicity (Franken et al., 2012; Antonelli et al., 2015).

Evidence Supporting this KER

Overall Weight of Evidence: High

Biological Plausibility

The biological rationale linking the direct deposition of energy on DNA with an increase in DSB formation is strongly supported by numerous literature reviews that are available on this topic (J .F. Ward, 1988; Lipman, 1988; Hightower, 1995; Valentin, 1998; ; UNSCEAR, 2000; Terato & Ide, 2005; Goodhead, 2006; Kim & Lee, 2007; Asaithamby et al., 2008; Hada & Georgakilas, 2008; Jeggo, 2009; Stewart, 2012; Okayasu, 2012b; M. E. Lomax et al., 2013; EPRI, 2014; Hamada, 2014; Moore et al., 2014; Desouky et al., 2015; Rothkamm et al., 2015; Ainsbury, 2016; Foray et al., 2016; Hamada & Sato, 2016; Hamada, 2017a; Sage & Shikazono, 2017; Chadwick, 2017; Wang et al., 2021; Nagane et al., 2021; Sylvester et al., 2018; Baselet et al., 2019). Ionizing radiation can be in the form of high energy particles (such as alpha particles, beta particles, or charged ions) or high energy photons (such as γ-rays or X-rays). Ionizing radiation can break the DNA within chromosomes both directly and indirectly, as shown through using velocity sedimentation of DNA through neutral and alkaline sucrose gradients. The most direct path entails a collision between a high-energy particle or photon and a strand of DNA.

Additionally, excitation of secondary electrons in the DNA allows for a cascade of ionization events to occur, which can lead to the formation of multiple damage sites (Joiner, 2009). As an example, high-energy electrons will traverse a DNA molecule in a mammalian cell within 10-18 s and 10-14 s, resulting in 100,000 ionizing events per 1 Gy dose in a 10 µm cell (Joiner, 2009). The amount of damage can be influenced by factors such as the cell cycle stage and chromatin structure. It has been shown that in more condensed, packed chromatin structures such as those present in intact cells and heterochromatin, it is more difficult for the DNA to be damaged (Radulescu et al., 2006; Agrawala et al., 2008; Falk et al., 2008; Venkatesh et al., 2016). In contrast, DNA damage is more easily induced in lightly-packed chromatin such as euchromatin and nucleoids, (Radulescu et al., 2006; Falk et al., 2008; Venkatesh et al., 2016).

Of the possible radiation-induced DNA damage types, DSB is considered to be the most harmful to the cell, as there may be severe consequences if this damage is not adequately repaired (Khanna & Jackson, 2001; Smith et al., 2003; Okayasu, 2012a; M. E. Lomax et al., 2013; Rothkamm et al., 2015).

A considerable fraction of DSBs can also be formed in cells through indirect mechanisms. In this case, deposited energy can split water molecules near DNA, which can generate a significant quantity of reactive oxygen species in the form of hydroxyl free radicals (Ward, 1988; Wolf, 2008; Desouky et al., 2015; Maier et al., 2016, Cencer et al., 2018; Bains, 2019; Ahmadi et al., 2021). Estimates using models and experimental results suggest that hydroxyl radicals may be present within nanoseconds of energy deposition by radiation (Yamaguchi et al., 2005). These short-lived but highly reactive hydroxyl radicals may react with nearby DNA. This will produce DNA damage, including single-strand breaks and DSBs (Ward, 1988; Sasaki, 1998; Desouky et al., 2015; Maier et al., 2016). DNA breaks are especially likely to be produced if the sugar moiety is damaged, and DSBs occur when two single-strand breaks are in close proximity to each other (Ward, 1988).

Empirical Evidence

Empirical data strongly supports this KER. The evidence presented below is summarized in table 1. The types of DNA damage produced by ionizing radiation and the associated mechanisms, including the induction of DSBs, are reviewed by Lomax et al. (2013) and documents produced by international radiation governing frameworks (Valentin, 1998; UNSCEAR, 2000). Other reviews also highlight the relationship between the deposition of energy by radiation and DSB induction, and discuss the various methods available to detect these DSBs (Terato & Ide, 2005; Rothkamm et al., 2015; Sage & Shikazono, 2017). A visual representation of the time frames and dose ranges probed by the dedicated studies discussed here is shown in Figures 1 & 2 below.

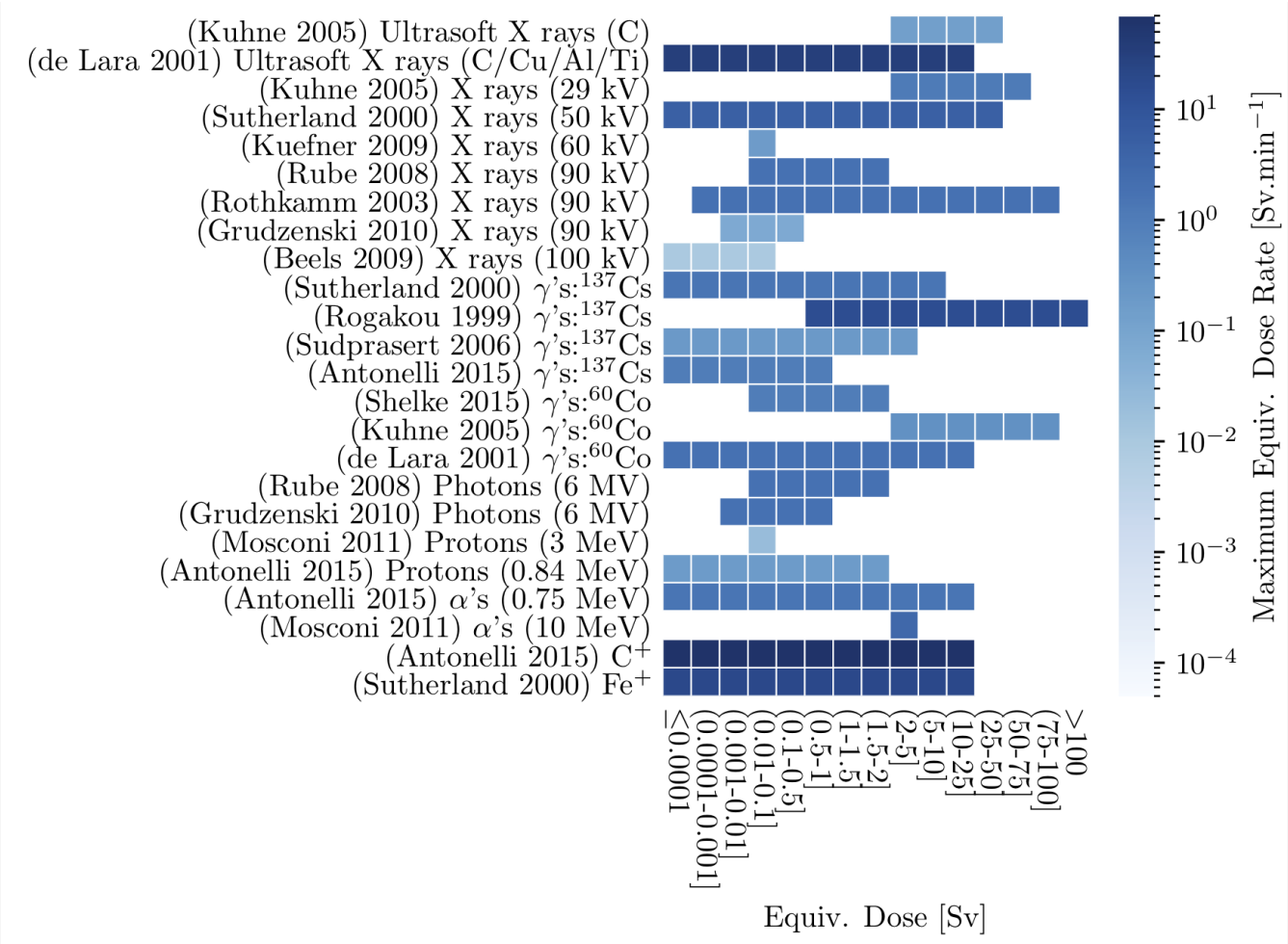


Figure 1: Plot of example studies (y-axis) against equivalent dose (Sv) used to determine the empirical link between direct deposition of energy and DSBs. The z-axis denotes the equivalent dose rate used in each study. The y-axis is ordered from low LET to high LET from top to bottom.

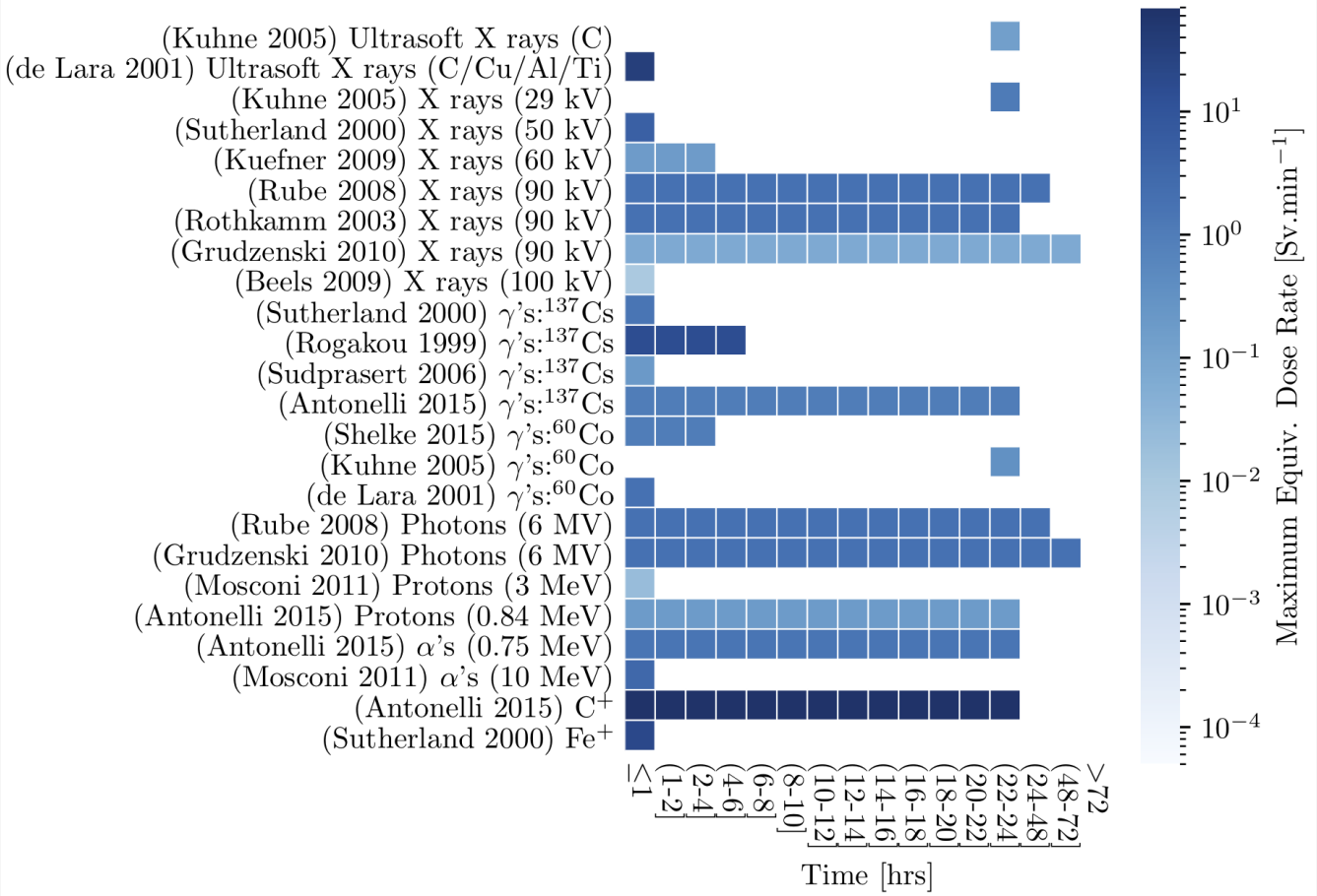


Figure 2: Plot of example studies (y-axis) against time scales used to determine the empirical link between direct deposition of energy and DSBs. The z-axis denotes the equivalent dose rate used in each study. The y-axis is ordered from low LET to high LET from top to bottom.

Dose Concordance

There is evidence in the literature suggesting a dose concordance between the direct deposition of energy by ionizing radiation and the incidence (Grudzenski et al., 2010) of DNA DSBs. Results from in vitro (Aufderheide et al., 1987; Sidjanin, 1993; Bucolo, 1994; Frankenberg et al., 1999; Rogakou et al., 1999; Belli et al., 2000; Sutherland et al., 2000; Lara et al., 2001; Rydberg et al., 2002; Baumstark-Kham et al., 2003; Rothkamm & Lo, 2003; Long, 2004; Kuhne et al., 2005; Sudprasert et al., 2006; Beels et al., 2009; Grudzenski et al., 2010; Liao, 2011; Franken et al., 2012; Bannik et al., 2013; Shelke & Das, 2015; Antonelli et al., 2015; Markiewicz et al., 2015; Allen, 2018; Dalke, 2018; Bains, 2019; Ahmadi et al., 2021; Sabirzhanov et al., 2020; Ungvari et al., 2013; Rombouts et al., 2013; Baselet et al., 2017), in vivo (Reddy, 1998; Sutherland et al., 2000; Rube et al., 2008; Beels et al., 2009; Grudzenski et al., 2010; Markiewicz et al., 2015; Barnard, 2018; Barnard, 2019; Barnard, 2022; Schmal et al., 2019; Barazzuol et al., 2017; Geisel et al., 2012), ex vivo (Rube et al., 2008; Flegal et al., 2015) and simulation studies (Charlton et al., 1989) suggest that there is a positive, linear, dose- dependent increase in DSBs with increasing deposition of energy across a wide range of radiation types (iron ions, X-rays, ultrasoft X-rays, gamma-rays, photons, UV light, and alpha particles) and radiation doses (1 mGy - 100 Gy) (Aufderheide et al., 1987; Sidjanin, 1993; Frankenberg et al., 1999; Sutherland et al., 2000; de Lara et al., 2001; Baumstark-Khan et al., 2003; Rothkamm & Lo, 2003; Kuhne et al., 2005; Rube et al., 2008; Grudzenski et al., 2010; Bannik et al., 2013; Shelke & Das, 2015; Antonelli et al., 2015; Dalke, 2018; Barazzuol et al., 2017; Ungvari et al., 2013; Rombouts et al., 2013; Baselet et al., 2017; Geisel et al., 2012). DSBs have been predicted to occur at energy deposition levels as low as 75 eV (Charlton et al., 1989).

Time Concordance

There is evidence suggesting a time concordance between the direct deposition of energy and the incidence of DSBs. A number of different models and experiments have provided evidence of ionizing radiation-induced foci (IRIF), which can be used to infer DSB formation seconds (Mosconi et al., 2011) or minutes after radiation exposure (Rogakou et al., 1999; Rothkamm & Lo, 2003; Rube et al., 2008; Beels et al., 2009; Kuefner et al., 2009; Grudzenski et al., 2010;

Antonelli et al., 2015; Acharya et al., 2010; Sabirzhanov et al., 2020; Rombouts et al., 2013; Nübel et al., 2006; Baselet et al., 2017; Zhang et al., 2017).

Essentiality

Deposition of energy is essential for DNA strand breaks. They can also be caused through other routes, such as oxidative stress (Cadet et al., 2012), but under normal physiological conditions deposition of energy is necessary. This was tested through many studies using various indicators such as 53BP1 foci/cell, γH2AX foci/cell, DNA migration, and the amount of DNA in tails for the comet assay. Various organisms such as humans, mice, rabbits, guinea pigs, and cattle were used. They showed that without the deposition of energy, there was only a negligible amount of DNA strand breaks (Aufderheide et al., 1987; Sidjanin, 1993; Bucolo, 1994; Reddy, 1998; Rogers, 2004; Bannik et al., 2013; Dalke, 2018; Bains, 2019; Barnard, 2019; Barnard, 2021).

Uncertainties and Inconsistencies

Uncertainties and inconsistencies in this KER are as follows:

- Studies have shown that dose-rates (Brooks et al., 2016) and radiation quality (Sutherland et al., 2000; Nikjoo et al., 2001; Jorge et al., 2012) are factors that can influence the dose-response relationship.
- Low-dose radiation has been observed to have beneficial effects and may even invoke protection against spontaneous genomic damage (Feinendegen, 2005; Day et al., 2007; Feinendegen et al., 2007; Shah et al., 2012; Nenoi et al., 2015; Dalke, 2018). This protective effect has been documented in in vivo and in vitro, as reviewed by ICRP (2007) and UNSCEAR (2008) and can vary depending on the cell type, the tissue, the organ, or the entire organism (Brooks et al., 2016).
- Depositing ionizing energy is a stochastic event; as such this can influence the location, degree and type of DNA damage imparted on a cell. As an example, studies have shown that mitochondrial DNA may also be an important target for genotoxic effects of ionizing radiation (Wu et al., 1999).

Quantitative Understanding of the Linkage

Quantitative understanding of this linkage suggests that DSBs can be predicted upon exposure to ionizing radiation. This is dependent on the biological model, the type of radiation and the radiation dose. In general, 1 Gy of radiation is thought to result in 3000 damaged bases (Maier et al., 2016), 1000 single-strand breaks, and 40 DSBs (Ward, 1988; Foray et al., 2016; Maier et al., 2016). The table below provides representative examples of the calculated DNA damage rates across different model systems, most of which are examining DNA DSBs.

Dose Concordance

The following tables provide representative examples of the relationship, unless otherwise indicated, all data is significantly significant.

Reference	Experiment Description	Result
Ward, 1988	In vitro. Cells containing approximately 6 pg of DNA were exposed to 1 Gy.	Under the assumption of 6 pg of DNA per cell. 60 eV of energy deposited per event over a total of 1 Gy. Deoxyribose (2.3 pg/cell): 14,000 eV deposited, 235 events. Bases (2.4 pg/cell): 14.7 keV deposited, 245 events. Phosphate (1.2 pg/cell): 7,300 eV deposited, 120 events. Bound water (3.1 pg/cell): 19 keV deposited, 315 events. Inner hydration shell (4.2 pg/cell): 25,000 eV deposited 415 events.
Charlton, 1989	In-silico. A computer simulation/model was used to test various types of radiation with doses from 0 to 400 eV (energy deposited) on the amount of DNA damage produced.	Simulated dose-concordance prediction of increase in number of DSBs/54 nucleotide pairs as direct deposition of energy increases in the range 75-400 eV. In the range 100 - 150 eV: 0.38 DSBs/54 nucleotide pairs and at 400 eV: ~0.80 DSBs per 64 nucleotide pairs.
Sutherland, 2000	In vitro. Human cells were exposed to 137Cs γ-rays (0 – 100 Gy, 0.16 – 1.6 Gy/min). The frequency of DSBs was determined using gel electrophoresis.	Using isolated bacteriophage T7 DNA and 0-100 Gy of γ rays, observed a response of 2.4 DSBs per megabase pair per Gy.
Rogakou et al., 1999	In vitro. Normal human fibroblasts (IMR90) and human breast cancer cells (MCF7) were exposed to 0.6 and 2 Gy 137Cs γ-rays delivered at 15.7 Gy/min. The number of DSBs were determined by immunoblotting for γ-H2AX.	Radiation doses of 0.6 Gy & 2 Gy to normal human fibroblasts (IMR90) and MCF7 cells resulted in 10.1 & 12.2 DSBs per nucleus on average (0.6 Gy), respectively; increasing to 24 & 27.1 DSBs per nucleus (2 Gy).

Kuhne et al., 2005	In vitro. Primary human skin fibroblasts (HSF2) were exposed to 0 – 70 Gy 60Co γ-rays (0.33 Gy/min), X-rays (29 kVp, 1.13 Gy/min), and CK X-rays (0.14 Gy/min). The number of DSBs were determined with pulsed-field gel electrophoresis.	γ-ray and X-ray irradiation of primary human skin fibroblasts (HSF2) at 0 - 70 Gy. γ-rays: (6.1 ± 0.2) x 10-9 DSBs per base pair per Gy, X-rays: (7.0 ± 0.2) x 10-9 DSBs per base pair per Gy. CK X -rays: (12.1 ± 1.9) x 10-9 DSBs per base pair per Gy.
Rothkamm, 2003	In vitro. Primary human fibroblast cell lines MRC-5 (lung), HSF1 and HSF2 (skin), and180BR (deficient in DNA ligase IV) were exposed to 1 mGy – 100 Gy X-rays (90 kV). Low doses were delivered at 6 – 60 mGy/min and high doses were delivered at 2 Gy/min. The number of DSBs were determined with pulsed-field gel electrophoresis.	X-ray irradiation of primary human fibroblasts (MRC-5) in the range 1 mGy - 100 Gy, 35 DSBs per cell per Gy.
Grudzenski et al, 2010	In vitro. Primary human fibroblasts (HSF1) and C57BL/6NCrl adult mice were exposed to X-rays (2.5 – 200 mGy, 70 mGy/min), and photons (10 mGy – 1 Gy, 2 Gy/min (100 mGy and 1 Gy), and 0.35 Gy/min (10 mGy)). γ-H2AX immunofluorescence was observed to determine DSBs.	X-rays irradiating primary human fibroblasts (HSF1) in the range 2.5 - 100 mGy yielded a response of 21 foci per Gy. When irradiating adult C57BL/6NCrl mice with photons a response of 0.07 foci per cell at 10 mGy was found. At 100 mGy the response was 0.6 foci per cell and finally, at 1 Gy; 8 foci per cell.
de Lara, 2001	In vitro. Chinese hamster cells (V79-4) were exposed to 0 – 20 Gy of 60Co γ-rays (2 Gy/min), and ultrasoft X-rays (0.7 – 35 Gy/min): carbon-K shell (0.28 keV), copper L-shell (0.96 keV), aluminum K-shell (1.49 keV), and titanium K-shell (4.55 keV). The number of DSBs were determined with pulsed-field gel electrophoresis.	V79-4 cells irradiated with γ-rays and ultrasoft X-rays (carbon K-shell, copper L-shell, aluminium K-shell and titanium K-shell) in the range 0 - 20 Gy. Response (DSBs per Gy per cell): γ-rays: 41, carbon K-shell: 112, copper L-shell: 94, aluminum K-shell: 77, titanium K-shell: 56.
Rübe et al., 2008	In vivo. Brain, lung, heart and small intestine tissue from adult SCID, A-T, BALB/c and C57BL/6NCrl mice; Whole blood and isolated lymphocytes from BALB/c and C57BL/6NCrl mice were exposed to 0.1 – 2 Gy of photons (whole body irradiation, 6 MV, 2 Gy/min) and X-rays (whole body irradiation, 90 kV, 2 Gy/min). γ-H2AX foci were determined with immunochemistry to measure DSBs.	Linear dose-dependent increase in DSBs in the brain, small intestine, lung and heart of C57BL/6CNrl mice after whole-body irradiation with 0.1 - 1.0 Gy of radiation. 0.8 foci per cell (0.1 Gy) and 8 foci per cell (1 Gy).
Antonelli et al., 2015	In vitro. Primary human foreskin fibroblasts (AG01522) were exposed to 0 – 1 Gy of 137Cs γ-rays (1 Gy/min), protons (0.84 MeV, 28.5 keV/µm), carbon ions (58 MeV/u, 39.4 keV/µm), and alpha particles (americium-241, 0.75 MeV/u, 0.08 Gy/min, 125.2 keV/µm). γ-H2AX foci were determined with immunochemistry to measure DSBs.	Linear dose-dependent increase in the number of DSBs from 0 - 1 Gy for γ-rays and alpha particles as follows: γ-rays: 24.1 foci per Gy per cell nucleus, alpha particles: 8.8 foci per Gy per cell nucleus.
Barnard et al., 2019	In vivo. 10-week-old female C57BL/6 mice were whole-body exposed to 0.5, 1, and 2 Gy of 60Co γ-rays at 0.3, 0.063, and 0.014 Gy/min. p53 binding protein 1 (53BP1) foci were determined via immunofluorescence.	Central LECs showed a linear increase in mean 53BP1 foci/cell with the maximum dose and dose-rate displaying a 78x increase compared to control. Peripheral LECs and lower dose rates displayed similar results, with slightly fewer foci.
Ahmadi et al., 2021	In vitro. Human LEC cells were exposed to 137Cs γ-rays at doses of 0, 0.1, 0.25, and 0.5 Gy and dose rates of 0.065 and 0.3 Gy/min. DNA strand breaks were measured using the comet assay.	Human LECs showed a gradual increase in the tail from the comet assay with the maximum dose and dose-rate displaying a 3.7x increase compared to control. Lower dose-rates followed a similar pattern with a lower amount of strand breaks.
Hamada et al., 2006	In vitro. Primary normal human diploid fibroblast (HE49) cells were exposed to 0.1, 0.5, and 4 Gy X-rays at 240 kV with a dose rate of 0.5 Gy/min. The number of γ- H2AX foci/cell, which represented DNA strand breaks, was determined 6 – 7 min after irradiation through fluorescence microscopy.	Cells displayed a linear increase in the number of γ-H2AX foci/cell, with the maximum dose displaying a 125x increase compared to control (32 foci/Gy).
Schmal et al., 2019	In vivo. Juvenile and adult C57BL/6 mice were exposed to whole body 6-MV photons at 2 Gy/min. Irradiations were done in 5x, 10x, 15x and 20x fractions of 0.1 Gy. Double staining for NeuN and 53BP1 was used to quantify DNA damage foci and the possible accumulation in the hippocampal dentate gyrus.	Only low 53BP1-foci levels (~0.03 foci/cell) were observed in non-irradiated controls. However, 0.1 h post-irradiation, directly after single dose exposure to 0.1 Gy, Approx 1 focus/cell was induced. Following fractioned low dose ionizing radiation, (20 × 0.1 Gy, 72 h post-IR) the number of persisting foci was higher in hippocampal neurons compared to non-irradiated wild-type (WT) mice.

Barazzuol et al., 2017	In vivo. C57BL/6 mice were exposed to 0.1 or 2 Gy of X-rays (250 kV) at a dose rate of 0.5 Gy/min. 53BP1 foci were quantified with immunofluorescence in neural stem cells and neuron progenitors in the lateral ventricle.	Both 0.1 and 2 Gy resulted in increased 53BP1 foci with a 10-fold increase at 0.1 Gy and an 80-fold increase at 2 Gy.
Sabirzhanov et al., 2020	In vitro. Rat cortical neurons were exposed to 2, 8 or 32 Gy of X rays (320 kV) at a dose rate of 1.25 Gy/min. Western blot was used to measure γ-H2AX, p-ataxia telangiectasia mutated (ATM) and p- ATM/RAD3-related (ATR) levels.	In rat cortical neurons, p-ATM increased at 2, 8, and 32 Gy, with a 15-fold increase at 8 and 32 Gy. γ-H2AX levels increased at 8 and 32 Gy.
Geisel et al., 2012	In vivo. Patients with suspected coronary artery disease receiving X-rays from computed tomography or conventional coronary angiography had levels of DSBs assessed in blood lymphocytes by γ-H2AX fluorescence.	There was a correlation between effective dose (in mSv) and DSBs. For both conventional coronary angiography and computed tomography, a dose of 10 mSv produced about 2-fold more DNA DSBs than a dose of 5 mSv.
Ungvari et al., 2013	In vitro. Rat cerebromicrovascular endothelial cells and hippocampal neurons were irradiated with 2-10 Gy of 137Cs gamma rays. DNA strand breaks were assessed with the comet assay.	DNA damage increased at all doses (2-10 Gy). In the control, less than 5% of DNA damage was in the tail, while by 6 Gy, 35% of the DNA damage was in the tail in cerebromicrovascular endothelial cells and 25% was in the tail in neurons.
Rombouts et al., 2013	In vitro. EA.hy926 cells and human umbilical vein endothelial cells were irradiated with various doses of X-rays (0.25 Gy/min). γ-H2AX foci were assessed with immunofluorescence.	More γ-H2AX foci were observed at higher doses in both cell types. In human umbilical vein endothelial cells, few foci/nucleus were observed at 0.05 Gy, with about 23 at 2 Gy. In EA.hy926 cells, few foci/nucleus were observed at 0.05 Gy, with about 37 at 2 Gy.
Baselet et al., 2017	In vitro. Human telomerase-immortalized coronary artery endothelial cells were irradiated with various doses of X-rays (0.5 Gy/min). Immunocytochemical staining was performed for γ-H2AX and 53BP1 foci.	Doses of 0.05 and 0.1 Gy did not increase the number of γ-H2AX foci, but 0.5 Gy increased foci number by 5-fold and 2 Gy by 15-fold. A dose of 0.05 Gy did not increase the number of 53BP1 foci, but 0.1 Gy, 0.5 Gy and 2 Gy increased levels by 3-fold, 7-fold and 8-fold, respectively.

Time Concordance

Reference	Experiment Description	Result
Rogakou et al., 1999	In vitro. Normal human fibroblasts (IMR90), human breast cancer cells (MCF7), human astrocytoma cells (SF268), Indian muntjac Muntiacus muntjak normal skin fibroblasts, Xenopus laevisA6 normal kidney cells, Drosophila melanogaster epithelial cells, and Saccharomyces cerevisiae were exposed to 0.6, 2, 20, 22, 100, and 200 Gy 137Cs γ-rays. Doses below 20 Gy were delivered at 15.7 Gy/min and other doses were delivered in 1 min. DNA breaks were visualized using immunofluorescence.	DSBs were present at 3 min and persisted from 15 - 60 min.
Hamada, 2017b	In vitro. human LECs were exposed to 0.025 Gy X-rays at 0.42 – 0.45 Gy/min. 53BP1 foci were measured via indirect immunofluorescence.	In cells immediately exposed to 0.025 Gy, the level of 53BP1 foci/cell increased to 3.3x relative to control 0.5 h post-irradiation.
Hamada et al., 2006	In vitro. Primary normal human diploid fibroblast (HE49) cells were exposed to 0.1, 0.5, and 4 Gy (deposition of energy) at 240 kV with a dose rate of 0.5 Gy/min. The number of H2AX foci/cell, which represented DNA strand breaks, was determined through fluorescence microscopy.	In cells immediately exposed to 0.5 Gy, 11% of cells had 18 foci six min post-irradiation, compared to 90% of controls having 0 foci.
Acharya et al., 2010	In vitro. Human neural stem cells were exposed to 1, 2 and 5 Gy of γ-rays at a dose rate of 2.2 Gy/min. The levels of γ-H2AX phosphorylation post irradiation were assessed by immunocytochemistry, fluorescence-activated cell sorting (FACS) analysis and γ-H2AX foci enumeration.	The number of cells positive for nuclear γ-H2AX foci peaked at 20 min post-irradiation. After 1h, this level quickly declined.

Schmal et al., 2019	In vivo. Juvenile and adult C57BL/6 mice were exposed to whole body 6-MV photons at 2 Gy/min. Irradiations were done in 5x, 10x, 15x and 20x fractions of 0.1 Gy. Double staining for NeuN and 53BP1 was used to quantify DNA damage foci and the possible accumulation in the hippocampal dentate gyrus.	To assess possible accumulation of persisting 53BP1-foci during fractionated radiation, juvenile and adult mice were examined 72 h after exposure to 5×, 10×, 15×, or 20× fractions of 0.1 Gy, compared to controls. The number of persisting 53BP1-foci increased significantly in both juvenile and adult mice during fractionated irradiation (maximum at 1 min post-IR).
Dong et al., 2015	In vivo. C57BL/6J mice were exposed to 2 Gy of X-rays at 2 Gy/min using a 6 MV source. γ-H2AX foci were assessed with immunofluorescence in the brain.	At 0.5 h, about 14 γ-H2AX foci/cell were present. This decreased linearly to about 2 foci/cell at 24 h, with no foci/cell from 48 h to 6 weeks.
Barazzuol et al., 2017	In vivo. C57BL/6 mice were exposed to 0.1 or 2 Gy of X-rays (250 kV) at a rate of 0.5 Gy/min. 53BP1 foci were quantified with immunofluorescence in neural stem cells and neuron progenitors in the lateral ventricle.	At both 0.5 and 6 h post-irradiation, increased 53BP1 foci were observed, with the highest level at 0.5 h.
Sabirzhanov et al., 2020	In vitro. Rat cortical neurons were exposed to 2, 8 or 32 Gy of X rays (320 kV) at a dose rate of 1.25 Gy/min. Western blot was used to measure γ-H2AX, p-ATM and p-ATR levels.	In rat cortical neurons, γ-H2AX, p-ATM and p-ATR all increased at 30 min post-irradiation, with a sustained increase until 6 h.
Zhang et al., 2017	In vitro. HT22 hippocampal neuronal cells were irradiated with X-rays (320 kVp) at 8 or 12 Gy at a dose rate of 4 Gy/min. The comet assay was preformed to assess the DNA double strand breaks in HT22 cells. Western blot was used to measure γ-H2AX and p-ATM.	At 8 Gy, the comet assay showed an increased tail moment at both 30 min and 24 h post-irradiation. At 12 Gy, p-ATM was increased over 4-fold at both 30 min and 1 h post-irradiation. γ-H2AX was increased over 3-fold at 30 min post-irradiation and almost 2-fold at 1 and 24 h.
Geisel et al., 2012	In vivo. Patients with suspected coronary artery disease receiving X-rays from computed tomography or conventional coronary angiography had levels of DSBs assessed in blood lymphocytes by γ-H2AX fluorescence.	DSBs were increased at 1 h post-irradiation and returned to pre-irradiation levels by 24 h.
Park et al., 2022	In vitro. Human aortic endothelial cells were irradiated with 137Cs gamma rays at 4 Gy (3.5 Gy/min). γ-H2AX was measured with western blot. p-ATM and 53BP1 were determined with immunofluorescence.	γ-H2AX, p-ATM, and 53BP1 increased at 1 h post-irradiation and slightly decreased for the rest of the 6 h but remained elevated above the control.
Kim et al., 2014	In vitro. Human umbilical vein endothelial cells were irradiated with 4 Gy of 137Cs gamma rays. γ-H2AX levels were determined with immunofluorescence.	γ-H2AX foci greatly increased at 1 and 6 h post-irradiation, with the greatest increase at 1 h.
Dong et al., 2014	In vitro. Human umbilical vein endothelial cells were irradiated with 2 Gy of 137Cs gamma rays. γ-H2AX levels were determined with immunofluorescence.	γ-H2AX foci increased 8-fold at 3 h, 7-fold at 6 h, and 2-fold at 12 and 24 h post-irradiation.
Rombouts et al., 2013	In vitro. EA.hy926 cells and human umbilical vein endothelial cells were irradiated with X-rays (0.25 Gy/min). γ-H2AX foci were assessed with immunofluorescence.	The greatest increase in γ-H2AX foci was observed 30 min post-irradiation, while levels were still slightly elevated at 24 h.
Nübel et al., 2006	In vitro. Human umbilical vein endothelial cells were irradiated with gamma rays at 20 Gy. DNA strand breaks were assessed with the comet assay and western blot for γ-H2AX.	The olive tail moment increased 5-fold immediately after irradiation and returned to control levels by 4 h. A large increase in γ-H2AX was observed at 0.5 h post-irradiation, with lower levels at 4 h but still above the control.
Baselet et al., 2017	In vitro. Human telomerase-immortalized coronary artery endothelial cells were irradiated with various doses of X-rays (0.5 Gy/min). Immunocytochemical staining was performed for γ-H2AX and 53BP1 foci.	Increased γ-H2AX and 53BP1 foci were observed at 0.5 h post-irradiation, remaining elevated at 4 h but returning to control levels at 24 h.
Gionchiglia et al., 2021	In vivo. Male CD1 and B6/129 mice were irradiated with X-rays at 10 Gy. Brain sections were single or double-stained with antibodies against γ-H2AX and p53BP1.	In the forebrain, cerebral cortex, hippocampus and subventricular zone (SVZ)/ rostral migratory stream (RMS)/ olfactory bulb (OB), γH2AX and p53BP1 positive cells increased at both 15 and 30 mins post-irradiation, with the greatest increase at 30 min.

Response-response relationship

There is evidence of a response-response relationship between the deposition of energy and the frequency of DSBs. In studies encompassing a variety of biological models, radiation types and radiation doses, a positive, linear relationship was found between the radiation dose and the number of DSBs (Aufderheide et al., 1987; Sidjanin, 1993; Frankenberg et al., 1999; Sutherland et al., 2000; de Lara et al., 2001; Baumstark-Khan et al., 2003; Rothkamm & Lo, 2003; Kuhne et al., 2005; Rube et al., 2008; Grudzenski et al., 2010; Bannik et al., 2013; Shelke & Das, 2015; Antonelli et al., 2015; Hamada, 2017b; Dalke, 2018; Barazzuol et al., 2017; Geisel et al., 2012; Ungvari et al., 2013; Rombouts et al., 2013; Baselet et al., 2017). There were, however, at least four exceptions reported. When human blood lymphocytes were irradiated with X-rays in vitro, a linear relationship was only found for doses ranging from 6 - 500 mGy; at low doses from 0 - 6 mGy, there was a quadratic relationship reported (Beels et al., 2009). Secondly, simulation studies predicted that there would be a non-linear increase in DSBs as energy deposition increased, with a saturation point at higher LETs (Charlton et al., 1989). Furthermore, primary normal human fibroblasts exposed to 1.2 – 5 mGy X-rays at 5.67 mGy/min showed a supralinear relationship, indicating at low doses, the DSBs are mostly due to radiation-induced bystander effects. Doses above 10 mGy showed a positive linear relationship (Ojima et al., 2008). Finally, in the human lens epithelial cell line SRA01/04, DNA strand breaks appeared immediately after exposure to UVB (0.14 J/cm2) and were repaired after 30 minutes. They then reappeared after 60 and 90 minutes. Both were once again repaired within 30 minutes. However, the two subsequent stages of DNA strand breaks did not occur when exposed to a lower dose of UVB (0.014 J/cm2) (Cencer et al., 2018).

Time-scale

Data from temporal response studies suggests that DSBs likely occur within seconds to minutes of energy deposition by ionizing radiation. In a variety of biological models, the presence of DSBs has been well documented within 10 - 30 minutes of radiation exposure (Rogakou et al., 1999; Rube et al., 2008; Beels et al., 2009; Kuefner et al., 2009; Grudzenski et al., 2010; Antonelli et al., 2015; Acharya et al., 2010; Dong et al., 2015; Barazzuol et al., 2017; Sabirzhanov et al., 2020; Rombouts et al., 2013; Nübel et al., 2006; Baselet et al., 2017; Zhang et al., 2017; Gionchiglia et al., 2021); there is also evidence that DSBs may actually be present within 3 - 5 minutes of irradiation (Kleiman, 1990; Rogakou et al., 1999; Rothkamm & Lo, 2003; Rube et al., 2008; Grudzenski et al., 2010; Cencer et al., 2018). Interestingly, one study that focussed on monitoring the cells before, during and after irradiation by taking photos every 5, 10 or 15 seconds found that foci indicative of DSBs were present 25 and 40 seconds after collision of the alpha particles and protons with the cell, respectively. The number of foci were found to increase over time until plateauing at approximately 200 seconds after alpha particle exposure and 800 seconds after proton exposure (Mosconi et al., 2011).

After the 30 minute mark, DSBs have been shown to rapidly decline in number. By 24 hours post-irradiation, DSB numbers had declined substantially in systems exposed to radiation doses between 40 mGy and 80 Gy (Aufderheide et al., 1987; Baumstark-Khan et al., 2003; Rothkamm & Lo, 2003; Rube et al., 2008; Grudzenski et al., 2010; Bannik et al., 2013; Markiewicz et al., 2015; Russo et al., 2015; Antonelli et al., 2015; Dalke, 2018; Bains, 2019; Barnard, 2019; Ahmadi et al., 2021; Dong et al., 2015; Dong et al., 2014; Sabirzhanov et al., 2020; Rombouts et al., 2013; Baselet et al., 2017; Gionchiglia et al., 2021), with the sharpest decrease documented within the first 5 h (Kleiman, 1990; Sidjanin, 1993; Rogakou et al., 1999; Rube et al., 2008; Kuefner et al., 2009; Grudzenski et al., 2010; Bannik, 2013; Markiewicz et al., 2015; Shelke & Das, 2015; Cencer et al., 2018; Acharya et al., 2010; Park et al., 2022; Kim et al., 2014; Nübel et al., 2006). Interestingly, DSBs were found to be more persistent when they were induced by higher LET radiation (Aufderheide et al., 1987, Baumstark-Khan et al., 2003; Antonelli et al., 2015).

Known modulating factors

Modulating Factor	Details	Effects on the KER	References
Nitroxides	Increased concentration	Decreased DNA strand breaks.	DeGraff et al., 1992; Citrin & Mitchel, 2014
5-fluorouracil	Increased concentration	Increased DNA strand breaks.	De Angelis et al., 2006; Citrin & Mitchel, 2014
Thiols	Increased concentration	Decreased DNA strand breaks.	Milligan et al., 1995; Citrin & Mitchel, 2014
Cisplatin	Increased concentration	Decreased DNA break repair.	Sears & Turchi; Citrin & Mitchel, 2014

Known Feedforward/Feedback loops influencing this KER

Not Identified.

References

Agrawala, P.K. et al. (2008), "Induction and repairability of DNA damage caused by ultrasoft X-rays: Role of core events.", Int. J. Radiat. Biol., 84(12):1093–1103. doi:10.1080/09553000802478083.

Ahmadi, M. et al. (2021), “Early responses to low-dose ionizing radiation in cellular lens epithelial models”, Radiation research, Vol.197/1, Radiation Research Society, United States, https://doi.org/10.1667/RADE-20-00284.1

Ainsbury, E. A. et al. (2016), “Ionizing radiation induced cataracts: Recent biological and mechanistic developments

and perspectives for future research", Mutation research. Reviews in mutation research, Vol. 770, Elsevier B.V., Amsterdam, https://doi.org/10.1016/j.mrrev.2016.07.010

Alexander, J. L. and Orr-Weaver, T. L. (2016), "Replication fork instability and the consequences of fork collisions from replication", Genes & Development, Vol. 30/20, Cold Spring Harbor Laboratory Press, https://doi.org/10.1101/gad.288142.116

Allen, C. H. et al. (2018), "Raman micro-spectroscopy analysis of human lens epithelial cells exposed to a low-dose-range of ionizing radiation", Physics in medicine & biology, Vol. 63/2, IOP Publishing, Bristol, https://doi.org/10.1088/1361-6560/aaa176

Antonelli, A.F. et al. (2015), "Induction and Repair of DNA DSB as Revealed by H2AX Phosphorylation Foci in Human Fibroblasts Exposed to Low- and High-LET Radiation: Relationship with Early and Delayed Reproductive Cell Death", Radiat. Res. 183(4):417-31, doi:10.1667/RR13855.1.

Acharya, M. et al. (2010), "Consequences of ionizing radiation-induced damage in human neural stem cells", Free Radical Biology and Medicine. 49(12):1846-1855, doi:10.1016/j.freeradbiomed.2010.08.021.

Asaithamby, A. et al. (2008), "Repair of HZE-Particle-Induced DNA Double-Strand Breaks in Normal Human Fibroblasts.", Radiat Res. 169(4):437–446. doi:10.1667/RR1165.1.

Aufderheide, E. et al. (1987), "Heavy ion effects on cellular DNA: Strand break induction and repair in cultured diploid lens epithelial cells", International journal of radiation biology and related studies in physics, chemistry and medicine, Vol. 51/5, Taylor & Francis, London, https://doi.org/10.1080/09553008714551071

Bannik, K. et al. (2013), "Are mouse lens epithelial cells more sensitive to γ-irradiation than lymphocytes?", Radiation and environmental biophysics, Vol. 52/2, Springer-Verlag, Berlin/Heidelberg, https://doi.org/10.1007/s00411-012-0451-8

Bains, S. K. et al. (2019), "Effects of ionizing radiation on telomere length and telomerase activity in cultured human lens epithelium cells", International journal of radiation biology, Vol. 95/1, Taylor & Francis, Abingdon, https://doi.org/10.1080/09553002.2018.1466066

Barazzuol, L et al. (2017), "A coordinated DNA damage response promotes adult quiescent neural stem cell activation. PLOS Biology, 15(5). https://doi.org/10.1371/journal.pbio.2001264

Barnard, S. G. R. et al. (2018), "Dotting the eyes: mouse strain dependency of the lens epithelium to low dose radiation-induced DNA damage", International journal of radiation biology, Vol. 94/12, Taylor & Francis, Abingdon, https://doi.org/10.1080/09553002.2018.1532609

Barnard, S. G. R. et al. (2019), "Inverse dose-rate effect of ionising radiation on residual 53BP1 foci in the eye lens", Scientific Reports, Vol. 9/1, Nature Publishing Group, England, https://doi.org/10.1038/s41598-019-46893-3

Barnard, S. G. R. et al. (2022), "Radiation-induced DNA damage and repair in lens epithelial cells of both Ptch1 (+/-) and Ercc2 (+/-) mutated mice", Radiation Research, Vol. 197/1, Radiation Research Society, United States, https://doi.org/10.1667/RADE-20-00264.1

Baselet, B. et al. (2019), "Pathological effects of ionizing radiation: endothelial activation and dysfunction", Cellular and molecular life sciences, Vol. 76/4, Springer Nature, https://doi.org/10.1007/s00018-018-2956-z

Baselet, B. et al. (2017), "Functional Gene Analysis Reveals Cell Cycle Changes and Inflammation in Endothelial Cells Irradiated with a Single X-ray Dose", Frontiers in pharmacology, Vol. 8, Frontiers, https://doi.org/10.3389/fphar.2017.00213

Baumstark-Khan, C., J. Heilmann, and H. Rink (2003), 'Induction and repair of DNA strand breaks in bovine lens epithelial cells after high LET irradiation", Advances in space research, Vol. 31/6, Elsevier Ltd, England, https://doi.org/10.1016/S0273-1177(03)00095-4

Beels, L. et al. (2009), "g-H2AX Foci as a Biomarker for Patient X-Ray Exposure in Pediatric Cardiac Catheterization", Are We Underestimating Radiation Risks?":1903–1909. doi:10.1161/CIRCULATIONAHA.109.880385.

Belli M, Cherunbini R, Vecchia MD, Dini V, Moschini G, Signoretti C, Simon G, Tabocchini MA, Tiveron P. 2000. DNA DSB induction and rejoining in V79 cells irradiated with light ions: a constant field gel electrophoresis study. Int J Radiat Biol. 76(8):1095-1104.

Brooks, A.L., D.G. Hoel & R.J. Preston (2016), "The role of dose rate in radiation cancer risk: evaluating the effect of dose rate at the molecular, cellular and tissue levels using key events in critical pathways following exposure to low LET radiation.", Int. J. Radiat. Biol. 92(8):405–426. doi:10.1080/09553002.2016.1186301.

Bucolo, C. et al. (1994), "The effect of ganglioside on oxidation-induced permeability changes in lens and in epithelial cells of lens and retina", Experimental eye research, Vol. 58/6, Elsevier Ltd, London, https://doi.org/10.1006/exer.1994.1067

Cabrera et al. (2011), "Antioxidants and the integrity of ocular tissues", in Veterinary medicine international, SAGE-Hindawi Access to Research, United States. DOI: 10.4061/2011/905153

Cadet, J. et al. (2012), “Oxidatively generated complex DNA damage: tandem and clustered lesions”, Cancer letters, Vol. 327/1, Elsevier Ireland Ltd, Ireland. https://doi.org/10.1016/j.canlet.2012.04.005

Cannan, W.J. & D.S. Pederson (2016), "Mechanisms and Consequences of Double-Strand DNA Break Formation in Chromatin.", J. Cell Physiol. 231(1):3–14. doi:10.1002/jcp.25048.

Cencer, C. S. et al. (2018), “PARP-1/PAR activity in cultured human lens epithelial cells exposed to two levels of UVB light”, Photochemistry and photobiology, Vol. 94/1, Wiley, Hoboken, https://doi.org/10.1111/php.12814

Chadwick, K.H., (2017), Towards a new dose and dose-rate effectiveness factor (DDREF)? Some comments., J Radiol Prot., 37:422-433. doi: 10.1088/1361-6498/aa6722.

Charlton, D.E., H. Nikjoo & J.L. Humm (1989), "Calculation of initial yields of single- and double-strand breaks in cell nuclei from electrons, protons and alpha particles.", Int. J. Rad. Biol., 53(3):353-365, DOI: 10.1080/09553008814552501

Christensen, D.M. (2014), "Management of Ionizing Radiation Injuries and Illnesses, Part 3: Radiobiology and Health Effects of Ionizing Radiation.", 114(7):556–565. doi:10.7556/jaoa.2014.109.

Citrin, D.E. & J.B. Mitchel (2014), "Public Access NIH Public Access.", 71(2):233–236. doi:10.1038/mp.2011.182.doi.

Dalke, C. et al. (2018), “Lifetime study in mice after acute low-dose ionizing radiation: a multifactorial study with special focus on cataract risk”, Radiation and environmental biophysics, Vol. 57/2, Springer Berlin Heidelberg, Berling/Heidelberg, https://doi.org/10.1007/s00411-017-0728-z

Day, T.K. et al. (2007), "Adaptive Response for Chromosomal Inversions in pKZ1 Mouse Prostate Induced by Low Doses of X Radiation Delivered after a High Dose.", Radiat Res. 167(6):682–692. doi:10.1667/rr0764.1.

De Angelis, P. M. et al. (2006), “Cellular response to 5-fluorouracil (5-FU) in 5-FU-resistant colon cancer cell lines during treatment and recovery”, Molecular Cancer, Vol. 5/20, BioMed Central, https://doi.org/10.1186/1476-4598-5-20

DeGraff, W. G. et al. (1992), “Nitroxide-mediated protection against X-ray- and neocarzinostatin-induced DNA damage”, Free Radical Biology and Medicine, Vol. 13/5, Elsevier, https://doi.org/10.1016/0891-5849(92)90142-4

Desouky, O., N. Ding & G. Zhou (2015), "ScienceDirect Targeted and non-targeted effects of ionizing radiation.", J. Radiat. Res. Appl. Sci. 8(2):247–254. doi:10.1016/j.jrras.2015.03.003.

Dong et al. (2015), “Relationship between irradiation-induced neuro-inflammatory environments and impaired cognitive function in the developing brain of Mice. International Journal of Radiation Biology, 91(3):224–239. https://doi.org/10.3109/09553002.2014.988895

Dong, X. et al. (2014), “NEMO modulates radiation-induced endothelial senescence of human umbilical veins through NF-κB signal pathway”, Radiation Research, Vol. 183/1, BioOne, https://doi.org/10.1667/RR13682.1

Dubrova, Y.E. & M.A. Plumb (2002), "Ionising radiation and mutation induction at mouse minisatellite loci The story of the two generations", Mutat. Res. 499(2):143–150.

EPRI (2014), Epidemiology and mechanistic effects of radiation on the lens of the eye: Review and scientific appraisal of the literature, EPRI, California

Falk, M., E. Lukášová & S. Kozubek (2008), "Chromatin structure influences the sensitivity of DNA to γ-radiation.", Biochim. Biophys. Acta. - Mol. Cell. Res. 1783(12):2398–2414. doi:10.1016/j.bbamcr.2008.07.010.

Feinendegen, L.E. (2005), "UKRC 2004 debate Evidence for beneficial low level radiation effects and radiation hormesis. Radiology.", 78:3–7. doi:10.1259/bjr/63353075.

Feinendegen, L.E., M. Pollycove & R.D. Neumann (2007), "Whole-body responses to low-level radiation exposure: New concepts in mammalian radiobiology.", Exp. Hematol. 35(4 SUPPL.):37–46. doi:10.1016/j.exphem.2007.01.011.

Flegal, M. et al. (2015), "Measuring DNA Damage and Repair in Mouse Splenocytes After Chronic In Vivo Exposure to Very Low Doses of Beta- and γ-Radiation.", (July):1–9. doi:10.3791/52912.

Foray, N., M. Bourguignon and N. Hamada (2016), “Individual response to ionizing radiation”, Mutation research. Reviews in mutation research, Vol. 770, Elsevier B.V., Amsterdam, https://doi.org/10.1016/j.mrrev.2016.09.001

Franken NAP, Hovingh S, Cate RT, Krawczyk P, Stap J, Hoebe R, Aten J, Barendsen GW. 2012. Relative biological effectiveness of high linear energy transfer alpha-particles for the induction of DNA-double-strand breaks, chromosome aberrations and reproductive cell death in SW-1573 lung tumour cells. Oncol reports. 27:769-774.

Frankenberg D, Brede HJ, Schrewe UJ, Steinmetz C, Frankenberg-Scwager M, Kasten G, Pralle E. 1999. Induction of DNA Double-Strand Breaks by 1H and 4He Ions in Primary Human Skin Fibroblasts in the LET range of 8 to 124 keV/µm. Radiat Res. 151:540-549.

Geisel, D. et al. (2012), “DNA double-strand breaks as potential indicators for the biological effects of ionising radiation exposure from cardiac CT and conventional coronary angiography: a randomised, controlled study”,

European Radiology, Vol. 22/8, Springer Nature, https://doi.org/10.1007/s00330-012-2426-1

Gionchiglia, N. et al. (2021), “Association of Caspase 3 Activation and H2AX γ Phosphorylation in the Aging Brain: Studies on Untreated and Irradiated Mice,” Biomedicines. 9(9):1166. doi: 10.3390/biomedicines9091166.

Goodhead, D.T. (2006), "Energy deposition stochastics and track structure: What about the target?", Radiat. Prot. Dosimetry. 122(1–4):3–15. doi:10.1093/rpd/ncl498.

Grudzenski, S. et al. (2010), "Inducible response required for repair of low-dose radiation damage in human fibroblasts.", Proc. Natl. Acad. Sci. USA. 107(32): 14205-14210, doi:10.1073/pnas.1002213107.

Hada, M. & A.G. Georgakilas (2008), "Formation of Clustered DNA Damage after High-LET Irradiation: A Review.", J. Radiat. Res., 49(3):203–210. doi:10.1269/jrr.07123.

Hamada, N. et al. (2006), “Histone H2AX phosphorylation in normal human cells irradiated with focused ultrasoft X rays: evidence for chromatin movement during repair”, Radiation Research, Vol. 166/1, Radiation Research Society, United States, https://doi.org/10.1667/RR3577.1

Hamada, N. (2014), “What are the intracellular targets and intratissue target cells for radiation effects?”, Radiation research, Vol. 181/1, The Radiation Research Society, Lawrence, https://doi.org/10.1667/RR13505.1

Hamada, N. and T. Sato (2016), “Cataractogenesis following high-LET radiation exposure”, Mutation Research. Reviews in mutation research, Vol. 770, Elsevier B.V., Amsterdam, https://doi.org/10.1016/j.mrrev.2016.08.005

Hamada, N. (2017a), “Ionizing radiation sensitivity of the ocular lens and its dose rate dependence”, International journal of radiation biology, Vol. 93/10, Taylor & Francis, Abingdon, https://doi.org/10.1080/09553002.2016.1266407

Hamada, N. (2017b), “Ionizing radiation response of primary normal human lens epithelial cells”, PloS ONE, Vol. 12/7, Public Library Science, San Francisco, https://doi.org/10.1371/journal.pone.0181530

Havas, M. (2017), “When theory and observation collide: Can non-ionizing radiation cause cancer?”, Environmental pollution, Vol. 221, Elsevier Ltd, England. https://doi.org/10.1016/j.envpol.2016.10.018

Hightower, K. R. (1995), “The role of the lens epithelium in development of UV cataract”, Current eye research, Vol. 14/1, Informal UK Ltd, Philadelphia, https://doi.org/10.3109/02713689508999916

Iliakis, G., T. Murmann & A. Soni (2015), "Alternative end-joining repair pathways are the ultimate backup for abrogated classical non-homologous end-joining and homologous recombination repair: Implications for the formation of chromosome translocations.", Mutat. Res. - Genet. Toxicol. Environ. Mutagen. 793:166–175. doi:10.1016/j.mrgentox.2015.07.001.

Joiner, M. (2009), "Basic Clinical Radiobiology", Edited by. [1] P.J. Sadler, Next-Generation Met Anticancer Complexes Multitargeting via Redox Modul Inorg Chem 52 21.:375. doi:10.1201/b13224.

Jorge, S.-G. et al. (2012), "Evidence of DNA double strand breaks formation in Escherichia coli bacteria exposed to alpha particles of different LET assessed by the SOS response.", Appl. Radiat. Isot. 71(SUPPL.):66–70. doi:10.1016/j.apradiso.2012.05.007.

Kadhim, M.A., M.A. Hill & S.R. Moore, (2006), "Genomic instability and the role of radiation quality.", Radiat. Prot. Dosimetry. 122(1–4):221–227. doi:10.1093/rpd/ncl445.

Khanna, K.K. & S.P. Jackson (2001), "DNA double-strand breaks: signaling, repair and the cancer connection.", Nature Genetics. 27(3):247-54. doi:10.1038/85798.

Kim, K. S. et al. (2014), “Characterization of DNA damage-induced cellular senescence by ionizing radiation in endothelial cells”, International Journal of Radiation Biology, Vol. 90/1, Informa, London, https://doi.org/10.3109/09553002.2014.859763

Kim, J. N. and B. M. Lee (2007), “Risk factors, health risks, and risk management for aircraft personnel and frequent flyers”, Journal of toxicology and environmental health. Part B, Critical reviews, Vol. 10/3, Taylor & Francis Group, Philadelphia, https://doi.org/10.1080/10937400600882103

Kleiman, N. J., R. Wang and A. Spector (1990), “Ultraviolet light induced DNA damage and repair in bovine lens epithelial cells”, Current eye research, Vol. 9/12, Informa UK Ltd, Oxford, https://doi.org/10.3109/02713689009003475

Kuefner, M.A. et al. (2009), "DNA Double-Strand Breaks and Their Repair in Blood Lymphocytes of Patients Undergoing Angiographic Procedures.", Investigative radiology. 44(8):440-6. doi:10.1097/RLI.0b013e3181a654a5.

Kuefner, M.A. et al. (2015), "Chemoprevention of Radiation-Induced DNA Double-Strand Breaks with Antioxidants.", Curr Radiol Rep (2015) 3: 81. https://doi.org/10.1007/s40134-014-0081-9

Kuhne, M., G. Urban & M. Lo, (2005), "DNA Double-Strand Break Misrejoining after Exposure of Primary Human Fibroblasts to CK Characteristic X Rays, 29 kVp X Rays and Co γ-Rays.", Radiation Research. 164(5):669-676. doi:10.1667/RR3461.1.

de Lara, C.M. et al. (2001), "Dependence of the Yield of DNA Double-Strand Breaks in Chinese Hamster V79-4 Cells on the Photon Energy of Ultrasoft X Rays.", Radiation Research. 155(3):440-8. doi:10.1667/0033-7587(2001)155[0440:DOTYOD]2.0.CO;2.

Liao, J. et al. (2011), “Anti-UVC irradiation and metal chelation properties of 6-benzoyl-5,7-dihydroxy-4-phenyl-chromen-2-one: An implications for anti-cataract agent”, International journal of molecular sciences, Vol. 12/10, MDPI, Basel. https://doi.org/10.3390/ijms12107059

Lipman, R. M., B. J. Tripathi, R. C. Tripathi (1998), “Cataracts induced by microwave and ionizing radiation”, Survey of ophthalmology, Vol. 33/3, Elsevier Inc, United States, https://doi.org/10.1016/0039-6257(88)90088-4

Lomax, M.E., L.K. Folkes & P.O. Neill (2013). "Biological Consequences of Radiation-induced DNA Damage: Relevance to Radiotherapy", Statement of Search Strategies Used and Sources of Information Why Radiation Damage is More Effective than Endogenous Damage at Killing Cells Ionising Radiation-induced Do. 25:578–585. doi:10.1016/j.clon.2013.06.007.

Long, A. C., C. M. H. Colitz, and J. A. Bomser (2001), “Apoptotic and necrotic mechanisms of stress-induced human lens epithelial cell death”, Experimental biology and medicine, SAGE Publications, London, https://doi.org/10.1177/153537020422901012

Lorat, Y. et al. (2015), "Nanoscale analysis of clustered DNA damage after high-LET irradiation by quantitative electron microscopy – The heavy burden to repair.", DNA Repair (Amst). 28:93–106. doi:10.1016/j.dnarep.2015.01.007.

Maier, P. et al. (2016), "Cellular Pathways in Response to Ionizing Radiation and Their Targetability for Tumor Radiosensitization.", Int. J. Mol. Sci., 14;17(1), pii:E102, doi:10.3390/ijms17010102.

Markiewicz, E. et al. (2015), “Nonlinear ionizing radiation-induced changes in eye lens cell proliferation, cyclin D1 expression and lens shape”, Open biology, Vol. 5/4, Royal society, London, https://doi.org/10.1098/rsob.150011

Milligan, J. R. et al. (1995), « DNA repair by thiols in air shows two radicals make a double-strand break”, Radiation Research, Vol 143/3, pp. 273-280

Moore, S., F.K.T. Stanley & A.A. Goodarzi (2014), "The repair of environmentally relevant DNA double strand breaks caused by high linear energy transfer irradiation – No simple task.", DNA repair (Amst), 17:64–73. doi: 10.1016/j.dnarep.2014.01.014.

Mosconi, M., U. Giesen & F. Langner (2011), "53BP1 and MDC1 foci formation in HT-1080 cells for low- and high-LET microbeam irradiations.", Radiat. Envrion. Biophys. 50(3):345–352. doi:10.1007/s00411-011-0366-9.

Nagane, M. et al. (2021), “DNA damage response in vascular endothelial senescence: Implication for radiation-induced cardiovascular disease”, Journal of Radiation Research, Vol. 62/4, https://doi.org/10.1093/jrr/rrab032

Nenoi, M., B. Wang & G. Vares (2015), "In vivo radioadaptive response: A review of studies relevant to radiation-induced cancer risk.", Hum. Exp. Toxicol. 34(3):272–283. doi:10.1177/0960327114537537.

Nikitaki, Z. et al. (2016), "Measurement of complex DNA damage induction and repair in human cellular systems after exposure to ionizing radiations of varying linear energy transfer (LET).", Free Radiac. Res. 50(sup1):S64-S78, doi:10.1080/10715762.2016.1232484.

Nikjoo, H. et al. (2001), "Computational approach for determining the spectrum of DNA damage induced by ionizing radiation.", Radiat. Res. 156(5 Pt 2):577–83.

Nübel, T. et al. (2006), “Lovastatin protects human endothelial cells from killing by ionizing radiation without impairing induction and repair of DNA double-strand breaks”, Clinical Cancer Research, Vol. 12/3, American Association for Cancer Research, https://doi.org/10.1158/1078-0432.CCR-05-1903

Ojima, M., N. Ban, and M. Kai (2008), “DNA double-strand breaks induced by very low X-ray doses are largely due to bystander effects”, Radiation Research, Vol. 170/3, Radiation Research Society, United States, https://doi.org/10.1667/RR1255.1

Okayasu, R. (2012a), "Repair of DNA damage induced by accelerated heavy ions-A mini review.", Int. J. Cancer. 130(5):991–1000. doi:10.1002/ijc.26445.

Okayasu, R. (2012b), "Heavy ions — a mini review.", 1000:991–1000. doi:10.1002/ijc.26445.

Park, J. W. et al. (2022), “Metformin alleviates ionizing radiation-induced senescence by restoring BARD1-mediated DNA repair in human aortic endothelial cells”, Experimental Gerontology, Vol. 160, Elsevier, Amsterdam, https://doi.org/10.1016/j.exger.2022.111706

Parris, C.N. et al. (2015), "Enhanced γ-H2AX DNA damage foci detection using multimagnification and extended depth of field in imaging flow cytometry.", Cytom. Part A. 87(8):717–723. doi:10.1002/cyto.a.22697.

Radulescu I., K. Elmroth & B. Stenerlöw (2006), "Chromatin Organization Contributes to Non-randomly Distributed

Double-Strand Breaks after Exposure to High-LET Radiation.", Radiat. Res. 161(1):1–8. doi:10.1667/rr3094.

Rastogi, R. P. et al. (2010), “Molecular mechanisms of ultraviolet radiation-induced DNA damage and repair”, Journal of nucleic acids, Hindawi Ltd, United States. https://doi.org/10.4061/2010/592980

Reddy, V. N. et al. (1998), “The effect of aqueous humor ascorbate on ultraviolet-B-induced DNA damage in lens epithelium”, Investigative ophthalmology & visual science, Vol. 39/2, Arvo, pp. 344-350

Rogakou, E.P. et al. (1999), "Megabase Chromatin Domains Involved in DNA Double-Strand Breaks In Vivo.", J. Cell Biol, 146(5):905-16. doi: 10.1083/jcb.146.5.905.

Rogers, C. S. et al. (2004), “The effects of sub-solar levels of UV-A and UV-B on rabbit corneal and lens epithelial cells”, Experimental eye research, Vol. 78/5, Elsevier Ltd, London, https://doi.org/10.1016/j.exer.2003.12.011

Rombouts, C. et al. (2013), “Differential response to acute low dose radiation in primary and immortalized endothelial cells”, International Journal of Radiation Biology, Vol. 89/10, Informa, London, https://doi.org/10.3109/09553002.2013.806831

Rothkamm, K. et al. (2015), "Review DNA Damage Foci: Meaning and Significance.", Environ. Mol. Mutagen., 56(6):491-504, doi: 10.1002/em.21944.

Rothkamm, K. & M. Lo (2003), "Evidence for a lack of DNA double-strand break repair in human cells exposed to very low x-ray doses.", PNAS, 100(9):5057-62. doi:10.1073/pnas.0830918100.

Rübe, C.E. et al. (2008), "Cancer Therapy: Preclinical DNA Double-Strand Break Repair of Blood Lymphocytes and Normal Tissues Analysed in a Preclinical Mouse Model: Implications for Radiosensitivity Testing.", Clin. Cancer Res., 14(20):6546–6556. doi:10.1158/1078-0432.CCR-07-5147.

Russo, A. et al. (2015), "Review Article Genomic Instability: Crossing Pathways at the Origin of Structural and Numerical Chromosome Changes.", Envrion. Mol. Mutagen. 56(7):563-580. doi:10.1002/em.

Rydberg B, Heilbronn L, Holley WR, Lobrich M, Zeitlin C et al. 2002. Spatial Distribution and Yield of DNA Double-Strand Breaks Induced by 3-7 MeV Helium Ions in Human Fibroblasts. Radiat Res. 158(1):32-42.

Sabirzhanov, et al. (2020), “Irradiation-Induced Upregulation of miR-711 Inhibits DNA Repair and Promotes Neurodegeneration Pathways.”, Int J Mol Sci. 21(15):5239. doi: 10.3390/ijms21155239.

Sage, E. & N. Shikazono (2017), "Free Radical Biology and Medicine Radiation-induced clustered DNA lesions: Repair and mutagenesis.", Free Radic. Biol. Med. 107(December 2016):125–135. doi:10.1016/j.freeradbiomed.2016.12.008.

Sasaki, H. et al. (1998), “TEMPOL protects against lens DNA strand breaks and cataract in the x-rayed rabbit”, Investigative ophthalmology & visual sciences, Vol. 39/3, Arvo, Rockville, pp. 544-552

Schmal, Z. et al. (2019), “DNA damage accumulation during fractionated low-dose radiation compromises hippocampal neurogenesis”, Radiotherapy and Oncology. 137:45-54. doi:10.1016/j.radonc.2019.04.021.

Sears, C. R. and J. J. Turchi (2012), “Complex cisplatin-double strand break (DSB) lesions directly impair cellular non-homologous end-joining (NHEJ) independent of downstream damage response (DDR) pathways”, Journal of biological chemistry, Vol 287/29, The American Society for Biochemistry and Molecular Biology, Inc, USA, https://doi.org/10.1074/jbc.M112.344911

Shah, D.J., R.K. Sachs & D.J. Wilson (2012), "Radiation-induced cancer: A modern view." Br. J. Radiol. 85(1020):1166–1173. doi:10.1259/bjr/25026140.

Shelke, S. & B. Das (2015), "Dose response and adaptive response of non- homologous end joining repair genes and proteins in resting human peripheral blood mononuclear cells exposed to γ radiation.", (December 2014):365–379. doi:10.1093/mutage/geu081.

Sidjanin, D., S. Zigman and J. Reddan (1993), “DNA damage and repair in rabbitlens epithelial cells following UVA radiation”, Current eye research, Vol. 12/9, Informa UK Ltd, Oxford, https://doi.org/10.3109/02713689309020382

Smith, J. et al. (2003), "Impact of DNA ligase IV on the delity of end joining in human cells.", Nucleic Acids Research. 31(8):2157-2167.doi:10.1093/nar/gkg317.

Smith, T.A. et al. (2017), "Radioprotective agents to prevent cellular damage due to ionizing radiation." Journal of Translational Medicine.15(1).doi:10.1186/s12967-017-1338-x.

Stewart, F. A. et al. (2012), “ICRP publication 118: ICRP statement on tissue reactions and early and late effects of radiation in normal tissues and organs – threshold doses for tissue reactions in a radiation protection context”, Annals of the ICRP, Vol, 41/1-2, Elsevier Ltd, London, https://doi.org/10.1016/j.icrp.2012.02.001

Sudprasert, W., P. Navasumrit & M. Ruchirawat (2006), "Effects of low-dose γ radiation on DNA damage, chromosomal aberration and expression of repair genes in human blood cells.", Int. J. Hyg. Envrion. Health, 209:503–511. doi:10.1016/j.ijheh.2006.06.004.

Sutherland, B.M. et al. (2000), "Clustered DNA damages induced in isolated DNA and in human cells by low doses of

ionizing radiation.", J. of Rad. Res. 43 Suppl(S):S149-52. doi: 10.1269/jrr.43.S149

Sylvester, C. B. et al. (2018), “Radiation-Induced Cardiovascular Disease: Mechanisms and Importance of Linear Energy Transfer”, Frontiers in cardiovascular medicine, Vol. 5, Frontiers, https://doi.org/10.3389/fcvm.2018.00005

Terato, H. & H. Ide (2005), "Clustered DNA damage induced by heavy ion particles.", Biol. Sci. Sp. 18(4):206–215. doi:10.2187/bss.18.206.

Ungvari, Z. et al. (2013), “Ionizing radiation promotes the acquisition of a senescence-associated secretory phenotype and impairs angiogenic capacity in cerebromicrovascular endothelial cells: role of increased DNA damage and decreased DNA repair capacity in microvascular radiosensitivity”, The journals of gerontology. Series A, Biological sciences and medical sciences, Vol. 68/12, Oxford University Press, Oxford, https://doi.org/10.1093/gerona/glt057

Valentin, J.D.J (1998), "Chapter 1. Ann ICRP.", 28(4):5–7. doi:10.1016/S0146-6453(00)00002-6. http://www.ncbi.nlm.nih.gov/pubmed/10882804.

Venkatesh, P. et al. (2016), "Effect of chromatin structure on the extent and distribution of DNA double strand breaks produced by ionizing radiation; comparative study of hESC and differentiated cells lines.", Int J. Mol. Sci. 17(1). doi:10.3390/ijms17010058.

Wang, et al. (2021), “Ionizing Radiation-Induced Brain Cell Aging and the Potential Underlying Molecular Mechanisms.”, Cells. 10(12):3570. doi: 10.3390/cells10123570

Ward, J. F. (1988), "DNA Damage Produced by Ionizing Radiation in Mammalian Cells: Identities, Mechanisms of Formation, and Reparability.", Prog. Nucleic Acid Res. Mol. Biol. 35(C):95–125. doi:10.1016/S0079-6603(08)60611-X.

Wolf, N. et al. (2008), “Radiation cataracts: Mechanisms involved in their long delayed occurrence but then rapid progression”, Molecular vision, Vol. 14/34-35, Molecular Vision, Atlanta, pp. 274-285

Wu, L.J. et al. (1999), "Targeted cytoplasmic irradiation with alpha particles induces mutations in mammalian cells.", Proc. Natl. Acad. Sci. 96(9):4959–4964. doi:10.1073/pnas.96.9.4959.

Yamaguchi, H. et al. (2005), "Estimation of Yields of OH Radicals in Water Irradiated by Ionizing Radiation.", J. of Rad. Res. 46(3):333-41. doi: 10.1269/jrr.46.333.

Zhang, L. et al. (2017), “The inhibitory effect of minocycline on radiation-induced neuronal apoptosis via AMPKα1 signaling-mediated autophagy.”, Sci Rep.7(1):16373. doi: 10.1038/s41598-017-16693-8.

Relationship: 2769: Energy Deposition leads to Oxidative Stress

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Deposition of energy leads to abnormal vascular remodeling	adjacent	High	High
Deposition of Energy Leading to Learning and Memory Impairment	adjacent	High	Moderate
Deposition of energy leading to occurrence of bone loss	adjacent	High	Moderate
Deposition of energy leading to occurrence of cataracts	adjacent	High	High

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	Moderate	NCBI
mouse	Mus musculus	Moderate	NCBI
rat	Rattus norvegicus	High	NCBI
rabbit	Oryctolagus cuniculus	Low	NCBI

Life Stage Applicability

Life Stage	Evidence
Juvenile	High
Adult	Moderate

Sex Applicability

Sex	Evidence
Male	High
Female	Moderate
Unspecific	High

Most evidence is derived from in vitro studies, predominately using rabbit models. Evidence in humans and mice is moderate, while there is considerable available data using rat models. The relationship is applicable in both sexes; however, males are used more often in animal studies. No studies demonstrate the relationship in preadolescent animals, while adolescent animals were used very often, and adults were used occasionally in in vivo studies.

Key Event Relationship Description

Energy deposited onto biomolecules stochastically in the form on ionizing and non-ionizing radiation can cause direct and indirect molecular-level damage. As energy is deposited in an aqueous solution, water molecules can undergo radiolysis, breaking bonds to produce reactive oxygen species (ROS) (Ahmadi et al., 2021; Karimi et al., 2017) or directly increase function of enzymes involved in ROS generation (i.e. catalaze). Various species of ROS can be generated with differing degrees of biological effects. For example, singlet oxygen, superoxide, and hydroxyl radical are highly unstable, with short half-lives and react close to where they are produced, while species like H2O2 are much more stable and membrane permeable, meaning they can travel from the site of production, reacting elsewhere as a much weaker oxidant (Spector, 1990). In addition, enzymes involved in reactive oxygen and nitrogen species (RONS) production can be directly upregulated following the deposition of energy (de Jager, Cockrell and Du Plessis, 2017). Although less common than ROS, reactive nitrogen species (RNS) can also be produced by energy deposition resulting in oxidative stress (Cadet et al., 2012; Tangvarasittichai & Tangvarasittichai, 2019), a state in which the amount of ROS and RNS, collectively known as RONS, overwhelms the cell's antioxidant defence system. This loss in redox homeostasis can lead to oxidative damage to macromolecules including proteins, lipids, and nucleic acids (Schoenfeld et al., 2012; Tangvarasittichai & Tangvarasittichai, 2019; Turner et al., 2002).

Evidence Supporting this KER

Overall weight of evidence: High

Biological Plausibility

A large body of literature supports the linkage between the deposition of energy and oxidative stress. Multiple reviews describe the relationship in the context of ROS production (Marshall, 1985; Balasubramanian, 2000; Jurja et al., 2014), antioxidant depletion (Cabrera et al., 2011; Fletcher, 2010; Ganea & Harding, 2006; Hamada et al., 2014; Spector, 1990; Schoenfeld et al., 2012; Wegener, 1994), and overall oxidative stress (Eaton, 1994, Tangvarasittichai & Tangvarasittichain, 2019). This includes investigations into the mechanism behind the relationship (Ahmadi et al., 2021; Balasubramanian, 2000; Cencer et al., 2018; Eaton, 1994; Fletcher, 2010; Jiang et al., 2006; Jurja et al., 2014; Padgaonkar et al., 2015; Quan et al., 2021; Rong et al., 2019; Slezak et al., 2015; Soloviev & Kizub, 2019; Tian et al., 2017; Tahimic & Globus, 2017; Varma et al., 2011; Venkatesulu et al., 2018; Wang et al., 2019a; Yao et al., 2008; Yao et al., 2009; Zigman et al., 2000).

Water radiolysis is a main source of free radicals. Energy ionizes water and free radicals are produced that combine to create more stable ROS, such as hydrogen peroxide, hydroxide, superoxide, and hydroxyl (Eaton, 1994; Rehman et al., 2016; Tahimic & Globus, 2017; Tian et al., 2017; Varma et al., 2011; Venkatesulu et al., 2018). ROS formation causes ensuing damage to the body, as ~80% of tissues are comprised of water (Wang et al., 2019a). Ionizing radiation (IR) is a source of energy deposition, it can also interact with molecules, such as nitric oxide (NO), to produce less common free radicals, including RNS (Slezak et al., 2015; Tahimic & Globus, 2017; Wang et al., 2019a). Free radicals can diffuse throughout the cell and damage vital cellular components, such as proteins, lipids, and DNA, as well as dysregulate cellular processes, such as cell signaling (Slezak et al., 2015; Tian et al., 2017).

ROS are also commonly produced by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX). Deposition of energy can activate NOX and induce expression of its catalytic and cytosolic components, resulting in increased intracellular ROS (Soloviev & Kizub, 2019). Intracellular ROS production can also be initiated through the expression of protein kinase C, which in turn activates NOX through phosphorylation of its cytosolic components (Soloviev & Kizub, 2019). Alternatively, ROS are often formed at the electron transport chain (ETC) of the mitochondria, due to IR-induced electron leakage leading to ionization of the surrounding O2 to become superoxide (Soloviev & Kizub, 2019). Additionally, energy reaching a cell can be absorbed by an unstable molecule, often NADPH, known as a chromophore, which leads to the production of ROS (Balasubramanian, 2000; Cencer et al., 2018; Jiang et al., 2006; Jurja et al., 2014; Padgaonkar et al., 2015; Yao et al., 2009; Zigman et al., 2000).

Energy deposition can also weaken a cell's antioxidant defence system through the depletion of certain antioxidant enzymes, such as superoxide dismutase (SOD) and catalase (CAT). Antioxidants are consumed during the process of neutralizing ROS, so as energy deposition stimulates the formation of ROS it begins to outpace the rate at which antioxidants are replenished; this results in an increased risk of oxidative stress when their concentrations are low (Belkacémi et al., 2001; Giblin et al., 2002; Ji et al., 2014; Kang et al., 2020; Karimi et al., 2017; Padgaonkar et al., 2015; Rogers et al., 2004; Slezak et al., 2015; Tahimic & Globus, 2017; Wang et al., 2019a; Wegener, 1994; Weinreb & Dovrat, 1996; Zhang et al., 2012; Zigman et al., 1995; Zigman et al., 2000). When the amount of ROS overwhelms

the antioxidant defence system, the cell will enter oxidative stress leading to macromolecular and cellular damage (Tangvarasittichai & Tangvarasittichai, 2019).

Empirical Evidence

The relationship between energy deposition and oxidative stress is strongly supported by primary research on the effects of IR on ROS and antioxidant levels (Bai et al., 2020; Cervelli et al., 2017; Hatoum et al., 2006; Huang et al., 2018; Huang et al., 2019; Karam & Radwan, 2019; Kook et al., 2015; Liu et al., 2018; Liu et al., 2019; Mansour, 2013; Philipp et al., 2020; Ramadan et al., 2020; Sharma et al., 2018; Shen et al., 2018; Soltani et al., 2016; Soucy et al., 2010; Soucy et al., 2011; Ungvari et al., 2013; Wang et al., 2016; Wang et al., 2019b; Zhang et al., 2018; Zhang et al., 2020). Of note is that the relationship is demonstrated across studies conducted using various cell types, models and using broad dose ranges as summarized below. Much evidence is available and described to help discern the quantitative understanding of the relationship, since it is well established.

Dose Concordance

It is well-accepted that any dose of radiation will deposit energy onto matter. Doses as low as 1 cGy support this relationship (Tseung et al., 2014). Following the deposition of energy, markers of oxidative stress are observed in the form of RONS, a change in levels of antioxidants, and oxidative damage to macromolecules. These effects have been shown across various organs/tissues and cell types as described below.

RONS

Cardiovascular tissue:

There is a considerable amount of evidence to support this relationship in cell types and tissues of relevance to the cardiovascular system. Recent studies have shown a linear increase in ROS in human umbilical vein endothelial cells (HUVECs) following 0-5 Gy gamma irradiation (Wang et al., 2019b). HUVECs irradiated with 0.25 Gy X-rays (Cervelli et al., 2017) and 9 Gy 250kV photons (Sharma et al., 2018) show increased ROS. Gamma ray irradiated rats at 5 Gy display increased ROS levels in the aorta (Soucy et al., 2010). A study using cerebromicrovascular endothelial cell (CMVECs) showed a dose-dependent increase in ROS from 0-8 Gy gamma irradiation (Ungvari et al., 2013). Additionally, telomerase-immortalized coronary artery endothelial (TICAE) and telomerase-immortalized microvascular endothelial (TIME) cells irradiated with 0.1 and 5 Gy of X-rays displayed increased ROS production (Ramadan et al., 2020). Gut arterioles of rats showed increased ROS following multiple fractions of 2.5 Gy X-ray rat irradiation (Hatoum et al., 2006). Additionally, rats irradiated with 1 Gy of 56Fe expressed increased ROS levels in the aorta (Soucy et al., 2011).

Brain tissue:

Markers of oxidative stress have also been consistently observed in brain tissue. Human neural stem cells subjected to 1, 2 or 5 Gy gamma rays showed a dose-dependent increase in RONS production (Acharya et al., 2010). A dose-dependent increase in ROS was observed in rat brains following 1-10 Gy gamma rays (Collins-Underwood et al., 2008). Neural precursor cells exposed to 0-10 Gy of X-irradiation showed increased ROS levels (Giedzinski et al., 2005; Limoli et al., 2004). Mice brain tissue displayed increased ROS following proton irradiation (Baluchamy et al., 2012; Giedzinski et al., 2005). Neural processor cells expressed linearly increased ROS levels following doses of 56Fe (Limoli et al., 2007). A dose-dependent increase in RONS was also observed after exposure to 1-15 cGy 56Fe irradiation in mice neural stem/precursor cell (Tseng et al., 2014). Human neural stem cells exposed to 5-100 cGy of various ions demonstrated a dose-dependent increase in RONs (Baulch et al., 2015).

Eye tissue:

The eye is also sensitive to the accumulation of free radicals, in a state of antioxidant decline. It has been shown in human lens epithelial cells (HLECs) and HLE-B3 following gamma irradiation of 0.25 and 0.5 Gy that ROS levels are markedly increased (Ahmadi et al., 2021). Exposure to non-ionizing radiation, such as ultraviolet (UV)-B, has also led to increased ROS in HLECs and mice lenses (Ji et al., 2015; Kubo et al., 2010; Rong et al., 2019; Yang et al., 2020)

Bone tissue:

Rat bone marrow-derived mesenchymal stem cell (bmMSCs) irradiated with 2, 5 and 10 Gy gamma rays and murine MC3T3-E1 osteoblast cells irradiated with 2, 4, and 8 Gy of X-rays have shown a dose-dependent increase in ROS levels (Bai et al., 2020; Kook et al., 2015). Murine RAW264.7 cells and rat bmMSC irradiated with 2 Gy of gamma rays displayed increased ROS levels (Huang et al., 2019; Huang et al., 2018; hang et al., 2020). Human bone marrow-derived mesenchymal stem cell (hBMMSCs) irradiated with 2 or 8 Gy X-rays showed increased ROS (Liu et al., 2018; Zhang et al., 2018). Similarly, murine MC3T3-E1 osteoblast-like cells irradiated with 6 Gy of X-rays also displayed increased ROS (Wang et al., 2016). Finally, whole-body irradiation of mice with 2 Gy of 31.6 keV although LET 12C heavy ions showed increased ROS (Liu et al., 2019)

Antioxidants

Blood:

Workers exposed to X-rays at less than 1 mSv/year for an average of 15 years showed around 20% decreased antioxidant activity compared to unexposed controls (Klucinski et al., 2008). Similarly, adults exposed to high background irradiation of 260 mSv/year showed about 50% lower antioxidant activity power compared to controls (Attar, Kondolousy and Khansari, 2007).

Cardiovascular tissue:

Heart tissue of rats following gamma irradiation of rats at 5 and 6 Gy resulted in a decrease in antioxidant levels (Karam & Radwan, 2019; Mansour, 2013). Similarly, HUVECs (Soltani, 2016) and TICAE cells (Philipp et al., 2020) irradiated at 2 Gy and 0.25-10 Gy gamma rays, respectively, displayed decreased antioxidant levels. Mice exposed to 18 Gy of X-ray irradiation showed decreased antioxidants in the aorta (Shen et al., 2018).

Brain tissue:

Mice brain tissue following 2, 10 and 50 cGy whole-body gamma irradiation revealed a dose-dependent change in SOD2 activity (Veeraraghan et al., 2011). Mice brain tissue showed decreased glutathione (GSH) and SOD levels following proton irradiation (Baluchamy et al., 2012)

Eye tissue:

Rats exposed to 15 Gy gamma rays demonstrated decreased antioxidants in the lens tissue (Karimi et al, 2017). Neutron irradiation of rats at 3.6 Sv resulted in a decrease in antioxidants in lens (Chen et al., 2021). A few studies found a dose concordance between UV irradiation and decreased antioxidant levels (Hua et al, 2019; Ji et al, 2015; Zigman et al., 2000; Zigman et al, 1995). HLECs following UVB exposure from 300 J/m2 to 14,400 J/m2 in HLECs showed linear decreases in antioxidant activity (Ji et al., 2015). Similarly, HLEC exposed to 4050, 8100 and 12,150 J/m2 found decreased antioxidant levels (Hua et al., 2019). Following UV irradiation of rabbit and squirrel lens epithelial cells (LECs) showed a linear decrease of antioxidant level, CAT (Zigman et al., 2000; Zigman et al., 1995). Mice exposed to UV irradiation found decreased antioxidant levels in lens (Zhang et al., 2012). Similarly, SOD levels decreased following 0.09 mW/cm 2 UVB exposure of HLECs (Kang et al., 2020).

Bone tissue:

Rat bmMSCs irradiated with 2, 5 and 10 Gy gamma rays and Murine MC3T3-E1 osteoblast cells irradiated with 2, 4, and 8 Gy of X- rays showed a dose-dependent decrease in antioxidant levels (Bai et al., 2020; Kook et al., 2015). hBMMSCs irradiated with 8 Gy X-rays also showed a decrease in antioxidant, SOD, levels (Liu et al., 2018).

Oxidative Damage

Cardiovascular tissue:

HUVECs and rat hearts irradiated by gamma rays at 2 and 6 Gy, respectively, resulted in increased levels of oxidative stress markers, such as malondialdehyde (MDA), and thiobarbituric reactive substances (TBARS) (Mansour, 2013; Soltani, 2016).

Brain tissue:

Mice brain tissue were shown to have increased lipid peroxidation (LPO) as determined by MDA measurements, following proton irradiation at 1 and 2 Gy (Baluchamy et al., 2012). Neural precursor cells from rat hippocampus exposed to 0, 1, 5 and 10 Gy of X- irradiation resulted in increased lipid peroxidation (Limoli et al., 2004).

Eye tissue:

Rats exposed to 15 Gy gamma rays demonstrated increased MDA in lens tissue (Karimi et al, 2017). Neutron irradiation of rats at 3.6 Sv resulted in an initial decrease, followed by an increase in MDA in lens (Chen et al., 2021). Following UV irradiation at 300, 4050, 8100 and 12,150 J/m2, there was an increase in LPO in human lens (Chitchumroonchokchai et al., 2004; Hua et al., 2019). Similarly, LPO increased following 0.09 mW/cm2 UVB exposure of HLECs (Kang et al., 2020).

Time Concordance

It is well-accepted that deposition of energy into matter results in immediate vibrational changes to molecules or ionization events. Deposition of energy is therefore an upstream event to all follow-on latent events like oxidative stress.

RONS

Cardiovascular tissue:

In TICAE and TIME cells, ROS increased at 45 minutes after X-ray irradiation (Ramadan et al., 2020). Superoxide and peroxide production were increased 1 day after 2-8 Gy of gamma irradiation in CMVECs (Unvari et al., 2013).

Bone tissue:

hBMMSCs irradiated with X-rays at 2 Gy showed peak ROS production at 2-8h post-irradiation (Zhang et al., 2018). Murine RAW264.7 cells (can undergo osteoclastogenesis) irradiated with 2 Gy of gamma rays showed increased ROS at 2-8h post- irradiation (Huang et al., 2018).

Brain tissue:

In human lymphoblast cells exposed to 2 Gy of X-rays, ROS were increased at various times between 13 and 29 days post- irradiation (Rugo and Schiestl, 2004). RONS were increased in human neural stem cells at 12-48h post-

irradiation with 2 and 5 Gy of gamma rays (Acharya et al., 2010). ROS levels were increased in rat neural precursor cells at 6-24h after irradiation with 1-10 Gy of protons (Giedzinksi et al., 2005). Both 56Fe (1.3 Gy) and gamma ray (2 Gy) irradiation of mice increased ROS levels after 2 months post-irradiation in the cerebral cortex (Suman et al., 2013). ROS were also increased 12 months after 56Fe irradiation (Suman et al., 2013). RONS increased as early as 12h post-irradiation continuing to 8 weeks with 2-200 cGy doses of 56Fe irradiation of mouse neural stem/precursor cells (Tseng et al., 2014). The same cell type irradiated with 1 and 5 Gy of 56Fe irradiation showed increased ROS at 6h post-irradiation, with the last increase observed 25 days post-irradiation (Limoli et al., 2004).

Eye tissue:

Mice exposed to 11 Gy of X-rays showed increased ROS at 9 months post-irradiation in lenses (Pendergrass et al., 2010). In human lens cells, ROS were found increased at 1h after 0.25 Gy gamma ray irradiation (Ahmadi et al., 2021), 15 minutes after 30 mJ/cm2 UV radiation (Jiang et al., 2006), 2.5-120 minutes after 0.014 and 0.14 J/cm2 UV radiation (Cencer et al., 2018), and 24h after 30 mJ/cm2 UVB-radiation (Yang et al., 2020).

Antioxidants

Cardiovascular tissue:

CAT antioxidant enzyme was decreased in mice aortas as early as 3 days post-irradiation, remaining decreased until 84 days after irradiation with 18 Gy of X-rays (Shen et al., 2018). The antioxidant enzymes peroxiredoxin 5 (PRDX5) and SOD were both shown to have the greatest decrease at 24h after 2 Gy gamma irradiation of TICAE cells (Philipp et al., 2020).

Eye tissue:

Bovine lenses irradiated with 44.8 J/cm2 of UVA radiation showed decreased CAT levels at 48-168h post-irradiation (Weinreb and Dovrat, 1996). UV irradiation of mice at 20.6 kJ/m2 led to decreased GSH at both 1 and 16 months post-irradiation in the lens (Zhang et al., 2012). Bovine lens cells exposed to 10 Gy of X-rays showed decreased levels of the antioxidant GSH at 24 and 120h after exposure (Belkacemi et al., 2001).

Oxidative damage markers

Cardiovascular tissue:

Oxidative damage markers 4-hydroxynonemal (4-HNE) and 3-Nitrotyosine (3-NT) were both significantly increased in the aorta of mice at 3 days post-irradiation, remaining increased until 84 days after irradiation with 18 Gy of X-rays (Shen et al., 2018).

Essentiality

Radiation has been found to induce oxidative stress above background levels. Many studies have shown that lower doses of ionizing radiation resulted in decreased levels in markers of oxidative stress in multiple cell types (Acharya et al., 2010; Ahmadi et al., 2021; Bai et al., 2020; Baluchamy et al., 2012 Chen et al., 2021; Collins-Underwood et al., 2008; Giedzinski et al., 2005; Kook et al., 2015; Kubo et al., 2010; Philipp et al., 2020; Ramadan et al., 2020; Ungvari et al., 2013; Veeraraghan et al., 2011; Wang et al., 2019b; Zigman et al., 2000; Zigman et al., 1995). The essentiality of deposition of energy can be assessed through the removal of deposited energy, a physical stressor that does not require to be metabolized in order to elicit downstream effects on a biological system. Studies that do not deposit energy are observed to have no downstream effects.

Uncertainties and Inconsistencies

There are several uncertainties and inconsistencies in this KER.

- Chen et al. (2021) found that radiation can have adaptive responses. The study used three neutron radiation doses, 0.4 and 1.2 Sv, and 3.6 Sv. After 0.4 and 1.2 Sv, the activity of antioxidant enzymes GSH and SOD increased, and the concentration of malondialdehyde, a product of oxidative stress, decreased. After 3.6 Sv, the opposite was true.
- While the concentration of most antioxidant enzymes decreases after energy deposition, there is some uncertainty with SOD. Certain papers have found that its concentration decreases with dose (Chen et al., 2021; Hua et al., 2019; Ji et al., 2015; Kang et al., 2020) while others found no difference after irradiation (Rogers et al., 2004; Zigman et al., 1995). Several studies have also found that higher levels of SOD do not increase resistance to UV radiation (Eaton, 1994; Hightower, 1995).
- At 1-week post-irradiation with 10 Gy of 60Co gamma rays, TICAE cells experienced a significant increase in levels of the antioxidant, PRDX5, contrary to the decrease generally seen in antioxidant levels following radiation exposure (Philipp et al., 2020).
- Various studies found an increase in antioxidant SOD levels within the brain after radiation exposure (Acharya et al., 2010; Baluchamy et al., 2012; Baulch et al., 2015; Veeraraghan et al., 2011).
- Chien et al. (2015) found no changes to ROS levels in hippocampal neurons five days after 0.2 Gy of electron radiation.
- Antioxidants that increase in expression are indicative of the presence of RONS. When antioxidants decrease in

expression/activity, this is most likely due to the overwhelming of the antioxidant defence mechanisms

- There is limited data to support an understanding of deposition of energy leading to oxidative stress at low doses.

Quantitative Understanding of the Linkage

The table below provides some representative examples of quantitative linkages between the two key events. It was difficult to identify a general trend across all the studies due to differences in experimental design and reporting of the data. All data is statistically significant unless otherwise stated.

Response-response relationship

Dose Concordance

Reference	Experiment Description	Result
Attar, Kondolousy and Khansari, 2007	In vivo. One hundred individuals between 20 and 50 years old in two villages in Iran exposed to background IR at 260 mSv/year had antioxidant levels measured. The control group was from two villages not exposed to the high background radiation. The total antioxidant levels in the blood were determined by the ferric reducing/antioxidant power assay.	The total antioxidant level was significantly reduced from 1187±199 µmol in the control to 686±170 µmol in the exposed group.
Klucinski et al., 2008	In vivo. A group of 14 men and 31 women aged 25–54 years working X-ray equipment (receiving doses of less than 1 mSv/year) for an average of 15.3 years (range of 2-33 years) were compared to a control group for antioxidant activity. Antioxidant activity of SOD, glutathione peroxidase (GSH-Px), and CAT in erythrocytes were measured in U/g of hemoglobin.	Enzymes (SOD, GSH, CAT) showed significantly decreased antioxidant activity in the workers. In the controls (U/g of Hb): • SOD: 1200 ± 300 • GSH-Px: 39 ± 7 • CAT: 300 ± 60 In the workers (U/g of Hb): • SOD: 1000 ± 200 • GSH-Px: 29 ± 4 • CAT: 270 ± 50
Limoli et al., 2007	In vitro. Neural precursor cells isolated from rat hippocampi was exposed to 0.25-5 Gy of 56Fe irradiation at dose rates of 0.5-1.0 Gy/min. ROS were measured 6h post-irradiation.	At a low dose of 0.25 Gy and 0.5 Gy, relative ROS levels were significantly elevated and showed a linear dose response (from ~1 to ~2.25 relative ROS levels) until 1 Gy, where it reached its peak (~3 relative ROS levels). At higher doses, the relative ROS levels decreased.
Tseng et al., 2014	In vitro. Neural stem/precursor cells isolated from mouse subventricular and hippocampal dentate subgranular zones were exposed to 1-15 cGy of 56Fe irradiation at dose rates ranging from 5-50 cGy/min. RONS levels were measured.	A dose-dependent and significant rise in RONS levels was detected after 56Fe irradiation. 12 h post-irradiation, a steady rise was observed and reached a 6-fold peak after 15 cGy.

Limoli et al., 2004	In vitro. Neural precursor cells from rat hippocampus were exposed to 0, 1, 5 and 10 Gy of X-irradiation at a dose rate of 4.5 Gy/min. ROS levels were measured. In vivo. MDA was used to quantify oxidative stress.	A dose-dependent increase in ROS levels was seen in the first 12 h post-irradiation, with relative maximums at 12 h after 5 Gy (35% increase) and 24 h after 1 Gy (31% increase). ROS levels measured 1 week after 5 Gy were increased by 180% relative to sham-irradiated controls. MDA levels increased significantly (approximately 1.3-fold) after exposure to 10 Gy.
Collins-Underwood et al., 2008	In vitro. Immortalized rat brain microvascular endothelial cells were exposed to 1-10 Gy of 137Cs-irradiation at a dose rate of 3.91 Gy/min. Intracellular ROS and O2- production were both measured.	Irradiation resulted in a significant dose-dependent increase in intracellular ROS generation from 1-10 Gy. At 5 Gy, there was an approximate 10-fold increase in ROS levels, and at 10 Gy there was an approximate 20-fold increase.
Giedzinski et al., 2005	In vitro. Neural precursor cells were irradiated with 1, 2, 5 and 10 Gy of 250 MeV protons (1.7-1.9 Gy/min) and X-irradiation (4.5 Gy/min). ROS levels were measured.	There was a rapid increase in ROS at 6, 12, 18 and 24h after proton irradiation, with an exception at the 1 Gy 18h point. Most notably, at 6h post-irradiation, a dose-dependent increase in relative ROS levels from 1 to 10 Gy was seen that ranged from 15% (at 1 Gy) to 65% (at 10 Gy). Linear regression analysis showed that at ≤2 Gy, ROS levels increased by 16% per Gy. The linear dose response obtained at 24h showed that proton irradiation increased the relative ROS levels by 3% per Gy.
Veeraraghan et al., 2011	In vivo. Adult mice were exposed to 2, 10 or 50 cGy of whole-body gamma irradiation at 0.81 Gy/min. Brain tissues were harvested 24h post-irradiation. SOD2 levels and activity were measured.	Compared to the controls, the levels of SOD2 expression increased in the brain after 2, 10 and 50 cGy. Analysis revealed a significant and dose-dependent change in SOD2 activity. More specifically, SOD2 activity showed significant increases after 10 (~25% increase above control) and 50 cGy (~60% increase above control), but not 2 cGy.
Baluchamy et al., 2012	In vivo. Male mice were exposed to whole-body irradiation with 250 MeV protons at 0.01, 1 and 2 Gy and the whole brains were dissected out. ROS, LPO, GSH and total SOD were measured.	Dose-dependent increases in ROS levels was observed compared to controls, with a two-fold increase at 2 Gy. A 2.5 to 3-fold increase in LPO levels was also seen at 1 and 2 Gy, respectively, which was directly correlated with the increase in ROS levels. Additionally, results showed a significant reduction in GSH (~70% decrease at 2 Gy) and SOD activities (~2-fold decrease) following irradiation that was dose-dependent.
Acharya et al., 2010	In vitro. Human neural stem cells were subjected to 1, 2 or 5 Gy of gamma irradiation at a dose rate of 2.2 Gy/min. RONS and superoxide levels were determined.	Intracellular RONS levels increased by approximately 1.2 to 1.3-fold compared to sham-irradiated controls and was found to be reasonable dose-responsive. At 12h, levels of superoxide increased 2 and 4-fold compared to control for 2 and 5 Gy, respectively. At 24h and 48h, there was a dose-dependent increase in RONS levels. At 7 days, levels of RONS increased approximately 3 to 7-fold for 2 and 5 Gy, respectively.
Baulch et al., 2015	In vitro. Human neural stem cells were exposed to 5-100 cGy of 16O, 28Si, 48Ti or 56Fe particles (600 MeV) at 10-50 cGy/min. RONS and superoxide levels were determined.	3 days post-irradiation, oxidative stress was found to increase after particle irradiation. Most notably, exposure to 56Fe resulted in a dose-dependent increase with 100% increase in RONS levels at 100 cGy. Dose-dependent increase was also seen in superoxide levels after 56Fe irradiation. At 7 days post-irradiation, 56Fe irradiation induced significantly lower nitric oxide levels by 47% (5 cGy), 55% (25 cGy) and 45% (100 cGy).

Bai et al., 2020	In vitro. bmMSCs were taken from 4-week-old, male Sprague-Dawley rats. After extraction, cells were then irradiated with 2, 5, and 10 Gy of 137Cs gamma rays. Intracellular ROS levels and relative mRNA expression of the antioxidants, SOD1, SOD2, and CAT2, were measured to assess the extent of oxidative stress induced by IR.	Cellular ROS levels increased significantly in a dose-dependent manner from 0-10 Gy. Compared to sham-irradiated controls, ROS levels increased by ~15%, ~55%, and ~105% after exposure to 2, 5, and 10 Gy, respectively. Antioxidant mRNA expression decreased in a dose-dependent manner from 0-10 Gy, with significant increases seen at doses 2 Gy for SOD1 and CAT2 and 5 Gy for SOD2. Compared to sham-irradiated controls, SOD1 expression decreased by ~9%, ~18%, and ~27% after exposure to 2, 5, and 10 Gy, respectively. SOD2 expression decreased by ~31% and ~41% after exposure to 5 and 10 Gy, respectively. CAT2 expression decreased by ~15%, ~33%, and ~58% after exposure to 2, 5, and 10 Gy, respectively.
Liu et al., 2018	In vitro. hBMMSCs were irradiated with 8 Gy of X-rays at a rate of 1.24 Gy/min. Intracellular ROS levels and SOD activity were measured to analyze IR-induced oxidative stress.	Compared to sham-irradiated controls, hBMMSCs irradiated with 8 Gy of X-rays experienced a significant increase to intracellular ROS levels. hBMMSCs irradiated with 8 Gy of X-rays experienced a ~46% reduction in SOD activity.
Kook et al., 2015	In vitro. Murine MC3T3-E1 osteoblast cells were irradiated with 2, 4, and 8 Gy of X-rays at a rate of 1.5 Gy/min. Intracellular ROS levels and the activity of antioxidant enzymes, including GSH, SOD, CAT, were measured to assess the extent of oxidative stress induced by IR exposure.	Compared to sham-irradiated controls, irradiated MC3T3-E1 cells experienced a dose-dependent increase in ROS levels, with significant increases at 4 and 8 Gy (~26% and ~38%, respectively). Antioxidant enzyme activity initially increased by a statistically negligible amount from 0-2 Gy and then decreased in a dose-dependent manner from 2-8 Gy. SOD activity decreased significantly at 4 and 8 Gy by ~29% and ~59%, respectively. GSH activity similarly decreased significantly at 4 and 8 Gy by ~30% and ~48%, respectively. CAT activity did not change by a statistically significant amount.
Liu et al., 2019	In vivo. 8–10-week-old, juvenile, female SPF BALB/c mice underwent whole-body irradiation with 2 Gy of 31.6 keV/μm 12C heavy ions at a rate of 1 Gy/min. ROS levels were measured from femoral bone marrow mononuclear cells of the irradiated mice to analyze IR-induced oxidative stress.	Compared to sham-irradiated controls, irradiated mice experienced a ~120% increase in ROS levels.
Zhang et al., 2020	In vitro. Murine RAW264.7 osteoclast precursor cells were irradiated with 2 Gy of 60Co gamma rays at a rate of 0.83 Gy/min. ROS levels were measured to determine the extent of oxidative stress induced by IR exposure.	Compared to sham-irradiated controls, ROS levels in irradiated RAW264.7 cells increased by ~100%.
Wang et al., 2016	In vitro. Murine MC3T3-E1 osteoblast-like cells were irradiated with 6 Gy of X-rays. Intracellular ROS production was measured to assess oxidative stress from IR exposure.	Compared to sham-irradiated controls, intracellular ROS production increased by ~81%.
Huang et al., 2018	In vitro. Murine RAW264.7 osteoblast-like cells were irradiated with 2 Gy of gamma rays at a rate of 0.83 Gy/min. ROS levels were measured to analyze IR-induced oxidative stress.	Compared to sham-irradiated controls, ROS levels in RAW264.7 cells increased by ~138% by 2 h post-irradiation.

Zhang et al., 2018	In vitro. hBMMSCs were irradiated with 2 Gy of X-rays at a rate of 0.6 Gy/min. Relative ROS concentration was measured to assess the extent of oxidative stress induced by IR.	Compared to sham-irradiated controls, irradiated hBMMSCs experienced a maximum increase of ~90% to ROS levels at 3 h post-irradiation.
Huang et al., 2019	In vitro. Rat bmMSC were irradiated with 2 Gy of 60Co gamma rays at a rate of 0.83 Gy/min. ROS levels were measured to assess IR-induced oxidative stress.	Compared to sham-irradiated controls, ROS levels in irradiated bone marrow stromal cells increased by approximately 2-fold.
Soucy et al., 2011	In vivo. 7- to 12-month-old, adult, male Wistar rats underwent whole-body irradiation with 1 Gy of 56Fe heavy ions. ROS production in the aorta was measured along with changes in activity of the ROS-producing enzyme xanthine oxidase (XO) to assess IR-induced oxidative stress.	Compared to sham-irradiated controls, irradiated mice experienced a 74.6% increase in ROS production (from 4.84 to 8.45) and XO activity increased by 36.1% (6.12 to 8.33).
Soucy et al., 2010	In vivo. 4-month-old, adult, male Sprague-Dawley rats underwent whole-body irradiation with 5 Gy of 137Cs gamma rays. Changes in XO activity and ROS production were measured in the aortas of the mice to assess IR-induced oxidative stress.	Compared to sham-irradiated controls, irradiated mice experienced a ~68% increase in ROS production and a ~46% increase in XO activity.
Karam & Radwan, 2019	In vivo. Adult male Albino rats underwent irradiation with 5 Gy of 137Cs gamma rays at a rate of 0.665 cGy/s. Activity levels of the antioxidants, SOD and CAT, present in the heart tissue were measured to assess IR-induced oxidative stress.	Compared to the sham-irradiated controls, SOD and CAT activity decreased by 57% and 43%, respectively, after irradiation.
Cervelli et al., 2017	In vitro. HUVECs were irradiated with 0.25 Gy of X-rays at a rate of 91 mGy/min. ROS production was measured to analyze IR-induced oxidative stress.	Compared to the sham-irradiated controls, irradiated mice experienced a ~171% increase in ROS production (not significant).
Mansour, 2013	In vivo. Male Wistar rats underwent whole-body irradiation with 6 Gy of 137Cs gamma rays at a rate of 0.012 Gy/s. MDA was measured from heart homogenate, along with the antioxidants: SOD, GSH, and GSH-Px.	Compared to sham-irradiated controls, MDA increased by 65.9%. SOD, GSH-Px, and GSH decreased by 33.8%, 42.4%, and 50.0%, respectively.
Soltani, 2016	In vitro. HUVECs were irradiated with 2 Gy of ^{60}Co gamma rays at a dose rate of 0.6 Gy/min. Markers of oxidative stress, including reduced GSH and TBARS, were measured to assess GSH depletion and LPO, respectively.	Compared to non-irradiated controls, sham-irradiated cells experienced a ~28% decrease in GSH and a ~433% increase in TBARS.

Wang et al., 2019b	In vitro. HUVECs were irradiated with 0.2, 0.5, 1, 2, and 5 Gy of 137Cs gamma rays. ROS production was measured to assess IR-induced oxidative stress.	Compared to sham-irradiated controls, ROS production increase significantly ~32% at 5 Gy. While changes to ROS production were insignificant at doses <2 Gy, following a linear increase from 0-5 Gy.
Sharma et al., 2018	In vitro. HUVECs were irradiated with 9 Gy of photons. ROS production was measured to determine the effects of IR on oxidative stress.	Compared to sham-irradiated controls, irradiated HUVECs displayed ~133% increase in ROS production.
Hatoum et al., 2006	In vivo. Sprague-Dawley rats were irradiated with 9 fractions of 2.5 Gy of X-rays for a cumulative dose of 22.5 Gy at a rate of 2.43 Gy/min. Production of the ROS superoxide and peroxide in gut arterioles were measured to determine the level of oxidative stress caused by irradiation.	ROS production started increasing compared to the sham-irradiated control after the second dose and peaked at the fifth dose. By the ninth dose, superoxide production increased by 161.4% and peroxide production increased by 171.3%.
Phillip et al., 2020	In vitro. Human TICAE cells were irradiated with 0.25, 0.5, 2, and 10 Gy of 60Co gamma rays at a rate of 0.4 Gy/min. Levels of the antioxidants, SOD1 and PRDX5 were measured to assess oxidative stress from IR exposure.	While SOD1 levels did not follow a dose-dependent pattern. At 2 Gy, SOD1 decreased about 0.5-fold. At 1 week post-irradiation, PRDX5 remained at approximately control levels for doses <2 Gy but increased by ~60% from 2-10 Gy. PRDX5 only decreased at 2 Gy and 24h post-irradiation.
Ramadan et al., 2020	In vitro. Human TICAE/TIME cells were irradiated with 0.1 and 5 Gy of X-rays at a dose rate of 0.5 Gy/min. Intracellular ROS production was measured to determine the extent of IR-induced oxidative stress.	ROS production saw a dose-dependent increase in both TICAE and TIME cells. By 45 min post-irradiation, 0.1 Gy of IR had induced increases to ROS production of ~3.6-fold and ~8-fold in TICAE and TIME cells, respectively, compared to sham-irradiated controls. 5 Gy of IR caused more significant increases to ROS production of ~18-fold and ~17-fold in TICAE and TIME cells, respectively, compared to sham-irradiated controls.
Shen et al., 2018	In vivo. 8-week-old, female, C57BL/6 mice were irradiated with 18 Gy of X-rays. Levels of the oxidative markers, 4-HNE and 3-NT, and the antioxidants, CAT and heme oxygenase 1 (HO-1) were measured in the aortas of the mice.	Compared to sham-irradiated controls, irradiated mice saw maximum increases of ~1.75-fold on day 14 and ~2.25-fold on day 7 to 4-HNE and 3-NT levels, respectively. While CAT levels decreased up to 0.33-fold on day 7, HO-1 levels increased by ~1.9-fold on day 7.
Ungvari et al., 2013	In vitro. The CMVECs of adult male rats were irradiated with 2, 4, 6, and 8 Gy of 137Cs gamma rays. Production of the reactive oxygen species, peroxide and O2.-, were measured to assess the extent of IR-induced oxidative stress.	Compared to sham-irradiated controls, production of peroxide in CMVECs of irradiated mice 1 day post exposure increased in a dose-dependent manner from 0-8 Gy, with significant changes observed at doses >4 Gy. At 8 Gy, peroxide production had increased ~3.25-fold. Production of O2.- followed a similar dose-dependent increase with significant observed at doses >6 Gy. At 8 Gy, O2.- production increased ~1.6-fold. 14 days post-exposure, IR-induced changes to ROS production were not significant for either peroxide or O2.- and did not show a dose-dependent pattern. ROS production progressively decreased from 0-4 Gy and then recovered from 6-8 Gy back to control levels.

Ahmadi et al., 2021	In vitro. HLEC and HLE-B3 cells were exposed to 0.1, 0.25 and 0.5 Gy of gamma irradiation at 0.3 and 0.065 Gy/min. Intracellular ROS levels were measured.	In HLE-B3 cells, there were about 7 and 17% ROS-positive cells 1 h after exposure to 0.25 and 0.5 Gy respectively at 0.3 Gy/min. 24 h after exposure there were about 10% ROS-positive cells after 0.5 Gy at 0.3 Gy/min. 1 h after exposure there were about 13 and 17% ROS-positive cells at 0.25 and 0.5 Gy and 0.065 Gy/min. 24 h after exposure there were 8% ROS-positive cells after 0.5 Gy and 0.065 Gy/min. In human lens epithelial cells 1 h after exposure there were about 10 and 19% ROS-positive cells after 0.25 and 0.5 Gy at 0.3 Gy/min. After exposure to 0.5 Gy at 0.065 Gy/min there were about 16 and 9% ROS-positive cells one and 24 h after exposure.
Ji et al, 2015	In vitro. HLECs were exposed to UVB irradiation (297 nm; 2 W/m2) for 0 – 120 min. Total antioxidative capability (T-AOC), ROS levels, MDA, and SOD were measured at various time points at 5-120 min.	HLECs exposed to 1 W/m2 UVB for 0 - 120 min (representative of dose) showed a gradual increase in ROS levels that began to plateau 105 min post-irradiation at an ROS level 750 000x control.
Hua et al, 2019	In vitro. HLECs were exposed to 4050, 8100 and 12,150 J/m2 of UVB-irradiation at 1.5, 3.0 and 4.5 W/m2. MDA, SOD, GSH-Px, and GSH were measured.	MDA activity as a ratio of the control increased about 1.5 at 3.0 W/m2 and about 3 at 4.5 W/m2. SOD activity as a ratio of the control decreased about 0.1 at 1.5 W/m2, 0.2 at W/m2, and 0.3 at 4.5 W/m2. GSH-Px activity as a ratio of the control decreased about 0.02 at 3.0 W/m2 and 0.2 at 4.5 W/m2. GSH activity as a ratio of the control decreased about 0.2 at 3.0 W/m2 and 0.7 at 4.5 W/m2.
Chen et al, 2021	In vivo. Male rats were irradiated with 0, 0.4, 1.2 and 3.6 Sv of neutron-irradiation at 14, 45 and 131 mSv/h. In rat lenses, MDA, GSH, and SOD, were measured.	MDA concentration decreased by about 1.5 nmol/mg protein at 1.2 Sv and increased by about 7.5 nmol/mg protein relative to the control at 3.6 Sv. GSH concentration increased by about 3.5 µg/mg protein and decreased by about 1 µg/mg protein relative to the control at 3.6 Sv (neutron radiation). SOD activity decreased by about 0.08 U/mg protein relative to the control at 3.6 Sv. It should be noted that Sv is not the correct unit when investigating animals and cultured cells, radiation should have been measured in Gy (ICRU, 1998).
Zigman et al., 2000	In vitro. Rabbit LECs were exposed to 3-12 J/cm2 of UVA-irradiation (300-400 nm range, 350 nm peak). CAT activity was assayed to demonstrate oxidative stress.	Rabbit LECs exposed to 3 – 12 J/cm2 UVA showed an approximately linear decrease in catalase activity (indicative of increased oxidative stress) with the maximum dose displaying a 3.8x decrease.
Chitchumroonchokchai et al, 2004	In vitro. HLECs were exposed to 300 J/m2 of UVB-irradiation at 3 mW/cm2. MDA and HAE were used to measure oxidative stress.	The concentration of MDA and HAE increased by about 900 pmol/mg protein compared to the control after irradiation with 300 J/m2 UVB.

Zigman et al, 1995	In vitro. Rabbit and squirrel LECs were exposed to 6, 9, 12, 15 and 18 J/m2 of UV-irradiation at 3 J/cm2/h (300-400 nm range, 350 nm peak). CAT was used to measure oxidative stress levels.	The CAT activity was 10% of the control activity at 6 J/cm2, and then decreased to 0% of the control activity at 18 J/cm2 (99.9% UV-A and 0.1% UV-B).
Karimi et al, 2017	In vivo. Adult rats were exposed to 15 Gy of gamma 60Co-irradiation at a dose rate of 98.5 cGy/min. In lens tissue, MDA, thiobarbituric acid (TBA), and GSH levels were used to indicate oxidative stress.	MDA concentration increased from 0.37 +/- 0.03 to 1.60 +/- 0.16 nmol/g of lens after irradiation. GSH concentration decreased from 0.99 +/- 0.06 to 0.52 +/- 0.16 µmol/g of lens after exposure.
Rong et al., 2019	In vitro. HLECs were exposed to UVB-irradiation (297 nm; 2 W/m^2 for 10 min). Intracellular H_2O_2 and superoxide levels were measured.	The amount of ROS was measured as the dicholofluoroscein (DCFH-DA) fluorescence density, which increased about 10-fold relative to the control. A similar test but with dihydroethidium (DHE) staining showed a fluorescence density increase of about 3-fold relative to the control.
Kubo et al., 2010	In vitro. Lenses isolated from mice were exposed to 400 or 800 J/m2 of UVB-irradiation. ROS levels were measured.	The ratio of ROS level/survived LECs increased from about 175 to 250% after exposure to 400 and 800 J/m2 UVB respectively.
Kang et al., 2020	In vitro. HLECs were exposed to 0.09 mW/cm2 UVB-irradiation (275-400 nm range, 310 nm peak) for 15 min. MDA and SOD activity were measured.	MDA activity increased about 30% compared to control after 15 min of 0.09 mW/cm2 UVB exposure. SOD activity decreased about 50% compared to control under the same conditions.
Yang et al., 2020	In vitro. HLEs were irradiated with 30 mJ/cm2 of UVB-irradiation. ROS levels were determined.	The level of ROS production in HLEs increased approximately 5-fold as determined by 2',7'-dichlorofluorescein diacetate after exposure to 30 mJ/cm2 UVB.
Zhang et al., 2012	In vivo. Adult mice were exposed to 20.6 kJ/m2 UV-irradiation (313 nm peak; 1.6 mW/cm2). GSH levels were measured in lens homogenates.	Decrease in GSH of about 1 and 2 µmol/g wet weight compared to control after 1 and 16 months respectively after 20.6 kJ/m2 UV (313 nm peak) at 1.6 mW/cm2.

Time-scale

Time Concordance

Reference	Experiment Description	Result
Tseng et al., 2014	In vitro. Neural stem/precursor cells isolated from mouse subventricular and hippocampal dentate subgranular zones were exposed to 1-200 cGy of 56Fe irradiation at dose rates ranging from 5-50 cGy/min. RONS were measured from 1 to 8 weeks post-irradiation.	Compared to sham-irradiated controls, a trend toward increasing oxidative stress was seen, particularly at 1- and 4-weeks post-irradiation where RONS levels showed dose-responsive increases. The greatest rise was also seen at 10 cGy where relative RONS levels increased ~2-fold from 1 to 4 weeks, ~3-fold from 4 to 6 weeks and ~2 fold from 6 to 8 weeks. RONS were also found increased at doses as low as 2 cGy at 12 and 24h post-irradiation.
Suman et al., 2013	In vivo. Female mice were exposed to either 1.3 Gy of 56Fe irradiation (1 GeV/nucleon; dose rate of 1 Gy/min) or 2 Gy of gamma irradiation (dose rate of 1 Gy/min). ROS were measured in cerebral cortical cells at 2 and 12 months.	ROS levels showed statistically significant increases after 56Fe irradiation at both 2 and 12 months, while gamma irradiation led to an increase at only 2 months. The percent fluorescence intensity of ROS levels for control, gamma irradiated and 56Fe-irradiated were approximately 100, 115 and 140 at 2 months, and 100, 90 and 125 at 12 months, respectively.

Limoli et al., 2004	In vitro. Neural stem/precursor cells isolated from mouse subventricular and hippocampal dentate subgranular zones were exposed to 1 or 5 Gy of 56Fe irradiation at dose rates ranging from 4.5 Gy/min. RONS were measured at various time points until 33 days post-exposure.	ROS levels exhibited statistically significant fluctuations, increasing over the first 12h before dropping at 18h and rising again at 24h. At 5 Gy, ROS levels fluctuated with a peak at 7 days, a decrease at 13 days, an increase at 25 days, and a decrease below control levels at 33 days. At 1 Gy, ROS levels peaked at 25 days and also decreased below control at 33 days.
Gledzinski et al., 2005	In vitro. Neural precursor cells derived from rats were irradiated with 1, 2, 5 and 10 Gy of proton (1.7-1.9 Gy/min). ROS levels were determined at 5-25h post-irradiation.	Proton irradiation led to a rapid rise in ROS levels, with the increase most marked at 6h (approximately 10-70% for 1 and 10 Gy, respectively). The increase in ROS persisted for 24h, mainly for 10 Gy where the ROS levels were around 30% above control at the 12, 18 and 24h mark.
Acharya et al., 2010	In vitro. Human neural stem cells were subjected to 1, 2 or 5 Gy of gamma irradiation at a dose rate of 2.2 Gy/min. RONS and superoxide levels were measured at various time points until 7 days.	Intracellular RONS and superoxide levels showed significant increase from 2- to 4-fold at 12h. At 7 days, levels of RONS increased and were dose-responsive, elevated by ~3- to 7-fold and 3- to 5-fold, respectively, over sham-irradiated controls.
Rugo and Schiestl, 2004	In vitro. Human lymphoblast cell lines (TK6 and TK6 E6) were irradiated with 2 Gy of X-irradiation at a dose rate of 0.72 Gy/min. ROS levels were measured at various time points until 29 days.	In the TK6 E6 clones, there was only a significant ROS increase at day 29 (45.7 DCF fluorescence units). In the TK6 clones, there were significant ROS increases at days 13 (26.0 DCF fluorescence units), 15 (26.3 DCF fluorescence units) and 20 (38.1 DCF fluorescence units), with a strong trend of increased ROS in the treated group at day 25. On day 18, ROS levels decreased in the irradiated group, and there was no significant difference at day 29.
Huang et al., 2018	In vitro. Murine RAW264.7 cells were irradiated with 2 Gy of gamma rays at a rate of 0.83 Gy/min. ROS levels were measured at 2 and 8 h post-irradiation.	ROS levels in irradiated RAW264.7 cells decreased by ~10% from 2 h post-exposure to 8 h post-exposure (from ~138% above control at 2 h to ~98% above control at 8).
Zhang et al., 2018	In vitro. hBMMSCs were irradiated with 2 Gy of X-rays at a rate of 0.6 Gy/min. Relative ROS concentration was measured at 0, 0.5, 2, 3, 6, 8, and 12 h post-irradiation.	ROS levels increased in time dependent manner until a peak of ~90% above control level at 3 h-post irradiation, and then steadily declined back to approximately control levels at 12 h post-irradiation.
Phillip et al., 2020	In vitro. Human TICAE cells were irradiated with 0.25, 0.5, 2, and 10 Gy of 60Co gamma rays at a rate of 400 mGy/min. Levels of the antioxidants, SOD1 and PRDX5 were measured at 4 h, 24 h, 48 h, and 1-week post-irradiation to assess oxidative stress from IR exposure.	SOD1 levels did not follow a time-dependent pattern. However, SOD1 decreased at 2 Gy for every timepoint post-irradiation. While PRDX5 levels stayed at approximately baseline levels for the first two days after exposure to 10 Gy of radiation, levels elevated by ~1.6-fold after 1 week.
Ramadan et al., 2020	In vitro. Human TICAE/TIME cells were irradiated with 0.1 and 5 Gy of X-rays at a rate of 0.5 Gy/min. Intracellular ROS production was measured at 45 min, 2 h, and 3 h post-irradiation.	After irradiation, ROS production saw time-dependent decreases in both TICAE and TIME cells from 45 min to 3 h post-exposure. ROS production was elevated at 45 min but returned to approximately baseline levels at 2 and 3 h.
Shen et al., 2018	In vivo. 8-week-old, female, C57BL/6 mice were irradiated with 18 Gy of X-rays. Levels of the oxidative markers, 4-HNE and 3-NT, and the antioxidants, CAT and heme HO-1 were measured the aortas of the mice at 3, 7, 14, 28, and 84 days post-irradiation.	Significant changes were observed in 4-HNE, 3-NT, CAT, and HO-1 levels of irradiated mice after 3 days. 3-NT and HO-1 levels increased from days 3 to 7 and then progressively decreased, while 4-HNE levels followed the same pattern but with a peak at day 14. CAT levels were at their lowest at day 3 and followed a time dependent increase until day 84.
Ungvari et al., 2013	In vitro. The CMVECs of adult male rats were irradiated with 2, 4, 6, and 8 Gy of 137Cs gamma rays. Production of the reactive oxygen species, peroxide and superoxide, were measured at 1- and 14-days post-irradiation.	ROS production was generally higher at day 1 than day 14, with the difference becoming progressively more significant from 2-8 Gy. Peroxide production was reduced from a ~3.25-fold increase compared to controls at day 1 back to baseline levels at day 14. Superoxide production had a ~1.6-fold increase at day 1 recover to baseline levels at day 14.

Ahmadi et al., 2021	In vitro. HLEC and HLE-B3 cells were exposed to 0.1, 0.25 and 0.5 Gy of gamma irradiation at 0.3 and 0.065 Gy/min. ROS levels were measured.	In human LECs immediately exposed to 0.25 Gy gamma rays, the level of ROS positive cells increased by 5%, relative to control, 1 h post-irradiation.
Jiang et al., 2006	In vitro. HLECs were exposed to UV-irradiation at a wavelength over 290 nm (30 mJ/cm2). ROS levels were measured.	Approximately 10-fold increase in ROS generation 15 min after exposure to 30 mJ/cm2 UV.
Pendergrass et al., 2010	In vivo. Female mice were irradiated with 11 Gy of X-irradiation at a dose rate of 2 Gy/min. ROS levels in the lenses were used to represent oxidative stress.	9 months after irradiation with 11 Gy X-rays at 2 Gy/min there's 2250% cortical ROS relative to the control. 3 months after there was no significant change.
Belkacemi et al., 2001	In vitro. Bovine lens cells were exposed to 10 Gy of X-irradiation at 2 Gy/min. GSH levels were measured.	The intracellular GSH pool was measured by a decrease of about 15% monobromobimane fluorescence relative to the control 24 h after exposure to 10 Gy X-rays at 2 Gy/min and there was a decrease of about 40% relative to the control by 120 h.
Weinreb and Dovrat, 1996	In vitro. Bovine lenses were irradiated with 22.4 J/cm2 (10 min) and 44.8 J/cm2 (100 min) of UVA-irradiation at 8.5 mW/cm2. CAT levels were determined.	CAT activity decreased from 1.75 (control) to 0.5 U/mg protein at 48-168 h after exposure to 44.8 J/cm2 UV-A.
Cencer et al., 2018	In vitro. HLECs were exposed to 0.014 and 0.14 J/cm^2 of UVB-irradiation at 0.09, 0.9 mW/cm^2 for 2 and 5 min. ROS levels (mainly H_2O_2) were measured.	About 5 min after exposure to both 0.09 and 0.9 mW/cm2 UVB for 2.5 min there is an increase of about 4 average brightness minus control (densitometric fluorescence scanning for ROS, mostly indicating H_2O_2). About 90 and 120 min after exposure to 0.9 mW/cm^2 the average brightness minus control is about 35 and 20 respectively.
Yang et al., 2020	In vitro. HLECs were irradiated with 30 mJ/cm2 of UVB-irradiation. Intracellular ROS levels were measured.	The level of ROS production in HLECs increased approximately 5-fold as determined by 2',7'-dichlorofluorescein diacetate 24 h after exposure to 30 mJ/cm2 UVB.
Zhang et al., 2012	In vivo. Adult mice were exposed to 20.6 kJ/m2 UV-irradiation (313 nm peak; 1.6 mW/cm2). GSH levels were measured in lens homogenates.	Decrease in GSH of about 1 and 2 µmol/g wet weight compared to control after 1 and 16 months respectively after 20.6 kJ/m2 UV (313 nm peak) at 1.6 mW/cm2.

Known modulating factors

Modulating Factors	MF details	Effects on the KER	References
Antioxidants	CAT, GSH-Px, SOD, PRDX, vitamin E, C, carotene, lutein, zeaxanthin, selenium, zinc, alpha-lipoic acid, melatonin, ginko biloba leaf, fermented ginkobiloba leaf, Nigella sativa oil, thymoquinone, and ferulic acid	Adding or withholding antioxidants will decrease or increase the level of oxidative stress respectively	(Zigman et al., 1995; Belkacémi et al., 2001; Chitchumroonchokchai et al., 2004; Fatma et al., 2005; Jiang et al., 2006; Fletcher, 2010; Karimi et al., 2017; Hua et al., 2019; Kang et al., 2020; Yang et al., 2020; Manda et al., 2008; Limoli et al., 2007; Manda et al., 2007; Taysi et al., 2012; Ismail et al., 2016; Demir et al., 2020; Chen et al., 2021)

Age	Increased age	Antioxidant levels are lower and show a greater decrease after radiation in older organisms. This compromises their defence system, resulting in ROS increases and therefore, an increased likelihood of oxidative stress	(Marshall, 1985; Spector, 1990; Giblin et al., 2002; Kubo et al., 2010; Pendergrass et al., 2010; Zhang et al., 2012; Hamada et al., 2014; Tangvarasittichai & Tangvarasittichai, 2019)
Oxygen	Increased oxygen levels	Higher oxygen concentrations increase sensitivity to ROS	(Hightower et al., 1992; Eaton, 1994; Huang et al., 2006; Zhang et al., 2010; Schoenfeld et al., 2012)

Known Feedforward/Feedback loops influencing this KER

The relationship between deposition of energy and increased oxidative stress leads to several feedforward loops. Firstly, ROS activates the transforming growth factor beta (TGF)-β, which increases the production of ROS. This process is modulated in normal cells containing PRDX-6, or cells with added MnTBAP, which will both prevent TGF-β from inducing ROS formation (Fatma et al., 2005). Secondly, ROS can damage human mitochondrial DNA (mtDNA), this can then cause changes to the cellular respiration mechanisms, leading to increased ROS production (Turner et al., 2002; Zhang et al., 2010; Tangvarasittichai & Tangvarasittichai, 2019, Ahmadi et al., 2021; Yves, 2000). Some other feedback loops through which deposition of energy causes oxidative stress are discussed by Soloviev & Kizub (2019).

References

Acharya, M. M. et al. (2010), “Consequences of ionizing radiation-induced damage in human neural stem cells”, Free radical biology & medicine, Vol. 49/12, Elsevier, Amsterdam, https://doi.org/10.1016/j.freeradbiomed.2010.08.021.

Ahmadi, M. et al. (2021), “Early responses to low-dose ionizing radiation in cellular lens epithelial models”, Radiation research, Vol. 197/1, Radiation Research Society, Bozeman, https://doi.org/10.1667/RADE-20-00284.1

Attar, M., Y. M. Kondolousy, N. Khansari, (2007), “Effect of High Dose Natural Ionizing Radiation on the Immune System of the Exposed Residents of Ramsar Town, Iran”, Iranian Journal of Allergy, Asthma and Immunology, Vol. 6/2, pp. 73-78.

Bai, J. et al. (2020), “Irradiation-induced senescence of bone marrow mesenchymal stem cells aggravates osteogenic differentiation dysfunction via paracrine signaling”, American Journal of Physiology - Cell Physiology, Vol. 318/5, American Physiological Society, Rockville, https://doi.org/10.1152/ajpcell.00520.2019.

Balasubramanian, D (2000), “Ultraviolet radiation and cataract”, Journal of ocular pharmacology and therapeutics, Vol. 16/3, Mary Ann Liebert Inc., Larchmont, https://doi.org/10.1089/jop.2000.16.285.

Baluchamy, S. et al. (2012), “Reactive oxygen species mediated tissue damage in high energy proton irradiated mouse brain”, Molecular and cellular biochemistry, Vol. 360/1-2, Springer, London, https://doi.org/10.1007/s11010-011-1056-2.

Baulch, J. E. et al. (2015), “Persistent oxidative stress in human neural stem cells exposed to low fluences of charged particles Redox biology, Vol. 5, Elsevier, Amsterdam, https://doi.org/10.1016/j.redox.2015.03.001.

Belkacémi, Y. et al. (2001), “Lens epithelial cell protection by aminothiol WR-1065 and anetholedithiolethione from ionizing radiation”, International journal of cancer, Vol. 96, John Wiley & Sons, Ltd., Hoboken, https://doi.org/10.1002/ijc.10346.

Cabrera M., R. Chihuailaf and F. Wittwer Menge (2011), “Antioxidants and the integrity of ocular tissues”, Veterinary medicine international, Vol. 2011, Hindawi, London, https://doi.org/10.4061/2011/905153.

Cadet, J. et al. (2012), “Oxidatively generated complex DNA damage: tandem and clustered lesions”, Cancer letters, Vol. 327, Elsevier, Amsterdam, https://doi.org/10.1016/j.canlet.2012.04.005.

Cencer, C. et al. (2018), “PARP-1/PAR activity in cultured human lens epithelial cells exposed to tow levels of UVB light”, Photochemistry and photobiology, Vol. 94, John Wiley & Sons, Ltd., Hoboken, https://doi.org/10.1111/php.12814.

Cervelli, T. et al. (2017), “A New Natural Antioxidant Mixture Protects against Oxidative and DNA Damage in Endothelial Cell Exposed to Low-Dose Irradiation”, Oxidative medicine and cellular longevity, Vol. 2017, Hindawi, London, https://doi.org/10.1155/2017/9085947.

Chen, Y. et al. (2021), “Effects of neutron radiation on Nrf2-regulated antioxidant defence systems in rat lens”, Experimental and therapeutic medicine, Vol. 21/4, Spandidos Publishing Ltd, Athens, https://doi.org/10.3892/etm.2021.9765.

Chien, L. et al. (2015) “Low-dose ionizing radiation induces mitochondrial fusion and increases expression of mitochondrial complexes I and III in hippocampal neurons”, Oncotarget, Vol. 6/31, Impact Journals, Orchard Park, https://doi.org/10.18632/oncotarget.5790

Chitchumroonchokchai, C. et al. (2004), “Xanthophylls and α-tocopherol decrease UVB-induced lipid peroxidation and stress signaling in human lens epithelial cells”, The Journal of Nutrition, Vol. 134/12, American Society for Nutritional Sciences, Bethesda, https://doi.org/10.1093/jn/134.12.3225.

Collins-Underwood, J. R. et al. (2008), “NADPH oxidase mediates radiation-induced oxidative stress in rat brain microvascular endothelial cells”, Free radical biology & medicine, Vol. 45/6, Elsevier, Amsterdam, https://doi.org/10.1016/j.freeradbiomed.2008.06.024.

de Jager, T.L., Cockrell, A.E., Du Plessis, S.S. (2017), “Ultraviolet Light Induced Generation of Reactive Oxygen Species”, in Ultraviolet Light in Human Health, Diseases and Environment. Advances in Experimental Medicine and Biology, Springer, Cham, https://doi.org/10.1007/978-3-319-56017-5_2

Demir, E. et al. (2020), “Nigella sativa oil and thymoquinone reduce oxidative stress in the brain tissue of rats exposed to total head irradiation”, International journal of radiation biology, Vol. 96/2, Informa, London, https://doi.org/10.1080/09553002.2020.1683636.

Eaton, J. W. (1994), “UV-mediated cataractogenesis: A radical perspective”, Documenta ophthalmologica, Vol. 88, Springer, London, https://doi.org/10.1007/BF01203677.

Fatma, N. et al. (2005), “Impaired homeostasis and phenotypic abnormalities in Prdx6-/- mice lens epithelial cells by reactive oxygen species: Increased expression and activation of TGFβ”, Cell death and differentiation, Vol. 12, Nature Portfolio, London, https://doi.org/10.1038/sj.cdd.4401597.

Fletcher, A. E (2010), “Free radicals, antioxidants and eye diseases: evidence from epidemiological studies on cataract and age-related macular degeneration”, Ophthalmic Research, Vol. 44, Karger International, Basel, https://doi.org/10.1159/000316476.

Ganea, E. and J. J. Harding (2006), “Glutathione-related enzymes and the eye”, Current eye research, Vol. 31/1, Informa, London, https://doi.org/10.1080/02713680500477347.

Giblin, F. J. et al. (2002), “UVA light in vivo reaches the nucleus of the guinea pig lens and produces deleterious, oxidative effects”, Experimental eye research, Vol. 75/4, Elsevier, Amsterdam, https://doi.org/10.1006/exer.2002.2039.

Giedzinski, E. et al. (2005), “Efficient production of reactive oxygen species in neural precursor cells after exposure to 250 MeV protons”, Radiation research, Vol. 164/4, Radiation Research Society, Bozeman, https://doi.org/10.1667/rr3369.1.

Hamada, N. et al. (2014), “Emerging issues in radiogenic cataracts and cardiovascular disease”, Journal of radiation research, Vol. 55/5, Oxford University Press, Oxford, https://doi.org/10.1093/jrr/rru036.

Hatoum, O. A. et al. (2006), “Radiation induces endothelial dysfunction in murine intestinal arterioles via enhanced production of reactive oxygen species”, Arteriosclerosis, Thrombosis, and Vascular Biology, Vol. 26/2, Lippincot Williams & Wilkins, Philadelphia, https://doi.org/10.1161/01.ATV.0000198399.40584.8C.

Hightower, K. and J. McCready (1992), “Mechanisms involved in cataract development following near-ultraviolet radiation of cultured lenses”, Current eye research, Vol. 11/7, Informa, London, https://doi.org/10.3109/02713689209000741.

Hightower, K. R. (1995), “The role of the lens epithelium in development of UV cataract”, Current eye research, Vol. 14/1, Informa, London, https://doi.org/10.3109/02713689508999916.

Hua, H. et al. (2019), “Protective effects of lanosterol synthase up-regulation in UV-B-induced oxidative stress”, Frontiers in pharmacology, Vol. 10, Frontiers Media SA, Lausanne, https://doi.org/10.3389/fphar.2019.00947.

Huang, L. et al. (2006), “Oxidation-induced changes in human lens epithelial cells 2. Mitochondria and the generation of reactive oxygen species”, Free radical biology & medicine, Vol. 41/6, Elsevier, Amsterdam, https://doi.org/10.1016/j.freeradbiomed.2006.05.023.

Huang, B. et al. (2019), “Amifostine suppresses the side effects of radiation on BMSCs by promoting cell proliferation and reducing ROS production”, Stem Cells International, Vol. 2019, Hindawi, London, https://doi.org/10.1155/2019/8749090.

Huang, B. et al. (2018), “Sema3a inhibits the differentiation of raw264.7 cells to osteoclasts under 2gy radiation by reducing inflammation”, PLoS ONE, Vol. 13/7, PLOS, San Francisco, https://doi.org/10.1371/journal.pone.0200000.

ICRU (1998), “ICRU report 57: conversion coefficients for use in radiological protection against external radiation”, Journal of the ICRU, Vol. 29/2, SAGE Publishing

Ismail, A. F. and S. M. El-Sonbaty (2016), “Fermentation enhances Ginkgo biloba protective role on gamma-irradiation induced neuroinflammatory gene expression and stress hormones in rat brain”, Journal of photochemistry and photobiology. B, Biology, Vol. 158, Elsevier, Amsterdam, https://doi.org/10.1016/j.jphotobiol.2016.02.039.

Ji, Y. et al. (2015), “The mechanism of UVB irradiation induced-apoptosis in cataract”, Molecular and cellular biochemistry, Vol. 401, Springer, London, https://doi.org/10.1007/s11010-014-2294-x.

Jiang, Q. et al. (2006), “UV radiation down-regulates Dsg-2 via Rac/NADPH oxidase-mediated generation of ROS in human lens epithelial cells”, International Journal of Molecular Medicine, Vol. 18/2, Spandidos Publishing Ltd, Athens, https://doi.org/10.3892/ijmm.18.2.381.

Jurja, S. et al. (2014), “Ocular cells and light: harmony or conflict?”, Romanian Journal of Morphology & Embryology, Vol. 55/2, Romanian Academy Publishing House, Bucharest, pp. 257–261.

Kang, L. et al. (2020), “Ganoderic acid A protects lens epithelial cells from UVB irradiation and delays lens opacity”, Chinese journal of natural medicines, Vol. 18/12, Elsevier, Amsterdam, https://doi.org/10.1016/S1875-5364(20)60037-1.

Karam, H. M. and R. R. Radwan (2019), “Metformin modulates cardiac endothelial dysfunction, oxidative stress and inflammation in irradiated rats: A new perspective of an antidiabetic drug”, Clinical and Experimental Pharmacology and Physiology, Vol. 46/12, Wiley-Blackwell, Hoboken, https://doi.org/10.1111/1440-1681.13148.

Karimi, N. et al. (2017), “Radioprotective effect of hesperidin on reducing oxidative stress in the lens tissue of rats”, International Journal of Pharmaceutical Investigation, Vol. 7/3, Phcog Net, Bengaluru, https://doi.org/10.4103/jphi.JPHI_60_17.

Kłuciński, P. et al. (2008), “Erythrocyte antioxidant parameters in workers occupationally exposed to low levels of ionizing radiation”, Annals of Agricultural and Environmental Medicine, Vol. 15/1, pp. 9-12.

Kook, S. H. et al. (2015), “Irradiation inhibits the maturation and mineralization of osteoblasts via the activation of Nrf2/HO-1 pathway”, Molecular and Cellular Biochemistry, Vol. 410/1-2, Springer, London, https://doi.org/10.1007/s11010-015-2559-z.

Kubo, E. et al. (2010), “Protein expression profiling of lens epithelial cells from Prdx6-depleted mice and their vulnerability to UV radiation exposure”, American Journal of Physiology, Vol. 298/2, American Physiological Society, Rockville, https://doi.org/10.1152/ajpcell.00336.2009.

Lee, J. et al. (2004), “Reactive oxygen species, aging, and antioxidative nutraceuticals”, Comprehensive reviews in food science and food safety, Vol. 3/1, Blackwell Publishing Ltd, Oxford, http://doi.org/10.1111/j.1541-4337.2004.tb00058.x.

Limoli, C. L. et al. (2007), “Redox changes induced in hippocampal precursor cells by heavy ion irradiation”, Radiation and environmental biophysics, Vol. 46/2, Springer, London, https://doi.org/10.1007/s00411-006-0077-9.

Limoli, C. L. et al. (2004), “Radiation response of neural precursor cells: linking cellular sensitivity to cell cycle checkpoints, apoptosis and oxidative stress”, Radiation research, Vol. 161/1, Radiation Research Society, Bozeman, https://doi.org/10.1667/rr3112.

Liu, F. et al. (2019), “Transcriptional response of murine bone marrow cells to total-body carbon-ion irradiation”, Mutation Research - Genetic Toxicology and Environmental Mutagenesis, Vol. 839, Elsevier, Amsterdam, https://doi.org/10.1016/j.mrgentox.2019.01.014.

Liu, Y. et al. (2018), “Protective effects of α2macroglobulin on human bone marrow mesenchymal stem cells in radiation injury”, Molecular Medicine Reports, Vol. 18/5, Spandidos Publishing Ltd, Athens, https://doi.org/10.3892/mmr.2018.9449.

Manda, K. et al. (2007), “Melatonin attenuates radiation-induced learning deficit and brain oxidative stress in mice”, Acta neurobiologiae experimentalis, Vol. 67/1, Nencki Institute of Experimental Biology, Warsaw, pp. 63 –70.

Manda, K., M. Ueno and K. Anzai (2008), “Memory impairment, oxidative damage and apoptosis induced by space

radiation: ameliorative potential of alpha-lipoic acid", Behavioural brain research, Vol. 187/2, Elsevier, Amsterdam, https://doi.org/10.1016/j.bbr.2007.09.033.

Mansour, H. H. (2013), "Protective effect of ginseng against gamma-irradiation-induced oxidative stress and endothelial dysfunction in rats", EXCLI Journal, Vol. 12, Leibniz Research Centre for Working Environment and Human Factors, Dortmund, pp. 766-777.

Marshall, J. (1985), "Radiation and the ageing eye", Ophthalmic & physiological optics, Vol. 5, Wiley-Blackwell, Hoboken, https://doi.org.10.1111/j.1475-1313.1985.tb00666.x.

Padgaonkar, V. A. et al. (2015) "Thioredoxin reductase activity may be more important than GSH level in protecting human lens epithelial cells against UVA light", Photochemistry and photobiology, Vol. 91/2, Wiley-Blackwell, Hoboken, https://doi.org/10.1111/php.12404.

Pendergrass, W. et al. (2010), "X-ray induced cataract is preceded by LEC loss, and coincident with accumulation of cortical DNA, and ROS; similarities with age-related cataracts", Molecular Vision, Vol. 16, Emory University, Atlanta, pp. 1496-513.

Philipp, J. et al. (2020), "Radiation Response of Human Cardiac Endothelial Cells Reveals a Central Role of the cGAS-STING Pathway in the Development of Inflammation", Proteomes, Vol. 8/4, Multidisciplinary Digital Publishing Institute, Basel, https://doi.org/10.3390/proteomes8040030.

Quan, Y. et al. (2021), "Connexin hemichannels regulate redox potential via metabolite exchange and protect lens against cellular oxidative damage", Redox biology, Vol. 46, Elsevier, Amsterdam, https://doi.org/10.1016/j.redox.2021.102102.

Ramadan, R. et al. (2020), "Connexin43 Hemichannel Targeting With TAT-Gap19 Alleviates Radiation-Induced Endothelial Cell Damage", Frontiers in Pharmacology, Vol. 11, Frontiers Media SA, Lausanne, https://doi.org/10.3389/fphar.2020.00212

Rehman, M. U. et al. (2016), "Comparison of free radicals formation induced by cold atmospheric plasma, ultrasound, and ionizing radiation", Archives of biochemistry and biophysics, Vol. 605, Elsevier, Amsterdam, https://doi.org/10.1016/j.abb.2016.04.005.

Rogers, C. S. et al. (2004), "The effects of sub-solar levels of UV-A and UV-B on rabbit corneal and lens epithelial cells", Experimental eye research, Vol. 78, Elsevier, Amsterdam, https://doi.org/10.1016/j.exer.2003.12.011.

Rong, X. et al. (2019), "TRIM69 inhibits cataractogenesis by negatively regulating p53", Redox biology, Vol. 22, Elsevier, Amsterdam, https://doi.org/10.1016/j.redox.2019.101157.

Rugo, R. E. and R. H. Schiestl (2004), "Increases in oxidative stress in the progeny of X-irradiated cells", Radiation research, Vol. 162/4, Radiation Research Society, Bozeman, https://doi.org/10.1667/rr3238.

Santos, A. L., S. Sinha, and A. B. Linder (2018), "The good, the bad, and the ugly of ROS: New insights on aging and aging-related diseases from eukaryotic and prokaryotic model organisms", Oxidative medicine and cellular longevity, Vol. 2018, Hindawi, London, https://doi.org/10.1155/2018/1941285.

Schoenfeld, M. P. et al. (2012), "A hypothesis on biological protection from space radiation through the use of new therapeutic gases as medical counter measures", Medical gas research, Vol. 2/8, BioMed Central Ltd, London, https://doi.org/10.1186/2045-9912-2-8.

Sharma, U. C. et al. (2018), “Effects of a novel peptide Ac-SDKP in radiation-induced coronary endothelial damage and resting myocardial blood flow”, Cardio-oncology, Vol. 4, BioMed Central Ltd, London, https://doi.org/10.1186/s40959-018-0034-1.

Shen, Y. et al. (2018), “Transplantation of bone marrow mesenchymal stem cells prevents radiation-induced artery injury by suppressing oxidative stress and inflammation”, Oxidative Medicine and Cellular Longevity, Vol. 2018, Hindawi, London, https://doi.org/10.1155/2018/5942916.

Slezak, J. et al. (2017), “Potential markers and metabolic processes involved in the mechanism of radiation-induced heart injury”, Canadian journal of physiology and pharmacology, Vol. 95/10, Canadian Science Publishing, Ottawa, https://doi.org/10.1139/cjpp-2017-0121.

Soloviev, A. I. and I.V. Kizub (2019), “Mechanisms of vascular dysfunction evoked by ionizing radiation and possible targets for its pharmacological correction”, Biochemical pharmacology, Vol. 159, Elsevier, Amsterdam, https://doi.org/10.1016/j.bcp.2018.11.019.

Soltani, B. (2016), “Nanoformulation of curcumin protects HUVEC endothelial cells against ionizing radiation and suppresses their adhesion to monocytes: potential in prevention of radiation-induced atherosclerosis”, Biotechnology Letters, Vol. 38, Springer, London, https://doi.org/10.1007/s10529-016-2189-x.

Soucy, K. G. et al. (2011), “HZE 56Fe-Ion Irradiation Induces Endothelial Dysfunction in Rat Aorta: Role of Xanthine Oxidase”, Radiation Research, Vol. 176/4, Radiation Research Society, Bozeman, https://doi.org/10.1667/RR2598.1.

Soucy, K. G. et al. (2010), “Dietary inhibition of xanthine oxidase attenuates radiation-induced endothelial dysfunction in rat aorta”, Journal of Applied Physiology, Vol. 108/5, American Physiological Society, Rockville, https://doi.org/10.1152/japplphysiol.00946.2009.

Spector, A. (1990), “Oxidation and aspects of ocular pathology”, The CLAO journal, Vol. 16, Contact Lens Association of Ophthalmologists, Colorado, pp. S8-10.

Stohs, S. (1995), “The role of free radicals in toxicity and disease”, Journal of Basic and Clinical Physiology and Pharmacology, Vol. 6/3-4, Walter de Gruyter GmbH, Berlin, pp. 205-228.

Suman, S. et al. (2013), “Therapeutic and space radiation exposure of mouse brain causes impaired DNA repair response and premature senescence by chronic oxidant production”, Aging, Vol. 5/8, Impact Journals, Orchard Park, https://doi.org/10.18632/aging.100587.

Tahimic, C. G. T., and R. K. Globus (2017), “Redox signaling and its impact on skeletal and vascular responses to spaceflight”, International Journal of Molecular Sciences, Vol. 18/10, Multidisciplinary Digital Publishing Institute, Basel, https://doi.org/10.3390/ijms18102153.

Tangvarasittichai, O. and S. Tangvarasittichai (2018), “Oxidative stress, ocular disease and diabetes retinopathy”, Current pharmaceutical design, Vol. 24/40, Bentham Science Publishers, Sharjah, https://doi.org/10.2174/1381612825666190115121531.

Taysi, S. et al. (2012), “Zinc administration modulates radiation-induced oxidative injury in lens of rat”, Pharmacognosy Magazine, Vol. 8/2, https://doi.org/10.4103/0973-1296.103646

Tian, Y. et al. (2017), “The Impact of Oxidative Stress on the Bone System in Response to the Space Special Environment”, International Journal of Molecular Sciences, Vol. 18/10, Multidisciplinary Digital Publishing Institute, Basel, https://doi.org/10.3390/ijms18102132.

Tseng, B. P. et al. (2014), “Functional consequences of radiation-induced oxidative stress in cultured neural stem cells

and the brain exposed to charged particle irradiation", Antioxidants & redox signaling, Vol. 20/9, Mary Ann Leibert Inc., Larchmont, https://doi.org/10.1089/ars.2012.5134.

Turner, N. D. et al. (2002), "Opportunities for nutritional amelioration of radiation-induced cellular damage", Nutrition, Vol. 18/10, Elsevier Inc, New York, http://doi.org/10.1016/S0899-9007(02)00945-0.

Ungvari, Z. et al. (2013), "Ionizing Radiation Promotes the Acquisition of a Senescence-Associated Secretory Phenotype and Impairs Angiogenic Capacity in Cerebromicrovascular Endothelial Cells: Role of Increased DNA Damage and Decreased DNA Repair Capacity in Microvascular Radiosensitivity", The Journals of Gerontology Series A: Biological Sciences and Medical Sciences, Vol. 68/12, Oxford University Press, Oxford, https://doi.org/10.1093/gerona/glt057.

Varma, S. D. et al. (2011), "Role of ultraviolet irradiation and oxidative stress in cataract formation-medical prevention by nutritional antioxidants and metabolic agonists", Eye & contact lens, Vol. 37/4, Lippincot Williams & Wilkins, Philadelphia, https://doi.org/10.1097/ICL.0b013e31821ec4f2.

Venkatesulu, B. P. et al. (2018), "Radiation-Induced Endothelial Vascular Injury: A Review of Possible Mechanisms", JACC: Basic to Translational Science, Vol. 3/4, Elsevier, Amsterdam, https://doi.org/10.1016/j.jacbts.2018.01.014.

Veeraraghavan, J. et al. (2011), "Low-dose gamma-radiation-induced oxidative stress response in mouse brain and gut: regulation by NFκB-MnSOD cross-signaling", Mutation research, Vol. 718/1-2, Elsevier, Amsterdam, https://doi.org/10.1016/j.mrgentox.2010.10.006.

Wang, C. et al. (2016), "Protective effects of cerium oxide nanoparticles on MC3T3-E1 osteoblastic cells exposed to X-ray irradiation", Cellular Physiology and Biochemistry, Vol. 38/4, Karger International, Basel, https://doi.org/10.1159/000443092.

Wang, H. et al. (2019a), "Radiation-induced heart disease: a review of classification, mechanism and prevention", International Journal of Biological Sciences, Vol. 15/10, Ivyspring International Publisher, Sydney, https://doi.org/10.7150/ijbs.35460.

Wang, H. et al. (2019b), "Gamma Radiation-Induced Disruption of Cellular Junctions in HUVECs Is Mediated through Affecting MAPK/NF-κB Inflammatory Pathways", Oxidative Medicine and Cellular Longevity, Vol. 2019, Hindawi, London, https://doi.org/10.1155/2019/1486232.

Wegener, A. R. (1994), "In vivo studies on the effect of UV-radiation on the eye lens in animals", Documenta ophthalmologica, Vol. 88, Springer, London, https://doi.org/10.1007/BF01203676.

Weinreb O. and A. Dovrat (1996), "Transglutaminase involvement in UV-A damage to the eye lens", Experimental eye research, Vol. 63/5, Elsevier, London, https://doi.org/10.1006/exer.1996.0150.

Yang, H. et al. (2020), "Cytoprotective role of humanin in lens epithelial cell oxidative stress-induced injury", Molecular medicine reports, Vol. 22/2, Spandidos Publishing Ltd, Athens, https://doi.org/10.3892/mmr.2020.11202.

Yao, K. et al. (2008), "The flavonoid, fisetin, inhibits UV radiation-induced oxidative stress and the activation of NF-κB and MAPK signaling in human lens epithelial cells", Molecular Vision, Vol. 14, Emory University, Atlanta, pp. 1865-1871.

Yao, J. et al. (2009), "UVB radiation induces human lens epithelial cell migration via NADPH oxidase-mediated generation of reactive oxygen species and up-regulation of matrix metalloproteinases", International Journal of Molecular Medicine, Vol. 24/2, Spandidos Publishing Ltd, Athens, https://doi.org/10.3892/ijmm_00000218.

Yves, C. (2000), "Oxidative stress and Alzheimer disease", The American Journal of Clinical Nutrition, Vol. 71/2, https://doi.org/10.1093/ajcn/71.2.621s.

Zhang, J. et al. (2012), “Ultraviolet radiation-induced cataract in mice: The effect of age and the potential biochemical mechanism”, Investigative ophthalmology & visual science, Vol. 53, Association for Research in Vision and Ophthalmology, Rockville, https://doi.org/10.1167/iovs.12-10482.

Zhang, L. et al. (2020), “Amifostine inhibited the differentiation of RAW264.7 cells into osteoclasts by reducing the production of ROS under 2 Gy radiation”, Journal of Cellular Biochemistry, Vol. 121/1, John Wiley & Sons, Ltd., Hoboken, https://doi.org/10.1002/jcb.29247.

Zhang, L. et al. (2018), “Astragalus polysaccharide inhibits ionizing radiation-induced bystander effects by regulating MAPK/NF-κB signaling pathway in bone mesenchymal stem cells (BMSCs)”, Medical Science Monitor, Vol. 24, International Scientific Information, Inc., Melville, https://doi.org/10.12659/MSM.909153.

Zigman, S. et al. (2000), “Effects of intermittent UVA exposure on cultured lens epithelial cells”, Current Eye Research, Vol. 20/2, Informa UK Limited, London, https://doi.org/10.1076/0271-3683(200002)2021-DFT095.

Zigman, S. et al. (1995), “Damage to cultured lens epithelial cells of squirrels and rabbits by UV-A (99.9%) plus UV-B (0.1%) radiation and alpha tocopherol protection”, Molecular and cellular biochemistry, Vol. 143, Springer, London, https://doi.org/10.1007/BF00925924.

Relationship: 2809: Energy Deposition leads to Modified Proteins

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Deposition of energy leading to occurrence of cataracts	adjacent	Moderate	Moderate

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	Moderate	NCBI
rat	Rattus norvegicus	High	NCBI
mouse	Mus musculus	Moderate	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Unspecific	High

This KER is plausible in all life stages, sexes, and organisms. The majority of the evidence is from in vivo male adult rats, and in vitro bovine models that do not specify sex.

Key Event Relationship Description

Energy deposition, such as that released from radiation (ionizing or non-ionizing) in sensitive lens cells can lead to protein modifications such as

phosphorylation, disulfide bond formation, D-Asp formation, and carbonylation, among other changes (Hamada et al., 2014; Lipman et al., 1988; Reisz et al., 2014). It is important to note that ionizing and non-ionizing radiation work by different mechanisms; ionizing radiation has enough energy to remove tightly bound electrons from atoms, leading to the formation of ions (charged particles), while the absorption of non-ionizing radiation leads to molecular vibrations and rotations, resulting in heat generation (Alcócer et al., 2020). The modifications arise as energy deposited onto a cell interacts with molecules (e.g. proteins, lipids, DNA), altering the redox balance of the cell, and resulting in amino acid modifications (Neves-Petersen et al., 2012). These changes cause structural and functional molecular-level damage to the proteins, such as aggregation (Reisz et al., 2014; Hamada et al., 2014). However, the extent of damage from different types of protein modifications would vary as these protein changes may be short-lived due to the cell life cycle and the associated regulation of the protein (Basisty et al., 2018).

Under homeostatic conditions, cells inherently have a set amount of total protein that are soluble (Pace et al., 2004). These properties can be disrupted by the deposition of energy. The interaction of a soluble protein with large amounts of energy can change its molecular weight and solubility through deamidation and the formation of disulfide bonds (Hanson et al., 2000; Reddy 1990; Miesbauer et al., 1994).

Other types of protein modification can also occur, including protein carbonylation and D-Asp formation (Reisz et al., 2014; Hamada et al., 2014). Protein carbonylation, a result of reactive oxygen species (ROS), is the post-translational addition of carbonyl to the protein’s side chain, these can observably be increased when a cell is exposed to ionizing radiation (Resiz et al., 2014). Inversion of amino acids from the L to D conformation can also occur in response to the ionization events or thermal energy released from radiation, this contributes to protein quaternary structure changes (Fujii et al., 2004).

Evidence Supporting this KER

Overall Weight of Evidence: Moderate

Biological Plausibility

The biological plausibility of the relationship between deposited energy leading to modified proteins is moderately supported by the literature. It is well accepted that deposition of energy, from ionizing sources (γ-rays, X-rays) and non-ionizing sources (ultraviolet (UV) radiation) can increase protein modifying events, resulting in structural changes to the protein (Hamada et al., 2014; Van Kuijk et al., 1991; Lipman et al., 1988; Reisz et al., 2014). These modifications include deamidation, oxidation, and disulfide bonds (Hanson et al., 2000; Kim et al., 2016; Kim et al., 2015; Lipman et al., 1988). Human, mouse, and rat models have been studied and prominent changes observed include increased cross-linking, altered water-solubility, and increased aggregation (Fochler & Durchschlag, 1997; Van Kuijk, 1991; Davies & Delsignore, 1987).

Deposition of energy can alter the protein profile within a cell leading to a decrease in water-soluble proteins and an increase in water-insoluble proteins. This arises from structural-level modifications to the protein amino acids. The amino acids that are particularly at risk are aromatic amino acids, as well as cysteine residues, which are known to have the lowest redox potential (Reisz et al., 2014). Aromatic amino acids can be converted into photosensitizers (Walrant & Santus, 1974). Tryptophan, which is present in alpha crystalline molecules, can also be converted into kynurenine when exposed to UV radiation, through the destabilization of its structural protein folds (Xia et al., 2013). Exposure to UV and photons, has been associated with the aggregation of water-soluble proteins and an increase in insoluble protein content (Van Kuijk, 1991; Wang et al., 2010; Hamada et al., 2014;). Stressors such as γ-rays can also lead to protein oxidation via reactive oxygen species (ROS), including protein cross-linking and hydrophobic protein interactions (Davies & Delsignore, 1987; Lee & Song, 2002). Additionally, at high concentrations, ROS from radiation can oxidize and cross-link proteins, producing insoluble protein clumps (Young, 1994).

Protein aggregation has also been shown to result from the formation of disulfide bonds. (Lipman et al., 1988). It is believed that when energy is deposited, it causes the protein molecule to unfold from its native structural conformation and aggregate through disulfide connections with other modified proteins (Chen et al., 2013). Treatment with a reducing agent that cleaves disulfide bonds results in the release of the aggregates, suggesting that the bonds between the sulfide sites have an impact on protein aggregation (Reddy, 1990).

Empirical Evidence

The empirical evidence relating to this KER moderately supports the relationship between the deposition of energy and modified proteins. A variety of protein changes have been used to measure this relationship, including molecular weight, solubility, and the presence of oxidation sites. Most of the data comes from high (>2 Gy) dose studies, with in vivo and in vitro models for taxa such as rodents, bovines, and humans (Zigler & Goosey, 1981; Anbaraki et al., 2016; Andley et al., 1990; Zigman et al., 1975; Abdelkawi et al., 2008; Shang et al. 1994; Shin et al., 2004).

Dose Concordance

Strong evidence is available in lens cells to support a dose response relationship between energy deposition and protein modification. A study showed, that lens crystallin proteins continuously irradiated in vitro for 24 hrs using UV from white light-daylight fluorescent lamps with a measurement of 500 ft-c (foot candles) contained high molecular weight proteins relative to controls (Zigler & Goosey, 1981). In another study, cross-linking of lens crystallin proteins was observed in vitro as early as 30 minutes and within 2-4 hrs following UV exposure. There was also a gradual decrease in native protein concentration with increasing dose of UV (Anbaraki et al., 2016). Other studies also show similar findings, whereby there is a shift in the percentage of high molecular weight proteins following in vitro exposure of lens cells to 140 J/m2 UV, but not 70 J/m2 (Andley et al., 1990). Zigman et al showed alterations in lens protein water-insolubility after 8 weeks of continuous UV exposure on in vivo eyes, with no significant change after 4 weeks (Zigman et al., 1975). In radon exposed whole lenses, no significant change to soluble lens protein content were observed until 6 weeks of continuous in vivo radon treatments, whereby, levels reached 0.85x control, and then continued decreasing at 8 weeks of exposure (Abdelkawi et al., 2008). Other studies using ionizing radiation have shown protein modifications as a result of oxidative by-products from radiation exposure. For example, Trp 69, Met 70, and Met 102 in γ-crystallins was shown to be oxidized after exposure to 5 Gy of γ-rays. Kim et al. (2015) observed naked lens cortex protein modifications from 5% in the control to 9% after 10 Gy of γ -rays and at the maximum dose tested of 50 Gy (Shang et al., 1994).

Time Concordance

No evidence available.

Essentiality

Radiation exposure is essential to increase levels of modified proteins above control levels. Studies that do not deposit energy are observed to have no downstream effects. One study found that the sham-exposed group exhibited less cross-linking of lens crystallin proteins compared to in vitro 4 hr UV exposed groups (Anbaraki et al., 2016). Additionally, relatively lower doses of X-ray exposures result in lower levels of protein alterations compared to lens cells receiving a higher dose. Water insoluble lens proteins remain at low levels, compared to cells exposed to 8 weeks of UV, where the levels were observed to rise above 1.4x from controls (Zigman et al., 1975). Similarly, in lenses exposed to radon gas for 6 weeks, there was a 1.2x fold increase in the levels of lens proteins compared to unexposed cells (Abdelkawi et al., 2008).

Uncertainties and Inconsistencies

Although the relationship is well- supported, the degree and type of modification can be variable depending on the exposure conditions. Significant increases in oxidized crystallin protein are seen anywhere from 5 Gy in vivo (Kim et al. 2015) to 50 Gy in vivo (Kim et al., 2016) to 270 Gy in vitro (Finley et al., 1998). This relationship is difficult to predict.

Quantitative Understanding of the Linkage

The following tables provide representative examples of the relationship, unless otherwise indicated, all data is significantly significant.

Dose Concordance

Reference	Experiment Description	Result
Abdelkawi et al., 2008	In vivo, two-month-old adult male Swiss albino mice received whole-body radon exposure to 3.54 mJ m^{-3} h for six continuous weeks (dose of 637.2 mJ m^{-3}) and the levels of soluble protein were measured using a Lowry assay.	Cells showed a decrease in soluble lens protein concentration (indicative of increased protein modification) to 0.85x control.
Abdelkwai, 2012	In vivo, male rats received whole-body exposure to 0.5 Gy/week of γ-rays and observed identified molecular weight changes in proteins using spectroscopy.	Cells showed an increase in crystallin molecular weight with each isoform, α, β-H, β-L, and γ increasing 28, 16, 27, and 54% relative to control.
Kim et al., 2015	In vitro, male rat lenses exposed to 2.8 Gy/h γ-ray and protein oxidation was detected using liquid chromatography-tandem mass spectroscopy.	A 5 Gy γ-ray treatment group had 10 sites of oxidation on water-soluble and water-insoluble γE- or γF-crystallin proteins.
Sherif & Abdelkawi, 2006	In vivo, male rat lenses received whole-body γ-ray exposure to 0.5 Gy/week and total soluble protein level was determined by the Lowry assay.	Rat lenses exposed to 0 - 4.0 Gy γ-rays showed a decrease in soluble lens protein (indicative of increased modified protein levels) with the maximum dose displaying a 1.6x decrease relative to control.
Shang et al., 1994	In vitro, bovine lens cortices exposed to 0-500 Gy at 3.96 Gy/min γ-rays and protein changes (β- and γ-crystallins) assessed using SDS-PAGE.	Cells exposed to 0-500 Gy displayed a linear increase in β-crystallin fragmentation above 10 Gy. They also displayed increased protein aggregates above 10 Gy, with the notable exception of β-crystallin which exhibited a slight drop below the trend line (but not below control) at 50 Gy.
Anbaraki et al., 2016	In vitro, bovine lens proteins exposed to 316 W/m^2 UV and protein modifications assessed using SDS-PAGE.	Increased cross-linking and oligomerization of UV-exposed lens proteins was observed Increase in dose caused an increase in higher molecular weight proteins, starting at 0.5 hr of light exposure, with another increase at 2 hrs of light. The non-native staining is relatively similar for 2-4hr exposures, but with increase dose, native staining decreases
Zigler & Goosey, 1981	In vitro, human lens proteins exposed to 12.5 W/m^2 UV and protein modification was detected using SDS-PAGE.	24 h exposure to UV resulted in increased molecular weight of crystallin proteins. This trend continued with the 48hr dose group.
Andley et al., 1990	In vitro, rabbit lens epithelial cells exposed to 70 or 140 Jm^{-2} UVB and protein modifications assayed via SDS-PAGE and autoradiographic scans.	UVB irradiation caused a decrease in the amount of 37 kD protein that was produced and expelled from the cells. Exposure to 70 J/m^2 led to a 7% decrease in 37 kD protein levels and exposure to 140 J/m^2 led to a 50% decrease in 37 kD protein levels. However, most of the other proteins remained unchanged.
Moran et al., 2013	In vitro, human crystallins exposed to 35 W/m^2 UVB for 6 h and protein weight changes detected using SDS-PAGE.	Exposure to 35 Wm-2 UVB for 6 h results in increased concentration of γD-crystallin proteins above 20 kDa molecular weight compared to control.
Fochler & Durchschlag, 1997	In vitro, calf crystallins exposed to UV (60, 100, 150 kJ/m2) or X-rays (1, 5, 10 kGy). Changes in protein weight were detected using SDS-PAGE.	At all doses measured, there is either a shift or a disappearance of the alpha and gamma crystallins on the SDS-PAGE.
Zigman et al., 1975	In vivo, mice received whole-body exposure to 450 $\mu W/cm^2$ long-wave UV and insoluble protein level was assessed using SDS-PAGE.	At 4 weeks of UV (12 hr on/off cycle), the treatment group and the control group were shown to diverge with a linear increase in the treatment group. At 8 weeks of UV (12 hr on/off cycle), the treatment group reached an insoluble protein level of 0.35 mg/lens, 1.4x control level.
Giblin et al., 2002	In vivo, male guinea pigs received whole-body exposure to 0.5 mW/cm^2 UVA and protein solubility changes were measured using the BCA protein assay.	Lens nucleus cells exposed to 4-5 months of UV-A had 276 mg/g water-soluble protein level. This is 20% less than the 343 mg/g seen in control groups. The cortex did not have significant differences.
Simpanya et al., 2008	In vivo, male guinea pigs received whole-body exposure to 0.5 mW/cm^2 UVA and protein changes were assayed using dynamic light scattering.	After 5 months exposure to UV-A, proteins in the nucleus had a higher average diameter compared to control. At 2.1 mm across the optical axis, the UV group had an average of 1020 diameter (arbitrary units), 5.67x the control's 180 average.
Fochler & Durchschlag, 1997	*In vitro*, sex calf crystallins exposed to UV (60, 100, 150 kJ/m2) or X-rays (1, 5, 10 kGy). Changes in protein weight were detected using SDS-PAGE.	At all doses measured, there is either a shift or a disappearance of the alpha and gamma crystallins on the SDS-PAGE.

Time Concordance

No evidence found.

Known modulating factors

Modulating Factor (MF)	MF Specification	Effect(s) on the KER	Reference(s)
Age	The absorption of radiation in the lens of the eye, such as UV, increases with age.	Free UV filters exist in the eye to help block UV from interacting with proteins in the lens. The filters, such as tryptophan metabolites, degrade as people age, reducing the protection for proteins in the lens.	Bron et al., 2000; Davies & Truscott, 2001; Truscott & Friedrich, 2016
Free Radical Scavengers	The addition of antioxidants attenuates the effect of energy deposition.	Sodium Azide (NaN_3) and Cystamine, free radical scavengers, reduce the amount of cross-linking of crystalline proteins.	Zigler & Goosey, 1981; Shin et al., 2004

Known Feedforward/Feedback loops influencing this KER

N/A

References

Abdelkawi, S. (2012), “Lens crystallin response to whole body irradiation with single and fractionated doses of gamma radiation”, International Journal of Radiation Biology, Vol.88/8, Informa UK Ltd, London, https://doi.org/10.3109/09553002.2012.695097.

Abdelkawi, S., M. Abo-Elmagd and H. Soliman. (2008), “Development of cataract and corneal opacity in mice due to radon exposure”, Radiation Effects and Defects in Solids, Vol.163/7, Taylor & Francis, Oxfordshire, https://doi.org/10.1080/10420150701249603.

Anbaraki, A. et al. (2016), “Preventive role of lens antioxidant defense mechanism against riboflavin-mediated sunlight damaging of lens crystallin”, International Journal of Biological Macromolecules, Vol.91, Elsevier, Amsterdam, https://doi.org/10.1016/j.ijbiomac.2016.06.047.

Andley, U. et al. (1990), “Effect of ultraviolet-b radiation on protein synthesis in cultured ens epithelial cells”, Current Eye Research, Vol.9/11, Taylor & Francis, Oxfordshire, https://doi.org/10.3109/02713689008997583.

Basisty, Nathan et al. (2018) “Protein Turnover in Aging and Longevity”, Proteomics, Vol.18, https://doi.org/10.1002/pmic.201700108

Bloemendal, H. et al. (2004), “Ageing and vision: structure, stability and function of lens crystallins”, Progress in Biophysics and Molecular Biology, Vol.86/3, Elsevier, Amsterdam, https://doi.org/10.1016/J.PBIOMOLBIO.2003.11.012.

Chen, Y. et al. (2013), “Ocular aldehyde dehydrogenases: Protection against ultraviolet damage and maintenance of transparency for vision”, Progress in Retinal and Eye Research, Vol.33/1, Elsevier, Amsterdam, https://doi.org/10.1016/j.preteyeres.2012.10.001.

Davies, K.J.A. and M.E. Delsignore. (1987), “Protein damage and degradation by oxygen radicals. 3. Modification of secondary and tertiary structure”, Journal of Biological Chemistry, Vol.262/20, American Society for Biochemistry and Molecular Biology, Rockville, https://doi.org/10.1016/s0021-9258(18)48020-9.

Davies, M. and R. Truscott. (2001), “Photo-oxidation of proteins and its role in cataractogenesis”, Journal of Photochemistry and Photobiology B: Biology, Vol.63/1-3, Elsevier, Amsterdam, https://doi.org/10.1016/s1011-1344(01)00208-1.

Finley, E. et al. (1998), “Radiolysis-induced oxidation of bovine alpha-crystallin", Photochemistry and Photobiology, Vol.68/1, Wiley-Blackwell. Hoboken, https://doi.org/10.1111/j.1751-1097.1998.tb03245.x.

Fochler, C. and H. Durchschlag. (1997), “Investigation of irradiated eye-lens proteins by analytical ultracentrifugation and other techniques”, Progress in Colloid and Polymer Science, Vol.107, Springer, Berlin, https://doi.org/10.1007/BFb0118020.

Fujii, N., H. Uchida, and T. Saito (2004), “The damaging effect of UV-C irradiation on lens alpha-crystallin”, Molecular Vision, Vol. 10, United States, pp. 814-820

Geiger, T. and S. Clarke. (1987), “Deamidation, isomerization, and racemization at asparaginyl and aspartyl residues in peptides: succinimide-linked reactions that contribute to protein degradation”, Journal of Biological Chemistry, Vol.262, American Society for Biochemistry and Molecular Biology, Rockville, https://doi.org/10.1016/s0021-9258(19)75855-4.

Giblin, F. et al. (2002), “UVA light in vivo reaches the nucleus of the guinea pig lens and produces deleterious, oxidative effects”, Experimental Eye Research, Vol.75/4, Academic Press Inc, Cambridge, https://doi.org/10.1006/exer.2002.2039.

Hamada, N. et al. (2014), “Emerging issues in radiogenic cataracts and cardiovascular disease”, Journal of Radiation Research, Vol.55/5, Oxford University Press, Oxford, https://doi.org/10.1093/jrr/rru036.

Hanson, S. et al. (2000), “The major in vivo modifications of the human water-insoluble lens crystallins are disulfide bonds, deamidation, methionine oxidation and backbone cleavage”, Experimental Eye Research, Vol.71/2, Academic Press Inc, Cambridge, https://doi.org/10.1006/EXER.2000.0868.

Kim, I. et al. (2016), “One-shot LC–MS/MS analysis of post-translational modifications including oxidation and deamidation of rat lens α- and β-crystallins induced by γ-irradiation”, Amino Acids, Vol.48/12, Springer, Berlin, https://doi.org/10.1007/s00726-016-2324-y.

Kim, I. et al. (2015), “Site specific oxidation of amino acid residues in rat lens γ-crystallin induced by low-dose γ-irradiation”, Biochemical and Biophysical Research Communications, Vol.466/4, Elsevier, Amsterdam, https://doi.org/10.1016/j.bbrc.2015.09.075.

Kozbenko, T. et al. (2022), “Deploying elements of scoping review methods for adverse outcome pathway development: a space travel case example”, International Journal of Radiation Biology, 1–12. https://doi.org/10.1080/09553002.2022.2110306

Kuan, Y. et al. (2013), “Radiation processing of food proteins – A review on the recent developments”, Trends in Food Science & Technology, Vol.30/2, pp.105–120.

Lee, Y. and K.B. Song. (2002), “Effect of gamma-irradiation on the molecular properties of myoglobin”, Journal of Biochemistry and Molecular Biology, Vol.35/6, Biochemical Society of the Republic of Korea, Seoul, https://doi.org/10.5483/bmbrep.2002.35.6.590.

Lipman, R., B. Tripathi and R. Tripathi. (1988), “Cataracts induced by microwave and ionizing radiation”, Survey of Ophthalmology, Vol.33/3, Elsevier, Amsterdam, https://doi.org/10.1016/0039-6257(88)90088-4.

Miesbauer, L. R. et al. (1994), “Post-translational modifications of the water soluble human lens crystallins from young adults”, Journal of Biological Chemistry, Vol.269, American Society for Biochemistry and Molecular Biology, Rockville, https://doi.org/10.1016/s0021-9258(18)99902-3.

Moran, S. et al. (2013), “Amyloid fiber formation in human γd-crystallin induced by UV-B photodamage”, Biochemistry, Vol.52/36, American Chemical Society Publications, Washington, https://doi.org/10.1021/bi4008353.

Pace, C.N. et al. (2004), “Protein structure, stability and solubility in water and other solvents”, Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences, Vol.359, Royal Society, London, https://doi.org/10.1098/RSTB.2004.1500.

Reddy, V.N. (1990), “Glutathione and its function in the lens—An overview”, Experimental Eye Research, Vol.50/6, Academic Press Inc, Cambridge, https://doi.org/10.1016/0014-4835(90)90127-G.

Reisz, J. et al. (2014), “Effects of ionizing radiation on biological molecules - mechanisms of damage and emerging methods of detection”, Antioxidants and Redox Signaling, Vol.21(2), Mary Ann Liebert Inc, Larchmont, https://doi.org/10.1089/ars.2013.5489.

Shang, F., L. Huang and A. Taylor. (1994), “Degradation of native and oxidized beta-and gamma-crystallin using bovine Lens epithelial cell and rabbit reticulocyte extracts”, Current Eye Research, Vol.13/6, Taylor & Francis, Oxfordshire, https://doi.org/10.3109/02713689408999870.

Sherif, S. and S. Abdelkawi. (2006). “Effect of fractionated dose of whole body gamma irradiation on eye lens proteins of rats”, Isotope and Radiation Research, Vol.38/3, Middle Eastern Regional Radioisotope Center for Arab Countries, Cario, pp.547-558.

Shin, D., et al. (2004), “Cell type-specific activation of intracellular transglutaminase 2 by oxidative stress or ultraviolet irradiation: Implications of transglutaminase 2 in age-related cataractogenesis”, Journal of Biological Chemistry, Vol.279/15, The American Society for Biochemistry and Molecular Biology, Rockville, https://doi.org/10.1074/jbc.M308734200.

Simpanya, F,M. et al. (2008), “Measurement of lens protein aggregation in vivo using dynamic light scattering in a guinea Pig/UVA model for nuclear cataract”, Photochemistry and Photobiology, Vol.84/6, Wiley-Blackwell, Hoboken, https://doi.org/10.1111/j.1751-1097.2008.00390.x.

Truscott, R. and M. Friedrich. (2016), “The etiology of human age-related cataract. Proteins don't last forever”, Biochimica et Biophysica Acta, Vol.1890/1, Elsevier, Amsterdam, https://doi.org/10.1016/j.bbagen.2015.08.016.

Van Kuijk, F. (1991), “Effects of ultraviolet light on the eye: Role of protective glasses”, Environmental Health Perspectives, Vol.96/30, National Institute of Environmental Health Sciences, Durham, https://doi.org/10.1289/ehp.9196177.

Walrant, P. and R. Santus. (1974), “Ultraviolet and N-Formyl-Kynurenine-Sensitized Photoinactivation of Bovine Carbonic Anhydrase: An Internal Photodynamic Effect”, Photochemistry and Photobiology, Vol.20/5, Wiley-Blackwell, Hoboken, https://doi.org/10.1111/j.1751-1097.1974.tb06600.x.

Wang, W., S. Nema and D. Teagarden (2010), “Protein aggregation- Pathways and influencing factors”, International Journal of Pharmaceutics, Vol.390/2, Elsevier, Amsterdam, https://doi.org/10.1016/j.ijpharm.2010.02.025.

Xia, Z. et al. (2013), “UV-radiation Induced Disruption of Dry-Cavities in Human γD-crystallin Results in Decreased Stability and Faster Unfolding”, Sci Rep Vol.3, https://doi.org/10.1038/srep01560.

Young, R. (1994), “The family of sunlight-related eye diseases”, Optometry and Vision Science, Vol.71/2, Lippincott Williams & Wilkins, Philadelphia, https://doi.org/10.1097/00006324-199402000-00013.

Zigler, S.J. and J. Goosey. (1981), “Photosensitized Oxidation in the Ocular Lens: Evidence for Photosensitizers Endogenous To the Human Lens”, Photochemistry and Photobiology, Vol.33/6, Wiley-Blackwell, Hoboken, https://doi.org/10.1111/j.1751-1097.1981.tb05505.x.

Zigman, S. et al. (1975), “The response of mouse ocular tissues to continuous near-UV light exposure”, Investigative Ophthalmology, Vol.14/September, Association for Research in Vision and Ophthalmology, Rockville, pp.710-713.

Relationship: 2810: Oxidative Stress leads to Increase, Oxidative DNA damage

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Deposition of energy leading to occurrence of cataracts	adjacent	Moderate	Low

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	Moderate	NCBI
mouse	Mus musculus	Moderate	NCBI
rat	Rattus norvegicus	Moderate	NCBI
bovine	Bos taurus	Moderate	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	Moderate

Sex Applicability

Sex	Evidence
Unspecific	Moderate

This KER is plausible in all life stages, sexes, and organisms with DNA. The majority of the evidence is from in vivo studies conducted in male and female adult mice and rats. No in vitro evidence was found to support the relationship.

Key Event Relationship Description

Oxidative stress refers to a state in which the amount of reactive oxygen (ROS) and nitrogen (RNS) species overwhelms the cell's antioxidant defense system. This loss in redox homeostasis can lead to oxidative damage to proteins, lipids, and nucleic acids (Schoenfeld et al., 2012; Tangvarasittichai & Tangvarasittichai, 2018; Turner et al., 2002). ROS are molecules with oxygen as the functional center and at least one unpaired electron in the outer orbits. Although less common than ROS, RNS can also induce oxidative stress (Cadet et al., 2012; Tangvarasittichai & Tangvarasittichai, 2018).

Organisms contain a defense system of antioxidants to help manage ROS levels. Antioxidant measures consist of antioxidant enzymes, vitamins and minerals that catalyze the conversion of ROS to non-toxic molecules such as water and O2. When an antioxidant system is overwhelmed by the amount of ROS, the cell can enter a state of oxidative stress (Balasubramanian, 2000; Ganea & Harding, 2006; Karimi et al., 2017).

Unmanaged oxidative stress can damage vital macromolecules such as DNA leading to oxidative DNA damage. This can be divided into two categories, damage caused by one ROS, and damage caused by at least two ROS associating with the DNA in the space of one to two helix turns. The first scenario initiates DNA-protein cross-links, inter and intrastrand links, and tandem base lesions, while the second scenario produces more complicated lesions, known as oxidatively generated clustered lesions (ODCLs). These can include single and double strand breaks, abasic sites, and oxidized bases (Cadet et al., 2012) which can cause chromosomal aberrations, cytotoxicity, and oncogenic transformations (Stohs, 1995) as well as structural changes to the DNA, such as blocking polymerases (Zhang et al., 2010).

8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) lesions are the most common and best-studied, as such they are often used as a marker of oxidative DNA damage (Tangvarasitichai & Tangvarasittichai, 2018).

Cells possess DNA repair mechanisms that help repair the damage, but these processes are not perfect (Eaton, 1995; Ainsbury et al., 2016; Markkanen, 2017). Furthermore, certain types of lesions, such as DNA double strand breaks, are more complex to repair (Schoenfeld et al, 2012), leading to increased oxidative DNA damage.

Evidence Supporting this KER

Overall Weight of Evidence: Moderate

Biological Plausibility

When a cell is exposed to oxidative stress, DNA lesions can be induced. There are various repair systems that will attempt to repair the damage sometimes successfully, and other times inadequately or inefficiently, in this case oxidative DNA damage will persist. Furthermore, if there are too many lesions, the DNA repair system may be overwhelmed. A low level of damage is always found in healthy cells, but this amount increases under oxidative stress (Lee et al., 2004). It has been estimated that human cells have 70 000 lesions per day, mostly due to ROS produced during normal metabolism and base hydrolysis (Amente et al., 2019). These lesions can be DNA breaks, but there are also other types such as oxidized bases. Furthermore, while ROS induces DNA breaks, it can also be caused by other processes, or be an intermediate in DNA repair. As a result, oxidized nucleotides are generally a more accurate indicator of oxidative stress (Collins, 2014).

Oxidative stress affects different nitrogenous bases differently. For example, guanine (G) has a lower redox potential, causing it to be more vulnerable to oxidation compared to other nitrogenous bases. This leads to increased amounts of oxidized G products, relative to other forms of damage, Furthermore, ribonucleotides can also be oxidized, to the point where dGTP is more vulnerable to oxidation than G (Markkanen, 2017). Certain compounds such as hydroxyl radical generation systems and adriamycin-iron complexes will bind to and form ROS in association with DNA, therefore inducing site-specific DNA damage (Stohs, 1995).

Additionally, cells that are actively dividing are more sensitive to oxidative DNA damage (Sacca et al., 2009). A few studies have also found that single stranded DNA (ssDNA) is more likely to be oxidized than double stranded DNA (dsDNA). This indicates that persistent ssDNA sites, such as Z-DNA, stable R-loops, cruciforms, quadruplexes, or intramolecular triplexes might have higher incidences of oxidative damage (Amente et al., 2019).

Cells use three main methods to repair and prevent oxidative DNA damage. Firstly, enzymes such as Mut homologue 1, 2, 3, and Nudix-type 5 (MTH1, MTH2, MTH3, and NUDT5) are used to remove oxidized nucleotides before they can be incorporated into DNA. Another method is switching between replicative polymerases and DNA polymerase γ (Polγ) during replication when an 8-oxo-G lesion is encountered. This allows the replicative machinery to bypass the lesion. The third method is the base excision repair (BER) pathway, which is the major DNA repair pathway for base damage and has two general sub paths. The first is the short patch, where only the damaged nucleotides are replaced. The other is the long patch, which replaces a group of 2 to 12 nucleotides (Markkanen, 2017). For mitochondrial DNA (mtDNA), which is more sensitive to oxidative damage than nuclear DNA (Yakes & Van Houten, 1997), BER involves three main enzymes. 8-oxoguanine DNA glycosylase 1 (OGG1) removes 8-OHdG lesions, which are caused by the incorporation of 8-oxodGTP. AP endonuclease 1 (APE1) is an AP endonuclease that increases OGG1 turnover and adds a nick to the DNA, preparing it for further repair processes. Finally, DNA polymerase γ (Polγ) adds new nucleotides where the older ones were removed (Zhang et al., 2010). Another kind of BER pathway is SSBR (single strand break repair). When two SSBs are in juxtaposition, they can form DSBs, which are detrimental (Caldecott, 2024; Pfeiffer et al., 2000).

Different lesions are also repaired differently and can cause varying amounts of damage. For example, DNA single strand breaks are usually repaired quickly (Collins, 2014), while double strand breaks are more complicated and are therefore, less likely to be repaired correctly (Schoenfeld et al, 2012). More details on these processes are reviewed in Markkanen (2017). Overall the mechanism to oxidative stress leading to oxidative DNA damage is well accepted and understood.

Empirical Evidence

There is limited evidence supporting time- or dose-concordance.

Dose Concordance

Zhang et al. (2010) exposed male rats in vivo to 10%, 21% (atmospheric level) and 60% O2 (to induce oxidative stress). This resulted in a 1.5x increase in 8-OHdG levels. It was assumed that 60% oxygen induced oxidative stress, however the study only measured the downstream KE.

Time Concordance

Although DNA damage induced by oxidative stress can be repaired rapidly, the accumulation of oxidative stress typically causes oxidative DNA damage after several months. Two studies show an increase in damage 1.5 and two months respectively after the induction of oxidative stress (Pendergrass et al., 2010 – 2.5x increase in 8-OH-dG positive DNA fragments, in vivo irradiation with 11 Gy X-rays at 2 Gy//min) (Zhang et al., 2010 – 1.6x increase in 8-OHdG, exposure to 60% O2).

Pendergrass et al. (2010) reported that the amount of oxidative DNA damage increased as the amount of time after irradiation increased. It was observed that DNA damage (represented by the number of nuclear fragments in the lens cortex after exposure to 11 Gy X-rays) increased from 100 to 750 fragments from the time of radiation to over 22 months after. It was also shown that the amount of 8-OH G positive DNA fragments increased from about 5 to 55 from the time of radiation (11 Gy X-rays) to 11 months post-exposure (Pendergrass et al., 2010).

Essentiality

No evidence.

Uncertainties and Inconsistencies

No evidence.

Quantitative Understanding of the Linkage

Available data suggests that increases in oxidative stress leads to increases in oxidative DNA damage. The following tables provide representative examples of the relationship, unless otherwise indicated, all data is significantly significant.

Dose Concordance

Reference	Experiment Description	Result
Zhang et al., 2010	In vivo. 72 male Wistar rats were exposed to 21%, and 60% O2 to induce oxidative stress. Oxidative DNA damage was measured by determining 8-hydroxy-2’-deoxy-guanosine (8-OHdG) via competitive ELISA assays.	In rats exposed in vivo, a 39% increase in atmospheric O2 concentration (indicative of oxidative stress) resulted in a 1.27x increase in 8-OHdG.

Time Concordance

Reference	Experiment Description	Result
Pendergrass et al., 2010	In vivo. Female, 3-month-old, C57BL/6 mice had their heads exposed to 11 Gy X-rays at 2 Gy/min to induce oxidative stress. Oxidative DNA damage was measured using antibody staining of fixed eyes and immunofluorescence.	In mice exposed in vivo to 11 Gy X-rays, oxidative stress increased 4.3x relative to control 6 months post-irradiation. The amount of 8-OH G positive DNA fragments increased to 2.7x control 6.5 months after the increase in oxidative stress.

Known modulating factors

Modulating Factor (MF)	MF Specification	Effect(s) on the KER	Reference(s)
Age	Increased age	Increased levels of oxidative DNA damage, partly due to decreased antioxidant levels, meaning that the removal of ROS occurs more slowly, increasing the level of oxidative damage. Moreover, in humans, after about forty to fifty years, a barrier forms in the lens of the eye that decreases intracellular antioxidant transportation. Normally, antioxidants circulate via a current in the cytoplasm of lens fiber cells. However, as the age of the organism increases, the cytoplasm of these cells becomes stiffer. Small molecules such as H_2O_2 and the superoxide anion can diffuse through, but larger molecules, such as glutathione, cannot enter the barrier. As a result, the core of the lens has a decreased antioxidant concentration, making it more vulnerable to oxidative damage. Furthermore, the amount of protein and mRNA corresponding to important mitochondrial BER enzymes decreases with age, causing a decrease in DNA repair ability and therefore an increase in DNA damage in the mitochondria.	Stohs, 1995; Lee et al., 2004; Martinez et al., 2010; Pendergrass et al., 2010; Zhang et al., 2010; Ainsbury et al., 2016; Tangvarasittichai & Tangvarasittichai, 2018
H_2	Increased concentration	Decreased level of oxidative DNA damage.	Schoenfeld et al., 2012
Antioxidants	Increased concentration	Reviews have found that about 50% of studies examined showed a decrease in base oxidation, but the other half show no change.	Turner et al., 2002; Møller & Loft, 2006; Hoelzl et al., 2009
Lipoic acid	Increased concentration	Decreased level of oxidative DNA damage.	Turner et al., 2002
Acetyl carnitine	Increased concentration	Decreased level of oxidative DNA damage.	Turner et al., 2002
Ubiquinone Q-9	Increased concentration	Decreased level of oxidative DNA damage.	Turner et al., 2002

Modulating Factor (MF)	MF Specification	Effect(s) on the KER	Reference(s)
Hydroquinone	Increased concentration	Decreased level of oxidative DNA damage.	Turner et al., 2002
Folate	Increased concentration	Decreased level of oxidative DNA damage.	Turner et al., 2002
Aged garlic extracts	Increased concentration	Decreased level of oxidative DNA damage.	Turner et al., 2002

Known Feedforward/Feedback loops influencing this KER

Not identified

References

Ainsbury, E. A. et al. (2016), “Ionizing radiation induced cataracts: Recent biological and mechanistic developments and perspectives for future research”, Reviews in mutation research, Vol. 770, Elsevier. https://doi.org/10.1016/j.mrrev.2016.07.010

Amente, S. et al. (2019), “Genome-wide mapping of 8-oxo-7,8-dihydro-2’-deoxyguanosine reveals accumulation of oxidatively-generated damage at DNA replication origins within transcribed long genes of mammalian cells”, Nucleic Acids Research 2019, Vol. 47/1, Oxford University Press, England, https://doi.org/10.1093/nar/gky1152

Cadet, J. et al. (2012), “Oxidatively generated complex DNA damage: tandem and clustered lesions”, Cancer letters, Vol. 327/1, Elsevier Ireland Ltd, Ireland, https://doi.org/10.1016/j.canlet.2012.04.005

Cadet, J., and& Davies, K.J. A (2017). “Oxidative DNA damage & repair: An introduction. Free radical biology & medicine” Vol.107, 2-12 https://doi.org/10.1016/j.freeradbiomed.2017.03.030

Caldecott K. W. (2024). “Causes and consequences of DNA single-strand breaks”. Trends in biochemical sciences, Vol 49/1, 68–78. https://doi.org/10.1016/j.tibs.2023.11.001.

Collins, A. R. (2014), “Measuring oxidative damage to DNA and its repair with the comet assay”, Biochimica et biophysica acta. General subjects, Vol. 1840/2, Elsevier B.V., https://doi.org/10.1016/j.bbagen.2013.04.022

Eaton, J. W. (1995), “UV-mediated cataractogenesis: a radical perspective”, Documenta ophthalmologica, Vol. 88/3-4, Springer, Dordrecht, https://doi.org/10.1007/BF01203677

Hoelzl, C. et al. (2009), “Use of single cell gel electrophoresis assays for the detection of DNA-protective effects of dietary factors in humans: Recent results and trends”, Mutation Research, Vol. 681/1, Elsevier, https://doi.org/10.1016/j.mrrev.2008.07.004

Kozbenko, T. et al. (2022), “Deploying elements of scoping review methods for adverse outcome pathway development: a space travel case example”, International Journal of Radiation Biology, 1–12. https://doi.org/10.1080/09553002.2022.2110306

Lee, J., N, Koo and D. B. Min (2004), “Reactive oxygen species, aging, and antioxidative nutraceuticals”, Comprehensive reviews in food science and food safety, Vol. 3/1, Blackwell Publishing Ltd, Oxford, https://doi.org/10.1111/j.1541-4337.2004.tb00058.x

Markkanen, E. (2017), “Not breathing is not an option: How to deal with oxidative DNA damage”, DNA repair, Vol. 59, Elsevier B.V., Netherlands, https://soi.org/10.1016/j.dnarep.2017.09.007

Martinez, G. and R. U. de Longh (2010), “The lens epithelium in ocular health and disease”, The international journal of biochemistry & cell biology, Vol. 42/12, Elsevier B. V, Netherlands, https://doi.org/10.1016/j.biocel.2010.09.012

Møller, P. and Loft, S. (2006), “Dietary antioxidants and beneficial effect on oxidatively damaged DNA”, Free Radical Biology and Medicine, Elsevier, https://doi.org/10.1016/j.freeradbiomed.2006.04.001

Pendergrass, W. et al. (2010), “X-ray induced cataract is preceded by LEC loss, and coincident with accumulation of cortical DNA, and ROS; similarities with age-related cataracts”, Molecular vision, Vol. 16, Molecular Vision, United States, pp. 1496-1513

Pfeiffer, P. et al. (2000),. “Mechanisms of DNA double-strand break repair and their potential to induce chromosomal aberrations”,. Mutagenesis, Vol.15(4). https://doi.org/10.1093/mutage/15.4.289

Sacca, S. C. et al. (2009), “Gene-environment interactions in ocular diseases”, Mutation research – fundamental and molecular mechanisms of mutagenesis, Vol. 667/1-2, Elsevier, Amsterdam, https://doi.org/10.1016/j.mrfmmm.2008.11.002

Schoenfeld, M. P. et al. (2012), “A hypothesis on biological protection from space radiation through the use of new therapeutic gases as medical counter measures”, Medical gas research, Vol. 2/1, BioMed Central Ltd, India, https://doi.org/10.1186/2045-9912-2-8

Stohs, S. J. (1995), “The role of free radicals in toxicity and disease”, Journal of Basic and Clinical Physiology and Pharmacology, Vol. 6/3-4, Freund Publishing House Ltd, https://doi.org/10.1515/JBCPP.1995.6.3-4.205

Tangvarasittichai O. and S. Tangvarasittichai (2018), “Oxidative stress, ocular disease and diabetes retinopathy”, Current Pharmaceutical Design, Vol. 24/40, https://doi.org/10.2174/1381612825666190115121531

Turner, N. D. et al. (2002), “Opportunities for nutritional amelioration of radiation-induced cellular damage”, Nutrition, Vol. 18/10, Elsevier Inc, New York, http://doi.org/10.1016/S0899-9007(02)00945-0

Yakes, F. M. and B. Van Houten (1997), “Mitochondrial DNA damage is more extensive and persists longer than nuclear DNA damage in human cells following oxidative stress”, Cell Biology, Vol. 94, The National Academy of Sciences of the USA, United States, pp. 514-519

Zhang, Y. et al. (2010), “Oxygen-induced changes in mitochondrial DNA and DNA repair enzymes in aging rat lens”, Mechanisms of ageing and development, Vol. 131/11, Elsevier Ireland Ltd, Clare, https://doi.org/10.1016/j.mad.2010.09.003

Relationship: 2811: Oxidative Stress leads to Increase, DNA strand breaks

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Deposition of energy leading to occurrence of cataracts	adjacent	Moderate	Low
Deposition of Energy Leading to Learning and Memory Impairment	adjacent	Moderate	Moderate
Deposition of energy leads to abnormal vascular remodeling	adjacent	High	Moderate

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	Low	NCBI
rat	Rattus norvegicus	Low	NCBI
rabbit	Oryctolagus cuniculus	Low	NCBI
bovine	Bos taurus	Low	NCBI
mouse	Mus musculus	Low	NCBI

Life Stage Applicability

Life Stage	Evidence
Adult	Low
Not Otherwise Specified	Low

Sex Applicability

Sex	Evidence
Unspecific	Low
Male	Low

This KER is plausible in all life stages, sexes, and organisms with DNA. The evidence is from human, rodent, rabbit and bovine in vitro studies that do not specify the sex, as well as an adult rat in vivo study.

Key Event Relationship Description

Oxidative stress is an event that involves both a reduction in free radical scavengers and enzymes, and an increase in free radicals (Brennan et al., 2012). Oxidative stress needs to be maintained within an organism to avoid an excess of damage to biological structures, such as DNA. A redox homeostasis between the radicals and the scavengers is necessary. Between reactive oxygen species (ROS) and reactive nitrogen species (RNS), collectively known as RONS, ROS is particularly significant to oxidative damage and disease states. Radicals such as singlet oxygen and hydroxyl radical are highly unstable and will react with molecules near their generation point, while radicals such as H2O2 are more stable and membrane permeable, meaning they can travel further to find electrons (Spector, 1990). Since DNA is mainly found in nucleus, ROS needs to reach the nucleus to induce breaks. Hydroxyl radicals, in addition to being highly reactive, are capable of causing DNA damage (Halliwell et al., 2021; Engwa et al., 2020). The regulation of these radicals is achieved by the antioxidant defense response (ADR), which includes enzymatic and non-enzymatic processes. The ADR is recruited to manage RONS levels, with antioxidants such as superoxide dismutase (SOD) functioning as the first line of defense (Engwa et al., 2020). These antioxidants act as scavengers to oxidants, reacting with them before reaching other structures within the cell such as DNA strands (Cabrera et al., 2011; Engwa et al., 2020). The backbone of DNA can fragment upon sustained exposure to ROS (Uwineza et al., 2019; Cannan et al., 2016). Due to low oxidation potentials, adenine and guanine are the DNA bases more prone to oxidation, with oxidation potentials (normal hydrogen electrode) at pH 7 of 1.3 eV and 1.42 eV compared to the 1.6 eV and 1.7 eV of cytosine and thymine (Fong, 2016; Halliwell et al., 2021; Poetsch, 2020). In fact, certain radicals even target guanine in a selective fashion, including carbonate anion radical (CO3•-) and singlet oxygen (1O2) (Halliwell et al., 2021).

Evidence Supporting this KER

Overall Weight of Evidence: Moderate

Biological Plausibility

The biological plausibility of the relationship between increased oxidative stress leading to increased DNA double strand breaks (DSBs) is highly supported by the literature. Evidence was collected from studies conducted using in vitro lens epithelial cell models and derived from humans, bovine and germ line cells (Spector, 1990; Stohs, 1995; Aitken et al., 2001; Spector, 1995). As this evidence is derived from studies using a human cell model it limits the ability to compare between different taxonomies (Ahmadi et al., 2022; Cencer et al., 2018; Liu et al., 2013; Meng et al., 2021; Smith et al., 2015; Zhou et al., 2016). Other evidence comes from human-derived and rodent models of neuronal and endothelial cells (Cervelli et al., 2014; El-Missiry et al., 2018; Huang et al., 2021; Sakai et al., 2017; Ungvari et al., 2013; Zhang et al., 2017).

ROS that are generated specifically as a result of radiation are highly localized, increasing the likelihood of clustered regions of damage. Naturally generated ROS are more widespread and as a result less capable of generating clusters of damage. ROS will act on DNA bases to oxidize or delete them from the sequence, which create nicks on the strand (Cannan et al., 2016). This damage can occur to any DNA base but bases such as guanine and adenine are most vulnerable due to their low oxidation potentials (Fong, 2016). The mechanism through which the strand break occurs is a result of base excision repair (BER) happening at multiple sites that are too close together, resulting in the spontaneous conversion to DSBs prior to completion of repair. ROS damage to bases clustered together means that multiple sites of BER are happening very close together and while the strand may be able to support the damaged area for one repair, concurrent repairs make surrounding areas more fragile and the strand breaks at the nick sites are under added strain (Cannan et al., 2016). Endogenous damage to DNA as a result of radicals appears over time and mainly as isolated lesions, a pattern understood to be due to the diffusion of the radicals resulting in homogenous distribution patterns. This differs from the specific situations where radiation acts as the stressor to increase oxidative stress, as the radiation track will be highly localized and form radicals within that hit space. This leads to non-homologous lesions and clustered damage to the DNA (Ward et al., 1985).

Empirical Evidence

This relationship is well supported through empirical evidence from studies using stressors such as H_2O_2, photons, γ- and X-ray, which cause an increase in markers of oxidative stress such as ROS-generating enzymes (lactate dehydrogenase, LDH), and a decrease in free radical scavengers, resulting in DNA strand fragmentation. These studies include both in vivo and in vitro human lens epithelial cells (LECs), mouse, rat and rabbit models, including neuronal cells lines and endothelial cells (Ahmadi et al., 2022; Cencer et al., 2018; Cervelli et al., 2014; El-Missiry et al., 2018; Huang et al., 2021; Liu et al., 2013; Meng et al., 2021; Spector et al., 1997; Ungvari et al., 2013; Zhang et al., 2017; Zhou et al., 2016; Sakai et al., 2017).

Dose/Incidence Concordance

There is high evidence to support a dose concordance between oxidative stress and DNA strand breaks. One in vitro study demonstrated that when ROS levels in LECs are 10% above control following 0.5 Gy gamma ray exposure, DNA strand breaks increased 15-20% above control (Ahmadi et al., 2021). Another study with ultraviolet (UV)B radiation demonstrated higher ROS levels after exposure to 0.14 J/cm2 on in vitro LECs as compared to a lower dose exposure (0.014 J/cm2) for the same time. This corresponded to DNA strand break levels also increasing following high dose rate exposure, but not with the low dose exposure (Cencer et al., 2018).

A 30 μM of H_2O_2 treatment of in vitro LECs is associated with a 1.4x increase in lactate dehydrogenase (LDH) and 55% more DNA strand breaks (Liu et al., 2013; Smith et al., 2015). Following exposure of in vitro LECs to 50 μM H_2O_2, increased ROS levels, 4x for LDH, and decreased antioxidant levels, 2x control for GSH-Px and SOD, are associated with a 3x increase in γ-H2AX, a marker of DNA strand breaks (Meng et al., 2021). SOD and GSH decreased by 2-fold following 100 μM H_2O_2 exposure on LECs with an in vitro model (Zhou et al., 2016). At 125 μM H_2O_2 intact DNA can be reduced to near 1% of pre-treatment levels for in vitro LECs (Spector et al., 1997). Following 400 μM H_2O_2 LDH increased to 1200% of control in neuroblastoma cells (Feng et al., 2016) and DNA strand breaks increased to over 150% of control in in vitro LECs (Li et al., 1998).

Exposure of in vitro mouse hippocampal neuronal cells (HT22 cell line) to 10 Gy of X-irradiation resulted in a 5x increase in ROS generation and 3x increase in γ-H2AX (Huang et al., 2021). Another study exposed the same cell line to 8 and 12 Gy of X-irradiation and found a ~2x increase in ROS at 8 Gy and a 4.4x and 3.2x increase in phosphorylation of ataxia telangiectasia mutated (ATM) and γ-H2AX, respectively, 30 minutes after 12 Gy (Zhang et al., 2017). A separate study exposed adult male rats to 4 Gy of γ-irradiation and found 2x increase in 4-hydroxy-2-nonenal (4-HNE) (lipid peroxidation marker) and 3x increase in protein carbonylation. Glutathione reductase decreased by approximately 5x, whereas glutathione and glutathione peroxidase levels decreased by approximately 3x each. Tail DNA %, tail length and tail moment (DNA strand break parameters) increased by approximately 2x, 3x and 6x, respectively (El-Missiry et al., 2018).

Endothelial cells exposed to irradiation also demonstrated the relation between oxidative stress and DNA strand breaks. Rat cerebromicrovascular endothelial cells (CMVECs) exposed to 8 Gy 137Cs gamma rays showed increased cellular peroxide production and mitochondrial oxidative stress. Tail DNA content indicating DNA damage was also increased from 0 to 45% (Ungvari et al., 2013). Human umbilical vein endothelial cells (HUVECs) were irradiated with single (0.125, 0.25, 0.5 Gy), or fractionated (2 × 0.125 Gy, 2 × 0.250 Gy) doses of X-rays. Intracellular ROS production increased in a dose-dependent manner following 0.125, 0.25, 0.5 Gy, and γ-H2AX foci positive cells were observed at all doses (Cervelli et al., 2014). Human aortic endothelial cells (HAECs) exposed to 100μM H_2O_2 showed 3.7-fold increase in intracellular ROS and a 3.4- and 4.7-fold increase in γ-H2AX and p-ATM, respectively (Sakai et al., 2017).

Time Concordance

There is low evidence to support a time concordance between oxidative stress to strand breaks on DNA. Non-protein-thiol levels, an antioxidant, in in vitro LECs decreased to near zero by 30 minutes post-exposure to 300 µM H_2O_2, before recovering to 70% of control by 120 minutes. At 60 minutes post-exposure to 125 µM H_2O_2 there was a start to a divergence from control level DNA fragmentation, one that increased logarithmically, with the treated group having a 14~18% reduction in intact DNA by 9 h post-exposure (Yang et al., 1998). Time response information is difficult to monitor for DNA strand breaks because repair will occur, reducing the number of breaks over time. At 0 minutes post in vitro exposure to 40 µM H_2O_2 LECs had ~145% of control level DNA strand breaks but that number dropped to ~105% by 30 minutes post-exposure (Li et al., 1998).

Essentiality

Oxidative stress has been found to increase levels of DNA strand breaks above background levels (Li et al., 1998; Liu et al., 2013; Cencer et al., 2018; Ahmadi et al., 2022; El-Missiry et al., 2018; Huang et al., 2021; Cervelli et al., 2017; Sakai et al., 2017). It has been shown that inhibition of oxidative stress leads to a reduction in DNA strand breaks. Sulforaphane (SFN) is an isothiocyanate, which provides chemical protection against ROS by activating the release of enzymatic scavengers. When SFN was added to in vitro LECs exposed to 30 µM H_2O_2, LDH decreased to near unexposed cell levels from the 1.4x control level without SFN. This LDH drop was associated with reducing the levels of DNA strand breaks induced by oxidative stress almost 3-fold as compared to cells without SFN (Liu et al., 2013). In another study, intact DNA levels were returned to control when treated with µPx-11 (peroxidase that breaks down H_2O_2), following exposure to 125 µM H_2O_2. This was a near 100% recovery compared to the drop seen in LECs that did not contain µPx-11 (Spector et al., 1997).

Within the brain of Wistar rats, epigallocatechin-3-gallate (EGCG) ameliorated radiation-induced increases in lipid peroxidation and protein carbonylation, as well as decreases in glutathione (GSH), glutathione peroxidase (GPx) and glutathione reductase (GR) and reverted the levels back to those similar to controls. DNA strand break parameters also returned to those similar to controls after treatment with EGCG (El-Missiry et al., 2018). Similar effects were also shown in another study using treatment mesenchymal stem cell-conditioned medium in mouse hippocampal cells exposed to 10 Gy of X-irradiation (Huang et al., 2021).

HUVECs pretreated with the antioxidant mixture RiduROS blunted ROS generation in a concentration-dependent manner by 65% ± 5.6% and 98% ± 2%, at 0.1 and 1 µg/mL, respectively, compared with cells irradiated without pretreatment. Low-dose irradiation also increased DSB-induced γ-H2AX foci compared with control cells and 24 h of RiduROS pretreatment reduced the γ-H2AX foci number by 41% (Cervelli et al., 2017). Additionally, HAECs treated with eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) found significantly reduced intracellular ROS at 100µM, as well as reduced γ-H2AX foci formation by 47% and 48% following EPA and DHA treatment respectively. (Sakai et al., 2017).

Uncertainties and Inconsistencies

N/A.

Quantitative Understanding of the Linkage

The following tables provide representative examples of the relationship, unless otherwise indicated, all data is significantly significant.

Dose Concordance

Reference	Experiment Description	Result
Cencer et al., 2018	In vitro, human LECs exposed to UVB and tested for 120 min post exposure with fluorescent probes to detect ROS production and mitochondrial superoxide, and tetramethylrhodamine-dUTP (TMR) red assay to detect strand breaks.	Both ROS and DNA strand breaks were increased by both 0.014 J/cm2 and 0.14 J/cm2 UVB radiation. At 0.014 J/cm2, cellular ROS increased a maximum of 15 fluorescence units above the control at 5 min post-UVB, while DNA strand breaks increased about 115 fluorescence units above the control at this time. At 0.14 J/cm2, cellular ROS increased a maximum of about 35 fluorescence units above the control at 90 min post-UVB, while mitochondrial superoxide increased about 30 fluorescence units above the control and DNA strand breaks increased about 125 fluorescence units above the control at this time.
Ahmadi et al., 2021	In vitro, human LECs exposed to 0.065-0.3 Gy/min gamma radiation, with dihydroethdium (DHE) fluorescent probes to measure ROS levels and comet assay to measure strand breaks.	Human LECs exposed in vitro to 0.1 - 0.5 Gy gamma rays showed a gradual increase in ROS levels and a corresponding gradual increase in DNA in the tail from the comet assay (indicative of increased DNA strand breaks) with the maximum dose displaying a 10% increase in ROS levels and a 17% increase in DNA strand damage.

Li et al., 1998	In vitro, bovine LECs were exposed to 40 and 400 µM H2O2 with an alkaline unwinding assay to determine strand break levels.	Immediately after LECs were exposed to 40 µM and 400 µM H2O2, there were ~145% and ~150% DNA strand breaks compared to the unexposed control level, respectively. The amounts of DNA strand breaks in cells exposed to both concentrations were reduced to ~105% of the unexposed control level after 30 min. After 400 µM H2O2, oxidative stress as measured by LDH was 1200% of control in neuroblastoma cells.
Spector et al., 1997	In vitro, rat LECs exposed to 100 and 125 µM H2O2 with alkaline elution assay to determine single strand break level.	Exposure to 125 µM of H2O2 to lens epithelial cells resulted in reduction of intact DNA to near 1% by 9 hr post-exposure. Exposure to 100 µM H2O2 reduced SOD and GSH levels by 2-fold.
El-Missiry et al., 2018	In vivo, albino Wistar rats were exposed to 4 Gy of γ radiation (137Cs source) at 0.695 rad/s. Kits were used to measure 4-HNE (secondary product of lipid peroxidation) and protein carbonyl group levels as markers of oxidative stress. Antioxidants including GSH, GPx and GR were also assessed. The comet assay was used to analyze DNA strand breaks by visualizing DNA tail %, tail length and tail moment.	4-HNE and protein carbonyl levels increased by approximately 2- and 3-fold after radiation exposure. GSH and GPx levels decreased by approximately 3-fold each, whereas GR levels decreased by approximately 5-fold. Tail DNA %, tail length and tail moment increased by approximately 2-, 3- and 6-fold after exposure to 4 Gy.
Ungvari et al., 2013	In vitro. CMVECs and rat hippocampal neurons were irradiated with 2-8 Gy 137Cs gamma rays. 5(and 6)-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate acetyl ester (CM-H2DCFDA) staining, and flow cytometry were used to measure ROS production. DNA damage was quantified by measuring the tail DNA content (as a percentage of total DNA) using the Comet Assay-IV software.	Day 1 post-irradiation showed increased cellular peroxide production and increased mitochondrial oxidative stress in CMVECs in a dose-dependent manner, increasing a maximum of ~3-fold at 8 Gy. Tail DNA content also increased in a dose-dependent manner with an approximate increase from 0 to 45% at 8 Gy.
Huang et al., 2021	In vitro, HT22 cells (mouse hippocampal neuronal cell line) were exposed to 10 Gy of X-irradiation at 6 Gy/min. ROS levels were measured using H2-DCFDA staining and fluorescence microscope analysis, whereas western blotting was used to detect γ-H2AX.	At 10 Gy, intracellular ROS generation increased by 5-fold and γ-H2AX increased by 3-fold.
Zhang et al., 2017	In vitro. HT22 cells were exposed to 8 and 12 Gy X-rays. Relative intracellular ROS levels were determined by DCFDA. p-ATM, γ-H2AX were measured with Western blot.	Following 8 Gy irradiation, intracellular ROS levels increased ~1.8-fold. Phosphorylation of ATM and γ-H2AX were increased 4.4-fold and 3.2-fold, respectively, 30 min after 12 Gy.
Cervelli et al., 2014	In vitro. HUVECs were irradiated with single doses (0.125, 0.25, 0.5 Gy), or fractionated doses (2 × 0.125 Gy, 2 × 0.250 Gy) of X-rays. Intracellular ROS generation was measured with a fluorescent dye, C-DCFDA, using a spectrofluorometer. Immunofluorescence microscopy was used to measure γ-H2AX foci.	Intracellular ROS production was significantly increased in a dose-dependent manner (1.6-, 2- and 2.8-fold at 0.125, 0.25, 0.5 Gy, respectively). When HUVECs were exposed to fractionated doses, no increase in ROS generation was observed, compared with respective single doses. 24h post-irradiation the percentage of foci-positive cells exposed to 0.125 Gy, 2 × 0.125 Gy, 0.250 Gy, 2 × 0.250 Gy and 0.5 Gy, was 1.68, 1.48, 3.53, 2.59, 8.74-fold over the control, respectively.
Sakai et al., 2017	In vitro. HAECs were exposed to 100uM H2O2. Intracellular ROS was measured by CM-H2DCFDA. DNA DSBs were detected by immunofluorescent analysis with γ-H2AX as a marker.	Intracellular ROS increased by ~3.7-fold p-ATM increased by ~4.7-fold. γ-H2AX increased by ~3.4-fold.

Incidence Concordance

Reference	Experiment Description	Result

Meng et al., 2021	In vitro, human LECs exposed to 50 µM H2O2 with DCFH-DA fluorescent probe to detect ROS levels and immunofluorescence and western blot assay to detect γ-H2AX.	50 µM H2O2 exposure to lens epithelial cells increased oxidative stress, with ROS measured by LDH, by 4-fold and decreased the level of antioxidants by 2-fold as measured by SOD and GSH-PX. This resulted in 3-fold increase in γ-H2AX.
Smith et al., 2015	In vitro, human LECs exposed to 30 µM H2O2 with alkaline comet assay to determine amount of strand breaks.	Treatment of lens epithelial cells to 30 µM H2O2 induced DNA strand breaks by 55% at 0.5 hr after exposure and increased the level of LDH by ~1.4 fold at 24 hr post-exposure.
Liu et al. 2013	In vitro, human LECs exposed to 30 µM H2O2 with alkaline comet assay determination of strand breaks.	LDH increased by ~1.4 fold at 24 hr post-exposure, with a 5x increase from control levels in DNA strand breaks.

Time Concordance

Reference	Experiment Description	Result
Yang et al., 1998	In vitro, rabbit LECs exposed to H2O2 with TCA addition and thiol assay to determine non-protein thiol (NP-SH) level and alkaline elusion assay to determine strand breaks.	In rabbit LECs exposed in vitro to 125 µM H2O2, non-protein thiol levels decreased to <5% control (indicates oxidative stress) 30 min post-irradiation, and % DNA retained using alkaline elution decreased by 1.6 log (indicates increased DNA fragmentation) within the next 8.5 h.

Known modulating factors

There is limited evidence demonstrating this relationship across different life stages/ages or sexes (Cencer et al., 2018; Li et al., 1998).

Modulating Factors	MF Details	Effects on the KER	References
Age	Reduced antioxidant capacities have been linked to aged lenses (in humans >30 years old). The development of a chemical barrier between the cortex and the nucleus is partially responsible, as it prevents GSH from protecting aged lens cells from ROS.	Prevention of RONS-mediated damage is primarily achieved by antioxidants, so a lowered capacity would likely lead to reduced damage mitigation abilities. 78% of lens over 30 had a low level of GSH in the center compared to 14% of lens under 30. Lens epithelial cells have an associated 3-fold increase in γ-H2AX (marker of DNA damage) when GSH-PX decreases by 2-fold.	Taylor & Davies, 1987; Cabrera & Chihuailaf, 2011; Quinlan & Hogg, 2018; Sweeney & Truscott, 1998; Meng & Fang, 2021
Free radical scavengers	ROS-scavengers are essential components of the body's natural defense against oxidative damage. Increased ROS production leads to increased incidence of electron donation by scavengers, thus reducing the overall level of free radical scavengers available to deal with ROS.	Isothiocyanates, such as sulforaphane (SFN), activate the release of more enzymatic scavengers. When SFN was added to in vitro LECs, LDH decreased to near unexposed cell levels and was associated with 3.3x less DNA strand breaks compared to the non-SFN cells following stressor exposure. Epigallocatechin-3-gallate (EGCG) also has antioxidant properties and was shown to alleviate radiation-induced increases in oxidative stress and DNA strand breaks within rat hippocampi.	Taylor et al., 1987; Cabrera et al., 2011; Liu et al., 2013; El-Missiry et al., 2018
Media	Mesenchymal stem cell-conditioned medium (MSC-CM), which has self-renewal, differential and proliferation capacities.	MSC-CM treatment has also been shown to improve ROS levels and decrease radiation-induced DNA strand breaks within mouse hippocampal neuronal cells.	Huang et al., 2021

Known Feedforward/Feedback loops influencing this KER

Not identified.

References

Ahmadi, M. et al. (2021), "Early Responses to Low-Dose Ionizing Radiation in Cellular Lens Epithelial Models", Radiation Research, Vol.197, Radiation Research Society, Indianapolis, https://doi.org/10.1667/RADE-20-00284.1.

Aitken, R.J. and C. Krausz. (2001), "Oxidative stress, DNA damage and the Y chromosome", Reproduction, Vol.122/2001, Bioscientifica, Bristol, https://doi.org/10.1530/rep.0.1220497.

Annesley, S.J. and P.R. Fisher. (2019), "Mitochondria in Health and Disease", Cells, Vol.8/7, MDPI, Basel, https://doi.org/10.3390/cells8070680.

Brennan, L., R. McGreal and M. Kantorow. (2012), "Oxidative stress defense and repair systems of the ocular lens",

Frontiers in Bioscience – Elite, Vol.4/E(1), Frontiers in Bioscience, Singapore, https://doi.org/10.2741/365.

Britton, S. et al. (2020), “ATM antagonizes NHEJ proteins assembly and DNA-ends synapsis at single-ended DNA double strand breaks”, Nucleic Acids Research, Vol.48/17, Oxford University Press, Oxford, https://doi.org/10.1093/nar/gkaa723.

Cabrera, M. and R. Chihuailaf. (2011), “Antioxidants and the integrity of ocular tissues”, Veterinary Medicine International, Vol.2011, Hindawi Limited, London, https://doi.org/10.4061/2011/905153.

Cannan, W. and D. Pederson. (2016), “Mechanisms and consequences of double-strand DNA break formation in chromatin”, Journal of Cell Physiology, Vol.231/1, Wiley, Hoboken, https://doi.org/10.1002/jcp.25048.

Cencer, C. et al. (2018), “PARP-1/PAR Activity in Cultured Human Lens Epithelial Cells Exposed to Two Levels of UVB Light”, Photochemistry and Photobiology, Vol.94/1, Wiley-Blackwell, Hoboken, https://doi.org/10.1111/php.12814.

Cervelli, T.et al. (2014), “Effects of single and fractionated low-dose irradiation on vascular endothelial cells”, Atherosclerosis, Vol.235/2, Elsevier, Amsterdam, https://doi.org/10.1016/j.atherosclerosis.2014.05.932.

Cervelli, T. et al. (2017), “A New Natural Antioxidant Mixture Protects against Oxidative and DNA Damage in Endothelial Cell Exposed to Low-Dose Irradiation”, Oxidative medicine and cellular longevity, Vol. 2017, Hindawi, London, https://doi.org/10.1155/2017/9085947.

Climent, M. et al. (2020), “MicroRNA and ROS Crosstalk in Cardiac and Pulmonary Diseases”, International Journal of Molecular Science, Vol.21/12, MDPI, Basel, https://doi.org/10.3390/ijms21124370.

Dahm-Daphie, J., C. Sass, and W. Alberti. (2000), “Comparison of biological effects of DNA damage induced by ionizing radiation and hydrogen peroxide in CHO cells”, International Journal Radiation Biology, Vol.76/1, Informa, London, https://doi.org/10.1080/095530000139023.

El-Missiry, M. A. et al. (2018), "Neuroprotective effect of epigallocatechin-3-gallate (EGCG) on radiation-induced damage and apoptosis in the rat hippocampus", International Journal of Radiation Biology, Vol. 94/9, https://doi.org/10.1080/09553002.2018.1492755.

Engwa, G.A., F.N. Nweke and B.N. Nkeh-Chungag. (2020), “Free Radicals, Oxidative Stress-Related Diseases and Antioxidant Supplementation”, Alternative therapies in health and medicine, Vol.28/1, InnoVision Health Media, Eagan, pp.114-128.

Feng, C. et al. (2016), “Lycopene protects human SH-SY5Y neuroblastoma cells against hydrogen peroxide-induced death via inhibition of oxidative stress and mitochondria-associated apoptotic pathways”, Molecular Medicine Reports, Vol.13/5, Spandidos Publications, Athens, https://doi.org/10.3892/mmr.2016.5056.

Fong, C.W. (2016), “Platinum anti-cancer drugs: Free radical mechanism of Pt-DNA adduct formation and anti-neoplastic effect”, Free Radical Biology and Medicine, Vol.95/June 2016, Elsevier, Amsterdam, https://doi.org/10.1016/j.freeradbiomed.2016.03.006.

Halliwell, B. et al. (2021), “Hydroxyl radical is a significant player in oxidative DNA damage in vivo”, Chemical Society Reviews, Vol.50, Royal Society of Chemistry, London, https://doi.org/10.1039/d1cs00044f.

Huang, Y. et al. (2021), "Mesenchymal Stem Cell-Conditioned Medium Protects Hippocampal Neurons From Radiation Damage by Suppressing Oxidative Stress and Apoptosis", Dose-Response, Vol. 19/1, https://doi.org/10.1177/1559325820984944.

Kay, J. et al. (2019), “Inflammation-induced DNA damage, mutations and cancer”, DNA Repair, Vol.83, Elsevier, Amsterdam, https://doi.org/10.1016/j.dnarep.2019.102673.

Jeggo, P.A., V. Geuting and M. Löbrich. (2011), “The role of homologous recombination in radiation-induced double-strand break repair”, Radiotherapy and Oncology, Vol.101/1, Elsevier, Amsterdam, https://doi.org/10.1016/j.radonc.2011.06.019.

Kurutas E. B. (2016), “The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state”, Nutrition journal, Vol.15/1, Biomed Central, London, https://doi.org/10.1186/s12937-016-0186-5.

Kruk, J., K. Kubasik-Kladna and H. Aboul-Enein. (2016), “The Role Oxidative Stress in the Pathogenesis of Eye Diseases: Current Status and a Dual Role of Physical Activity”, Mini-Review in Medicinal Chemistry, Vol.16/3, Bentham Science Publishers, Sharjah, https://doi.org/10.2174/1389557516666151120114605.

Li, Y. et al. (1998), “Response of lens epithelial cells to hydrogen peroxide stress and the protective effect of caloric restriction”, Experimental Cell Research, Vol.239/2, Elsevier, Amsterdam, https://doi.org/10.1006/excr.1997.3870.

Liu, H. et al. (2013), “Sulforaphane can protect lens cells against oxidative stress: Implications for cataract prevention”, Investigative Ophthalmology and Visual Science, Vol.54/8, Association for Research in Vision and Ophthalmology, Rockville, https://doi.org/10.1167/iovs.13-11664.

Meng, K. and C. Fang. (2021), “Knockdown of Tripartite motif-containing 22 (TRIM22) relieved the apoptosis of lens epithelial cells by suppressing the expression of TNF receptor-associated factor 6 (TRAF6)”, Bioengineered, Vol.12/1,

Taylor & Francis, Oxfordshire, https://doi.org/10.1080/21655979.2021.1980645.

Nishida, M. et al. (2005), “Ga12/13- and Reactive Oxygen Species-dependent Activation of c-Jun NH2-terminal Kinase and p38 Mitogen-activated Protein Kinase by Angiotensin Receptor Stimulation in Rat Neonatal Cardiomyocytes”, Journal of Biological Chemistry, Vol.280/18, American Society for Biochemistry and Molecular Biology, Rockville, https://doi.org/10.1074/jbc.M409710200.

Poetsch, A.R. (2020), “The genomics of oxidative DNA damage, repair, and resulting mutagenesis”, Computational and Structural Biotechnology Journal, Vol.18, Elsevier, Amsterdam, https://doi.org/10.1016/j.csbj.2019.12.013.

Quinlan, R.A., and P.J. Hogg. (2018), “γ-Crystallin redox–detox in the lens”, Journal of Biological Chemistry, Vol.293/46, American Society for Biochemistry and Molecular Biology, Rockville, https://doi.org/10.1074/jbc.H118.006240.

Sakai, C. et al. (2017), “Fish oil omega-3 polyunsaturated fatty acids attenuate oxidative stress-induced DNA damage in vascular endothelial cells”, PloS one, Vol.12/11, https://doi.org/10.1371/journal.pone.0187934.

Scully, R. and A. Xie. (2013), “Double strand break repair functions of histone H2AX”, Mutation Research, Vol.750/1-2, Elsevier, Amsterdam, https://doi.org/10.1016/j.mrfmmm.2013.07.007.

Smith, A. et al. (2015), “Ku80 counters oxidative stress-induced DNA damage and cataract formation in the human lens”, Investigative Ophthalmology and Visual Science, Vol.56/13, Association for Research in Vision and Ophthalmology, Rockville, https://doi.org/10.1167/iovs.15-18309.

Spector, A. et al. (1997), “Microperoxidases catalytically degrade reactive oxygen species and may be anti-cataract agents”, Experimental Eye Research, Vol.65/4, Academic Press Inc, Cambridge, https://doi.org/10.1006/exer.1997.0336.

Spector, A. et al. (1996), “Variation in cellular glutathione peroxidase activity in lens epithelial cells, transgenics and knockouts does not significantly change the response to H2O2 stress”, Experimental Eye Research, Vol.62/5, Academic Press Inc, Cambridge, https://doi.org/10.1006/exer.1996.0063.

Spector, A. (1995), “Oxidative stress-induced cataract: mechanism of action”, The FASEB Journal, Vol.9/12, Federation of American Societies for Experimental Biology, Bethesda, https://doi.org/10.1096/fasebj.9.12.7672510.

Spector, A. (1990), “Oxidation and Aspects of Ocular Pathology”, CLAO Journal, Vol.16/1, Lippincott, Williams and Wilkins Ltd, Philadelphia, S8-S10.

Stohs, S. (1995), “The role of free radicals in toxicity and disease”, Journal of Basic Clinical Physiology and Pharmacology, Vol.6/3-4, Walter de Gruyter GmbH, Berlin, https://doi.org/10.1515/jbcpp.1995.6.3-4.205.

Sweeney, M.H.J. and R.J.W. Truscott. (1998), “An Impediment to Glutathione Diffusion in Older Normal Human Lenses: a Possible Precondition for Nuclear Cataract”, Experimental Eye Research, Vol.67, Academic Press Inc, Cambridge, https://doi.org/10.1006/exer.1998.0549.

Taylor, A. and K. J. A. Davies (1987), “Protein oxidation and loss of protease activity may lead to cataract formation in the aged lens”, Free Radical Biology & Medicine, Vol. 3, Pergamon Journals Ltd, United States of America, pp. 371-377

Ungvari, Z. et al. (2013), "Ionizing Radiation Promotes the Acquisition of a Senescence-Associated Secretory Phenotype and Impairs Angiogenic Capacity in Cerebromicrovascular Endothelial Cells: Role of Increased DNA Damage and Decreased DNA Repair Capacity in Microvascular Radiosens", The Journals of Gerontology Series A: Biological Sciences and Medical Sciences, Vol. 68/12, https://doi.org/10.1093/gerona/glt057.

Uwineza, A. et al. (2019), “Cataractogenic load – A concept to study the contribution of ionizing radiation to accelerated aging in the eye lens”, Mutation Research - Reviews in Mutation Research, Vol.779, Elsevier, Amsterdam, https://doi.org/10.1016/j.mrrev.2019.02.004.

Ward, J.F., W.F. Blakely & E.I. Joner. (1985), “Mammalian Cells Are Not Killed by DNA Single-Strand Breaks Caused by Hydroxyl Radicals from Hydrogen Peroxide”, Radiation Research, Vol.103/3, Radiation Research Society, Indianapolis, hptts://doi.org/10.2307/3576760.

Wu, H. et al. (2021), “Lactate dehydrogenases amplify reactive oxygen species in cancer cells in response to oxidative stimuli”, Signal Transduction and Targeted Therapy, Vol.6/1, Nature Portfolio, Berlin, https://doi.org/10.1038/s41392-021-00595-3.

Yang, Y. et al. (1998), “The effect of catalase amplification on immortal lens epithelial cell lines”, Experimental Eye Research, Vol.67/6, Academic Press Inc, Cambridge, https://doi.org/10.1006/exer.1998.0560.

Yuan, J., R. Adamski and J. Chen. (2010), “Focus on histone variant H2AX: To be or not to be”, FEBS Letters, Vol.584/17, Wiley, Hoboken, https://doi.org/10.1016/j.febslet.2010.05.021.

Zhang, L. et al. (2017), "The inhibitory effect of minocycline on radiation-induced neuronal apoptosis via AMPKα1 signaling-mediated autophagy", Scientific Reports, Vol. 7/1, https://doi.org/10.1038/s41598-017-16693-8.

Zhou, Y. et al. (2016), “Protective Effect of Rutin Against H2O2-Induced Oxidative Stress and Apoptosis in Human Lens Epithelial Cells”, Current Eye Research, Vol.41/7, Taylor & Francis, Oxfordshire,

https://doi.org/10.3109/02713683.2015.1082186.

Relationship: 2812: Oxidative Stress leads to Modified Proteins

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Deposition of energy leading to occurrence of cataracts	adjacent	Moderate	Low

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	Low	NCBI
guinea pig	Cavia porcellus	Moderate	NCBI
rabbit	Oryctolagus cuniculus	Low	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	Moderate

Sex Applicability

Sex	Evidence
Unspecific	Moderate

This KER is plausible in all life stages, sexes, and organisms. The majority of the evidence is from in vivo male adult guinea pigs and rabbit in vitro models that do not specify sex.

Key Event Relationship Description

Oxidative stress refers to production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), and a reduction/insufficiency in radical-clearing enzymes (Brennan et al., 2012; Engwa et al., 2022; Cabrera & Chihuailaf, 2011). Under normal conditions, radicals are kept at a sustainable level by the body's antioxidant defense system but if the radicals exceed the defense threshold, it can lead to protein oxidation (Taylor & Davies, 1987; Cabrera & Chihuailaf, 2011; Engwa et al., 2022).ROS and RNS, collectively known was RONS, have subdivisions of radicals and non-radicals, with the former being the more reactive (Cabrera & Chihuailaf, 2011; Engwa et al., 2022). The superoxide ion radical works to oxidize biological structures such as proteins and DNA, as well as helping to generate other types of radicals. Superoxide ion can oxidize the amino acids arginine into glutamic semialdehyde and methionine into disulphides. Ozone, another ROS, specifically oxidizes proteins by reacting with their alcohol, amine, and sulfhydryl functional groups (Engwa et al., 2022). Furthermore, H_2O_2 is able to travel further than other ROS as it is more stable (Spector, 1990), it can also interact with transition metal ions (Cu+ or Fe2+) that are often bound to proteins such as ferritin and ceruloplasmin. This interaction oxidizes the protein, converting H_2O_2 into a hydroxyl radical. (Engwa et al., 2022). Another example of non-radical oxidation of proteins is peroxynitrite's action on tryptophan and methionine. These amino acids are oxidized, tryptophan into nitrotryptophan and methionine into methionine sulfoxide or ethylene (Engwa et al., 2022; Perrin & Koppenol, 2000). There is also evidence to support H_2O_2 leading to protein modifications, however singlet oxygen or hydroxyl radicals seem to not be involved (Hightower, 1995). Targets of free radicals can include lipids, DNA, and proteins (Engwa et al., 2022).

Antioxidants stabilize radicals by facilitating an electron donation (Cabrera & Chihuailaf, 2011). This reduces the number of radicals available to oxidize other macromolecules like proteins, thus reducing the number of molecules sustaining modifications (Engwa et al., 2022). Proteins are particularly good targets of free radicals because of their abundance of amino acids containing sulfur and aromatics, as well as the fact that following proline oxidation, peptide bonds are at risk of free radical attack (Cabrera & Chihuailaf, 2011). Free radicals have an affinity for sulfur-containing amino acids, such as cysteine and methionine, due to their ability to readily react with most ROS, making the proteins containing them the most susceptible to oxidative modifications. This quality of the amino acids makes them act in an antioxidant capacity for the other structures in the area (Bin et al., 2017).

Proteins that interact with RONS will undergo bond alterations that can lead to aggregation. Free radicals can modify proteins in both reversible and irreversible ways. Redox-response proteins get oxidized as part of the protective mechanism against oxidizing radicals but will be repaired once the threat is over. In this instance, modifications are reversible, and homeostasis is maintained via antioxidative action. These proteins function as buffer, reducing free radicals before that can oxidize other proteins. Irreversible oxidation, on the other hand, occurs when there is oxidation on important functional or structural sites (Chen et al., 2013). These sites are important to a certain function of a protein or help maintain its specific structural configuration. This damage can result in loss of function and/or misfolding of proteins. The amino acids of proteins are very susceptible to ROS attacks, with methionine, tryptophan, histidine, and cysteine residues being the most at risk (Chen et al., 2013; Balasubramanian, 2000). Once the amino acids get oxidized by the ROS, they become oxidation products and are no longer useful for the originally intended function within the protein (Engwa et al., 2022).

Protein carbonyl level is changed by ROS exposure through the post-translational modification called carbonylation, where carbonyl groups are added to the protein (Grimsrud et al., 2008). ROS accomplishes this by interacting with amino acids such as proline and lysine, on the protein side chains, which tend to create carbonyl derivatives (Engwa et al., 2022). In proteins attacked by radicals, there is also a tendency to form cross-links between the proteins. These connections affect water solubility of the proteins. Normal proteins have a balance of protein-protein and protein-water interactions that maintain structure and solubility, however following the oxidation of the amino side chains of the proteins, they become thermodynamically preferred to have more protein-protein interactions. This causes an increase in cross-linking and aggregation, which leads to decreased water solubility (Xiong, 2000).

Evidence Supporting this KER

Overall Weight of Evidence: Moderate

Biological Plausibility

The biological plausibility of the relationship between increased oxidative stress leading to modified proteins is strongly supported by the literature. Studies show that increase of oxidative stress, leads to protein modifications (Uwineza et al., 2019; Taylor & Davies, 1987; Truscott, 2005; Brennan et al., 2012; Davies & Truscott, 2001). This relationship has been observed in rabbit and guinea pig models (Shang et al., 2001; Giblin et al., 2002). Excessive ROS generation can lead to oxidization of amino acid side chains, cross-link formation, and conformational changes (Uwineza et al., 2019). Radical oxygen species can modify protein molecules chemically, and act to increase proteolytic activity of cellular enzymes by inactivating proteolytic enzyme inhibitors (Balasubramanian, 2000; Stohs, 1995). Oxidation via radical species-protein interactions can also lead to increased insolubility of the proteins due to their modified structure and inability to interact with unmodified proteins (Kim et al., 2015).

Proteins aggregation can be exacerbated by protein oxidation. For example, in lens cells, the presence of free radicals can attack abundant proteins such as alpha crystalline. The thiol groups on the crystallin proteins then become oxidized and increase the number of disulfide adducts, increasing protein aggregates (Cabrera & Chihuailaf, 2011; Moreau et al., 2012). Amino acid side chains are particularly susceptible to damage from oxidative stress, resulting in cross-linking and conformational changes which can culminate in protein accumulation. The accumulation is a result of the cells being denucleated and therefore, unable to reverse the sustained damage via protein turnover (Uwineza et al., 2019). Oxidative conditions can contribute to the loss of protein function leading to the generation of high molecular weight aggregates. This change is hypothesised to be a result of methionine oxidation, which is more likely to happen when GSH levels are low resulting in an increase in hydroxyl radical formation (Brennan et al., 2012; Truscott, 2005). The hydroxyl radical also results in covalently bound protein aggregates, and alongside superoxide ion it leads to protein fragmentation (Stohs, 1995).

Empirical Evidence

Empirical evidence to support increased oxidative stress leading to modified proteins is low. Experimental studies include in vitro lens epithelial cells of rabbits, as well as in vivo whole lens of guinea pigs (Shang et al., 2001; Giblin et al., 2002).

Dose/Incidence Concordance

There is low evidence to support dose concordance between oxidative stress and modified proteins. ROS clearing enzyme levels such as GSH in lens cortices decreased significantly at both doses of H_2O_2: 0.65x control at 4 h of 20 µM and 0.16x control at 4 h of 60 µM. Change in protein carbonyl concentration was 1.25x control after 4 h of 20 µM but reached 3.67x control following 4 h of 60 µM H_2O_2 in vitro exposure (Shang et al., 2001). Following 4-5 months UVA, in vivo lenses experienced a 29% reduction in GSH associated with 20% reduction in soluble proteins (Giblin et al., 2002).

Time Concordance

No data available

Essentiality

No data available

Uncertainties and Inconsistencies

N/A

Quantitative Understanding of the Linkage

The following tables provide representative examples of the relationship, unless otherwise indicated, all data is significantly significant.

Dose Concordance

Reference	Experiment Description	Result
Shang et al., 2001	In vitro, rabbit lens epithelial cells exposed to 0-60 µM H_2O_2 with Western blot assay used to assay protein carbonyl levels and HPLC used to determine GSH levels.	Rabbit LECs exposed to 0-60 µM H_2O_2 showed a gradual decrease in GSH levels (indicative of oxidative stress) and a corresponding gradual increase in protein carbonyl concentration with the maximum dose displaying a 1.6x decrease in GSH and a 3.67x increase in protein carbonyl concentration.

Incidence Concordance

Reference	Experiment Description	Result
Giblin et al., 2002	In vivo, guinea pigs received whole body exposure to UVA radiation at a dose rate of 0.5 mW/cm2, 24 h a day, over a 4-5-month period with protein solubility changes measured by BCA protein assay and GSH measured using Ellman’s reagent.	Guinea pig lens cells exposed to 5 months of 0.5 mW/cm2 UVA (indicative of dose) displayed a 29% decrease in GSH levels and a 20% increase in water-insoluble proteins relative to controls.

Known modulating factors

Modulating Factor (MF)	MF Specification	Effect(s) on the KER	Reference(s)

Modulating Factor (MF)	MF Specification	Effect(s) on the KER	Reference(s)
Free Radical Scavengers	Antioxidant supplementation has been linked to reduced oxidative damage. The scavengers work to reduce the reactivity of ROS in the cell by donating one of their own electrons, resulting in a matching pair on the radical.	Lower levels of free radical scavengers would result in a limited ability to reduce RONS-mediated damage. Reduced GSH levels are associated with protein modifications, including changes to water-solubility (29% decrease GSH ≥ 20% decrease soluble proteins) and protein carbonyl concentration (84% decrease GSH ≥ 367% increase carbonyl concentration).	Taylor & Davies, 1987; Cabrera & Chihuailaf, 2011; Giblin et al., 2002; Shang et al., 2001
Age	Older lenses have reduced antioxidant capacities (in humans >30 years old). This is due in part to the development of a chemical barrier between the cortex and the nucleus of the lens that prevents GSH from protecting the oldest lens cells from oxidative damage.	Antioxidants function to prevent RONS-mediated damage, so proteins in older lenses, with reduced antioxidant capacities, will be more likely to undergo oxidative modifications.	Taylor & Davies, 1987; Cabrera & Chihuailaf, 2011; Quinlan & Hogg, 2018; Sweeney & Truscott, 1998

Known Feedforward/Feedback loops influencing this KER

N/A

References

Balasubramanian, D. (2000), “Ultraviolet radiation and cataract”, Journal of Ocular Pharmacology and Therapeutics, Vol.16/3, Mary Ann Liebert Inc, Larchmont, https://doi.org/10.1089/jop.2000.16.285.

Bin, P., R. Huang and X. Zhou. (2017). “Oxidation Resistance of the Sulfur Amino Acids: Methionine and Cysteine”, BioMed Research International, Vol.2017, Hindawi Limited, London, https://doi.org/10.1155/2017/9584932.

Brennan, L., R. McGreal and M. Kantorow. (2012), “Oxidative stress defense and repair systems of the ocular lens”, Frontiers in Bioscience – Elite, Vol.4/E(1), Frontiers in Bioscience, Singapore, https://doi.org/10.2741/365.

Cabrera, M., & R. Chihuailaf. (2011), “Antioxidants and the integrity of ocular tissues”, Veterinary Medicine International, Vol.2011, Hindawi Limited, London, https://doi.org/10.4061/2011/905153.

Chen, Y. et al. (2013), “Ocular aldehyde dehydrogenases: Protection against ultraviolet damage and maintenance of transparency for vision”, Progress in Retinal and Eye Research, Vol.33/1, Elsevier Ltd, London, https://doi.org/10.1016/j.preteyeres.2012.10.001.

Davies, M. and R. Truscott. (2001), “Photo-oxidation of proteins and its role in cataractogenesis”, Journal of Photochemistry and Photobiology B: Biology, Vol.63/1-3, Elsevier, Amsterdam, https://doi.org/10.1016/s1011-1344(01)00208-1.

Engwa, G.A., F.N. Nweke and B.N. Nkeh-Chungag. (2022), “Free Radicals, Oxidative Stress-Related Diseases and Antioxidant Supplementation”, Alternative therapies in health and medicine, Vol.28/1, InnoVision Health Media, Eagan, pp.114-128.

Giblin, F. et al. (2002), “UVA Light In vivo Reaches the Nucleus of the Guinea Pig Lens and Produces Deleterious, Oxidative Effects”, Experimental Eye Research, Vol.75/4, Academic Press Inc, Cambridge, https://doi.org/10.1006/exer.2002.2039.

Grimsrud, P.A. et al. (2008), “Oxidative Stress and Covalent Modification of Protein with Bioactive Aldehydes”, Journal of Biological Chemistry, Vol.283/32, American Society for Biochemistry and Molecular Biology, Rockville, https://doi.org/10.1074/jbc.R700019200.

Hightower, K. (1995), “The role of the Lens epithelium in development of UV cataract”, Current Eye Research, Vol.14/1, Taylor & Francis, Oxfordshire, https://doi.org/10.3109/02713689508999916.

Kim, I. et al. (2015), “Site specific oxidation of amino acid residues in rat lens γ-crystallin induced by low-dose γ-irradiation”, Biochemical and Biophysical Research Communications, Vol.466/4, Elsevier, Amsterdam, https://doi.org/10.1016/j.bbrc.2015.09.075.

Kozbenko, T. et al. (2022), “Deploying elements of scoping review methods for adverse outcome pathway development: a space travel case example”, International Journal of Radiation Biology, 1–12. https://doi.org/10.1080/09553002.2022.2110306

Perrin, D. and W.H. Koppenol. (2000), “The Quantitative Oxidation of Methionine to Methionine Sulfoxide by Peroxynitrite”, Archives of Biochemistry and Biophysics, Vol.377/2, Elsevier, Amsterdam, https://doi.org/10.1006/abbi.2000.1787.

Quinlan, R.A., and P.J. Hogg. (2018), “γ-Crystallin redox–detox in the lens”, Journal of Biological Chemistry, Vol.293/46, American Society for Biochemistry and Molecular Biology, Rockville, https://doi.org/10.1074/jbc.H118.006240.

Shang, F., T. Nowell and A. Taylor. (2001), “Removal of oxidatively damaged proteins from lens cells by the ubiquitin-proteasome pathway”, Experimental Eye Research, Vol.73/2, Academic Press Inc, Cambridge, https://doi.org/10.1006/exer.2001.1029.

Spector, A. (1990), “Oxidation and Aspects of Ocular Pathology”. CLAO Journal, Vol.16/1, Lippincott, Williams and Wilkins Ltd, Philadelphia, S8-S10.

Stohs, S. (1995), “The role of free radicals in toxicity and disease”, Journal of Basic Clinical Physiology and Pharmacology, Vol.6/3-4, Walter de Gruyter GmbH, Berlin, https://doi.org/10.1515/jbcpp.1995.6.3-4.205.

Sweeney, M.H.J. and R.J.W. Truscott. (1998), “An Impediment to Glutathione Diffusion in Older Normal Human Lenses: a Possible Precondition for Nuclear Cataract”, Experimental Eye Research, Vol.67, Academic Press Inc, Cambridge, https://doi.org/10.1006/exer.1998.0549.

Taylor, A. and K. Davies. (1987), “Protein oxidation and loss of protease activity may lead to cataract formation in the aged lens”, Free Radical Biology and Medicine, Vol.3/6, Elsevier, Amsterdam, https://doi.org/10.1016/0891-5849(87)90015-3.

Truscott, R. (2005), “Age-related nuclear cataract - Oxidation is the key”, Experimental Eye Research, Vol.80/5, Academic Press Inc, Cambridge, https://doi.org/10.1016/j.exer.2004.12.007.

Uwineza, A. et al. (2019), “Cataractogenic load- A concept to study the contribution of ionizing radiation to accelerated aging in the eye lens”, Mutation Research - Reviews in Mutation Research, Vol.779, Elsevier, Amsterdam, https://doi.org/10.1016/j.mrrev.2019.02.004.

Xiong, Y.L. (2000), “Chapter 4: Protein Oxidation and Implications for Muscle Food Quality”, in Antioxidants in Muscle Foods: Nutritional Strategies to Improve Quality, Wiley, Hoboken, pp.85-111.

Relationship: 1909: Increase, Oxidative DNA damage leads to Inadequate DNA repair

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Oxidative DNA damage leading to chromosomal aberrations and mutations	adjacent	High	Low
Deposition of energy leading to occurrence of cataracts	adjacent	Moderate	Low

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	Moderate	NCBI
mouse	Mus musculus	Moderate	NCBI
rat	Rattus norvegicus	Low	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	Moderate

Sex Applicability

Sex	Evidence
Unspecific	Moderate

This KER is plausible in all life stages, sexes, and organisms with DNA. The majority of the evidence is from in vivo mice studies of all ages with no specification on sex. No in vitro evidence was found to support the relationship.

Key Event Relationship Description

Oxidative DNA lesions are present in the cell at steady state due to low levels of reactive oxygen species (ROS) and other free radicals generated by endogenous processes involving redox reactions. The most prominent examples of oxidative DNA lesions include 7, 8-dihydro-8oxo-deoxyGuanine (8-oxo-dG), 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FaPydG), and thymidine glycol (Tg). Under homeostatic conditions, cells are able to regulate the level of free radicals and readily repair oxidized DNA bases using basal repair mechanisms to prevent irreversible damage (Swenberg et al., 2011). Oxidative DNA lesions are mainly repaired by base excision repair (BER) initiated by DNA glycosylases such as oxoguanine glycosylase 1 (OGG1), endonuclease III homologue 1 (NTH1), and Nei-like DNA glycosylases (NEIL 1/2), which detect and remove damaged bases. Abasic sites are then cleaved by endonucleases or lyases, resulting in transient single-strand breaks (SSB) that enter either short-patch or long-patch repair. Nucleotide excision repair (NER) and single-strand base repair is also involved in repairing oxidized bases to a lesser extent (Shafirovich et al., 2016; Hedge et al., 2012) Increase in free radicals or exposure to oxidizing agents can increase the level of oxidative DNA lesions and overwhelm the repair pathways, compromising the quality of repair. If the repair mechanisms are compromised, oxidative lesions may accumulate (insufficient repair) and cause incorrect base pairing during replication or incomplete repair (indicated by accumulation of repair intermediates) (Markkanen, 2017).

Evidence Supporting this KER

Overall Weight of Evidence: Moderate

Inadequate repair of oxidative lesions is indicated by an increase in oxidative lesions above background, activation of repair enzymes, increase in repair intermediates (abasic sites and SSBs), and incorrect base insertion opposite lesion during replication (lesion bypass by translesion DNA synthesis).

Biological Plausibility

The mechanism of repair of oxidative DNA lesions in humans is well-established and numerous literature reviews are available on this topic (Berquist and Wilson III, 2012; Cadet and Wagner, 2013). As described above, oxidative DNA lesions are mostly repaired via BER and, to a lesser extent, NER. Previous studies have reported thresholded dose-response curves in oxidative DNA damage and attributed these observations to exceeded repair capacity at the inflection point on the curve (Gagne et al., 2012; Seager et al., 2012). In vivo, increase and accumulation of oxidative DNA lesions despite the activation of BER have been observed following chemical exposures, demonstrating insufficient repair of oxidative DNA lesions past a certain level (Ma et al., 2008).

OGG1 and NTH1, the glycosylases that initiate the BER of 8-oxo-dG and thymine glycol (Tg) lesions, respectively, are bifunctional, containing both glycosylase and lyase activities. The glycosylase removes the oxidized guanine by cleaving the glycosidic bond, giving rise to an apurinic site. The lyase then cleaves the phosphodiester bond 5’ to the AP site; a transient SSB is created for further processing in BER (Delaney et al., 2012). Abasic sites created by OGG1 and other glycosylases are also processed by apuric/apyrimidinic endonucleases (APE1) to create the 5’ nick (Allgayer et al., 2016). The repair process can be inhibited when non-DSB oxidative DNA damage results in altered nuclease or glycosylase activity, making the area resistant to repair following radiation exposure (Georgakilas et al., 2013).

Previous studies have demonstrated that an imbalance in any one of the multiple steps of BER can lead to an accumulation of repair intermediates and failed repair. Given that OGG1 is relatively slower in releasing its catalytic product than other glycosylases, it is highly likely that a disproportionate increase in oxidative DNA lesions compared to the level of available OGG1 would lead to an imbalance between lesions and the initiating step of BER (Brenerman et al., 2014). Accumulation of oxidative lesions would be observed as a result. Moreover, studies have reported accumulation of SSB due to OGG1 and NTH1 overexpression, demonstrating that the imbalanced lyase activity generates excessive SSB intermediates (Yang et al., 2004; Yoshikawa et al., 2015; Wang et al., 2018).

Increases in oxidative lesions may produce more lesions and repair intermediates in close proximity to each other. Previous studies in mammalian cell extracts have reported reduction in repair efficiency when oxidative lesions are in tandem or opposite each other. For example, OGG1 showed reduced binding to 8-oxo-dG near an AP site incision. Furthermore, the OGG1-8-oxo-dG complex has been observed to hinder the repair of neighbouring AP site incision, delaying the completion of BER; this interaction between BER enzymes has been suggested to cause an accumulation of oxidative lesions and repair intermediates (Pearson et al., 2004; Budworth et al., 2005; Bellon et al., 2009; Yoshikawa et al., 2015; Sharma et al., 2016; Georgakilas et al., 2013).

If oxidative lesions persist in the genome due to insufficient repair, incorrect base insertion opposite unrepaired oxidative DNA lesions may occur during replication. This is a well-established event. For example, 8-oxo-dG and FaPydG, the two most prominent oxidative DNA lesions, are able to form base pairs with dATP, giving rise to G:C→T:A transversions after subsequent DNA synthesis (Freudenthal et al., 2013; Gehrke et al., 2013; Markkanen, 2017). Replicative DNA polymerases such as DNA polymerase α, δ, and ε (pol α, δ, ε) have a poor ability to extend the DNA strand past 8-oxo-dG:dCTP base pairs and may cause replication to stall or incorrectly insert dATP opposite 8-oxo-dG (Hashimoto et al., 2004; Markkanen et al., 2012). In stalled replication forks, repair polymerases may be recruited to perform translesion DNA synthesis (TLS). Human Y-family DNA polymerases (Rev 1, pol κ, ι, and η) are DNA repair polymerases mainly involved in TLS in stalled replication forks. However, TLS is not free of error and its accuracy differs for each repair polymerase. For example, it is known that pol κ and η perform TLS across 8-oxo-dG and preferentially insert dATP opposite the lesion, generating G:C→T:A transversions. The error-prone nature of bypassing unrepaired oxidative lesions has been described in many previous studies and reviews (Greenberg, 2012; Maddukuri et al., 2014; Taggart et al., 2014; Shah et al., 2018). There is also risk associated with repairing the lesions, that the process could lead to increased genomic instability and mutation potential. A balance needs to be achieved between the risk posed by repair and that by residual oxidative damage (Poetsch, 2020).

Repair by OGG1 requires 8-oxo-dG:dC base pairing, thus, it is unable to repair 8-oxo-dG:dA mispairing in newly synthesized strands. The repair of 8-oxo-dG:dA base pairs post-replication is performed by MUT Y homologue, MYH, an adenine DNA glycosylase. However, the removal of dA instead of the damaged guanine may lead to futile cycles of BER because: 1) another dA is often inserted opposite the lesion, or 2) BER ligases have a poor ability of ligating the 3’end of dC opposite 8-oxo-dG (Hashimoto et al., 2004; Caglayan and Wilson, 2015). Accumulated 8-oxo-dG may be more resistant to repair post-replication due to this futile BER.

Empirical Evidence

Example in vitro studies demonstrating dose and temporal concordance, or essentiality

- Human normal hepatocytes (HL-7702) were exposed to N,N-dimethylformamide for 24 hours at increasing concentrations (C. Wang et al., 2016)
    - Concentration-dependent increase in ROS was observed; the increase was statistically significant compared to control at all concentrations (6.4, 16, 40, 100 mM)
    - No significant increase in 8-oxodG was observed until the highest two concentrations (40 and 100 mM) indicating insufficient repair at these concentrations
    - Significant up-regulation of excision repair genes (XRCC2 and XRCC3) occurred at 6.4 and 16 mM, below the concentrations that significantly induced 8-oxodG, supporting sufficient DNA repair at these low concentrations.
    - These results demonstrate that repair is sufficient at low concentrations (rapidly removing 8-oxodG) and not until higher concentrations is repair overwhelmed (i.e., insufficient), where 8-oxo-dG significantly increases.

- AS52 Chinese hamster ovary cells (wild type and OGG1-overexpressing (OGG1+)) were exposed to varying doses of ultraviolet A (UVA) radiation (Dahle et al., 2008)
  - Formamidopyrimidine glycosylase (Fpg)-sensitive sites were quantified using alkaline elution after increasing repair times (0, 1, 2, 3, 4 h) following 100 kJ/m^2 UVA irradiation
  - OGG1-overexpressing AS52 cells (OGG1+): Fpg-sensitive sites reduced to 71% within half an hour and down to background levels at 4h
  - Wild type AS52 cells: at 4h, 70% of the Fpg-sensitive sites remained, indicating accumulation of oxidative lesions
  - The above results demonstrated that excess OGG1 was able to prevent the accumulation of oxidative lesions, while the amount of OGG1 in wild type was insufficient to handle the amount of lesions induced by the same magnitude of UVA irradiation.
  - Mutations in the *Gpt* gene was quantified in both wild type and OGG1+ cells by sequencing after 13-15 days following 400 kJ/m^2 UVA irradiation
    - G:C→T:A mutations in UVA-irradiated OGG1+ cells were completely eliminated (thus, repair was sufficient when repair overexpressed).
    - G:C→T:A mutation frequency in wild type cells increased from 1.8 mutants/million cells to 3.8 mutants/million cells following irradiation – indicating incorrect repair or lack of repair of accumulated 8-oxo-dG.
    - The above result also demonstrates the essentiality of 8-oxo-dG formation in the oxidative DNA damage-induced G to T transversion mutations.

- HL-60 human leukemia cells were irradiated with X-rays at a rate of 0.5 Gy/min for increasing durations (i.e., increasing doses). 8-OHdG levels were quantified by HPLC as number of 8-OHdG per 10^6 deoxyguanosine (Li et al., 2013)
  - No increase in 8-OHdG was observed up to 2 Gy (sufficient repair at low doses), above which the level of lesions increased linearly up to 20 Gy (insufficient repair)
  - This thresholded dose-response curve, indicative of overwhelmed repair processes, was also observed in mouse liver in the same study described below.

In vivo studies demonstrating dose or time concordance

- Two groups of 5-week-old C57BL/6J mice were exposed to increasing doses of X-rays at a rate of 0.5 Gy/min (200 kV, 12 mA). The livers were collected from one group immediately after exposure and urine samples were collected over 24 hours following irradiation in the second group of mice (Li et al., 2013).
  - 8-OHdG in the mouse liver DNA were quantified by HPLC and expressed as 8-OHdG per 10^6 deoxyguanosine
  - Between 0 and 0.5 Gy, no increase in lesions was observed
  - Between 0.5 and 30 Gy, a linear dose-response in 8-OHdG was observed
  - The thresholded dose-response curve was concordant in the urine samples; no increase in urinary 8-OHdG (8-OHdG/creatinine (ng/mg)) was observed between 0 and 0.1 Gy but between 0.1 and 5 Gy, the number of lesions increased linearly with dose

- Male Sprague-Dawley rats were fed 0.5 mmol aniline/kg/day for 30 days. Genomic DNA, nuclear extracts, and mitochondrial extracts were collected from spleen tissues (Ma et al., 2008).
  - 8-OHdG was quantified using enzyme-linked immunosorbent assay (ELISA) on digested genomic DNA. There was a significant 2.8-fold increase in lesions in aniline-fed rats than in control rats.
  - Both the nuclear extracts and mitochondrial extracts were tested for OGG1 activity, where 1.32-fold and 1.15-fold increase in enzyme activity (both significant; $p<0.05$) were observed in the respective extracts of aniline-treated rats.
  - The OGG1 enzyme content in the extracts was detected using Western blotting; the increase in OGG1 content in aniline-treated rats was consistent with the OGG1 activity assay.
  - Despite the increase in OGG1 enzyme content and activity, the quantity of 8-OHdG increased.
  - Together, these results demonstrate that repair is sufficient at low concentrations because 8-oxodG adducts are rapidly removed. At higher concentrations, 8-oxo-dG begins to significantly increase indicating repair is overwhelmed (i.e., insufficient).

- Two groups of C57BL/6J mice received lens-specific irradiation in vivo with 3 mJ/cm2 UVB a week apart, with one group being sacrificed 7 days after exposure and the other sacrificed immediately. Immunofluorescence was used to observe cyclobutane pyrimidine dimers (CPD) (Mesa & Bassnett, 2013).
  - Exposed lenses showed a 25% decrease in cyclobutane pyrimidine dimer levels seven days post-exposure.

Uncertainties and Inconsistencies

Although the dual functionality of OGG1 as a glycosylase and lyase has been widely accepted and demonstrated experimentally, there are studies showing that the cleavage of phosphodiester bond 5’ to the lesion is mainly

performed by apurinic endonuclease 1 (APE1) (Allgayer et al., 2016; R. Wang et al., 2018) In some cases, APE1 may be the main factor driving the accumulation of BER intermediates. Some studies suggest that OGG1 is involved in the repair of non-transcribed strands and is not required for transcription-coupled repair of 8-oxo-dG; Le Page et al. reported efficient repair of 8-oxo-dG in the transcribed sequence in *Ogg1* knockout mouse cells (Le Page et al., 2000). Moreover, the repair of 8-oxo-dG is also affected by the neighbouring sequence; the position of the lesions may have a negative effect on repair efficiency (Pastoriza-Gallego et al., 2007). We note that the study by Allgayer et al. was investigating the fate and effect of 8-oxo-dG during transcription; repair mechanism may vary by situation and availability of repair enzymes at the time.

Quantitative Understanding of the Linkage

The precise relationship between levels of oxidative DNA lesions and when repair can be considered inadequate have not been fully defined; this relationship will very likely differ between cell types and tissues and, thus, difficult to define. There are computational models of repair kinetics of 8-oxo-dG.

Sokhansanj and Wilson III [2004] applied a quantitative model of BER and the literature value for the rate of formation of endogenous 8-oxo-dG to investigate the rate of clearance of BER repair intermediates (Sokhansanj and Wilson III, 2004).

- The BER model used Michaelis-Menten enzyme kinetics and included the activities of OGG1, AP lyases, polymerases, and ligases.
- The model assumed the formation rate of endogenous oxidative lesions to be 500 8-oxo-dG/day
- Based on the above, it was estimated that following a sudden spike in 8-oxo-dG up to 20,000 8-oxo-dG/cell, the total level of repair intermediates would return to baseline within 4000 seconds (less than 1 hour)
    - This model also assumed that OGG1 was available in excess
- When APE1 (AP site endonuclease) is present, glycosylase reaction kinetics of OGG1(a bifunctional glycosylase/lyase) was observed to increase
    - Suggested to be due to the coordinated action of the two enzymes
- A 10-fold reduction in OGG1 kinetics led to 10-fold increase in 8-oxo-dG, while no other repair intermediates increased.

Known modulating factors

N/A

Modulating Factor (MF)	MF Specification	Effect(s) on the KER	Reference(s)

Known Feedforward/Feedback loops influencing this KER

N/A

References

Allgayer, J., Kitsera, N., Bartelt, S., Epe, B., Khobta, A. (2016), Widespread transcriptional gene inactivation initiated by a repair intermediate of 8-oxoguanine, Nucleic Acids Res, 44:7267-7280.

Bellon, S., Shikazono, N., Cunniffe, S., Lomax, M., O'Neill, P. (2009), Processing of thymine glycol in a clustered DNA damage site: mutagenic or cytotoxic, Nucleic Acids Res, 37:4430-4440.

Berquist, B., Wilson III, D. (2012), Pathways for Repairing and Tolerating the Spectrum of Oxidative DNA Lesions, Cancer Lett, 327:61-72.

Brenerman, B., Illuzzi, J., Wilson III, D. (2014), Base excision repair capacity in informing healthspan, Carcinogenesis, 35:2643-2652.

Budworth, H., Matthewman, G., O'Neill, P., Dianov, G. (2005), Repair of Tandem Base Lesions in DNA by Human Cell Extracts Generates Persisting Single-strand Breaks, J Mol Biol, 351:1020-1029.

Cadet, J., Wagner, J.R. (2013), DNA Base Damage by Reactive Oxygen Species, Oxidizing Agents, and UV Radiation, Cold Spring Harb Perspect Biol, 5:a012559.

Caglayan, M., Wilson, S. (2015), Oxidant and environmental toxicant-induced effects compromise DNA ligation during base excision DNA repair, DNA Repair, 35:85-89.

Dahle, J., Brunborg, G., Svendsrud, D., Stokke, T., Kvam, E. (2008), Overexpression of human OGG1 in mammalian cells decreases ultraviolet A induced mutagenesis, Cancer Lett, 267:18-25.

Delaney, S., Jarem, D., Volle, C., Yennie, C. (2012), Chemical and Biological Consequences of Oxidatively Damaged Guanine in DNA, Free Radic Res, 46:420-441.

Freudenthal, B., Beard, W., Wilson, S. (2013), DNA polymerase minor groove interactions modulate mutagenic bypass of a templating 8-oxoguanine lesion., Nucleic Acids Res, 41:1848-1858.

Gagne, J., Rouleau, M., Poirier, G. (2012), PARP-1 Activation— Bringing the Pieces Together, Science, 336:678-279.

Gehrke, T., Lischke, U., Gasteiger, K., Schneider, S., Arnold, S., Muller, H., Stephenson, D., Zipse, H., Carell, T. (2013), Unexpected non-Hoogsteen–based mutagenicity mechanism of FaPy-DNA lesions, Nat Chem Biol, 9:455-461.

Greenberg, M. (2012), Purine Lesions Formed in Competition With 8-Oxopurines From Oxidative Stress, Acc Chem Res, 45:588-597.

Georgakilas, A., P. O'Neill, and R. Stewart. (2013), “Induction and repair of clustered DNA lesions: What do we know so far?”, Radiation Research, Vol.180/1, Radiation Research Society, Indianapolis, https://doi.org/10.1667/RR3041.1.

Hashimoto, K., Tominaga, Y., Nakabeppu, Y., Moriya, M. (2004), Futile short-patch DNA base excision repair of adenine:8-oxoguanine mispair, Nucleic Acids Res, 32:5928-5934.

Hegde, M. L. et al. (2012). “Oxidized base damage and single-strand break repair in mammalian genomes: role of disordered regions and posttranslational modifications in early enzymes”, Progress in molecular biology and translational science, 110, 123–153, https://doi.org/10.1016/B978-0-12-387665-2.00006-7

Kozbenko, T. et al. (2022), “Deploying elements of scoping review methods for adverse outcome pathway development: a space travel case example”, International Journal of Radiation Biology, 1–12. https://doi.org/10.1080/09553002.2022.2110306

Le Page, F., Klunglund, A., Barnes, D., Sarasin, A., Boiteux, S. (2000), Transcription coupled repair of 8-oxoguanine in murine cells: The Ogg1 protein is required for repair in nontranscribed sequences but not in transcribed sequences, Proc Natl Acad Sci USA, 97:8397-8402.

Li, Y., Song, M., Kasai, H., Kawai, K. (2013), Generation and threshold level of 8-OHdG as oxidative DNA damage elicited by low dose ionizing radiation, Genes Environ, 35:88-92.

Ma, H., Wang, J., Abdel-Rahman, S., Boor, P., Firoze, M. (2008), Oxidative DNA damage and its repair in rat spleen following subchronic exposure to aniline, Toxicol Appl Pharmacol, 233:247-253.

Maddukuri, L., Ketkar, A., Eddy, S., Zafar, M., Eoff, R. (2014), The Werner syndrome protein limits the error-prone 8-oxo-dG lesion bypass activity of human DNA polymerase kappa, Nucleic Acids Res, 42:12027-12040.

Markkanen, E. (2017), Not breathing is not an option: How to deal with oxidative DNA damage, DNA Repair, 59:82-105.

Markkanen, E., Castrec, B., Vilani, G., Hubscher, U. (2012), A switch between DNA polymerases δ and λ promotes error-free bypass of 8-oxo-G lesions, Proc Natl Acad Sci USA, 27:931-940.

Mesa, R. and S. Bassnett. (2013), “UV-B-induced DNA damage and repair in the mouse lens”, Investigative Ophthalmology and Visual Science, Vol.54/10, Association for Research in Vision and Ophthalmology, Rockville, https://doi.org/10.1167/iovs.13-12644.

Pastoriza-Gallego, M., Armier, J., Sarasin, A. (2007), Transcription through 8-oxoguanine in DNA repair-proficient and Csb−/Ogg1− DNA repair-deficient mouse embryonic fibroblasts is dependent upon promoter strength and sequence context, Mutagenesis, 22:343-351.

Pearson, C., Shikazono, N., Thacker, J., O’Neill, P. (2004), Enhanced mutagenic potential of 8-oxo-7,8-dihydroguanine when present within a clustered DNA damage site, Nucleic Acids Res, 32:263-270.

Poetsch, A. (2020), “The genomics of oxidative DNA damage, repair, and resulting mutagenesis”, Computational and Structural Biotechnology Journal, Vol.18, Elsevier, Amsterdam, https://doi.org/10.1016/j.csbj.2019.12.013.

Seager, A., Shah, U., Mikhail, J., Nelson, B., Marquis, B., Doak, S., Johnson, G., Griffiths, S., Carmichael, P., Scott, S., Scott, A., Jenkins, G. (2012), Pro-oxidant Induced DNA Damage in Human Lymphoblastoid Cells: Homeostatic Mechanisms of Genotoxic Tolerance, Toxicol Sci, 128:387-397.

Shafirovich, V., Kropachev, K., Anderson, T., Li, Z., Kolbanovskiy, M., Martin, B., Sugden, K., Shim, Y., Min, J., Ceacintov, N. (2016), Base and Nucleotide Excision Repair of Oxidatively Generated Guanine Lesions in DNA, J Biol Chem, 291:5309-5319.

Shah, A., Gray, K., Figg, N., Finigan, A., Starks, L., Bennett, M. (2018), . Defective Base Excision Repair of Oxidative DNA Damage in Vascular Smooth Muscle Cells Promotes Atherosclerosis, Circulation, 138:1446-1462.

Sharma, V., Collins, L., Chen, T., Herr, N., Takeda, S., Sun, W., Swenberg, J., Nakamura, J. (2016), Oxidative stress at low levels can induce clustered DNA lesions leading to NHEJ mediated mutations, Oncotarget, 7:25377-25390.

Sokhansanj, B., Wilson III, D. (2004), Oxidative DNA damage background estimated by a system model of base excision repair, Free Rad Biol Med, 37:433-427.

Swenberg, J., Lu, K., Moeller, B., Gao, L., Upton, P., Nakamura, J., Starr, T. (2011), Endogenous versus Exogenous DNA Adducts: Their Role in Carcinogenesis, Epidemiology, and Risk Assessment, Toxicol Sci, 120:S130-S145.

Taggart, D., Fredrickson, S., Gadkari, V., Suo, Z. (2014), Mutagenic Potential of 8-Oxo-7,8-dihydro-2′-

deoxyguanosine Bypass Catalyzed by Human Y-Family DNA Polymerases, Chem Res Toxicol, 27:931-940.

Wang, C., Yang, J., Lu, D., Fan, Y., Zhao, M., Li, Z. (2016), Oxidative stress-related DNA damage and homologous recombination repairing induced by N,N-dimethylformamide , J Appl Toxicol, 36:936-945.

Wang, R., Li, C., Qiao, P., Xue, Y., Zheng, X., Chen, H., Zeng, X., Liu, W., Boldogh, I., Ba, X. (2018), OGG1-initiated base excision repair exacerbates oxidative stress-induced parthanatos, Cell Death and Disease, 9:628.

Yang, N., Galick, H., Wallace, S. (2004), Attempted base excision repair of ionizing radiation damage in human lymphoblastoid cells produces lethal and mutagenic double strand breaks, DNA Repair, 3:1323-1334.

Yoshikawa, Y., Yamasaki, A., Takatori., K., Suzuki, M., Kobayashi, J., Takao, M., Zhang-Akiyama, Q. (2015), Excess processing of oxidative damaged bases causes hypersensitivity to oxidative stress and low dose rate irradiation, Free Radic Res, 49:1239-1248.

Relationship: 1911: Increase, DNA strand breaks leads to Inadequate DNA repair

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Oxidative DNA damage leading to chromosomal aberrations and mutations	adjacent	High	Low
Deposition of energy leading to lung cancer	adjacent	Moderate	Moderate
Deposition of energy leading to occurrence of cataracts	adjacent	Moderate	Moderate

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	High	NCBI
mouse	Mus musculus	High	NCBI
rat	Rattus norvegicus	High	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Unspecific	High

This KER is plausible in all life stages, sexes, and organisms with DNA. The majority of the evidence is from in vivo adult mice with no specification on sex, and in vitro human models that do not specify sex.

Key Event Relationship Description

The maintenance of DNA integrity is essential for genomic stability; for this reason cells have multiple response mechanisms that enable the repair of damaged DNA. Thus when DNA double strand breaks (DSBs) occur, the most detrimental type of lesion, the cell will initiate repair machinery. These mechanisms are not foolproof, and emerging evidence suggests that closely spaced lesions can compromise the repair machinery. The two most common DSB repair mechanisms are non-homologous end joining (NHEJ) and homologous recombination (HR). The latter predominates in stem cells as they are frequently in the replicative phase of the cell cycle (Choi et al., 2020). NHEJ is initiated in G1 and early S phases of the cell cycle (Lieber et al., 2003) and is preferentially used to repair DSB damage (Godwint et al., 1994), as it is rapid and more efficient than HR (Lliakis, 1991; Jeggo, 1998; Mao et al., 2008). In higher-order eukaryotes such as humans, NHEJ is the favoured DNA repair mechanism because of the large non-coding regions within the genome. However, when other repair mechanisms (e.g., NHEJ, HR) are compromised, single strand annealing, which is a low fidelity mechanism may be involved (Chang et al., 2017). NHEJ can occur through one of two subtypes: canonical NHEJ (C-NHEJ) or alternative non-homologous end joining (alt-NHEJ). C-NHEJ, as the name suggests, simply ligates the broken ends back together. In contrast, alt-NHEJ occurs when one strand of the DNA on either side of the break is resected to repair the lesion (Bétermier et al., 2014). All repair mechanisms are error-prone, meaning that insertions and deletions are sometimes formed due to the DSBs being repaired imperfectly (Thurtle-Schmidt and Lo, 2018). However, alt-NHEJ is considered more error-prone than C-NHEJ, as studies have shown that it more often leads to chromosomal aberrations (Zhu et al., 2002; Guirouilh-Barbat et al., 2007; Simsek & Jasin, 2010). HR is operative during late S and G2 phases where the sister chromatid can be used as template for error-free repair (Van Gent et al 2001). Because of the reliance on the undamaged sister chromatid to repair the DSB, HR is less error-prone than NEHJ. Nevertheless, defects in HR are known to contribute to genomic instability and the formation of chromosomal aberrations (Deans et al 2000)

There is extensive evidence that DNA repair capacity can be overwhelmed or saturated in the presence of high numbers of strand breaks. For

example, with multiple single strand breaks (SSBs) in close proximity that can lead to DSBs (Caldecott, 2024). This is demonstrated by decades of studies showing dose-related increases in chromosomal exchanges, chromosomal breaks and micronuclei following exposure to double-strand break inducers. Additionally, the loss of heterozygosity (LOH) is an example of how during the repair of incorrect DNA that uses HR, there may be a loss of an allele during repair (Smukowski et al., 2023). Inadequate repair not only refers to overwhelming of DNA repair machinery, but also the use of repair mechanisms that are error-prone (i.e., misrepair is considered inadequate repair).

Evidence Supporting this KER

Biological Plausibility

The biological rationale linking increased DNA DSB formation with inadequate DSB repair is supported strongly by literature. This is evident from the number of review articles that have been published on the subject. Of particular relevance is a recent review that focuses particularly on DSBs induced by ionizing radiation and extensively details the processes involved in repairing DSBs, including discussions of entire pathways and individual proteins involved in DNA repair (Thompson, 2012). Multiple other shorter reviews are also available on the subject, which cover such topics as: the mechanisms of DSB formation and repair, how to quantify these two events, and the biological consequences of unrepaired or misrepaired DNA damage (Lett, 1996; van Gent et al., 2001; Khanna & Jackson, 2001; Vignard et al., 2013; Moore et al., 2014; Rothkamm et al., 2015; Chang et al., 2017; Löbrich and Jeggo, 2017; Sage and Shikazono, 2017). A brief overview of the biological plausibility of this KER is given below; for more detail, please consult the above-cited reviews.

When confronted with DSBs, there are two common repair pathways employed by the cell: homologous recombination (HR) and non-homologous end-joining (NHEJ). In HR, a homologous sequence on a sister chromatid is used as a template, ensuring that no sequence information is lost over the course of repair (e.g., Ferguson & Alt, 2001; van Gent et al., 2001; Jeggo & Markus, 2015; Schipler & Iliakis, 2013). Due to being inherently error-prone, NHEJ is commonly used in repairing DSBs in multicellular eukaryotic organisms, especially in humans (Feldmann et al., 2000). Due to being inherently error-prone, this repair process is used to generate genetic variation within antigen receptor axons through VDJ recombination, a process that leads to the careful breakage and repair of DNA (Murakami & Keeney, 2008; Malu et al., 2012). Genetic variation is also often generated during the repair of highly toxic DSB lesions. Repair to these DSB sites normally triggers cell cycle delay. NHEJ is most active in the following order of the cell cycle: G1 > S > G2/M (Mao et al., 2008). Since most somatic mammalian cells are in the G1 pre-replicative phase, DSBs also usually appear in this phase and thus are often repaired using the error-prone NHEJ (Jeggo et al., 1995). Cells in other phases of the cell cycle (S or G2) use HR (Ceccaldi et al., 2016). In addition,) and damaged cells in G0 also appear to use NHEJ repair (Frock et al., 2021).

The two broken ends of DNA DSBs are bridged by overlapping single-strand microhomology termini (Anderson, 1993; Getts & Stamato, 1994; Rathmell & Chu, 1994; Jeggo et al., 1995; Miller et al., 1995; Kirchgessner et al., 1995). The microhomology termini are ligated only when complementary base pairs are overlapped and, depending on where this match is found on the termini, it can lead to deletions and other rearrangements. With increasing DSBs, the probability of insufficient or incorrect repair of these breaks increases proportionately. It has been suggested that clustered DNA damage is less easily repairable than any other form of DNA damage (United Nations, 2000; Stenerlöw et al., 2000). With multiple lesions in close proximity within a damaged cluster, the probability of misrepair is high. This leads to an increased number of misrepaired termini (Goodhead et al., 1994; Goodhead, 1980; Tsao, 2007; Blakely, 2012), as the presence of multiple damage sites interferes with the ability of the repair enzymes to recognize and bind to the DNA accurately (Harrison et al., 1999; Tsao, 2007).

Empirical Evidence

Empirical data obtained for this KER strongly supports the idea that an increase in DNA DSBs will increase the frequency of inadequate DSB repair. The evidence presented below is summarized in table 4,here (click link). Much of the evidence comes from work with radiation stressors, which directly cause DNA DSBs in the genome (Pinto & Prise, 2005; Dong et al., 2017) in a dose-dependent fashion (Aufderheide, 1987; Frankenburg-Schwager et al., 1994; Rydberg et al., 1994; Durante et al., 1998; Dikomey & Brammer, 2000; Kuhne et al., 2000; Löbrich et al., 2000; Baumstark-Khan et al., 2003; Rothkamm & Lo, 2003; Kuhne et al., 2005; Asaithamby & Chen, 2009; Bracalente et al., 2013).This is a very data-rich area and it is not possible to summarize all of the evidence. However, some examples of key studies are provided below. We also direct the reader to the key event relationships 1939 (DNA strand breaks leading to chromosomal aberrations) and 1931 (DNA strand breaks leading to mutations).

The formation of DSBs by ionizing radiation, the repair process, the various methods used to analyze this repair process, and the biological consequences of unrepaired or misrepaired DNA damage are reviewed in Sage & Shikazono (2017).

Dose and Incidence Concordance

There is evidence in the literature suggesting a dose/incidence concordance between the occurrence of DSBs and the incidence of inadequate DNA repair upon exposure to radiation. Inadequate DNA repair appears to occur at the same radiation dose as DSBs. Visually, immunofluorescence has demonstrated a colocalization of DNA repair proteins with DSB foci in response to a radiation stressor (Paull et al., 2000; Asaithamby & Chen, 2009; Dong et al., 2017). In studies examining cellular responses to increasing doses of radiation, which is known to evoke a dose-dependent increase in DNA DSBs (Aufderheide, 1987; Durante et al., 1998; Dikomey & Brammer, 2000; Kuhne et al., 2000; Löbrich et al., 2000; Rothkamm & Löbrich, 2003; Kuhne et al., 2005; Asaithamby & Chen, 2009; Bracalente et al., 2013; Bernard et al., 2021), there were resulting dose-dependent increases in non-repaired DSBs (Aufderheide, 1987; Rydberg et al., 1994; Dikomey & Brammer, 2000; Baumstark-Khan et al., 2003), DSB misrepair rates (Mcmahon et al., 2016), and misrejoined DSBs (Durante et al., 1998; Kuhne et al., 2000; Kuhne et al., 2005; Rydberg et al., 2005), as well as a dose-dependent decrease in the total DSB rejoining (Löbrich et al., 2000). Furthermore, only 50% of the rejoined DSBs were found to be correctly repaired (Kuhne et al., 2000; Löbrich et al., 2000); 24 hours after being irradiated with an 80 Gy dose of alpha particles, this frequency of misrejoining increased to and remained constant at 80% (Kuhne et al., 2000). Furthermore, delivering radiation doses in fractionated increments also showed a dose-dependent change in the percentage of misrejoinings, such that larger fractionated doses (for example, 2 x 40 Gy) had a higher rate of DSB misrejoining than smaller fractionated doses (for example, 4 x 10 Gy) (Kuhne et al., 2000).

Temporal Concordance

There is evidence suggesting a time concordance between DSBs and DNA repair. DSBs and DNA repair have both been observed within minutes to hours of radiation exposure (Paull et al., 2000; Rothkamm & Löbrich, 2003; Pinto & Prise, 2005; Asaithamby & Chen, 2009; Barnard et al., 2021; Barnard et al., 2018). Single strand breaks (SSBs) and DNA. repair has also been observed minutes to hours post-irradiation (Sidjanin et al., 1993).

Essentiality

There is evidence from inhibition studies and knock-out/knock down studies suggesting that there is a strong relationship between DSBs and DNA repair. When an inhibitor of a DNA repair protein was added to cells prior to exposure to a radiation stressor, DNA repair foci were not formed post-irradiation (Paull et al., 2000), and there were significant increases in DSBs at 6 hours and 12 hours after the radiation treatment (Dong et al., 2017).

Similarly, there have been several knock-out/knock-down studies in which cells lacking a DNA repair protein have been exposed to a radiation stressor. As a result, DSBs were found to persist in these cells longer than in the wild-type cells (Coquerelle et al., 1987; Rothkamm and Löbrich, 2003; Bracalente et al., 2013; Mcmahon et al., 2016; Dong et al., 2017), and there was an increase in incorrectly rejoined DSBs (Löbrich et al., 2000). In one striking example, a human cell line lacking DNA ligase IV had DSBs that were still present approximately 240 - 340 hours post-irradiation (Mcmahon et al., 2016). Interestingly, there were also increased levels of DSBs in these cells prior to being exposed to a radiation stressor (Paull et al., 2000) . Similarly, a study examining DSB repair kinetics after irradiation found that DSBs persisted for a longer time period in two repair-deficient mouse strains relative to a repair-proficient mouse strain; this pattern was found in lymphocytes, as well as tissues from the brains, lungs, hearts and intestines of these mice (Rube et al., 2008). The roles of various DNA repair proteins in the context of DSBs are highlighted in reviews by Chang et al. (2001) and Van Gent et al. (2001) with discussions focusing on the consequences of losing some of these proteins in cells, mice and humans (Van Gent et al., 2001)

Uncertainties and Inconsistencies

Uncertainties and inconsistencies in this KER are as follows:

- There is controversy surrounding how error-prone NHEJ truly is. Recent studies suggest that the process may be quite accurate (reviewed in (Bétermier et al. 2014)). The accuracy of NHEJ may actually be dependent on the structure of the termini. Thus, the termini processing rather than the NHEJ mechanism itself is argued to be the error-prone process (Bétermier et al. 2014).
- There may be different cellular responses associated with low-dose radiation exposure and high-dose radiation exposure; these differences may also be dependent on a DSB threshold being exceeded prior to initiation repair. It has been suggested that DNA repair may not be activated at low doses of radiation exposure in order to prevent the risk of mutations from error-prone repair mechanisms (Marples 2004).
- DSB repair fidelity varies in terms of confounding factors and the genetic characteristics of individuals (Scott 2006). For example, individuals who smoke have a 50% reduction in the mean level of DSB repair capacity relative to the non-smokers; this is due to an increased methylation index in smokers. A higher methylation index indicates more inactivation of gene expression. It is thus possible that expression of DNA repair proteins in smokers is decreased due to increased methylation of the genes encoding for repair proteins. In terms of individual genetics, single nucleotide polymorphisms (SNPs) within the MRE11A, CHEK2, XRCC3, DNA-PKcs, and NBN repair genes have been highly associated with the methylation index (Leng et al. 2008). SNPs can critically affect the function of these core proteins, varying the fidelity of DNA repair from person to person.
- Cells containing DNA damaged may be eliminated by apoptotic pathways, therefore not undergo repair, alternatively evidence has also shown that damaged cells can propagate due to lack of detection by repair machinery (Valentin 2005).
- The focus of this KER was on DSBs because there is lack of data to support that SSBs lead to inadequate repair. Multiple SSBs can lead to DSBs. Thus, DSBs are the focus as they can drive the cell towards genomic instability, apoptosis or tumorigenesis. Further quantitative evidence to define the extent of SSBs leading to DSBs and the relationship with repair is necessary.
- Ercc2+/- mice have a mutation in a gene involved in the nucleotide excision repair (NER) pathway, leading to DNA repair deficiency. However, when compared to wild type mice Ercc2+/- mice had fewer DNA strand breaks. This was true of both central and peripheral lens cells, as well as 4 and 24 h after irradiation (60Co γ-rays, 0.3, 0.063 Gy/min) (Barnard et al., 2021).
- DNA damage repair times can vary depending on the stressors that instigate the DNA damage. For example, it has been found that some types of radiation i.e., high linear energy transfer (LET) increases the amount of time required to repair DNA breaks (Aufderheide, 1987; Frankenburg-Schwager et al., 1994; Rydberg et al., 1994; Baumstark-Khan et al., 2003; Tsao, 2007; Blakely, 2012), however Stenerlöw et al. (2000) found that repair half-times were independent of LET.

Quantitative Understanding of the Linkage

Quantitative understanding of this linkage suggests that DSB repair can be predicted from the presence of DSBs. The following tables provide representative examples of the relationship, unless otherwise indicated, all data is significantly significant. In terms of DNA repair in response to radiation-induced DSBs, one study suggests that complete DNA DSB repair occurs starting at a threshold dose of 5 mGy (0.005 Gy), as measured by the presence of γ-H2AX (Lobrich et al., 2005) and presence of 53BPI foci (Asaithamby & Chen, 2009). After a 10 Gy dose of radiation, approximately 10 - 15% of DSBs were found to be misrepaired (Mcmahon et al., 2016); at a dose of 80 Gy, the relative percentage of DSBs incorrectly repaired was estimated at 50 - 60% (Kuhne et al., 2000; Lobrich et al., 2000; Mcmahon et al., 2016). Twenty-four hours post-irradiation, this rate increased to approximately 80% for alpha particle irradiation at 80 Gy, and remained constant until the end of the assay (10 days) (Kuhne et al., 2000).

Dose Concordance

Reference	Experiment Description	Result
Rydberg et al., 1994	In vitro. Human VA13 lung fibroblast and GM38A skin fibroblast cells were exposed to neon ions (425 MeV/u, 1 – 5 Gy/min, 80 Gy), iron ions (600 MeV/u, 1 – 5 Gy/min, 50 Gy), and X rays (425 MeV/u, 1 – 2 Gy/min, 80 Gy) to induce DNA strand breaks. Initial breaks after exposure were measured via the fraction of activity released (FAR) assay, with an increased FAR value indicating an increased number of breaks. Repair was measured using the FAR assay after a period of incubation.	Exposure to X-rays, neon, and iron ions led to a 90, 70, and 50% FAR increase relative to control respectively, indicating the highest level of breaks in samples exposed to X-rays. Four h later, 15, 20, and 73% of the DNA strand breaks had not been repaired.
Kuhne et al., 2000	In vitro. Human lung fibroblast cells were exposed to X-rays (23 Gy/min) at doses from 0 - 320 Gy. Following this, both correct (measured via hybridization assay), and total (measured via FAR assay) breaks remaining were measured. Therefore, allowing for calculation of the amount of misrepaired breaks.	Cells exposed to 0 - 320 Gy X-rays displayed an approximately linear increase in DSBs. This led to a gradual increase in the % DSBs misrejoined, which began to plateau after 80 Gy at a misrejoining frequency of 50%.

Baumstark-Khan et al., 2003	In vitro. Bovine LECs were exposed to X-rays (5 Gy/min, 0 to 50 Gy), 16O (3.4, 8.7 MeV/u, 230.5 to 642.9 Gy), 40Ar (2.7, 6.2, 10.5, 19.3 MeV/u, 0 to 190 Gy), 132Xe (5.4, 10.1, 16.5 MeV/u, 0 to 80 Gy), 208Pb (3.0, 6.8, 15.4 MeV/u, 0 to 50 Gy), 238U (1.5, 1.9, 2.6, 4.0 MeV/u, 0 to 150 Gy). This led to the induction of both SSBs and DSBs, whose repair was measured using a method similar to the hydroxyapatite chromatography of alkaline unwound DNA.	Irradiation below 10 000 keV/µm led to almost 100% rejoining of SSBs and DSBs. At LETs above 10 000 keV/µm the rejoining capacity varied depending on the original level of damage. After irradiation with 238U (LET ~ 20 000 keV/µm) rejoining capacity as t -> ∞ ranged from 50 to 100%. After irradiation with 208Pb (LET ~ 18 000 keV/µm) rejoining capacity as t -> ∞ ranged from 15 to 28%. 48Ti was an exception, with an LET of 1440 keV/µm that resulted in a rejoining capacity of only 65% rather than almost 100% as t -> ∞.
Aufderheide, 1987	In vitro. Bovine lens epithelial cells (LECs) were exposed to 238U (5, 10, 20 x 106 P/cm2), 132Xe (3, 5, 7, 12, 20 x 106 P/cm2), 84Kr (9, 21 x 106 P/cm2), 40Ar (24 x 106 P/cm2), 16O (80 x 106 P/cm2), and X-rays (20, 40, 200 Gy). The radiation exposure induced DNA breaks were measured using the DNA unwinding method described by Rydberg (1975). The DNA then underwent a period of repair incubation lasting between 5 to 40 h, after which any remaining DNA damage was measured using the same method as before.	Bovine LECs exposed to 21 x 106 P/cm2 84Kr displayed a 1.3x increase in DNA breaks and a 5% decrease in the level of breaks repaired compared to cells exposed to 9 x 106 P/cm2.
Stenerlöw et al., 2000	In vitro. Human skin fibroblast cells were exposed to 100 Gy of photons (60Co, < 0.5 keV/um), nitrogen ions (80, 125, 175, 225 keV/um), and helium ions (40 keV/um), resulting in the formation of DSBs. Their number was calculated by fragment analysis, based upon the fraction of DNA less than 5.7 Mbp, under the assumption that the breaks were evenly distributed. DNA repair was also measured via fragment analysis.	Exposure to increasing LET of radiation at 100 Gy led to increasing DSBs, in general, with about 600 DSBs/Gbp after γ-ray irradiation and about 700 DSBs/Gbp after 225 keV/um nitrogen ion irradiation. A dose of 100 Gy also led to decreased repair at increased LET. About 20-22 h after γ-ray irradiation, 4% of DSBs were unrepaired, while 20-22 h after 225 keV/um nitrogen ion irradiation, 12% of DSBs were unrepaired.
Coquerelle et al., 1987	*In vitro*. Human skin fibroblast cells were exposed to ^{60}Co (1.5, 0.35 Gy/min) and alpha particles (120 keV/µm). Alkaline elution assay was used to detect DNA strand breaks. Repair of breaks were determined over time. The % rejoined DNA was calculated from the mean values of the entire elution profile.	Exposure to 25 Gy gamma rays or alpha particles resulted in ~20% strand breaks. 80% of these breaks were repaired 30 mins after the exposure.

Incidence Concordance

No studies were found.

Time Concordance

Reference	Experiment Description	Result
Durante et al., 1998	In vitro. Human, male, lymphocyte cells were exposed to either iron ions (140 keV/µm, 2 Gy), or carbon ions (42 keV/µm, 5 Gy) to induce DNA strand breaks. Misrepair was measured by producing chromosome spreads and evaluating them using a microscope and the PAINT classification code.	Exposure to 2 Gy iron particles resulted in about 0.45 breaks/cell, of which 50% were repaired 10 h later. However, there were 0.1 translocations/cell, 0.08 incomplete exchanges/cell, 0.075 complex exchanges/cell, and 0.07 dicentrics/cell. Exposure to 5 Gy carbon ions resulted in 1.15 breaks/cell, of which 25% were repaired 10 h later. However, there were 0.35 translocations/cell, 0.28 incomplete exchanges/cell, 0.43 complex exchanges/cell, and 0.29 dicentrics/cell.
Rydberg et al., 1994	In vitro. Human VA13 lung fibroblast and GM38A skin fibroblast cells were exposed to neon ions (425 MeV/u, 1 – 5 Gy/min, 80 Gy), iron ions (250, 400, 600 MeV/u, 1 – 5 Gy/min, 50 Gy), and X rays (425 MeV/u, 1 – 2 Gy/min, 80 Gy) to induce DNA breaks. Their repair was measured using pulsed-field gel electrophoresis and determining the amount of DNA released from the gel plug (fraction of activity released – FAR).	In GM38A cells, exposure to 80 Gy of all three radiation types led to DNA breaks. Repair was observed between 0.5 and 4 h after this. The most breaks remained after exposure to iron ions (75% of breaks remained), 25 – 42% remained after neon exposure, and only 15 – 20% remained after X ray irradiation.

Response-response relationship

There is evidence of a response-response relationship for DNA repair of radiation-induced DSBs. The frequency of DSBs has been shown to increase linearly with radiation dose (Löbrich et al., 2000; Rothkamm & Lo, 2003; Kuhne et al., 2005; Asaithamby & Chen, 2009). For DNA repair, increasing doses of a radiation stressor were found to cause a linear-quadratic relationship between the radiation dose and the number of misrejoined DSBs per cell (Kuhne et al., 2005). Interestingly, the relationships between radiation and DNA repair were found to vary depending on the type of radiation. There was a more linear response between radiation dose and the number of misrejoined DSBs for high LET particles relative to a more curvilinear relationship for lower LET particles (Rydberg et al., 2005). Additionally, a linear relationship was defined for low dose-rate radiation and the number of non-repaired DNA DSBs, but a linear-quadratic equation was described for high dose-rate radiation (Dikomey & Brammer, 2000).

Time-scale

Data from temporal response studies suggests that DSB repair may occur within 15 - 30 minutes of a DSB-inducing radiation stressor (Paull et al., 2000; Rothkamm & Lo, 2003; Pinto & Prise, 2005; Dong et al., 2017), with foci documented as early as 3-5 minutes post-irradiation (Asaithamby & Chen, 2009). The majority of DSB repair has been reported to occur within the first 3 - 6 hours following DSB induction (Rothkamm & Lo, 2003; Pinto & Prise, 2005; Asaithamby & Chen, 2009; Dong et al., 2017), with complete or near-complete DSB repair within 24 hours of the radiation stressor (Dikomey & Brammer, 2000; Lobrich et al., 2000; Rothkamm & Lo, 2003; Asaithamby & Chen, 2009; Mcmahon et al., 2016). In one 48-hour time-course experiment for DSB repair using two different types of radiation, the following repair progression was found at 30 minutes, 1 hour, 3 hours, 24 hours and 48 hours, respectively: 40 - 55%, 55 - 70%, 85%, 97 - 98% and 98% repair for X-rays and 30%, 45 - 50%, 65 - 70%, 85 - 90% and 90 - 96%

repair for alpha particles (Pinto & Prise, 2005). Twenty-four hours post-irradiation, the frequency of DSB misrejoining was found to remain constant at approximately 80% for the 10 days that the DSB repair was monitored (Kuhne et al., 2000).

Known modulating factors

Modulating Factor	Details	Effects on the KER	References
Linear energy transfer (LET)	Increased LET	As the LET of the stressor increases, the amount of misrepaired and unrejoined DSBs also increases. One possible explanation for this is that DSB free ends are closer together at higher LETs, making it easier for misrepair to occur. Furthermore, higher LET stressors produce more complex, clustered breaks which also increasing repair difficulty. At very high LET values (over 10 000 keV/um), no significant DNA repair is detected.	Aufderheide, 1987; Rydberg et al., 1994; Durante et al., 1998; Kuhne et al., 2000; Stenerlöw et al., 2000; Baumstark-Khan et al., 2003; Tsao, 2007; Mukherjee et al., 2008; Blakely, 2012; Hamada, 2017
Oxygen	Decreased oxygen levels	Cells in an anoxic environment will rejoin DNA breaks more quickly than those in an oxic environment because oxygen can attach to the broken ends of DNA, fixing the damage and making it unrepairable.	Frankenburg-Schwager et al., 1994

Known Feedforward/Feedback loops influencing this KER

Not identified.

References

Anderson, C.W. 1993, "DNA damage and the DNA-activated protein kinase.", Trends Biochem. Sci. 18(11):433–437. doi:10.1016/0968-0004(93)90144-C.

Antonelli, A.F. et al. (2015), "Induction and Repair of DNA DSB as Revealed by H2AX Phosphorylation Foci in Human Fibroblasts Exposed to Low- and High-LET Radiation: Relationship with Early and Delayed Reproductive Cell Death", Radiat. Res. 183(4):417-31, doi:10.1667/RR13855.1.

Asaithamby, A. & D.J. Chen (2009), "Cellular responses to DNA double-strand breaks after low-dose c-irradiation.", Nucleic Acids Res. 37(12):3912–3923. doi:10.1093/nar/gkp237.

Aufderheide, E. (1987), “Heavy ion effects on cellular DNA: strand break induction and repair in cultured diploid lens epithelial cells”, International journal of radiation biology and related studies in physics, chemistry and medicine, Vol. 51/5, Taylor & Francis, London, https://doi.org/10.1080/09553008714551071

Barnard, S. G. R. (2018), “Dotting the eyes: mouse strain dependency of the lens epithelium to low dose radiation-induced DNA damage”, International Journal of Radiation Biology, Vol. 94/12, https://doi.org/10.1080/09553002.2018.1532609

Barnard, S. G. R. (2021), “Radiation-induced DNA damage and repair in lens epithelial cells of both Ptch1(+/-) and Ercc2(+/-) mutated mice”, Radiation Research, Vol. 197/1, Radiation Research Society, United States, https://doi.org/10.1667/RADE-20-00264.1

Baumstark-Khan, C. et al. (2003), “Induction and repair of DNA strand breaks in bovine lens epithelial cells after high LET irradiation”, Advances in Space Research, Vol. 31/6, Elsevier Ltd, England, https://doi.org/10.1016/S0273-1177(03)00095-4

Bétermier, M., P. Bertrand & B.S. Lopez (2014), "Is Non-Homologous End-Joining Really an Inherently Error-Prone Process?", PLoS Genet. 10(1). doi:10.1371/journal.pgen.1004086.

Blakely, E. A. (2012), “Lauriston S. Taylor lecture on radiation protection and measurements: what makes particle radiation so effective?”, Health Physics, Vol. 103/5, Health Physics Society, United States, https://doi.org/10.1097/HP.0b013e31826a5b85

Bracalente, C. et al. (2013), "Induction and Persistence of Large g H2AX Foci by High Linear Energy Transfer Radiation in DNA-Dependent protein kinase e Deficient Cells.", Int. J. Radiat. Oncol. Biol. Phys. 87(4). doi:10.1016/j.ijrobp.2013.07.014.

Caldecott K. W. (2024). “Causes and consequences of DNA single-strand breaks”. Trends in biochemical sciences, 49(1), 68–78. https://doi.org/10.1016/j.tibs.2023.11.001.

Chang, H.H.Y. et al. (2017), "Non-homologous DNA end joining and alternative pathways to double - strand break repair.", Nat. Publ. Gr. 18(8):495–506. doi:10.1038/nrm.2017.48.

Choi, E. H., Yoon, S., Koh, Y. E., Seo, Y. J., & Kim, K. P. (2020),. “Maintenance of genome integrity and active homologous recombination in embryonic stem cells”,. Experimental & molecular medicine, 52(8), 1220–1229. https://doi.org/10.1038/s12276-020-0481-2

Coquerelle, T. M., K. F. Weibezahn and C. Lücke-Huhle (1987), “Rejoining of double strand breaks in normal human and ataxia-telangiectasia fibroblasts after exposure to 60Co gamma-rays, 241Am alpha-particles or bleomycin”, International journal of radiation biology and related studies in physics, chemistry, and medicine, Vol. 51/2, https://doi.org/10.1080/09553008714550711

Deans, B., Griffin, C. S., Maconochie, M. & Thacker, J. (2000), Xrcc2 is required for genetic stability, embryonic neurogenesis and viability in mice. EMBO J. 19, 6675–6685.

Dikomey, E. & I. Brammer (2000), "Relationship between cellular radiosensitivity and non-repaired double-strand breaks studied for di Ú erent growth states, dose rates and plating conditions in a normal human broblast line.", Int. J. Radiat. Biol., 76(6). doi:10.1080/09553000050028922.

Dong, J. et al. (2017), "Inhibiting DNA-PKcs in a non-homologous end-joining pathway in response to DNA double-strand breaks.", Oncotarget. 8(14):22662–22673. doi: 10.18632/oncotarget.15153.

Dubrova, Y.E. et al. (2002), "Elevated Minisatellite Mutation Rate in the Post-Chernobyl Families from Ukraine.", Am. J. Hum. Genet. 71(4):801–809. doi:10.1086/342729.

Durante, M. et al. (1998), “Rejoining and misrejoining of radiation-induced chromatin breaks. IV. Charged particles”, Radiation Research, Vol. 149/5, Radiation Research Society, Oak Brook, https://doi.org/10.2307/3579784

Feldmann, E. et al. (2000), "DNA double-strand break repair in cell-free extracts from Ku80-deficient cells: implications for Ku serving as an alignment factor in non-homologous DNA end joining.", Nucleic Acids Res. 28(13):2585–2596. doi:10.1093/nar/28.13.2585.

Ferguson, D.O. & F.W. Alt (2001), "DNA double strand break repair and chromosomal translocation: Lessons from animal models.", Oncogene, 20(40):5572–5579. doi: 10.1038/sj.onc.1204767.

Frankenburg-Schwager, M. et al. (1994), “Half-life values for DNA double-strand break rejoining in yeast can vary by more than an order of magnitude depending on the irradiation conditions”, International Journal of Radiation Biology, Vol. 66/5, Informa UK Ltd, London, https://doi.org/10.1080/09553009414551591

Frock, R. L., Sadeghi, C., Meng, J., & Wang, J. L. (2021),. “DNA End Joining: G0-ing to the Core”,. Biomolecules, 11(10), 1487. https://doi.org/10.3390/biom11101487.

van Gent D.C., J.H.J. Hoeijmakers & R. Kanaar (2001), "Chromosomal stability and the DNA double-stranded break connection.", Nat. Rev. Genet. 2(3):196–206. doi:10.1038/35056049. http://www.ncbi.nlm.nih.gov/pubmed/11256071.

Getts, R.C. & T.D. Stamato (1994), "Absence of a Ku-like DNA end binding activity in the xrs double-strand DNA repair-deficient mutant.", J. Biol. Chem. 269(23):15981–15984.

Godwin, A.R. et al. (1994), "Spontaneous and restriction enzyme-induced chromosomal recombination in mammalian cells.", PNAS 91(December):12554–12558. doi: 10.1073/pnas.91.26.12554

Goodhead, D.T. (1994), "Initial events in the cellular effects of ionizing radiations: clustered damage in DNA.", Int. J. Radiat. Biol. 65(1):7–17. doi:10.1080/09553009414550021. http://www.ncbi.nlm.nih.gov/pubmed/7905912.

Goodhead, D.T. et al. (1980), "Mutation and inactivation of cultured mammalian cells exposed to beams of accelerated heavy ions. IV. Biophysical interpretation.", Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med. 37(2):135–67. doi:10.1080/09553008014550201.

Gorbunova, V. 1997, "Non-homologous DNA end joining in plant cells is associated with deletions and filler DNA insertions.", Nucleic Acids Res. 25(22):4650–4657. doi:10.1093/nar/25.22.4650.

Guirouilh-Barbat, J. et al. (2007), "Defects in XRCC4 and KU80 differentially affect the joining of distal nonhomologous ends.", Proc Natl Acad Sci. 104(52):20902–20907. doi:10.1073/pnas.0708541104.

Grudzenski, S. et al. (2010), "Inducible response required for repair of low-dose radiation damage in human fibroblasts.", Proc. Natl. Acad. Sci. USA. 107(32): 14205-14210, doi:10.1073/pnas.1002213107.

Guirouilh-barbat, J. et al. (2014), "Is homologous recombination really an error-free process?", Front Genet. 5:175. doi:10.3389/fgene.2014.00175.

Hamada, N. (2017), “Ionizing radiation sensitivity of the ocular lens and its dose rate dependence”, International Journal of Radiation Biology, Vol. 93/10, Taylor & Francis, England, https://doi.org/10.1080/09553002.2016.1266407

Harrison, L., Z. Hatahet & S.S. Wallace (1999), "In vitro repair of synthetic ionizing radiation-induced multiply damaged DNA sites 1 1Edited by J. H. Miller.", J. Mol. Biol. 290(3):667–684. doi:10.1006/jmbi.1999.2892.

Hartlerode, A.J. & R. Scully (2009), "Mechanisms of double-strand break in somatic mammalian cells.", Biochem J. 423(2):157–168. doi:10.1042/BJ20090942.Mechanisms.

Jeggo, P.A. (1998), "DNA breakage and repair.", Adv. Genet. 38:185–218. doi:DOI: 10.1016/S0065-2660(08)60144-3. doi: DOI: 10.1016/S0065-2660(08)60144-3.

Jeggo, P.A. & L. Markus (2015), "How cancer cells hijack DNA double-strand break repair pathways to gain genomic instability.", Biochem. J., 471(1):1–11. doi:10.1042/BJ20150582.

Khanna, K.K. & S.P. Jackson (2001), "DNA double-strand breaks: signaling , repair and the cancer connection.", 27(march):247–254. doi: 10.1038/85798.

Kirchgessner, C. et al. (1995), "DNA-dependent kinase (p350) as a candidate gene for the murine SCID defect.", Science (80-). 267(5201):1178–1183. doi:10.1126/science.7855601.

Kozbenko, T. et al. (2022), “Deploying elements of scoping review methods for adverse outcome pathway development: a space travel case example”, International Journal of Radiation Biology, 1–12. https://doi.org/10.1080/09553002.2022.2110306

Kuhne, M., K. Rothkamm & M.Löbrich (2000), "No dose-dependence of DNA double-strand break misrejoining following a -particle irradiation.", Int. J. Radiat. Biol. 76(7):891-900

Kuhne, M., G. Urban & M. Lo (2005), "DNA Double-Strand Break Misrejoining after Exposure of Primary Human Fibroblasts to CK Characteristic X Rays, 29 kVp X Rays and 60Co γ Rays", Radiat. Res., 164(5):669–676. doi:10.1667/RR3461.1.

de Lara, C.M. et al. (2001), "Dependence of the Yield of DNA Double-Strand Breaks in Chinese Hamster V79-4 Cells on the Photon Energy of Ultrasoft X Rays.", Radiation Research. 155(3):440-8. doi:10.1667/0033-7587(2001)155[0440:DOTYOD]2.0.CO;2.

Leng, S. et al. (2008), "Public Access NIH Public Access. PLoS One.", 32(7):736–740. doi:10.1371/journal.pone.0178059.

Lieber, M.R. (2008), "The mechanism of human nonhomologous DNA End joining.", J Biol Chem. 283(1):1–5. doi:10.1074/jbc.R700039200.

Lobrich, M. and P. Jeggo, (2017), A Process of Resection-Dependent Nonhomologous End Joining Involving the Goddess Artemis., Trends Biochem Sci. 42(9): 690-701. doi: 10.1016/j.tibs.2017.06.011.

Lobrich, M. et al. (2000), "Joining of Correct and Incorrect DNA Double-Strand Break Ends in Normal Human and Ataxia Telangiectasia Fibroblasts.", 68(July 1999):59–68. doi:DOI: 10.1002/(SICI)1098-2264(200001)27:1<59::AID-GCC8>3.0.CO;2-9.

Lobrich, M. et al. (2005), "In vivo formation and repair of DNA double-strand breaks after computed tomography examinations.", Proc. Natl. Acad. Sci. 102(25):8984–8989. doi:10.1073/pnas.0501895102.

Lett, J. T. (1996), “Experimental models for cellular radiation targets: LET, RBE and radioprotectors”, Advances in Space Research, Vol. 18/1, Elsevier Ltd, England, https://doi.org/10.1016/0273-1177(95)00786-E

Malu, S. et al. (2012), "Role of non-homologous end joining in V(D)J recombination.", Immunol. Res. 54(1–3):233–246. doi:10.1007/s12026-012-8329-z.

Mao, Z. et al. (2008), "DNA repair by nonhomologous end joining and homologous recombination during cell cycle in human cells.", Cell Cycle. 7(18):2902–2906. doi:10.4161/cc.7.18.6679.

Marples, B. (2004), "Is low-dose hyper-radiosensitivity a measure of G2-phase cell radiosensitivity?", Cancer Metastasis Rev. 23(3–4):197–207.

doi:10.1023/B:CANC.0000031761.61361.2a.

McMahon, S.J. et al. (2016), "Mechanistic Modelling of DNA Repair and Cellular Survival Following Radiation-Induced DNA Damage.", Nat. Publ. Gr. (April):1–14. doi:10.1038/srep33290.

Miller, R.C. et al. (1995), "The Biological Effectiveness of Radon-Progeny Alpha Particles.", Radiat. Res. 142(1):61–69. doi:10.2307/3578967.

Moore, S., F.K.T. Stanley & A.A. Goodarzi (2014), "The repair of environmentally relevant DNA double strand breaks caused by high linear energy transfer irradiation – No simple task.", DNA repair (Amst), 17:64–73. doi: 10.1016/j.dnarep.2014.01.014.

Mukherjee, B. et al. (2008), “Modulation of the DNA-damage response to HZE particles by shielding”, DNA Repair, Vol. 7/10, Elsevier B.V, Amsterdam, https://doi.org/10.1016/j.dnarep.2008.06.016

Murakami, H. & S. Keeney (2008), "Regulating the formation of DNA double-strand breaks in meiosis.", Genes Dev. 22(3):286–292. doi:10.1101/gad.1642308.

Paull, T.T. et al. (2000), "A critical role for histone H2AX in recruitment of repair factors to nuclear foci after DNA damage.", Curr. Biol. 10(15):886–895. doi:10.1016/S0960-9822(00)00610-2

Pinto, M. & K. Prise (2005), "Evidence for Complexity at the Nanometer Scale of Radiation-Induced DNA DSBs as a Determinant of Rejoining Kinetics Evidence for Complexity at the Nanometer Scale of Radiation-Induced DNA DSBs as a Determinant of Rejoining Kinetics.", Radiat. Res. 164(1):73-85 doi:10.1667/RR3394.

Puchta, H. (2005), "The repair of double-strand breaks in plants: Mechanisms and consequences for genome evolution.", J. Exp. Bot. 56(409):1–14. doi:10.1093/jxb/eri025.

Thurtle-Schmidt, D.M. & T-W. Lo (2018), "Molecular biology at the cutting edge: A review on CRISPR/CAS9 gene editing for undergraduates.", Biochem. Mol. Biol. Educ. 46(2):195–205. doi:10.1002/bmb.21108.

Rathmell, W,K. & G. Chu (1994), "Involvement of the Ku autoantigen in the cellular response to DNA double-strand breaks.", Proc. Natl. Acad. Sci. 91(16):7623–7627. doi:10.1073/pnas.91.16.7623.

Rogakou, E.P. et al. (1999), "Megabase Chromatin Domains Involved in DNA Double-Strand Breaks In Vivo.", J. Cell Biol, 146(5):905-16. doi: 10.1083/jcb.146.5.905.

Rothkamm, K. et al. (2015), "Review DNA Damage Foci: Meaning and Significance.", Environ. Mol. Mutagen., 56(6):491-504, doi: 10.1002/em.21944

Rothkamm, K. & M. Lo (2003), "Evidence for a lack of DNA double-strand break repair in human cells exposed to very low x-ray doses.", PNAS, 100(9):5057-62. doi:10.1073/pnas.0830918100.

Rube, C.E. et al. (2008), "Cancer Therapy: Preclinical DNA Double-Strand Break Repair of Blood Lymphocytes and Normal Tissues Analysed in a Preclinical Mouse Model: Implications for Radiosensitivity Testing.", Clin. Cancer Res., 14(20):6546–6556. doi:10.1158/1078-0432.CCR-07-5147.

Rydberg, B. (1975), “The rate of strand separation in alkali of DNA of irradiated mammalian cells”, Radiation Research, Vol. 178/2, United States, pp. 190-197

Rydberg, B. et al. (1994), “DNA double-strand breaks induced by high-energy neon and iron ions in human fibroblasts. I. Pulsed-filed gel electrophoresis method”, Radiation Research, Vol. 139/2, Radiation Research Society, Oak Brook, https://doi.org/10.2307/3578657

Rydberg, B. et al. (2005), "Dose-Dependent Misrejoining of Radiation-Induced DNA Double-Strand Breaks in Human Fibroblasts: Experimental and Theoretical Study for High- and Low-LET Radiation.", Radiat. Res. 163(5):526–534. doi:10.1667/RR3346.

Sage, E. & N. Shikazono (2017), "Free Radical Biology and Medicine Radiation-induced clustered DNA lesions: Repair and mutagenesis.", Free. Radic. Biol. Med. 107(December 2016):125–135. doi:10.1016/j.freeradbiomed.2016.12.008.

Schipler, A. & G. Iliakis (2013), "DNA double-strand – break complexity levels and their possible contributions to the probability for error-prone processing and repair pathway choice.", Nucleic Acids Res., 41(16):7589–7605. doi:10.1093/nar/gkt556.

Scott, B. (2006), "Stochastic Thresholds: A Novel Explanation of Nonlinear Dose-Response Relationships for Stochastic Radiobiological Effects.", Dose-Response, 3(4):547–567. doi:10.2203/dose-response.003.04.009.

Shuman, S. & M.S. Glickman (2007), "Bacterial DNA repair by non-homologous end joining.", Nat. Rev. Microbiol. 5(11):852–861. doi:10.1038/nrmicro1768.

Sidjanin, D. et al. (1993), “DNA damage and repair in rabbit lens epithelial cells following UVA radiation, Taylor & Francis, Vol. 12/9, Informa UK Ltd, Lisse, https://doi.org/10.3109/02713689309020382

Simsek, D. & M. Jasin (2010), "HHS Public Access.", 118(24):6072–6078. doi:10.1002/cncr.27633.

Stenerlöw, E. H. et al. (2000), “Rejoining of DNA fragments produced by radiations of different linear energy transfer”, International Journal of Radiation Biology, Vol. 76/4, Informa UK Ltd, London, https://doi.org/10.1080/095530000138565

Sutherland, B.M. et al. (2000), "Clustered DNA damages induced in isolated DNA and in human cells by low doses of ionizing radiation.", J. of Rad. Res. 43 Suppl(S):S149-52. doi: 10.1269/jrr.43.S149

Thompson, L.H. (2012), "Recognition, signaling, and repair of DNA double-strand breaks produced by ionizing radiation in mammalian cells : The molecular choreography.", Mutat Res., 751(2):158–246. doi: 10.1016/j.mrrev.2012.06.002.

Tsao, D. et al. (2007), “Induction and processing of oxidative clustered DNA lesions in 56Fe-ion-irradiated human monocytes”, Radiation Research, Vol.168/1, United States, https://doi.org/10.1667/RR0865.1

Valentin J. (2005), "Low-dose Extrapolation of Radiation-related Cancer Risk.", Ann. ICRP, 35(4):1-140

Vignard, J., G. Mirey & B. Salles (2013), "Ionizing-radiation induced DNA double-strand breaks: A direct and indirect lighting up.", Radiother. Oncol. 108(3):362–369. doi:10.1016/j.radonc.2013.06.013.

Wang, H. et al, (2004),. “ATR affecting cell radiosensitivity is dependent on homologous recombination repair but independent of nonhomologous end joining”,. Cancer Research, 64(19), 7139–7143. https://doi.org/10.1158/0008-5472.can-04-1289.

Ward, J. F. (1988), "DNA Damage Produced by Ionizing Radiation in Mammalian Cells: Identities, Mechanisms of Formation, and Reparability.", Prog. Nucleic Acid Res. Mol. Biol. 35(C):95–125. doi:10.1016/S0079-6603(08)60611-X.

Wilson, T.E. & M.R. Lieber (1999), "Efficient Processing of DNA Ends during Yeast Nonhomologous End Joining.", J. Biol. Chem. 274(33):23599–23609. doi:10.1074/jbc.274.33.23599.

Zhu, C. et al. (2002), "Unrepaired DNA breaks in p53-deficient cells lead to oncogenic gene amplification subsequent to translocations.", Cell. 109(7):811–21. doi:10.1016/s0092-8674(02)00770-5.

Relationship: 164: Inadequate DNA repair leads to Increase, Mutations

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Alkylation of DNA in male pre-meiotic germ cells leading to heritable mutations	adjacent	High	Moderate
Alkylation of DNA leading to cancer 2	adjacent	High	Moderate
Alkylation of DNA leading to cancer 1	non-adjacent	High	Moderate
Oxidative DNA damage leading to chromosomal aberrations and mutations	adjacent	High	Low
Deposition of energy leading to lung cancer	adjacent	Moderate	Moderate
Bulky DNA adducts leading to mutations	adjacent		
DNA damage and mutations leading to Metastatic Breast Cancer	adjacent	High	High
Deposition of energy leading to occurrence of cataracts	adjacent	High	Low

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
mouse	Mus musculus	High	NCBI
human	Homo sapiens	High	NCBI
rat	Rattus norvegicus	High	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Unspecific	High

This KER is plausible in all life stages, sexes, and organisms with DNA. The majority of the evidence is from in vivo adult mice and male human, and mice in vitro models.

All organisms, from prokaryotes to eukaryotes, have DNA repair systems. Indeed, much of the empirical evidence on the fundamental principles described in this KER are derived from prokaryotic models. DNA adducts can occur in any cell type with DNA, and may or may not be repaired, leading to mutation. While there are differences among DNA repair systems across eukaryotic taxa, all species develop mutations following excessive burdens of DNA lesions like DNA adducts. Theoretically, any sexually reproducing organism (i.e., producing gametes) can also acquire DNA lesions that may or may not be repaired, leading to mutations in gametes.

Key Event Relationship Description

The described Key Event Relationship (KER) outlines a sequence of events related to DNA repair and its consequences. The upstream event is characterized by "Inadequate DNA repair," indicating that the cellular mechanisms responsible for repairing DNA damage are compromised or insufficient. This could result from various factors, such as genetic mutations, environmental exposures, or other cellular processes.

The downstream event in this KER is an "Increase in Mutations." As a consequence of inadequate DNA repair, the

accumulation of unrepaired or incorrectly repaired DNA damage can lead to an elevated rate of mutations in the genome. These mutations can involve changes in the DNA sequence, structure, or arrangement, which may have various implications for cellular function, including potential disruptions to normal processes and pathways.

This KER highlights the critical role of DNA repair mechanisms in maintaining genomic stability and preventing the buildup of mutations that can contribute to various biological outcomes, including disease development and other adverse effects.

Insufficient repair results in the retention of damaged DNA that is then used as a template during DNA replication. During replication of damaged DNA, incorrect nucleotides may be inserted, and upon replication these become 'fixed' in the cell. Further replication propagates the mutation to additional cells.

For example, it is well established that replication of alkylated DNA can cause insertion of an incorrect base in the DNA duplex (i.e., mutation). Replication of non-repaired O4 thymine alkylation leads primarily to A:T→G:C transitions. Retained O6 guanine alkylation causes primarily G:C→A:T transitions.

For repairing DNA double strand breaks (DSBs), non-homologous end joining (NHEJ) is one of the repair mechanisms used in human somatic cells (Petrini et al., 1997; Mao et al., 2008). However, this mechanism is error-prone and may create mutations during the process of DNA repair (Little, 2000). NHEJ is considered error-prone because it does not use a homologous template to repair the DSB. The NHEJ mechanism involves many proteins that work together to bridge the DSB gap by overlapping single-strand termini that are usually less than 10 nucleotides long (Anderson, 1993; Getts & Stamato, 1994; Rathmell & Chu, 1994). Inherent in this process is the introduction of errors that may result in mutations such as insertions, deletions, inversions, or translocations.

Furthermore, other repair mechanisms such as a loss in the mismatch repair (MMR) system can lead to a buildup of errors such as base-base mismatches and insertion-deletion errors in repetitive DNA sequences which are known as microsatellites. This could occur if an MMR gene (e.g. MLH1, PMS2) is inactivated through mutations or epigenetic silencing (Wang et al., 2022).

Evidence Supporting this KER

Overall Weight of Evidence: High

Biological Plausibility

If DNA repair is able to correctly and efficiently repair DNA lesions introduced by a genotoxic stressor, then no increase in mutation frequency will occur.

For example, for alkylated DNA, efficient removal by O^6-alkylguanine DNA alkyltransferase will result in no increases in mutation frequency. However, above a certain dose AGT becomes saturated and is no longer able to efficiently remove the alkyl adducts. Replication of O-alkyl adducts leads to mutation. The evidence demonstrating that replication of unrepaired O-alkylated DNA causes mutations is extensive in somatic cells and has been reviewed (Basu and Essigmann 1990; Shrivastav et al. 2010); specific examples are given below.

It is important to note that not all DNA lesions will cause mutations. It is well documented that many are bypassed error-free. For example, N-alkyl adducts can quite readily be bypassed error-free with no increase in mutations (Philippin et al., 2014).

Inadequate repair of DSB

Collective data from tumors and tumor cell lines has emerged that suggests that DNA repair mechanisms may be error-prone (reviewed in Sishc et al., 2017) (Sishc & Davis, 2017). NHEJ, the most common pathway used to repair DSBs, has been described as error-prone. The error-prone nature of NHEJ, however, is thought to be dependent on the structure of the DSB ends being repaired, and not necessarily dependent on the NHEJ mechanism itself (Bétermier et al., 2014). Usually when perfectly cohesive ends are formed as a result of a DSB event, ligase 4 (LIG4) will have limited end processing to perform, thereby keeping ligation errors to a minimum (Waters et al., 2014). When the ends are difficult to ligate, however, the resulting repair may not be completed properly; this often leads to point mutations and other chromosomal rearrangements. It has been shown that approximately 25 - 50% of DSBs are misrejoined after exposure to ionizing radiation (Löbrich et al., 1998; Kuhne et al., 2000; Lobrich et al., 2000). Defective repair mechanisms can increase sensitivity to agents that induce DSBs and lead eventually to genomic instability (reviewed in Sishc et al., (2017)).

Activation of mutagenic DNA repair pathways to withstand cellular or replication stress either from endogenous or exogenous sources can promote cellular viability, albeit at a cost of increased genome instability and mutagenesis (Fitzgerald et al., 2017). These salvage DNA repair pathways including, Break-induced Replication (BIR) and Microhomology-mediated Break-induced Replication (MMBIR). BIR repairs one-ended DSBs and has been extensively studied in yeast as well as in mammalian systems. BIR and MMBIR are linked with heightened levels of mutagenesis, chromosomal rearrangements and ensuing genome instability (Deem et al., 2011; Sakofsky et al., 2015; Saini et al., 2017; Kramara et al., 2018). In mammalian genomes BIR-like synthesis has been proposed to be involved in late-stage Mitotic DNA Synthesis (MiDAS) that predominantly occurs at so-called Common Fragile Sites (CFSs) and maintains telomere length under s conditions of replication stress that serve to promote cell viability (Minocherhomji et al., 2015; Bhowmick et al., 2016; Dilley et al., 2016).

Empirical Evidence

INSUFFICIENT REPAIR OF ALKYLATED DNA

Evidence in somatic cells

Empirical evidence to support this KER is primarily from studies in which synthetic oligonucleotides containing well-characterized DNA lesions were genetically engineered in viral or plasmid genomes and subsequently introduced into bacterial or mammalian cells. Mutagenicity of each lesion is ascertained by sequencing, confirming that replication of alkylated DNA (i.e., unrepaired DNA) causes mutations in addition to revealing the important DNA repair pathways and polymerases involved in the process. For example, plasmids containing O6-methyl or O6-ethylguanine were introduced into AGT deficient or normal Chinese hamster ovary cells (Ellison et al. 1989). Following replication, an increase in mutant fraction to 19% for O6-methylguanine and 11% for O6-ethylguanine adducts was observed in AGT deficient cells versus undetectable levels for control plasmids. The relationship between input of alkylated DNA versus recovered mutant fractions revealed that a large proportion of alkyl adducts were converted to mutations in the AGT deficient cells (relationship slightly sublinear, with more adducts than mutations). The primary mutation occurring was G:C-A:T transitions. The results indicate that replication of the adducted DNA caused mutations and that this was more prevalent with reduced repair capacity. The number of mutations measured is less than the unrepaired alkyl adducts transfected into cells, supporting that insufficient repair occurs prior to mutation. Moreover, the alkyl adducts occur prior to mutation formation, demonstrating temporal concordance.

Various studies in cultured cells and microorganisms have shown that the expression of O^6-methylguanine DNA methyltransferase (AGT/MGMT) (repair machinery – i.e., decrease in DNA strand breaks) greatly reduces the incidence of mutations caused by exposure to methylating agents such as MNU and MNNG (reviewed in Kaina et al. 2007; Pegg 2011). Thomas et al. (2013) used O6-benzylguanine to specifically inhibit MGMT activity in AHH-1 cells. Inhibition was carried out for one hour prior to exposure to MNU, a potent alkylating agent. Inactivation of MGMT resulted in increased MNU-induced HPRT (hypoxanthine-guanine phosphoribosyltransferase) mutagenesis and shifted the concentrations at which induced mutations occurred to the left on the dose axis (10 fold reduction of the lowest observed genotoxic effect level from 0.01 to 0.001 µg/ml). The ratio of mutants recovered in DNA repair deficient cells was 3-5 fold higher than repair competent cells at concentrations below 0.01 µg/ml, but was approximately equal at higher concentrations, indicating that repair operated effectively to a certain concentration. Only at this concentration (above 0.01 µg/ml when repair machinery is overwhelmed and repair becomes deficient) do the induced mutations in the repair competent cells approach those of repair deficient. Thus, induced mutation frequencies in wild type cells are suppressed until repair is overwhelmed for this alkylating agent. The mutations prevented by MGMT are predominantly G:C-A:T transitions caused by O6-methylguanine.

Evidence in germ cells

That saturation of repair leads to mutation in spermatogonial cells is supported by work using the OECD TG488 rodent mutation reporter assay in sperm. A sub-linear dose-response was found using the lacZ MutaMouse assay in sperm exposed as spermatogonial stem cells, though the number of doses was limited (van Delft and Baan 1995). This is indirect evidence that repair occurs efficiently at low doses and that saturation of repair causes mutations at high doses. Lack of additional data motivated a dose-response study using the MutaMouse model following both acute and sub-chronic ENU exposure by oral gavage (O'Brien et al. 2015). The results indicate a linear dose-response for single acute exposures, but a sub-linear dose-response occurs for lower dose sub-chronic (28 day) exposures, during which mutation was only observed to occur at the highest dose. This is consistent with the expected pattern for dose-response based on the hypothetical AOP. Thus, this sub-linear curve for mutation at low doses following sub-chronic ENU exposure suggests that DNA repair in spermatogonia is effective in preventing mutations until the process becomes overwhelmed at higher doses.

Mutation spectrum: Following exposure to alkylating agents, the most mutagenic adducts to DNA in pre-meiotic male germ cells include O6-ethylguanine, O4-ethylthymine and O2-ethylthymine (Beranek 1990; Shelby and Tindall 1997). Studies on sperm samples collected post-ENU exposure in transgenic rodents have shown that 70% of the observed mutations are at A:T sites (Douglas et al. 1995). The mutations observed at G:C base pairs are almost exclusively G:C-A:T transitions, presumably resulting from O6-ethylguanine. It is proposed that the prevalence of mutations at A:T basepairs is the result of efficient removal of O6-alkylguanine by AGT in spermatogonia, which is consistent with observation in human somatic cells (Bronstein et al. 1991; Bronstein et al. 1992). This results in the majority of O6-ethylguanine adducts being removed, leaving O4- and O2-ethylthymine lesions to mispair during replication. Thus, lack of repair predominantly at thymines and guanines at increasing doses leads to mutations in these nucleotides, consistent with the concordance expected between diminished repair capabilities at these adducts and mutation induction (i.e., concordance relates to seeing these patterns across multiple studies, species and across the data in germ cells and offspring).

Inadequate repair of oxidative DNA lesions: In vitro studies

- AS52 Chinese hamster ovary cells (wild type and OGG1-overexpressing) were exposed to kJ/m^2 UVA radiation (Dahle et al., 2008).
    - Mutations in the gpt gene were quantified in both wild type and OGG1+ cells by sequencing after 13-15 days following 400 kJ/m^2 UVA irradiation
        - G:C-A:T mutations in UVA-irradiated OGG1+ cells were completely eliminated
        - G:C-A:T mutation frequency in wild type cells increased from 1.8 mutants/million cells to 3.8 mutants/million cells following irradiation – indicating incorrect repair or lack of repair of accumulated 8-oxo-dG
        - Elevated levels of OGG1 was able to prevent G:C-A:T mutations, while the OGG1 levels in wild type cells was insufficient, leading to an increase in mutants (demonstrates inadequate repair leading to mutations)
- Xeroderma pigmentosum complementation group A (XPA) knockout (KO) and wild type TSCER122 human lymphoblastoid cells were transfected with TK gene-containing vectors with no adduct, a single 8-oxo-dG, or two 8-oxo-dG adducts in tandem (Sassa et al., 2015).
    - XPA is a key protein in nucleotide excision repair (NER) that acts as a scaffold in the assembly the repair complex.
    - Mutation frequency was determined by the number of TK-revertant colonies
    - Control vector induced a mutation frequency of 1.3% in both WT and XPA KO
    - Two 8-oxo-dG in tandem on the transcribed strand were most mutagenic in XPA KO, inducing 12% mutant frequency compared to 7% in WT
    - For both XPA KO and WT, G:C-A:T transversion due to 8-oxo-dG was the most predominant point mutation in the mutants
    - The lack of a key factor in NER leading to increased 8-oxo-dG-induced transversions demonstrates insufficient repair leading to increase in mutations

Inadequate repair of oxidative DNA lesions: In vivo studies in mice

- Spontaneous mutation frequencies in the liver of Ogg1-deficient (-/-) Big Blue mice was measured at 10 weeks of age (Klungland et al., 1999).
    - Mutation frequencies were 2- to 3-fold higher in the *Ogg1*-/- mice than in wild type
    - Of the 16 base substitutions detected in *Ogg1* -/- mutant plaques analyzed by sequencing, 10 indicated G:C-A:T transversions consistent with the known spectrum of mutation
    - The results support that insufficient repair of oxidized bases leads to mutation.
- *Ogg1* knockout (*Ogg1*-/-) in C57BL/6J mice resulted in 4.2-fold and 12-fold increases in the amount of 8-oxo-dG in the liver compared to wild type at 9 and 14 weeks of age, respectively (Minowa et al., 2000).
    - In these mice, there was an average of 2.3-fold increase in mutation frequencies in the liver (measured between 16-20 weeks)
        - 57% of the observed base substitutions were G:C-A:T transversions, while 35% in wild type mice corresponded to this transversion.
        - Approximately 70% of the increase in mutation frequency was due to G to T transversions.
    - Concordantly, KBrO3 treatment resulted in a 2.9-fold increase in mutation frequency in the kidney of *Ogg1* -/- mice compared to KBrO3-treated wild type (Arai et al., 2002).
        - G:C-A:T transversions made up 50% of the base substitutions in the *Ogg1-/-* mice.
    - Heterozygous *Ogg1* mutants (*Ogg1*+/-) retained the original repair capacity, where no increase in 8-oxo-dG lesions was observed in the liver at 9 and 14 weeks (Minowa et al., 2000).
        - This observation was consistent even after KBrO3 treatment of the mice (Arai et al., 2002).
    - From these results, we can infer that OGG1 proteins are present in excess and that one functional copy of the gene is sufficient in

addressing endogenous and, to a certain degree, chemical-induced oxidative DNA lesions.

Inadequate Repair of DSB

Empirical data obtained for this KER moderately supports the idea that inadequate DNA repair increases the frequency of mutations. The evidence presented below related to the inadequate repair of DSBs is summarized in table 5, here (click link). The review article by Sishc & Davis (2017) provides an overview of NHEJ mechanisms with a focus on the inherently error-prone nature of DSB repair mechanisms, particularly when core proteins of NHEJ are knocked-out. Although NHEJ is predominantly the preferred repair mechanism throughout the cell cycle, homologous recombination (HR) and single-stranded annealing (SSA) are favored during the S and G2 phases in scenarios where the NHEJ repair pathway is inhibited. The absence of HR leading to an increase in SSA activity is still a matter to debate (Ceccaldi et al., 2016). Another review also provides an overview of DSB induction, the repair process and how mutations may result, as well as the biological relevance of misrepaired or non-repaired DNA damage (Sage & Shikazono, 2017).

Dose and Incidence Concordance

There is evidence in the literature suggesting a dose/incidence concordance between inadequate DNA repair and increases in mutation frequencies. Evidence presented below related to the dose-response of mutation frequencies is summarized in table 2, here (click link). In response to increasing doses from a radiation stressor, dose-dependent increases in both measures of inadequate DNA repair and mutation frequency have been found. In an analysis that amalgamated results from several different studies conducted using in vitro cell-lines, the rate of DSB misrepair was revealed to increase in a dose-dependent fashion from 0 - 80 Gy, with the mutation rate also similarly increasing from 0 - 6 Gy (Mcmahon et al., 2016). Additionally, using a plant model, it was shown that increasing radiation dose from 0 - 10 Gy resulted in increased DNA damage as a consequence of inadequate repair. Mutations were observed 2 - 3 weeks post-irradiation (Ptácek et al., 2001). Moreover, increases in mutation densities were found in specific genomic regions of cancer samples (namely promoter DNAse I-hypersensitive sites (DHS) and 100 bp upstream of transcription start sites (TSS)) that were also found to have decreased DNA repair rates attributable to inadequate nucleotide excision repair (NER) (Perera et al., 2016).

Interestingly, mutation rates have been shown to increase as the required DNA repair becomes more complex. Upon completion of DSB repair in response to radiation and treatment with restriction enzymes, more mutations were found in cases where the ends were non-complementary and thus required more complex DNA repair (1 - 4% error-free) relative to cases where ends were complementary (34 - 38% error-free) (Smith et al., 2001).

Temporal Concordance

There is evidence in the literature suggesting a time concordance between the initiation of DNA repair and the occurrence of mutations. For simple ligation events, mutations were not evident until 12 - 24 hours, whereas DSB repair was evident at 6 -12 hours. For complex ligation events, however, mutations and DSB repair were both evident at 12 - 24 hours. As the relative percent of DNA repair increased over time, the corresponding percent of error-free rejoining decreased over time in both ligation cases, suggesting that overall DNA repair fidelity decreases with time ((Smith et al., 2001).

Essentiality

Inadequate DNA repair has been found to increase mutations above background levels. There is evidence from knock-out/knock-down studies suggesting that there is a strong relationship between the adequacy of DNA repair and mutation frequency. In all examined cases, deficiencies in proteins involved in DNA repair resulted in altered mutation frequencies relative to wild-type cases. There were significant decreases in the frequency and accuracy of DNA repair in cell lines deficient in LIG4 (DNA ligase 4, a DNA repair protein) (Smith et al., 2003) and Ku80 (Feldmann et al., 2000). Rescue experiments performed with these two cell lines further confirmed that inadequate DNA repair was the cause of the observed decreases in repair frequency and accuracy (Feldmann et al., 2000; Smith et al., 2003). In primary Nibrin-deficient mouse fibroblasts, there was increased spontaneous DNA damage relative to wild-type controls, suggestive of inadequate DNA repair. Using the corresponding Nibrin-deficient and wild-type mice, in vivo mutation frequencies were also found to be elevated in the Nibrin-deficient animals (Wessendorf et al., 2014). Furthermore, mutation densities were differentially affected in specific genomic regions in cancer patients depending on their Xeroderma pigmentosum group C (XPC) gene status. Specifically, mutation frequencies were increased in XPC-wild-type patients at DNase I-hypersensitive site (DHS) promoters and 100 bp upstream of TSS relative to cancer patients lacking functional XPC (Perera et al., 2016). Lastly, in a study using WKT1 cells with less repair capacity, radiation exposure induced four times more mutations in these cells than in TK6 cell, which had a normal repair capacity (Amundson and Chen, 1996).

Uncertainties and Inconsistencies

Repair of alkylated DNA

There were no inconsistencies in the empirical data reviewed or in the literature relating to biological plausibility. Much of the support for this KER comes predominantly from data in somatic cells and in prokaryotic organisms. We note that all of the data in germ cells used in this KER are produced exclusively from ENU exposure. Data on other chemicals are required. We consider the overall weight of evidence of this KER to be strong because of the obvious biological plausibility of the KER, and documented temporal association and incidence concordance based on studies over-expressing and repressing DNA repair in somatic cells.

Repair of oxidative lesions

- Thresholded concentration-response curve of mutation frequency was observed in AHH-1 human lymphoblastoid cells after treatment with pro-oxidants (H_2O_2 and $KBrO_2$) known to cause oxidative DNA damage (Seager et al., 2012), suggesting that cells are able to tolerate low levels of DNA damage using basal repair. However, increase in 8-oxo-dG lesions and up-regulation of DNA repair proteins were not observed under the same experimental condition.
- Mutagenicity of oxidative DNA lesions other than 8-oxo-dG, such as FaPydG and thymidine glycol, has not been as extensively studied and there are mixed results regarding the mutagenic outcome of these lesions.

Repair of double strand breaks

- One review paper found that DNA DSBs are repaired more efficiently at low dose (≤0.1 Gy) compared to high dose (>1 Gy) X-rays, but delayed mutation induction and genomic instability have also been demonstrated to occur at low doses (<1 cGy) of ionizing radiation (Preston et al., 2013).

Overall

- Mutation induction is stochastic, spontaneous, and dependent on the cell type as well as the individual's capability to repair efficiently (NRC, 1990; Pouget & Mather, 2001).

Quantitative Understanding of the Linkage

Thresholds for mutagenicity indicate that the response at low doses is modulated by the DNA repair machinery, which is effectively able to remove alkylated DNA at low doses [Gocke and Muller 2009; Lutz and Lutz 2009; Pozniak et al. 2009]. Kinetics of DNA repair saturation in somatic cells is described in Muller et al. [Muller et al. 2009].

For O-methyl adducts, once the primary repair process is saturated, in vitro data suggest that misreplication occurs almost every time a polymerase encounters a methylated guanine [Ellison et al. 1989; Singer et al. 1989]; however, it should be noted that this process can be modulated by flanking sequence. This conversion of adducts to mutations also appears to be reduced substantially in vivo [Ellison et al. 1989]. The probability of mutation will also depend on the type of adduct (e.g., O-alkyl adducts are more mutagenic than N-alkyl adducts; larger alkyl groups are generally more mutagenic, etc.). Overall, a substantive number of factors must be considered in developing a quantitative model.

Inadequate repair of oxidative lesions

The relationship between the quantity/activity of repair enzymes such as OGG1 in the cell and the quantity of oxidative lesions need to be better understood to define a threshold on the quantity of oxidative lesions exceeding basal repair capacity. Moreover, the proportion of oxidative lesions formed that lead to mutation versus strand breaks is not clearly understood.

Mutations resulting from oxidative DNA damage can occur via replicative polymerases and translesion synthesis (TLS) polymerases during replication, and during attempted repair. However, an in vitro study on TLS in yeast has shown that bypass of 8-oxo-dG by TLS polymerases during replication is approximately 94-95% accurate. Therefore, the mutagenicity of 8-oxo-dG and other oxidative lesions may depend on their abundance, not on a single lesion (Rodriguez et al., 2013). Applicability of this observation in mammalian cells needs further investigation. Information on the accuracy of 8-oxo-dG bypass in mammalian cells is limited.

The most notable example of mutation arising from inadequate repair of DNA oxidation is G to T transversion due to 8-oxo-dG lesions. Previous studies have demonstrated higher mutation frequency of this lesion compared to other oxidative lesions; for example, Tan et al. (1999) compared the mutation rate of 8-oxo-dG and 8-oxo-dA in COS-7 monkey kidney cells and reported that under similar conditions, 8-oxo-dG was observed to be four times more likely to cause base substitution (Tan et al., 1999).

Inadequate Repair of DSB

Quantitative understanding of this linkage is derived from the studies that examined DSB misrepair rates or mutation rates in response to a radiation stressor. In general, combining results from these studies suggests that increased mutations can be predicted when DNA repair is inadequate. At a radiation dose of 10 Gy, the rate of DSB misrepair was found to be approximately 10 - 15% (Lobrich et al., 2000); this rate increased to 50 - 60% at a radiation exposure of 80 Gy (Kuhne et al., 2000; Lobrich et al., 2000; McMahon et al., 2016). For mutation rates in response to radiation across a variety of models and radiation doses, please refer to the example table below.

Reference	Summary
Matuo et al., 2018	Yeast cells (saccharomyces cerevisiae) exposed to high LET cardbon ions (25 keV/um) and low LET carbon ions (13 keV/um) between 0-200 Gy induces a 24-fold increase overbaseline of mutations (high LET) and 11-fold increase over baseline mutations (low LET).
Nagashima et al., 2018	Hamster cells (GM06318-10) exposed to x-rays in the 0-1 Gy. Response of 19.0 ± 6.1 mutants per 10^9 survivors.
Albertini et al., 1997	T-lymphcytes isolated from human peripheral blood exposed to low LET gamma-rays (0.5-5 Gy) and high LET radon gas (0-1 Gy). Response of $7.0x10^{-6}$ mutants/Gy (Gamma-rays 0-2 Gy), $54x10^6$ mutants/Gy (Gamma-rays 2-4 Gy) and $63x10^{-6}$ mutants/Gy (0-1 Gy).
Dubrova et al., 2002	Observation of paternal ESTR mutation rates in CBAH mice following exposure to acute low LET X-rays (0-1 Gy), chronic low LET gamma-rays (0-1 Gy) and chronic high LET neutrons (0-0.5 Gy). Modelled response of y = mx + C, values of (m,C): X-rays: (0.338, 0.111), Gamma-rays: (0.373±0.082, 0.110), Neutrons: (1.135±0.202, 0.136).
McMahon et al., 2016	Study of HPRT gene in Chinese hamster cells following exposure to radiation of 1-6 Gy. Observation of 0.2 mutations in HPRT gene per 10^4 cells and 0.1 point mutations per 10^4 cells (1 Gy). At 6 Gy, observation of 1.5 mutations in the HPRT gene per 10^4 cells and 0.4 point mutations per 10^4 cells.

Response-response relationship

Inadequate Repair of DSB

There is evidence of a response-response relationship between inadequate DNA repair and increased frequency of mutations. When exposed to a radiation stressor, there was a positive relationship between the radiation dose and the DSB misrepair rate, and between the mutation rate and the radiation dose (Mcmahon et al., 2016). Similarly, there was a negative correlation found between NER and the mutation densities at specific genomic regions in cancer patients. Specifically, inadequate NER resulted in more mutations in the promoter DHS and the TSS, but normal NER at DHS flanking regions resulted in fewer mutations (Perera et al., 2016).

Time-scale

Inadequate Repair of DSB

Two studies were used to provide data regarding the time scale of DNA repair and the appearance of mutations. In a study using plants, DNA damage was evident immediately following radiation with 30 Gy of radiation; 50% of repairs were complete by 51.7 minutes, 80% by 4 hours, and repair was completed by 24 hours post-irradiation. Although no mutational analysis was performed during the period of repair, irradiated plants were found to have increased mutations when they were examined 2 - 3 weeks later (Ptácek et al., 2001). Both DNA repair and mutation frequency were examined at the same time in a study comparing simple and complex ligation of linearized plasmids. In this study, repaired plasmids were first detected between 6 - 12 hours for simple ligation events and between 12 - 24 hours for more complex ligation events; this first period was when the most error-free rejoining occurred in both cases. After this initial period of repair until its completion at 48 hr, repair became increasingly more erroneous such that mutations were found in more than half of the repaired plasmids at 48 hr regardless of the type of required ligation (Smith et al., 2001).

Known modulating factors

Not identified.

Known Feedforward/Feedback loops influencing this KER

Not identified.

References

Ainsbury, E. A. et al. (2016), “Ionizing radiation induced cataracts: recent biological and mechanistic developments and perspectives for future research”, Mutation research. Reviews in mutation research, Vol. 770, Elsevier B.V., https://doi.org/10.1016/j.mrrev.2016.07.010.

Albertini, R.J. et al. (1997), "Radiation Quality Affects the Efficiency of Induction and the Molecular Spectrum of HPRT Mutations in Human T Cells", 148(5 Suppl):S76-86.

Amundson, S.A. & D.J. Chen (1996), "Ionizing Radiation-Induced Mutation of Human Cells With Different DNA Repair Capacities.", Adv. Space Res. 18(1-2):119-126.

Anderson, C.W. 1993, "DNA damage and the DNA-activated protein kinase.", Trends Biochem. Sci. 18(11):433–437. doi:10.1016/0968-0004(93)90144-C.

Arai, T., Kelly, V.P., Minowa, O., Noda, T., Nishimura, S. (2002), High accumulation of oxidative DNA damage, 8-hydroxyguanine, in Mmh/Ogg1 deficient mice by chronic oxidative stress, Carcinogenesis, 23:2005-2010.

Basu, A.K. and J.M. Essigmann (1990), "Site-specific alkylated oligodeoxynucleotides: Probes for mutagenesis, DNA repair and the structure effects of DNA damage", *Mutation Research*, 233: 189-201.

Beranek, D.T. (1990), "Distribution of methyl and ethyl adducts following alkylation with monofunctional alkylating agents"*Mutation Research*, 231(1): 11-30.

Bétermier, M., P. Bertrand & B.S. Lopez (2014), "Is Non-Homologous End-Joining Really an Inherently Error-Prone Process?", PLoS Genet. 10(1). doi:10.1371/journal.pgen.1004086.

Bhowmick, R., S. Minocherhomji & I.D. Hickson (2016), "RAD52 Facilitates Mitotic DNA Synthesis Following Replication Stress", Mol. Cell., 64(6):1117-1126.

Ceccaldi, R. et al. (2016), “Repair Pathway Choices and Consequences at the Double-Strand Break.” Trends in cell biology, Vol. 26/1, Elsevier, Amsterdam, https://doi.org/10.1016/j.tcb.2015.07.009

Dahle, J., Brunborg, G., Svendsrud, D., Stokke, T., Kvam, E. (2008), Overexpression of human OGG1 in mammalian cells decreases ultraviolet A induced mutagenesis, Cancer Lett, 267:18-25.

Deem, A. et al. (2011), "Break-Induced Replication Is Highly Inaccurate", PLoS Biol., 9(2):e1000594, doi: 10.1371/journal.pbio.1000594.

Dilley, R.L. et al. (2016), "Break-induced telomere synthesis underlies alternative telomere maintenance", Nature, 539:54-58.

Douglas, G.R., J. Jiao, J.D. Gingerich, J.A. Gossen and L.M. Soper (1995), "Temporal and molecular characteristics of mutations induced by ethylnitrosourea in germ cells isolated from seminiferous tubules and in spermatozoa of lacZ transgenic mice", *Proc. Natl. Acad. Sci. USA*, 92(16): 7485-7489.

Dubrova, Y.E. et al. (2002), "Elevated Minisatellite Mutation Rate in the Post-Chernobyl Families from Ukraine.", Am. J. Hum. Genet. 71(4): 801-809.

Ellison, K.S., E. Dogliotti, T.D. Connors, A.K. Basu and J.M. Essigmann (1989), "Site-specific mutagenesis by O6-alkyguanines located in the chromosomes of mammalian cells: Influence of the mammalian O6-alkylguanine-DNA alkyltransferase", *Proc. Natl. Acad. Sci. USA*, 86: 8620-8624.

Feldmann, E. et al. (2000), "DNA double-strand break repair in cell-free extracts from Ku80-deficient cells : implications for Ku serving as an alignment factor in non-homologous DNA end joining.", Nucleic Acids Res. 28(13):2585–2596.

Fitzgerald, D.M., P.J. Hastings, and S.M. Rosenberg (2017), "Stress-Induced Mutagenesis: Implications in Cancer and Drug Resistance", Ann. Rev. Cancer Biol., 1:119-140, doi: 10.1146/annurev-cancerbio-050216-121919.

Garrett, J et al. (2020), “The protective effect of estrogen against radiation cataractogenesis is dependent upon the type of radiation”, Radiation Research, Vol. 194/5, Radiation Research Society, United States, https://doi.org/10.1667/RADE-20-00015.1.

Getts, R.C. & T.D. Stamato (1994), "Absence of a Ku-like DNA end binding activity in the xrs double-strand DNA repair-deficient mutant.", J. Biol. Chem. 269(23):15981–15984.

Gocke, E. and L. Muller (2009), "In vivo studies in the mouse to define a threhold for the genotoxicity of EMS and ENU"*Mutat. Res.*, 678, 101-107.

Gorbunova, V. (1997), "Non-homologous DNA end joining in plant cells is associated with deletions and filler DNA insertions.", Nucleic Acids Res. 25(22):4650–4657. doi:10.1093/nar/25.22.4650.

Hartlerode, A.J. & R. Scully (2009), "Mechanisms of double-strand break in somatic mammalian cells.", Biochem J. 423(2):157–168. doi:10.1042/BJ20090942.Mechanisms.

Kaina, B., M. Christmann, S. Naumann and W.P. Roos (2007), "MGMT: Key node in the battle against genotoxicity, carcinogenicity and apoptosis induced by alkylating agents", *DNA Repair*, 6: 1079–1099.

Klungland, A., Rosewell, I., Hollenbach, S., Larsen, E., Daly, G., Epe, B., Seeberg, E., Lindahl, T., Barnes, D. (1999), Accumulation of premutagenic DNA lesions in mice defective in removal of oxidative base damage, Proc Natl Acad Sci USA, 96:13300-13305.

Kramara, J., B. Osia & A. Malkova (2018), "Break-Induced Replication: The Where, The Why, and The How", Trends Genet. 34(7):518-531, doi: 10.1016/j.tig.2018.04.002.

Kuhne, M., K. Rothkamm & M.Löbrich (2000), "No dose-dependence of DNA double-strand break misrejoining following a -particle irradiation.", Int. J.

Radiat. Biol. 76(7):891-900

Lieber, M.R. (2008), "The mechanism of human nonhomologous DNA End joining.", J Biol Chem. 283(1):1–5. doi:10.1074/jbc.R700039200.

Little, J.B. (2000), "Radiation carcinogenesis.", Carcinogenesis 21(3):397-404 doi10.1093/carcin/21.3.397.

Lobrich, M. et al. (2000), "Joining of Correct and Incorrect DNA Double-Strand Break Ends in Normal Human and Ataxia Telangiectasia Fibroblasts.", 68(July 1999):59–68. doi:DOI: 10.1002/(SICI)1098-2264(200001)27:1<59::AID-GCC8>3.0.CO;2-9.

Mao Z, Bozzella M, Seluanov A, Gorbunova V. 2008. DNA repair by nonhomologous end joining and homologous recombination during cell cycle in human cells. Cell Cycle. 7(18):2902–2906. doi:10.4161/cc.7.18.6679.

Matuo Y, Izumi Y, Furusawa Y, Shimizu K. 2018. Mutat Res Fund Mol Mech Mutagen Biological e ff ects of carbon ion beams with various LETs on budding yeast Saccharomyces cerevisiae. Mutat Res Fund Mol Mech Mutagen. 810(November 2017):45–51. doi:10.1016/j.mrfmmm.2017.10.003.

Mcmahon SJ, Schuemann J, Paganetti H, Prise KM. 2016. Mechanistic Modelling of DNA Repair and Cellular Survival Following Radiation-Induced DNA Damage. Nat Publ Gr.(April):1–14. doi:10.1038/srep33290.

Minocherhomji, S. et al. (2015), "Replication stress activates DNA repair synthesis in mitosis", Nature, 528(7581):286-290.

Minowa, O., Arai, T., Hirano, M., Monden, Y., Nakai, S., Fukuda, M., Itoh, M., Takano, H., Hippou, Y., Aburatani, H., Masumura, K., Nohmi, T., Nishimura, S., Noda, T. (2000), Mmh/Ogg1 gene inactivation results in accumulation of 8-hydroxyguanine in mice, Proc Natl Acad Sci USA, 97:4156-4161.

Muller, L., E. Gocke, T. Lave and T. Pfister (2009), "Ethyl methanesulfonate toxicity in Viracept – A comprehensive human risk assessment based on threshold data for genotoxicity", *Toxicology Letters*, 190: 317-329.

Nagashima, H. et al. (2018), "Induction of somatic mutations by low-dose X-rays : the challenge in recognizing radiation-induced events.", J. Radiat. Res., Na 59(October 2017):11–17. doi:10.1093/jrr/rrx053.

NRC (1990), "Health Effects of Exposure to Low Levels of Ionizing Radiation", (BEIR V).

O'Brien, J.M., A. Williams, J. Gingerich, G.R. Douglas, F. Marchetti and C.L. Yauk CL. (2013), "No evidence for transgenerational genomic instability in the F1 or F2 descendants of Muta™Mouse males exposed to N-ethyl-N-nitrosourea", *Mutat. Res.*, 741-742:11-7

O'Brien, J.M., M. Walker, A. Sivathayalan, G.R. Douglas, C.L. Yauk and F. Marchetti (2015), "Sublinear response in lacZ mutant frequency of Muta™ Mouse spermatogonial stem cells after low dose subchronic exposure to N-ethyl-N-nitrosourea", Environ. Mol. Mutagen., 56(4): 347-55.

Pegg, A.E., (2011), "Multifaceted roles of alkyltransferase and related proteins in DNA repair, DNA damage, resistance to chemotherapy, and research tools", *Chem. Res. Toxicol.*, 24(5): 618-639.

Perera, D. et al. (2016), "Differential DNA repair underlies mutation hotspots at active promoters in cancer genomes.", Nature 532, 259-263.

Petrini, J.H.J., D.A. Bressan & M.S. Yao (1997), "The RAD52 epistasis group in mammalian double strand break repair.", Semin Immunol. 9(3):181–188. doi:10.1006/smim.1997.0067

Philippin, G., J. Cadet, D. Gasparutto, G. Mazon, R.P. Fuchs (2014), "Ethylene oxide and propylene oxide derived N7-alkylguanine adducts are bypassed accurately in vivo", *DNA Repair (Amst)*, 22:133-6.

Pouget, J.P. & S.J. Mather (2001), "General aspects of the cellular response to low- and high-LET radiation.", Eur. J. Nucl. Med. 28(4):541–561. doi:10.1007/s002590100484

Preston, R. et al. (2013), "Uncertainties in estimating health risks associated with exposure to ionising radiation", Journal of Radiological Protection, Vol.33/3, IOP Publishing, Bristol, https://doi.org/10.1088/0952-4746/33/3/573.

Ptácek, O. et al. (2001), "Induction and repair of DNA damage as measured by the Comet assay and the yield of somatic mutations in gamma-irradiated tobacco seedlings.", Mutat Res. 491(1-2):17–23

Puchta, H. (2005), "The repair of double-strand breaks in plants: Mechanisms and consequences for genome evolution.", J. Exp. Bot. 56(409):1–14. doi:10.1093/jxb/eri025

Pzoniak, A., L. Muller, M. Salgo, J.K. Jone, P. Larson and D. Tweats (2009), "Elevated ethyl methansulfonate in nelfinavir mesylate (Viracept, Roche): overview", *Aids Research and Therapy*, 6: 18.

Rathmell, W.K. & G. Chu (1994), "Involvement of the Ku autoantigen in the cellular response to DNA double-strand breaks.", Proc. Natl. Acad. Sci. 91(16):7623–7627. doi:10.1073/pnas.91.16.7623

Rodriguez, G.P., Song, J.B., Crouse, G.F. (2013), In Vivo Bypass of 8-oxodG, PLoS Genetics, 9:e1003682.

Sage, E. & N. Shikazono (2017), "Free Radical Biology and Medicine Radiation-induced clustered DNA lesions : Repair and mutagenesis ☆.", Free Radic. Biol. Med. 107(December 2016):125–135. doi:10.1016/j.freeradbiomed.2016.12.008

Saini, N. et al. (2017), "Migrating bubble during break-induced replication drives conservative DNA synthesis", Nature, 502:389-392.

Sakofsky, C.J. et al. (2015), "Translesion Polymerases Drive Microhomology-Mediated Break-Induced Replication Leading to Complex Chromosomal Rearrangements", Mol. Cell, 60:860-872.

Sassa, A., Kamoshita, N., Kanemaru, Y., Honma, M., Yasui, M. (2015), Xeroderma Pigmentosum Group A Suppresses Mutagenesis Caused by Clustered Oxidative DNA Adducts in the Human Genome, PLoS One, 10:e0142218.

Seager, A., Shah, U., Mikhail, J., Nelson, B., Marquis, B., Doak, S., Johnson, G., Griffiths, S., Carmichael, P., Scott, S., Scott, A., Jenkins, G. (2012), Pro-oxidant Induced DNA Damage in Human Lymphoblastoid Cells: Homeostatic Mechanisms of Genotoxic Tolerance, Toxicol Sci, 128:387-397.

Shelby, M.D. and K.R. Tindall (1997), "Mammalian germ cell mutagenicity of ENU, IPMS and MMS, chemicals selected for a transgenic mouse collaborative study. Mutation Research 388(2-3):99-109.

Shrivastav, N., D. Li and J.M. Essignmann (2010), "Chemical biology of mutagenesis and DNA repair: cellular response to DNA alkylation", *Carcinogenesis*, 31(1): 59-70.

Shuman, S. & M.S. Glickman (2007), "Bacterial DNA repair by non-homologous end joining.", Nat. Rev. Microbiol. 5(11):852–861. doi:10.1038/nrmicro1768.

Singer, B., F. Chavez, M.F. Goodman, J.M. Essigman and M.K. Dosanjh (1989), "Effect of 3' flanking neighbors on kinetics of pairing of dCTP or dTTP opposite O6-methylguanine in a defined primed oligonucleotide when Escherichia coli DNA polymerase I is used", *Proc. Natl. Acad. Sci. USA*, 86(21): 8271-8274.

Sishc-Brock J. & A.J. Davis (2017), "The role of the core non-homologous end joining factors in carcinogenesis and cancer.", Cancers (Basel). 9(7). doi:10.3390/cancers9070081.

Smith, J. et al. (2001), "The influence of DNA double-strand break structure on end-joining in human cells.", Nucleic Acids Res. 29(23):4783–4792

Smith, J. et al. (2003), "Impact of DNA ligase IV on the ® delity of end joining in human cells.", Nucleic Acids Res., 31(8):2157-67. doi:10.1093/nar/gkg317

Tan, X., Grollman, A., Shibutani, S. (1999), Comparison of the mutagenic properties of 8-oxo-7,8-dihydro-2'-deoxyadenosine and 8-oxo-7,8-dihydro-2'-deoxyguanosine DNA lesions in mammalian cells, Carcinogenesis, 20:2287-2292.

Thomas, A.D., G.J. Jenkins, B. Kaina, O.G. Bodger, K.H. Tomaszowski, P.D. Lewis, S.H. Doak and G.E. Johnson (2013), "Influence of DNA repair on nonlinear dose-responses for mutation", *Toxicol. Sci.*, 132(1): 87-95.

van Delft, J.H. and R.A. Baan (1995), "Germ cell mutagenesis in lambda lacZ transgenic mice treated with ethylnitrosourea; comparison with specific-locus test", *Mutagenesis*, 10(3): 209-214.

Wang, C. et al. (2022), “Detecting mismatch repair deficiency in solid neoplasms: immunohistochemistry, microsatellite instability, or both?”, Mod Pathol, 35, 1515–1528. https://doi.org/10.1038/s41379-022-01109-4

Waters, C.A. et al. (2014), "The fidelity of the ligation step determines how ends are resolved during nonhomologous end joining.", Nat Commun. 5:1–11. doi:10.1038/ncomms5286.

Wessendorf P. et al. (2014), "Mutation Research / Fundamental and Molecular Mechanisms of Mutagenesis Deficiency of the DNA repair protein nibrin increases the basal but not the radiation induced mutation frequency in vivo.", Mutat. Res. - Fundam. Mol. Mech. Mutagen. 769:11–16. doi:10.1016/j.mrfmmm.2014.07.001.

Wilson, T.E. & M.R. Lieber (1999), "Efficient Processing of DNA Ends during Yeast Nonhomologous End Joining.", J. Biol. Chem. 274(33):23599–23609. doi:10.1074/jbc.274.33.23599.

Relationship: 1912: Inadequate DNA repair leads to Increase, Chromosomal aberrations

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Oxidative DNA damage leading to chromosomal aberrations and mutations	adjacent	High	Low
Deposition of energy leading to lung cancer	adjacent	High	Low
Deposition of energy leading to occurrence of cataracts	adjacent	Low	Low

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
rat	Rattus norvegicus	Low	NCBI
mouse	Mus musculus	Low	NCBI
human	Homo sapiens	Low	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	Low

Sex Applicability

Sex	Evidence
Unspecific	Low

This KER is plausible in all life stages, sexes, and organisms with chromosomes. The majority of the evidence is from in vitro fetal human male models. No in vivo evidence was found to support the relationship.

Key Event Relationship Description

Cells are exposed to many insults, both endogenous and exogenous, that may cause damage to their DNA. In response to this constant threat, cells have accordingly evolved many different pathways for repairing DNA damage (Pfeiffer & Goedecke, 2000; Hoeijmakers, 2001; Jeggo & Markus, 2015; Rode et al., 2016). When confronted with double strand breaks (DSBs), there are two common repair pathways employed by the cell: homologous recombination (HR) and non-homologous end-joining (NHEJ). In HR, a homologous sequence on the sister chromatid is used as a template, ensuring that no sequence information is lost over the course of repair (Ferguson & Alt, 2001; van Gent et al., 2001; Hoeijmakers, 2001; Jeggo & Markus, 2015; Schipler & Iliakis, 2013; Venkitaraman, 2002). However, this method of DNA repair may result in a loss of an allele leading to heterozygosity. This may occur if a non-homologous chromosome with an erronous sequence is used as the template instead of the homologous chromosome, thus leading to a loss of genetic information (Ferguson & Alt, 2001). Despite this possible error, HR is generally considered to be one of the more accurate methods of DNA repair because it does make use of a template (van Gent et al., 2001; Schipler & Iliakis, 2013; Venkitaraman, 2002) . NHEJ, however, does not use a template and is generally described as being error-prone. This repair process allows for the direct religation of broken DNA ends without using template DNA as a guide (van Gent et al., 2001; Ferguson & Alt, 2001; Hoeijmakers, 2001; Venkitaraman, 2002; Schipler & Iliakis, 2013; Jeggo & Markus, 2015; Rode et al., 2016). In lieu of a template, NHEJ utilizes rapid repair kinetics to relegate the broken ends before they have time to diffuse away from each other (Schipler & Iliakis, 2013), thus fitting two ‘sticky’ DNA ends back together (Danford, 2012). There is not, however, an inherent quality control check; as such, sections of DNA may be gained or lost, or the wrong ends may be rejoined (Schipler & Iliakis, 2013). There are two versions of this error-prone DNA repair: classical or canonical NHEJ (c-NHEJ), and alternative NHEJ (alt-NHEJ) (Schipler & Iliakis, 2013). It is not well understood when or why one pathway is selected over another (Venkitaraman, 2002; Schipler & Iliakis, 2013). It has been proposed that the phase of the cell cycle may influence repair pathway choice (Ferguson & Alt, 2001; Vodicka et al., 2018); for instance, HR is generally more common than NHEJ when sister chromatids are available in S and G2 phases of the cell cycle (Hoeijmakers, 2001; Venkitaraman, 2002). If both HR and c-NHEJ are compromised, alt-NHEJ, which is slower and more error-prone than c-NHEJ, is thought to be the stand-by repair mechanism (Schipler & Iliakis, 2013). As BRCA2 is involved in both the NHEJ and HR repair pathways, it has recently been observed in BRCA2 deficient cells that single-strand annealing (SSA) may be triggered (Han et al. 2017).

If these repair processes are not able to properly and adequately repair the DNA, this may lead to the formation of chromosomal aberrations (CAs). CAs are defined as abnormalities in the chromosome structure, often due to losses or gains of chromosome sections or the entire chromosomes itself (van Gent et al., 2001; Durante & Cucinotta, 2008). These abnormalities can take many different forms and can be classified according to several different schemes. CAs can be defined as breaks, which occur when DSBs are not rejoined, or as exchanges, where the presence of multiple DSBs results in misrejoining of the DNA ends (Danford, 2012; Registre et al., 2016). CA classes can be further subdivided into chromosome-type aberrations (CSAs) that affect both sister chromatids, and chromatid-type aberrations (CTAs), affecting only one chromatid (Danford, 2012) . Examples of CSAs include chromosome-type breaks, centric ring chromosomes, and dicentric chromosomes (which have two centromeres), while CTAs refer to chromatid-type breaks and chromatid exchanges (Hagmar et al., 2004; Bonassi et al., 2008). Other types of CAs that may occur include micronuclei (MN; small nucleus-like structures containing chromosome fragments enclosed by a nuclear membrane (Fenech & Natarajan, 2011; Doherty et al., 2016)), nucleoplasmic bridges (NPBs; a stretch of chromatin enclosed by a nuclear membrane that is attached to two centromeres (Fenech & Natarajan, 2011; Russo et al., 2015)), nuclear buds (NBUDs; a MN that is still connected to the nucleus by nucleoplasmic material (Fenech & Natarajan, 2011)), and copy number variants (CNVs; base pair to megabase pair deletions or duplications of chromosomal segments (Russo et al., 2015)). CAs may also be classified as stable aberrations (translocations, inversions, insertions and deletions) and unstable aberrations (dicentric chromosomes, acentric fragments, centric rings and MN) (Hunter & Muirhead, 2009; Qian et al., 2016).

Evidence Supporting this KER

Overall Weight of Evidence: Low

Biological Plausibility

There is strong biological plausibility for a relationship between inadequate repair of DNA damage and a corresponding increase in CAs. This is evident in a variety of reviews on the topic (van Gent et al., 2001; Hoeijmakers, 2001; Povirk, 2006; Weinstock et al., 2006; Lieber et al., 2010; Rode et al., 2016).

The two most common methods used to repair DSBs, which are one of the most dangerous types of DNA lesions, are HR and NHEJ. Mechanisms for these two methods of DNA repair are well-established and have been thoroughly reviewed (Van Gent et al. 2001; Hoeijmakers 2001; Lieber et al. 2010; Jeggo and Markus 2015; Sishc and Davis 2017). Briefly, HR requires a template DNA strand to repair damage and thus facilitates the invasion of the damaged strand with matching sequences on homologous chromosomes or sister chromatids (Ferguson and Alt 2001; van Gent et al. 2001; Hoeijmakers 2001; Jeggo and Markus 2015; Schipler and Iliakis 2013; Venkitaraman 2002). Proteins involved in the HR pathway include the RAD50 proteins, MRE11, BRCA1, and BRCA2 (Ferguson and Alt 2001; van Gent et al. 2001; Hoeijmakers 2001; Jeggo and Markus 2015; Venkitaraman 2002). In contrast to this relatively accurate form of DNA repair (van Gent et al. 2001; Schipler and Iliakis 2013; Venkitaraman 2002), NHEJ is more error-prone. It does not require a template to guide repair, but simply re-ligates broken DNA ends back together (Van Gent et al. 2001; Ferguson and Alt 2001; Hoeijmakers 2001; Lieber et al. 2010; Schipler and Iliakis 2013; Jeggo and Markus 2015; Rode et al. 2016; Sishc and Davis 2017) Proteins used during NHEJ include the DNA-PK complex (encompassing Ku70, Ku80 and DNA-PK_{CS}), and the XRCC4-DNA ligase IV complex (Ferguson & Alt, 2001; van Gent et al., 2001; Hoeijmakers, 2001; Jeggo & Markus, 2015; Sishc & Davis, 2017).Interestingly, NHEJ is used in the biological V(D)J recombination process because its error-prone mechanism allows immune cells to develop a wide range of unique receptors for

antigen detection (Ferguson & Alt, 2001; van Gent et al., 2001; Lieber, 2010).

Damaged DNA in the form of DSBs can follow three possible outcomes: the DSB is rejoined accurately, with no changes made to the genome; the DSB is left unrepaired and the broken ends diffuse away from each other; or the DSB is repaired incorrectly such that the repaired version is different from the original version (Danford, 2012). These latter two errors in repair (the complete absence of repair or inaccurate repair) could arise due to interruptions to the repair process that allow time for the broken ends to move away from each other before they can be rejoined, mis-rejoining of the wrong DNA ends, or post-repair alterations that modify the junction point and lead to nucleotide losses (Schipler and Iliakis 2013). Unrepaired DSBs are the direct origin of micronuclei and unrepaired chromosomes correlated with radiosensitivity (Foray et al., 2016). Errors occurring during repair may be particularly detrimental if they interrupt or modify key genes, or if chromosome structures are created that cannot undergo proper mitosis (Schipler and Iliakis 2013).

The classic model of CA formation has centered around misrepair of DSBs. Exposing DNA to an endogenous or exogenous DSB-inducing agent directly results in DSBs, which may either persist or be misrepaired by inadequate repair mechanisms; in the event of this erroneous repair, CAs often eventually result (Bignold, 2009; Danford, 2012; Schipler & Iliakis, 2013) . Another model has been proposed that suggests CAs may actually be due to failure of enzymes that tether the DNA strands during the repair of enzyme-induced breaks in the DNA; the various pathways in the cell would likely employ assorted tethering enzymes. The numerous types of CAs would thus result from different kinds of tethering errors (Bignold 2009).

The type of CA that results may be dependent on the timing of inadequate repair. For example, DSBs may result in CSAs or CTAs depending on when during the cell cycle the DSB was incurred. DSBs that are not repaired before DNA duplication in the S-phase will be replicated and result in CTAs. If DSBs are incurred after the S-phase and are improperly repaired, CSAs will result (Danford, 2012; Registre et al., 2016; Vodicka et al., 2018). Similarly, CNVs are thought to be induced during the DNA replication phase. DNA replication stops can also be problematic for repair. Although the mechanism is not well studied, it has been suggested that stress during replication, in particular stalling replication forks, prompt microhomology-mediated mechanisms to overcome the replication stall, which often results in duplications or deletions. Two models that have been proposed to explain this mechanism include the Fork Stalling and Template Switching (FoSTeS) model, and the Microhomology-Mediated Break-Induced Replication (MMBIR) model (Lee et al. 2007; Hastings et al. 2009; Arlt et al. 2012; Arlt et al. 2014; Wilson et al. 2015).

The type of CA may also be dependent on the type of erroneous repair that occurs. Deletions or chromosome breaks may occur when DSBs are left unrepaired (Danford 2012). Deletions may also occur when nucleotides are removed at the junctions (Schipler and Iliakis 2013) or when the wrong DNA ends are religated (Venkitaraman 2002). Ligation of the incorrect ends of DNA DSBs may also lead to translocations or dicentrics (Ferguson & Alt, 2001; Lieber, 2010; Povirk, 2006; Venkitaraman, 2002). This type of error may occur when there are two or more DSBs in close proximity to each other that are misrejoined, thus resulting in the exchange of genetic material between two chromosomes (Ferguson and Alt 2001; Povirk 2006). NHEJ has been shown to play a significant role in the generation of chromosomal exchanges (Lieber 2010; Povirk 2006; Weinstock et al. 2006). Evidence for this comes from analysis of breakpoint junctions, which typically have little to no chromosomal homology when NHEJ repair is used (Povirk 2006; Weinstock et al. 2006); this was demonstrated in studies using translocation reporters (reviewed in Weinstock et al., 2006). There are, however, two types of NHEJ. c-NHEJ has been shown to suppress exchanges (Simsek and Jasin 2010) , which may be due to its relatively rapid repair kinetics (Schipler and Iliakis 2013). Chromosomal exchanges are thus suggested to originate more often from alt-NHEJ (Simsek and Jasin 2010; Zhang and Jasin 2011; Schipler and Iliakis 2013) .

NHEJ is also thought to mediate the formation of other types of CAs. Based on analysis of breakpoint junctions in lung adenocarcinoma samples where reciprocal inversions were found between genes *RET* and *KIF5B/CCDC6*, the majority of the inversions were thought to be induced by NHEJ (Mizukami et al. 2014). Chromothripsis, which refers to a single event that results in a massive number of CAs localized to a single or very few chromosomes (Russo et al. 2015; Leibowitz et al. 2015; Rode et al. 2016), may also be linked to NHEJ. The single catastrophic event sparking chromothripsis likely induces a large quantity of DSBs, essentially shattering the chromosome(s). These DSBs are then processed mainly by the error-prone NHEJ, which results in a large number of CAs, including chromosomal rearrangements, CNVs, and loss of heterozygosity (Leibowitz et al. 2015; Rode et al. 2016).

Fusing two broken chromosomes may lead to the formation of dicentric chromosomes, which are characterized by the presence of two centromeres. Dicentrics may also be formed by telomere-to-telomere end fusions (Fenech and Natarajan 2011; Rode et al. 2016). Telomeres, composed of TTAGGG repeats, are important structures that protect the ends of chromosomes and ensure accurate replication (Ferguson and Alt 2001; Hoeijmakers 2001; Vodicka et al. 2018); these nucleoprotein structures are shortened (Vodicka et al. 2018) by approximately 100 base pairs after each division, and are only replenished in cell types expressing the enzyme telomerase (Hoeijmakers 2001). If the

telomeres become critically short, they can be mistaken for broken DNA ends by DNA repair machinery, and thus may be ‘repaired’ by fusing the ends of two chromosomes together (Ferguson and Alt 2001; Vodicka et al. 2018).

Dicentrics can also contribute to other types of CAs. During mitosis, the two centromeres of a dicentric chromosome may be pulled to opposite ends of the cell by mitotic spindle (Ferguson and Alt 2001; Fenech and Natarajan 2011; Leibowitz et al. 2015; Rode et al. 2016). Because the ends of the chromosomes are fused, this can lead to the formation of an anaphase chromatin bridge between the daughter cells (Russo et al. 2015; Leibowitz et al. 2015; Rode et al. 2016). If this bridge persists beyond anaphase, it may become enclosed in a nucleoplasmic membrane along with the nucleus, thus generating a NPB (Fenech and Natarajan 2011). Eventually, however, these bridges do break (Ferguson and Alt 2001; Fenech and Natarajan 2011; Russo et al. 2015; Leibowitz et al. 2015; Rode et al. 2016); the break is nearly always uneven, meaning that one daughter cell will be missing genetic material and one will have extra genetic material (Fenech and Natarajan 2011). These fragments, with their ‘sticky’ ends from the break, may further propagate the formation of CAs by being ligated inappropriately to another chromosome. Thus the cycle, known as the breakage-fusion-bridge (BFB) cycle, is propagated and further contributes to chromosomal instability (Ferguson and Alt 2001; Fenech and Natarajan 2011; Russo et al. 2015; Leibowitz et al. 2015; Rode et al. 2016) .

MN may also be formed during this BFB cycle. When the anaphase bridges break, the remaining chromosome fragments may be packaged by a nuclear membrane into its own mini nucleus, thus, forming an MN. MN may also enclose acentric chromosome fragments, chromatid fragments, or even entire chromosomes that were not properly segregated during mitosis (Fenech and Natarajan 2011; Doherty et al. 2016). Similar to MN in structure are NBUDs; the only difference between these two structures is that NBUDs are still attached to the nucleus by nucleoplasmic material. A NBUD is formed if there is amplified DNA that needs to be removed; this amplified material is often segregated from the other DNA at the periphery of the nuclear membrane and excluded from the nucleus by budding, resulting in a NBUD. Additionally, NBUDs may also result from NPB breakages (Fenech and Natarajan 2011).

Empirical Evidence

There is moderate empirical evidence supporting the relationship between inadequate DNA repair and the frequency of CAs. The evidence presented below is summarized in table 6,here (click link). Several reviews discuss evidence that associates these two events (Ferguson and Alt 2001; van Gent et al. 2001; Sishc and Davis 2017; Venkitaraman 2002). Overall, however, there is weak empirical evidence available supporting a dose and incidence concordance, little empirical evidence supporting a temporal concordance, and strong empirical evidence supporting essentiality for this KER.

Dose and Incidence Concordance

There is weak empirical evidence available that directly examines the dose and incidence concordance between DNA repair and CAs within the same study. There are, however, studies that use an ionizing radiation stressor to examine dose concordance of either inadequate DNA repair in response to radiation exposure, or CA frequencies in response to irradiation. In an analysis that amalgamated results from several different studies conducted using *in vitro* experiments, the rate of DSB misrepair was revealed to increase in a dose-dependent fashion from 0 - 80 Gy (Mcmahon et al. 2016). Similarly, there was a clear correlation between radiation dose (i.e., increasing amounts of energy deposition) between 0 - 10 Gy and different clastogenic endpoints (Thomas et al. 2003; Tucker et al. 2005A; George et al. 2009; Arlt et al. 2014; Balajee et al. 2014; Lin et al. 2014; Suto et al. 2015; Mcmahon et al. 2016) . Overall, this suggests that exposure to radiation may increase both inadequate repair of DNA damage and the frequency of CAs in a dose-dependent fashion. More studies, however, are required to better assess the dose and incidence concordance of this KER.

Temporal Concordance

Temporal concordance between inadequate DNA repair and CA frequency is not well established. One study using cells pretreated with a DNA-PK inhibitor and irradiated with gamma rays found that DNA repair and MN were evident when they were assessed at 3 hours post-irradiation and 24 hours post-irradiation, respectively (Chernikova et al. 1999). This study does therefore suggest that there may be temporal concordance between these two events. Other radiation-based studies examining these two events separately, however, do not provide clear evidence of temporal concordance between DNA repair and CA frequency.

Essentiality

Numerous studies demonstrate that simply knocking-out one gene involved in DNA repair, without any other added stressor, is enough to increase the frequency of CAs in several types of cells (Karanjawala et al. 1999; Patel et al. 1998; Wilhelm et al. 2014). Further strengthening this relationship, addition of a DSB-inducing stressor to these DNA repair knock-out cells also significantly increases CA levels relative to wild-type cells receiving the same treatment (Cornforth and Bedford 1985; Simsek and Jasin 2010; Lin et al. 2014; Mcmahon et al. 2016). Essentiality is also

supported by looking at patients with the recessive genetic disorder ataxia-telangiectasia (AT), in which mutations in the gene encoding the ATM protein results in defects in DNA damage repair signaling. One recent study showed that in comparison to control patients, patients with AT had increased levels of several types of CAs. Upon exposure to a DSB-inducing stressor such as ionizing radiation, these patients showed further increases in these aberrations as well as a significant increase in the levels of complex aberrations as compared to controls (Bucher et al. 2021).

Inhibitor studies have also found similar results. Two strains of wild-type cells that were treated with hydroxyurea, which is known to inhibit DNA repair, both had increased CAs relative to untreated wild-type cells (Wilhelm et al. 2014). Similarly, immortalized myeloid cell lines, cells from patients with myeloid leukemia, and cells from healthy donors were all found to have dose-dependent decreases in ligation efficiency after being treated with increasing doses of antibodies against various NHEJ proteins (Heterodimer et al. 2002). In addition, cells that were pretreated with DNA-PK inhibitor wortmannin prior to being irradiated were found to have not only increased levels of MN, but also decreased rates of DNA rejoining (Chernikova et al. 1999). A study by White et al. (2010) reported similar results under ATM and DNA-PK inhibition, where IR-exposed human lung cells treated for 1 hour with a reversible inhibitor of either enzyme exhibited an elevated level of CAs at all tested doses of IR, compared to the non-inhibited, IR-exposed cells 48 hours post-exposure. These findings demonstrated that even a transient inhibition of ATM or DNA-PK can sufficiently disrupt DNA damage repair and lead to CAs (White et al., 2010).

Functional defects in the factors involved in NER due to mutations or knock-down/out have shown concordant results that are supportive of this KER. For example, UV61 Chinese hamster ovary cells (homogolous to human Cockayne syndrome group B cells), which have a defective ERCC6 gene, are incapable of repairing UV-induced cyclobutane pyrimidine dimers due to the compromised transcription-coupled NER (TCR). Following UV exposure, a significantly higher percentage of TCR-defective UV61 cells contained CAs than another Chinese hamster ovary cell line that is TCR-proficient (Proiettis de Santis et al., 2001). Down-regulation of xeroderma pigmentosum group A–complementing protein (XPA) by RNA interference (RNAi) in human bladder cancer cells was observed to significantly increase the baseline frequency of MN, nucleoplasmic bridges, and nuclear buds, while overexpression of XPA by transfection in the same cell line reduced these levels below that in the control cells (Zhi et al., 2017). Both studies support the essentiality of inadequate repair in the occurence of chromosomal aberrations.

A rescue experiment provided further evidence of the essential role DNA repair plays in relation to CA frequencies. Inhibition of NHEJ through knocking out either Ku70 or Xrcc4 resulted in higher CA frequencies in the form of translocations; when Xrcc4 was transiently expressed in Xrcc4-/- cells, translocations were significantly decreased by 5-fold(Simsek and Jasin 2010) . This provides strong evidence that the NHEJ repair pathway plays an important role in the formation of CAs, specifically translocations.

Uncertainties and Inconsistencies

Uncertainties in this KER are as follows:

1. In an experiment using both wild-type and *Ku70*-/- cells, knock-down of alt-NHEJ protein CtIP resulted in significantly decreased translocations in both cell types. When CtIP expression was rescued, translocation frequencies in these cells also returned to normal levels. This however, is opposite to results obtained in a similar study, where knock-out of Ku70 or Xrcc4 led to increased translocation frequency, and Xrcc4 rescue experiments resulted in decreased translocations (Simsek and Jasin 2010). It should be noted that alt-NHEJ is thought to be the major repair pathway responsible for generating translocations (Simsek and Jasin 2010; Zhang and Jasin 2011; Schipler and Iliakis 2013).
2. There is currently discussion regarding the accuracy of HR relative to NHEJ. Traditionally HR has been considered the more accurate type of DNA repair, while NHEJ is classically described as error-prone. There is emerging evidence, however, suggesting that HR may in fact be a mutagenic process. Evidence supporting HR as an error-prone repair pathway has been reviewed (Guirouilh-barbat et al. 2014).

Quantitative Understanding of the Linkage

Quantitative understanding of this linkage is lacking. Most data are derived from studies that examined DSB misrepair rates or CA rates in response to a radiation stressor. In terms of inadequate DNA repair, the rate of DSB misrepair was found to be approximately 10 - 15% at 10 Gy of radiation (Lobrich et al. 2000); this rate increased to 50 - 60% at a radiation exposure of 80 Gy (Kuhne et al. 2000; Lobrich et al. 2000; Mcmahon et al. 2016). It is not known, however, how this rate of inadequate repair directly relates to CA frequency. Overall, more studies are required that directly assess this relationship.

Response-response relationship

Studies directly examining the response-response relationship between inadequate repair and CA frequency are lacking. One study examined both DNA repair and CA frequency in cells exposed to DNA-PK inhibitor wortmannin. There was a negative, approximately linear relationship between DNA repair and increasing wortmannin dose, and a positive, approximately linear relationship between MN frequency and increasing wortmannin dose; this suggests that as adequate DNA repair declines, CA frequency increases (Chernikova et al. 1999). More studies are required, however, that directly quantify the response-response relationship between inadequate DNA repair and CAs.

Time-scale

The time scale between inadequate DNA repair and the increased frequency of CAs has not been well-established. Most data come from studies that assess only one of these events in relation to a radiation stressor rather than assessing the timing of the events relative to each other. More studies are thus required that directly assess this relationship.

Known modulating factors

DNA repair is a modulating factor in this KER. The progression from “Inadequate DNA repair” to “Increase, Chromosomal aberrations” only occurs when "Increase, DNA strand breaks" (KE 1635) precedes "Inadequate DNA repair", which indicates that DNA strand breaks could not be repaired.

Known Feedforward/Feedback loops influencing this KER

Not identified.

References

Arlt, M.F. et al. (2014), "NIH Public Access", 55(2):103–113. doi:10.1002/em.21840.

Arlt, M.F., T.E. Wilson & T.W. Glover (2012), "Replication Stress and Mechanisms of CNV Formation.", Curr. Opin. Genet. Dev. 22(3):204–210. doi:10.1016/j.gde.2012.01.009.

Balajee, A.S. (2014), "Multicolour FISH analysis of ionising radiation induced micronucleus formation in human lymphocytes.", Mutagenesis, 29(6):447–455. doi:10.1093/mutage/geu041.

Bignold, L.P. (2009), "Mechanisms of clastogen-induced chromosomal aberrations : A critical review and description of a model based on failures of tethering of DNA strand ends to strand-breaking enzymes.", Mutat. Res. 681:271–298. doi:10.1016/j.mrrev.2008.11.004.

Bonassi, S. (2008), "Chromosomal aberration frequency in lymphocytes predicts the risk of cancer: results from a pooled cohort study of 22 358 subjects in 11 countries.", Carninogenesis, 29(6):1178–1183. doi:10.1093/carcin/bgn075.

Bucher, M. et al., (2021), Analysis of chromosomal aberrations and γH2A.X foci to identify radiation-sensitive ataxia-telangiectasia patients., Mutat Res., Jan-Feb;861-862

Chernikova, S.B., R.L. Wells & M.M. Elkind (1999), "Wortmannin Sensitizes Mammalian Cells to Radiation by Inhibiting the DNA-Dependent Protein Kinase-Mediated Rejoining of Double-Strand Breaks.", Radit. Res. 151(2):159–166. doi: 10.2307/3579766.

Cornforth, M. & J. Bedford (1985), "On the Nature of a Defect in Cells from Individuals with Ataxia-Telangiectasia.", Science 227(4694):1589–1591. doi:10.1126/science.3975628.

Danford, N. (2012), "The Interpretation and Analysis of Cytogenetic Data.", Methods Mol. Biol. 817:93-120, doi:10.1007/978-1-61779-421-6.

Doherty, A., S.M. Bryce & J.C. Bemis (2016), "The In Vitro Micronucleus Assay.", Elsevier Inc.

Durante, M. and F. Cucinotta. (2008), “Heavy ion carcinogenesis and human space exploration”, Nature Reviews Cancer, Vol.8/6, Nature Portfolio, Berlin, https://doi.org/10.1038/nrc2391.

Fenech, M. & A.T. Natarajan (2011), "Molecular mechanisms of micronucleus, nucleoplasmic bridge and nuclear bud formation in mammalian and human cells. 26(1):125–132. doi:10.1093/mutage/geq052.

Ferguson, D.O. & F.W. Alt (2001), "DNA double strand break repair and chromosomal translocation: Lessons from animal models.", Oncogene, 20(40):5572–5579. doi: 10.1038/sj.onc.1204767.

Foray, N., M. Bourguignon and N. Hamada. (2016), “Individual response to ionizing radiation”, Mutation Research - Reviews in Mutation Research, Vol.770, Elsevier, Amsterdam, https://doi.org/10.1016/j.mrrev.2016.09.001.

van Gent D.C., J.H.J. Hoeijmakers & R. Kanaar (2001), "Chromosomal stability and the DNA double-stranded break connection.", Nat. Rev. Genet. 2(3):196–206. doi:10.1038/35056049.

George, K.A. et al. (2009), "Dose Response of γ Rays and Iron Nuclei for Induction of Chromosomal Aberrations in Normal and Repair-Deficient Cell Lines Dose Response of c Rays and Iron Nuclei for Induction of Chromosomal Aberrations in Normal and Repair-Deficient Cell Lines." Radit. Res., 171(6):752–763. doi:10.1667/RR1680.1.

Guirouilh-barbat, J. et al. (2014), "Is homologous recombination really an error-free process?", Front Genet. 5:175. doi:10.3389/fgene.2014.00175.

Hagmar, L. et al. (2004), "Impact of Types of Lymphocyte Chromosomal Aberrations on Human Cancer Risk: Results from Nordic and Italian Cohorts.", Cancer Res. 64(6):2258–2263. doi: 10.1158/0008-5472.CAN-03-3360.

Han, J., Ruan, C., Huen, M.S.Y. et al. (2017) “BRCA2 antagonizes classical and alternative nonhomologous end-joining to prevent gross genomic instability”. Nat Commun 8, 1470 https://doi.org/10.1038/s41467-017-01759-y.

Hastings, P.J., G. Ira & J.R. Lupski (2009), "A Microhomology-Mediated Break-Induced Replication Model for the Origin of Human Copy Number Variation.", 5(1). doi:10.1371/journal.pgen.1000327.

Heterodimer, K. et al. (2002), "Myeloid Leukemias Have Increased Activity of the Nonhomologous End-Joining Pathway and Concomitant DNA Misrepair that Is Dependent on the Ku70/86 Heterodimer.", Cancer Res. 62(10):2791-7.

Hunter, N. & C.R. Muirhead (2009), "Review of relative biological effectiveness dependence on linear energy transfer for low-LET radiations Review of relative biological effectiveness dependence.", J. Radiol. Prot. doi:10.1088/0952-4746/29/1/R01.

Jeggo, P.A. & L. Markus (2015), "How cancer cells hijack DNA double-strand break repair pathways to gain genomic instability.", Biochem. J., 471(1):1–11. doi:10.1042/BJ20150582.

Karanjawala, Z.E. et al. (1999), "The nonhomologous DNA end joining pathway is important for chromosome stability in primary fibroblasts.", Curr. Biol. 9(24):1501-4. doi: 10.1016/S0960-9822(00)80123-2.

Kozbenko, T. et al. (2022), “Deploying elements of scoping review methods for adverse outcome pathway development: a space travel case example”, International Journal of Radiation Biology, 1–12. https://doi.org/10.1080/09553002.2022.2110306

Kuhne, M., K. Rothkamm & M. Lobrich (2000), "No dose-dependence of DNA double-strand break misrejoining following a -particle irradiation.", Int. J. Radiat. Biol. 76(7):891-900

Lee, J.A., C.M.B. Carvalho & J.R. Lupski (2007), "A DNA Replication Mechanism for Generating Nonrecurrent Rearrangements Associated with Genomic Disorders.", Cell. 131(7):1235–1247. doi:10.1016/j.cell.2007.11.037.

Leibowitz, M.L., C. Zhang & D. Pellman (2015), "Chromothripsis: A New Mechanism for Rapid Karyotype Evolution.", Annu. Rev. Genet. 49:183-211, doi:10.1146/annurev-genet-120213-092228.

Lieber, M.R. et al. (2010), "Nonhomologous DNA End Joining (NHEJ) and Chromosomal Translocations in Humans.", Subcell. Biochem., 50:279-96 doi:10.1007/978-90-481-3471-7.

Lin, Y. et al. (2014), "Differential Radiosensitivity Phenotypes of DNA-PKcs Mutations Affecting NHEJ and HRR Systems following Irradiation with Gamma-Rays or Very Low Fluences of Alpha Particles.", PLoS One. 9(4):2–11. doi:10.1371/journal.pone.0093579.

Lobrich, M. et al. (2000), "Joining of Correct and Incorrect DNA Double-Strand Break Ends in Normal Human and Ataxia Telangiectasia Fibroblasts.", 68(July 1999):59–68. doi:DOI: 10.1002/(SICI)1098-2264(200001)27:1<59::AID-GCC8>3.0.CO;2-9.

Manova, V. & D. Gruszka (2015), "DNA damage and repair in plants - from models to crops.", Front Plant Sci. 6(October):885. doi:10.3389/fpls.2015.00885.

McMahon, S.J. et al. (2016), "Mechanistic Modelling of DNA Repair and Cellular Survival Following Radiation-Induced DNA Damage.", Nat. Publ. Gr.(April):1–14. doi:10.1038/srep33290.

Mizukami, T. et al. (2014), "Molecular Mechanisms Underlying Oncogenic RET Fusion in lung adenocarcinoma", J. Thorac. Oncol. 9(5):622–630. doi:10.1097/JTO.0000000000000135.

Patel, K.J. et al. (1998), "Involvement of Brca2 in DNA Repair.", Mol. Cell. 1(3):347-57. doi: 10.1016/S1097-2765(00)80035-0.

Pfeiffer, P. & W. Goedecke (2000), "Mechanisms of DNA double-strand break repair and their potential to induce chromosomal aberrations.", Mutagenesis 15(4):289-302. doi: http://dx.doi.org/10.1093/mutage/15.4.289.

Povirk, L.F. (2006), "Biochemical mechanisms of chromosomal translocations resulting from DNA double-strand breaks.", DNA Repair (Amst.) 5(9-10):1199–1212. doi:10.1016/j.dnarep.2006.05.016.

Proietti De Santis, L., C. L. Garcia, A. S. Balajee, G. T. Brea Calvo, L. Bassi, & F. Palitti (2001), "Transcription coupled repair deficiency results in increased chromosomal aberrations and apoptotic death in the UV61 cell line, the Chinese hamster homologue of Cockayne's syndrome B", Mutat Res, 485(2): 121–132.

Qian, Q. et al. (2016), "Effects of Ionising Radiation on Micronucleus Formation and Chromosomal Aberrations in Chinese.", Radiat. Prot. Dosimetry 168(2):197–203. doi: 10.1093/rpd/ncv290

Registre, M., R. Proudlock & N. Carolina (2016), "The In Vitro Chromosome Aberration Test.", Elsevier Inc. Genetic Toxicology Testing, pp.207-267. doi: 10.1016/B978-0-12-800764-8.00007-0.

Rode, A. et al. (2016), "Chromothripsis in cancer cells: An update.", Int. J. Cancer, 2333:2322–2333. doi:10.1002/ijc.29888.

Russo, A. et al. (2015), "Review Article Genomic Instability: Crossing Pathways at the Origin of Structural and Numerical Chromosome Changes.", Envrion. Mol. Mutagen. 56(7):563-580. doi:10.1002/em.

Schipler, A. & G. Iliakis (2013), "DNA double-strand – break complexity levels and their possible contributions to the probability for error-prone processing and repair pathway choice.", Nucleic Acids Res., 41(16):7589–7605. doi:10.1093/nar/gkt556.

Simsek, D. & M. Jasin (2010), "Alternative end-joining is suppressed by the canonical NHEJ component Xrcc4/ligase IV

during chromosomal translocation formation", Nat. Struct. Mol. Bio. 17(4):410–416. doi:10.1038/nsmb.1773.

Sishc, B.J. & A.J. Davis (2017), "The Role of the Core Non-Homologous End Joining Factors in Carcinogenesis and Cancer.", Cancers (Basel), 9(7) pii E81, doi:10.3390/cancers9070081.

Suto, Y. et al. (2015), "Construction of a cytogenetic dose – response curve for low-dose range gamma-irradiation in human peripheral blood lymphocytes using three-color FISH", Mut. Res. / Gen. Tox. and Environ. Mut. 794:32–38.

Thomas, P., K. Umegaki & M. Fenech (2003), "Nucleoplasmic bridges are a sensitive measure of chromosome rearrangement in the cytokinesis-block micronucleus assay.", Mutagenesis, 18(2):187-194, doi:10.1093/mutage/18.2.187.

Tucker, J.D. et al. (2005), "Persistence of Chromosome Aberrations Following Acute Radiation: I, PAINT Translocations, Dicentrics, Rings, Fragments, and Insertions.", Environ. Mol. Mutagen, 45(2-3):229-249. doi:10.1002/em.20090.

Varga, T. & P.D. Aplan (2005), "Chromosomal aberrations induced by double strand DNA breaks.", DNA Repair (Amst). 4(9):1038–1046. doi:10.1016/j.dnarep.2005.05.004.

Venkitaraman, A.R. (2002). "Cancer susceptibility and the Functions of BRCA1 and BRCA2.", Cell 108(2):171–182.

Vodicka, P. et al. (2018), "Genetic variation of acquired structural chromosomal aberrations.", Mutat. Res. Gen. Tox. En. 836(May):13–21. doi:10.1016/j.mrgentox.2018.05.014.

Weinstock, D.M. et al. (2006), "Modeling oncogenic translocations: Distinct roles for double-strand break repair pathways in translocation formation in mammalian cells.", DNA Repair (Amst.) 5(9-10):1065–1074. doi:10.1016/j.dnarep.2006.05.028.

White, J. S., S. Choi, & C. J. Bakkenist (2010), "Transient ATM kinase inhibition disrupts DNA damage-induced sister chromatid exchange", Sci Signal, 3(124): ra44. https://doi.org/10.1126/scisignal.2000758.

Wilhelm, T. et al. (2014), "Spontaneous slow replication fork progression elicits mitosis alterations in homologous recombination-deficient mammalian cells.", Proc. Natl. Acad. Sci. 111(2):763-768. doi:10.1073/pnas.1311520111.

Wilson, J.W. et al. (2015), "The effects of extremely low frequency magnetic fields on mutation induction in mice.", Mutat Res - Fundam Mol Mech Mutagen. 773:22–26. doi:10.1016/j.mrfmmm.2015.01.014.

Zhang, Y. & M. Jasin (2011), "An essential role for CtIP in chromosomal translocation formation through an alternative end-joining pathway.", Nat Publ Gr. 18(1):80–84. doi:10.1038/nsmb.1940.

Zhi, Y., H. Ji, J. Pan, P. He, X. Zhou, H. Zhang, Z. Zhou, & Z. Chen (2017), "Downregulated XPA promotes carcinogenesis of bladder cancer via impairment of DNA repair", Tumour Biol, 39(2): 1010428317691679.

Relationship: 1978: Increase, Mutations leads to Increase, Cell Proliferation

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Deposition of energy leading to lung cancer	adjacent	High	Low
Deposition of energy leading to occurrence of cataracts	adjacent	Moderate	Low

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	High	NCBI
rat	Rattus norvegicus	High	NCBI
mouse	Mus musculus	High	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence

Sex	Evidence
Unspecific	High

The domain of applicability pertains to all multicellular organisms, as cell proliferation and death regulate tissue homeostasis (Pucci et al. 2000).

Key Event Relationship Description

Mutations are defined as changes in the DNA sequence, which could occur in the form of deletions, insertions, missense mutations, nonsense mutations or frameshift mutations (Bertram, 2001; Danesi et al., 2003; Lodish, 2000). Elevated mutation frequencies may impact cellular activities by activating or inhibiting essential processes that control the natural course of cell proliferation (Bertram, 2001; Vogelstein and Kinzler, 2004; Lodish, 2000). Increased rates of cellular proliferation may arise due to mutations that activate proto-oncogenes, which results in sustained signaling for cell growth (Bertram, 2001; Vogelstein and Kinzler, 2004; Larsen and Minna, 2011; Lodish, 2000) and due to mutations that inactivate tumour suppressor genes (TSGs), resulting in the removal of cell cycle inhibition and/or decreased cell death signaling (Bertram, 2001; Vogelstein and Kinzler, 2004; Lodish, 2000). Mutations altering gene expression or protein activity can enable cells to escape growth inhibition by increasing resistance to apoptosis, or other inhibitory signals, or by escape of cell cycle checkpoints. Alternatively, mutations can stimulate growth by activating proliferative pathways such as EGFR.

Evidence Supporting this KER

Biological Plausibility

There is a strong biological plausibility for a relationship between increasing mutation frequencies and increasing cellular proliferation. This relationship is especially evident when examining the molecular biology of carcinogenesis. It is well-known that exposure of cells to a DNA-damaging agent, such as ionizing radiation, may result in damage to the DNA that manifests as genomic instability, including mutations. If enough mutations accumulate in critical genes, cells may begin to proliferate uncontrollably. This, alongside other events, may eventually result in tumourigenesis and cancer (reviewed in Bertram, 2001; Vogelstein and Kinzler, 2004; Panov, 2005; Lodish, 2000). In fact, one of the hallmarks of cancer is sustained proliferative signalling, and one of the enabling characteristics of this increased proliferation is genomic instability/mutations (Hanahan and Weinberg, 2011).

To prevent the propagation of erroneous DNA, there are specific cell cycle checkpoints that must be passed before DNA replication and mitosis can proceed. One of the most important checkpoints for committing to cell proliferation occurs during late G1 (Bertram, 2001; Lodish, 2000). This checkpoint is managed by retinoblastoma protein (RB), transcription factor E2F, and transcription factor p53. In a resting cell, RB is tightly bound to E2F; when growth factor signals are present, proteins are activated that phosphorylate RB, resulting in a conformation change and the release of E2F. This transcription factor then initiates transcription of genes required for DNA synthesis and thus cell proliferation. If there is damage to the DNA, p53 is upregulated and binds to unphosphorylated RB, thereby preventing the dissociation of RB and E2F (Bertram, 2001). This gives the cell enough time to repair the damaged DNA prior to DNA replication, and thus minimizes the propagation of the DNA errors. Existing mutations in the checkpoint genes, however, may compromise this process. For example, if mutations in p53 render it non-functional, damaged DNA will not be stopped at the checkpoint and will continue to be synthesized, despite the damage. Although the majority of DNA damage is addressed through the activation of repair mechanisms, if the cells fail to prevent DNA synthesis prior to repairing DNA damage (eg. ATM mutant cells), erroneous repair accumulates which could lead to the activation of cell proliferation or cell death (Levine and Holland, 2018). Accumulation of mutations in this manner may affect genes that impact cell proliferation rates (Bertram, 2001; Lodish, 2000). There are three categories of genes that, if mutated, may allow for uncontrolled cell proliferation: proto-oncogenes, TSGs, and caretaker/stability genes.

Proto-oncogenes are defined as genes that, when activated, promote cellular proliferation (Bertram, 2001; Lodish, 2000); they have been likened to the gas pedal of the car (Vogelstein and Kinzler, 2004). These genes are particularly dangerous if they are rendered abnormally active by gain-of-function (GOF) mutations; this may result in cellular proliferation being aberrantly activated (Bertram, 2001; Vogelstein and Kinzler,, 2004; Larsen and Minna 2011; Lodish, 2000). Two common examples of mutated proto-oncogenes that contribute to increased cell proliferation rates are *EGFR* and *KRAS*. The *EGFR* gene encodes the epidermal growth factor receptor (EGFR), a trans-membrane protein with tyrosine kinase activity. Binding of growth factors to EGFRs results in receptor dimerization, autophosphorylation, and propagation of pro-proliferative signals to the nucleus (Danesi et al., 2003; Santos et al., 2010; Larsen and Minna, 2011; NIH, 2018 EGFR). *KRAS* is responsible for making the KRAS protein, which is a G-protein with GTPase activity that is used in the RAS/MAPK signalling pathway. When a signal that promotes cellular growth is detected, KRAS binds to GTP and activates downstream signalling molecules, thus facilitating signal propagation to the nucleus (Adjei, 2001; Panov, 2005; Jancik et al., 2010; NIH, 2018 KRAS). Mutations that render these receptors constitutively active would thus result in increased rates of cellular proliferation (Sanders and Albitar, 2010).

TSGs, which are analogous to the brakes in a car (Vogelstein and Kinzler, 2004; Lodish, 2000), are genes that negatively regulate cellular growth by preventing proliferation and in some cases, promoting apoptosis (Bertram, 2001; Vogelstein and Kinzler, 2004; Panov, 2005; Sanders and Albitar, 2010; Lodish, 2000). Many of the cell cycle checkpoint proteins and proteins controlling cell death are TSGs (Bertram, 2001; Lodish, 2000). Loss-of function (LOF) mutations that result in the inactivation of these TSGs may thus promote cellular proliferation (Bertram, 2001; Vogelstein and Kinzler, 2004; Lodish, 2000). A common example of a mutated TSG is *TP53*, which encodes the p53 protein. As mentioned above, p53 is a cell checkpoint protein that delays replication when damaged DNA is present; if damage is severe enough, p53 may also activate an apoptotic pathway (Bertram, 2001; Danesi et al., 2003; Panov, 2005; Larsen and Minna, 2011; Lodish, 2000, NIH 2018c). Inactivating mutations in p53 thus allow for unhindered progression through the cell cycle, resulting in higher cell proliferation rates (Danesi et al., 2003; Fernandez-Antoran et al., 2019).

Finally, caretaker/stability genes encode for proteins involved in the detection, repair and prevention of DNA damage (Vogelstein and Kinzler 2004; Hanahan and Weinberg 2011). Genes involved in mismatch repair (MMR), nucleotide excision repair (NER) and base-excision repair (BER) pathways are examples of caretaker/stability genes (Vogelstein and Kinzler, 2004). Mutations in these genes may compromise aspects of DNA repair—the detection of damage, the initiation of repair, the repair process itself, or the removal of mutagens that could possibly damage DNA—thus allowing for more mutations to accumulate in the genome than usual (Hanahan and Weinberg, 2011). Although all genes may suffer from increased mutation rates when caretaker/stability genes are improperly functioning, mutations in TSGs and proto-oncogenes are the main contributors to increased cellular proliferation (Vogelstein and Kinzler, 2004). Caretaker/stability genes are similar to TSGs in that disruption of both alleles must occur for the gene function to be compromised (Vogelstein and Kinzler, 2004; Hanahan and Weinberg, 2011).

Empirical Evidence

There is moderate empirical evidence supporting the relationship between mutations and the cellular proliferation. The evidence presented below is summarized in table 7, here (click link). There are some available reviews that provide evidence for this relationship in the context of carcinogenesis (Welcker 2008, Kim 2018, Iwakuma 2007, Muller 2011), as one of the hallmarks of this disease is high levels of cellular proliferation (Hanahan and Weinberg 2011). Another review article explores the relationship between mutation accumulation and cellular proliferation through discussion of the stem cell division theory of cancer, and how it compares to the somatic mutation theory of cancer (López-lázaro 2018). Overall, however, there is little empirical evidence available supporting dose and incidence concordance, little empirical evidence supporting temporal concordance, and strong empirical evidence supporting essentiality for this KER. Some evidence from human epidemiology association and genetic studies also provides support for this KER.

Dose and Incidence Concordance

There are few studies available that assess the dose and incidence concordance between mutations and cell proliferation. One study providing dose information on this particular relationship analyzed the effect of sequentially adding mutations to mouse lung epithelial cells. Addition of mutations in the form of LT (suppression of p53 and pRB) or Kras(G12V) (an activated oncogene) on their own to lung epithelial cells did not increase tumour volume, but a combination of these genetic manipulations resulted in increasing tumour volume (suggestive of increased cell proliferation) over 40 days. The same results for LT and EGFR(ex19del) genetic manipulations were also achieved. This suggests that addition of multiple mutations increases cell proliferation (Sato et al. 2017). More studies, however, are required to directly assess this particular aspect of the relationship between mutations and cellular proliferation.

Time Concordance

Few studies are available that study the time concordance between mutations and cell proliferation. The timing between these two events is explored in a review that discusses theories of carcinogenesis. The somatic mutation theory of cancer states that accumulation of mutations results in higher rates of cellular proliferation, which eventually leads to cancer. A component of the stem cell division theory of cancer also states that an increased mutation burden may elevate rates of stem cell divisions in late carcinogenesis; however, a high frequency of stem cell division in the initial stages of cancer development is thought to be a key factor that contributes to mutation accumulation (López-lázaro 2018). More research is thus required to definitively determine whether mutations occur prior to increased rates of cellular proliferation.

Essentiality

There is strong evidence for the essentiality component of this KER. Numerous studies indicate that cellular proliferation is increased in biological systems with genetically manipulated TSGs and/or proto-oncogenes. It is important to note that uncontrolled cellular proliferation is a hallmark of human cancers (Hanahan and Weinberg 2011); the Catalogue of Somatic Mutations in Cancer (COSMIC) includes over 136,000 coding mutations in over 500,000 tumour samples (83 major cancer genes and 49 fusion gene pairs) and this number is continually increasing (Forbes et al. 2011). The managers of COSMIC note that key amongst all of these genes is *TP53*. Several review articles that focussed on genetic manipulations of *TP53* demonstrated that mutant or knocked-out p53 increased carcinogenesis across a variety of biological systems (Iwakuma and Lozano 2007; Muller et al. 2011; Kim and Lozano 2018). Furthermore, a number of studies that measured cellular proliferation directly found that both cells and mice lacking p53 had increased rates of cell proliferation (Hundley et al. 1997; Lang et al. 2004; Ventura et al. 2007; Duan et al. 2008; Li and Xiong 2017), in addition to modifications to the cell cycle such that more cells were found in the S- and G2/M phases and less in the G1 phase (Hundley et al. 1997). Some p53 mutations, including 515A, may also result in increased cellular proliferation (Lang et al. 2004). Further underlining the importance of p53 in controlling cellular proliferation, restoration of p53 in a p53-/- mouse model resulted in a significant size reduction in 7 out of 10 tumours, with some tumours disappearing altogether (Ventura et al. 2007).

Manipulations to other genes have also been shown to affect cellular proliferation. A review article centred on the tumour suppressor FBW7, which is a ubiquitin ligase that plays a role in degrading proto-oncogene products and thus controlling cellular proliferation, demonstrated that mutations to *FBW7* may contribute to carcinogenesis (Welcker and Clurman 2008). Knock-out of prostate SPOP (an E3 ubiquitin ligase adaptor commonly mutated in primary prostate adenocarcinoma) in $Spop^{fl/fl}$*;PBCre(+)* mice resulted in prostates with significantly higher masses, significantly more cellular proliferation, and increased expression of c-MYC protein relative to prostates from $Spop^{fl/fl}$*;PBCre(-)* controls with normal prostate SPOP expression. Furthermore, there was a strong inverse correlation between c-MYC activity and SPOP mRNA levels in two independent prostate cancer patient cohorts, suggesting that c-MYC upregulation in the absence of SPOP may be responsible for the increased cellular proliferation (Geng et al. 2017). Similarly, mouse embryonic fibroblasts lacking Cul9, a scaffold protein for assembly of E3 ubiquitin ligases, had an increased cellular proliferation rate and an increased number of cells in the S-phase of the cell cycle relative to wild-type controls. Cul9 mutant cells also showed similar cellular proliferation rates to Cul9-/- cells. In contrast, Arf-/- cells, p53-/- cells, and Cul9-/-p53-/- double knock-out cells had significantly higher cellular proliferation rates relative to the Cul9-/- and Cul9 mutant cells; all of these mutant cells, however, showed increased proliferation relative to wild-type cells (Li and Xiong 2017).

Inhibitor studies further highlight the role of mutations in increasing cellular proliferation. Mouse lung epithelial cells transformed with both Large T-antigen (LT; suppresses TSGs p53 and pRB) and activated oncogene Kras(G12V) or EGFR(ex19del) resulted in increased tumour volumes, which is suggestive of cell proliferation. Increasing concentrations of MEK inhibitor, which blocks the signalling pathway downstream of both Kras and EGFR, caused declines in cell number in the two transformed cell lines and in the parental lung epithelial cells. An EGFR inhibitor, which blocks signalling downstream of EGFR but upstream of Kras, had no effect on the transformed cells with activated Kras, but caused rapid declines in cell proliferation of transformed cells with activated EGFR. Altogether, these inhibitor studies suggest that the activated oncogene has an important role in promoting high rates of cell proliferation (Sato et al. 2017).

Human epidemiology association and genetic studies

Association studies in humans clearly show the correlation between mutations in specific genes and the proliferative status of human tumours. Human lung adenocarcinoma tumours were assessed for mutational status of *KRAS, TP53* and *STK11,* and cellular proliferation levels were measured in the mutant tumours relative to the wild-type tumours. Overall, mutations in *TP53* were associated with significantly increased proliferation levels regardless of the mutational status of *KRAS.* In contrast, mutations in *STK11*, either alone or in combination with *KRAS* mutations, were not associated with increased proliferation (Schabath et al. 2016). Assessment of breast cancer tumours demonstrated that those with low BRCA1 expression displayed increased cellular proliferation relative to those with high BRCA1 expression, as measured by nuclear Ki-67 levels (Jarvis et al. 1998).

Uncertainties and Inconsistencies

Uncertainties in this KER are as follows:

1. The location of the mutation will be critical in determining the downstream effects. This can also be modulated by an individual's susceptibility (Loewe and Hill 2010).
2. Although activating mutations in oncogenes such as RAS and MYC may induce abnormally high rates of cellular proliferation, extremely high levels of these proteins may actually lead to the opposite—cells may enter into a state of senescence and cease proliferation (Hanahan and Weinberg 2011).\
3. Cellular proliferation may be impacted by circadian cycles, such that disruptions to this natural circadian rhythm may also affect the cell cycle (Shostak 2017).

Quantitative Understanding of the Linkage

Data establishing a quantitative understanding between mutation frequency and cellular proliferation was not identified. More research is required to establish the quantitative relationship between these two events.

Response-response relationship

Data establishing a response-response relationship between mutation frequency and cellular proliferation was not identified. More research is required to establish the response-response relationship between these two events.

Time-scale

Although the time scale is not well-established for this KER, there are a few studies that have examined how cellular proliferation changes overtime in the presence of mutations. In Cul9-/- mouse embryonic fibroblasts, a higher proliferation rate relative to Cul9+/+ cells was evident by 3 days in culture (Li and Xiong 2017). A similar relationship was observed in mouse embryonic fibroblasts with p53 manipulations. Increased proliferation in p53-/-, p53 515A/+ and p53 515A/515A relative to p53+/- and p53+/+ cells was present by the fourth day in culture (Lang et al. 2004). Examination of population doublings in various cell lines found that Cul9-/- and Cul9 mutant cells had higher population doublings than wild-type cells by approximately passage 7; Arf-/-, p53-/-, and Cul9-/-p53-/- cells, however, displayed even higher rates of population doublings by passage 6 (Li and Xiong 2017). Additionally, tumour growth in mice inoculated with lung epithelial cells engineered with LT (suppresses p53 and pRB) and an activated oncogene (either EGFR or KRAS) was monitored over 40 days post-injection. Relative to mice inoculated with either LT-lung epithelial cells or activated oncogene-lung epithelial cells, mice inoculated cells containing both mutations had detectable tumours by approximately day 10 - 12 post-injection; the volumes of these tumours continued increasing until the end of the experiment (Sato et al. 2017).

There were also differences in the rate of DNA synthesis over time, which could possibly indicate higher rates of cell division. In all cell types examined (p53-/-, p53+/- and p53+/+, p53 515A/+, and p53 515A/515A), DNA synthesis declined over the first 6 days in culture, though the mutant p53 lines always had higher synthesis rates than p53-/-, p53+/- and p53+/+ cells. During culture days 6 - 10, DNA synthesis in the mutant p53 lines drastically increased, while the other p53 lines remained at the same relatively low level of synthesis (Lang et al. 2004).

Known Feedforward/Feedback loops influencing this KER

Proliferation increases the likelihood that existing DNA damage will result in mutation and creates new mutations through errors in replication.

It is generally accepted that proliferation increases the risk of mutation and cancer (Preston-Martin, Pike et al. 1990). DNA damage that has not been completely or correctly repaired when a cell undergoes mitosis can be fixed in the genome permanently as a mutation, to be propagated to future daughter cells. Incomplete DNA repair can also cause additional DNA damage when encountered by replicative forks. Therefore, in the presence of any DNA damage (and there is a background rate of damage in addition to any other genotoxic stimuli) mutations will increase with cell division (Kiraly, Gong et al. 2015). Mutation-prone double strand breaks can also arise from replicative stress in hyperplastic cells including hyperplasia arising from excess growth factor stimulation (Gorgoulis, Vassiliou et al. 2005). This relationship between proliferation and mutation is thought to drive a significant portion of the risk of cancer from estrogen exposure since breast cells proliferate in response to estrogen or estrogen plus progesterone and risk increases with cumulative estrogen exposure (Preston-Martin, Pike et al. 1990).

Not all proliferating tissue shows replicative stress and DSBs - tissue with a naturally high proliferative index like colon cells don't show any sign of damage (Halazonetis, Gorgoulis et al. 2008). Additional factors are therefore required beyond replication for damage and mutation from replicative stress, but replication is essential for the expression of these factors.

References

Adjei, A.A. (2001), "Blocking Oncogenic Ras Signaling for Cancer Therapy.", Journal of the National Cancer Institute, 93(14):1062–1074. doi:10.1093/jnci/94.13.1032

Bertram, J.S. (2001), "The molecular biology of cancer.", Mol. Aspects. Med. 21:166–223. doi:10.1016/S0098-2997(00)00007-8.

Danesi, R. et al. (2003), "Pharmacogenetics of Anticancer Drug Sensitivity in Non-Small Cell Lung Cancer." 55(1):57-103. doi:10.1124/pr.55.1.4.57.

Duan, W. et al. (2008), "Lung specific expression of a human mutant p53 affects cell proliferation in transgenic mice.", Transgenic Res. 17(3):355–366. doi:10.1007/s11248-007-9154-3.

Fernandez-Antoran, D. et al., (2019), Outcompeting p53-mutant cells in the normal esophagus by redox manipulation., Cell Stem Cell. 25(3):329-341. doi: 10.1016/j.stem.2019.06.011.

Forbes, S.A. et al. (2011), "COSMIC: Mining Complete Cancer Genomes in the Catalogue of Somatic Mutations in Cancer.", Nucleic Acids Res., 39(Database issue):945–950. doi:10.1093/nar/gkq929.

Geng, C. et al. (2017), "SPOP regulates prostate epithelial cell proliferation and promotes ubiquitination and turnover of c-MYC oncoprotein.", Oncogene. 36(33):4767–4777. doi:10.1038/onc.2017.80.

Hanahan, D. & R.A. Weinberg (2011), "Review Hallmarks of Cancer: The Next Generation.", Cell. 144(5):646–674. doi:10.1016/j.cell.2011.02.013.

Hundley, J.E. et al. (1997), "Increased Tumor Proliferation and Genomic Instability without Decreased Apoptosis in MMTV- ras Mice Deficient in p53.", Mol. Cell. Biol. 17(2):723–731. doi:10.1128/MCB.17.2.723.

Iwakuma, T. & G. Lozano (2007), "Crippling p53 activities via knock-in mutations in mouse models.", Oncogene, 26(15):2177–2184. doi:10.1038/sj.onc.1210278.

Jancik, S. et al. (2010), "Clinical Relevance of KRAS in Human Cancers", J. BioMed. & BioTech. 2010. doi:10.1155/2010/150960.

Jarvis, E.M., J.A. Kirk & C.L. Clarke (1998), "Loss of Nuclear BRCA1 Expression in Breast Cancers Is Associated with a Highly Proliferative Tumor Phenotype.", Cancer Genet Cytogenet. 101(97):101–115. doi:10.1016/S0165-4608(97)00267-7.

Kim, M.P. & G. Lozano (2018), "Mutant p53 partners in crime.", Nat Publ Gr. 25(1):161–168. doi:10.1038/cdd.2017.185.

Lang, G.A. et al. (2004), "Gain of Function of a p53 Hot Spot Mutation in a Mouse Model of Li-Fraumeni Syndrome.", Cell. 119(6):861–872. doi:10.1016/j.cell.2004.11.006.

Larsen, J.E. & J. Minna (2011), "Molecular Biology of Lung Cancer: Clinical Implications.", Clin. Chest Med., 32(4):703–740. doi:10.1016/j.ccm.2011.08.003.

Levine, M. S., & A. J. Holland, A. J. (2018),. “The impact of mitotic errors on cell proliferation and tumorigenesis.”, Genes & development, 32(9-10):, 620–638. https://doi.org/10.1101/gad.314351.118

Li, Z. & Y. Xiong (2017), "Cytoplasmic E3 ubiquitin ligase CUL9 controls cell proliferation, senescence, apoptosis and genome integrity through p53.", Oncogene, 36(36):5212–5218. doi:10.1038/onc.2017.141.

Lodish, H. et al. "Molecular Cell Biology.", 4th edition. New York: W. H. Freeman; 2000. Available from: https://www.ncbi.nlm.nih.gov/books/NBK21475.

Loewe, L. & W.G. Hill (2010), "The population genetics of mutations: Good, bad and indifferent." Philos Trans R Soc B Biol Sci. 365(1544):1153–1167. doi:10.1098/rstb.2009.0317.

López-lázaro M. (2018), "The stem cell division theory of cancer.", Crit. Rev. Oncol. Hematol. 123:95–113. doi:10.1016/j.critrevonc.2018.01.010.

Muller, P.A., K.H. Vousden & J.C. Norman (2011), "p53 and its mutants in tumor cell migration and invasion.", J. Cell. Biol. 192(2):209–218. doi:10.1083/jcb.201009059.

NIHa, EGFR gene epidermal growth factor receptor Normal. 2018. Genetics Home Reference EGFR gene.

NIHb, KRAS proto-oncogene, GTPase Normal. 2018. Genetics Home Reference KRAS gene.

NIHc, TP53 gene tumor protein p53 Normal. 2018. Genetics Home Reference TP53 gene.

Beir V. 1999. Health Effects of Exposure to Radon. National Academies Press.

Panov, S.Z. (2005), "Molecular biology of the lung cancer.", Radiology and Oncology 39(3):197–210.

Pucci, B., M. Kasten & A. Giordano (2000), "Cell Cycle and Apoptosis 1", Neoplasia, 2(4):291–299.doi: 10.1038/sj.neo.7900101

Sanders, H.R. & M. Albitar (2010), "Somatic mutations of signaling genes in non-small-cell lung cancer.", Cancer Genet Cytogenet. 203(1):7–15. doi:10.1016/j.cancergencyto.2010.07.134.

da Cunha Santos, G., F.A. Shepherd & M.S. Tsao (2010), "EGFR Mutations and Lung Cancer.", Annu Rev. Pathol., doi:10.1146/annurev-pathol-011110-130206.

Sato, T. et al. (2017), "Ex vivo model of non-small cell lung cancer using mouse lung epithelial cells.", Oncol. Lett. :6863–6868. doi:10.3892/ol.2017.7098.

Schabath, M.B. et al. (2016), "Differential association of STK11 and TP53 with KRAS.", Oncogene, 35(24):3209–3216. doi:10.1038/onc.2015.375.

Shostak, A. (2017), "Circadian Clock, Cell Division, and Cancer: From Molecules to Organism.", Int. J. Mol. Sci., doi:10.3390/ijms18040873.

Ventura, A. et al. (2007), "Restoration of p53 function leads to tumour regression in vivo.", Nature 445(7128):661-5. doi:10.1038/nature05541.

Vogelstein, B. & K.W. Kinzler (2004), "Cancer genes and the pathways they control.", Nat. Med, 10(8):789–799. doi:10.1038/nm1087.

Welcker, M. & B.E. Clurman (2008), "FBW7 ubiquitin ligase: a tumour suppressor at the crossroads of cell division, growth and differentiation.", Nature Publishing Group. doi:10.1038/nrc2290.

Relationship: 1979: Increase, Chromosomal aberrations leads to Increase, Cell Proliferation

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Deposition of energy leading to lung cancer	adjacent	Moderate	Low
Deposition of energy leading to occurrence of cataracts	adjacent	Moderate	Low

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links

Term	Scientific Term	Evidence	Links
human	Homo sapiens	High	NCBI
rat	Rattus norvegicus	High	NCBI
mouse	Mus musculus	High	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Unspecific	High

The domain of applicability pertains to all multicellular organisms, as cell proliferation and death regulate tissue homeostasis (Pucci et al., 2000).

Key Event Relationship Description

CAs are defined as abnormalities in the chromosome structure, often due to losses or gains of chromosome sections or the entire chromosomes itself, or chromosomal rearrangements (van Gent et al., 2001). These aberrant structures can come in a multitude of different forms. Types of CAs include: inversions, insertions, deletions, translocations, dicentric chromosomes (chromosomes that contain two centromeres, often resulting from telomere end fusions (Fenech & Natarajan 2011; Rode et al., 2016), centric ring chromosomes, acentric chromosome fragments, micronuclei (MN; small nucleus-like structures containing entire chromosomes or chromosome fragments (Fenech & Natarajan, 2011; Doherty et al., 2016), nucleoplasmic bridges (NBPs; a corridor of nucleoplasmic material containing chromatin that is attached to both daughter cell nuclei), nuclear buds (NBUDs; small MN-type structures that are still connected to the main nucleus (Fenech & Natarajan, 2011), and copy number variants (CNVs; deletions or duplications of chromosome segments (Russo et al., 2015).

If these CAs affect genes involved in controlling the cell cycle, this may result in increased cellular proliferationCAs arising from cell transformation can lead to stalling in cell replication to initiate repair (Jackson et al., 2009). CAs can also cause a loss of cell cycle checkpoints resulting in cell proliferation due to the entry into S-phase of the cell cycle (Jackson et al., 2009; Hanahan & Weinburg, 2011). There are three types of genes that, if modified, may result in high rates of proliferation: proto-oncogenes, tumour suppressor genes (TSGs), and caretaker/stability genes (Vogelstein & Kinzler, 2004; Hanahan & Weinberg, 2011). Furthermore, gene fusions that result from CAs have also been implicated in augmenting cellular proliferation (Sanders & Albitar, 2010; Ghazavi et al., 2015; Kang et al., 2016).

Evidence Supporting this KER

Biological Plausibility

There is a strong biological plausibility for a relationship between CAs and rates of cellular proliferation. This is particularly emphasized in the context of carcinogenesis, as high cellular proliferation is a known hallmark of cancer, and an enabling characteristic of increased proliferation is genomic instability (Hanahan & Weinberg, 2011).Topical reviews are available documenting the contribution of CAs to cellular proliferation and/or cancer development (Mes-Masson & Witte, 1987; Bertram, 2001; Vogelstein & Kinzler, 2004; Ghazavi et al. ,2015; Kang et al., 2016). The link between chromosomal instability (CIN), which describes the rate of chromosome gains and losses, and cancer development has also been well documented (Thompson et al., 2017; Gronroos, 2018; Targa & Rancati, 2018; Lepage et al., 2019).

Many CAs are thought to be formed through two main mechanisms: inadequate repair of DNA damage, and errors in mitosis. If there is damage to the DNA that the cell is unable to properly repair, the unrepaired lesion may translate into a CAs (Bignold, 2009; Danford, 2012; Schipler & Iliakis, 2013); the type of resulting CA is often influenced by the cell cycle stage when the damage occurred (Danford, 2012; Registre et al., 2016; Vodicka et al., 2018), and the type of erroneous repair (Ferguson & Alt, 2001; Povirk, 2006; Bignold, 2009; Danford, 2012; Schipler & Iliakis, 2013). Errors made during repair may be particularly detrimental if they interrupt or modify critical genes, or if chromosome structures are created that cannot undergo mitosis (Schipler & Iliakis, 2013). Similarly, errors in mitosis that prevent chromosomes from being properly segregated may also lead to CAs. These errors could be due to by improper timing of centrosome separation, the presence of extra centrosomes, inappropriate mitotic spindle assembly and attachment to kinetochores (found on the centromeres), and incorrect sister-chromatid cohesion (Levine & Holland, 2018).

The presence of CAs in cells may be particularly detrimental if they alter the rate of cellular proliferation by affecting genes that control the cell cycle, namely proto-oncogenes, TSGs (Bertram, 2001; Vogelstein & Kinzler, 2004) or caretaker/stability genes (Vogelstein & Kinzler, 2004). Proto-oncogenes are genes that, when activated, promote cellular proliferation. CAs that increase activation of these genes may aberrantly boost cell cycling and therefore increase proliferation (Bertram, 2001; Vogelstein & Kinzler, 2004). Activation of proto-oncogenes have also been implicated in the cancer stem cell theory of carcinogenesis (Vicente-duen et al., 2013). Examples or proto-oncogenes include *EGFR* and *KRAS* (Sanders & Albitar, 2010). TSGs refer to genes that actively suppress cell proliferation and, in some cases, promote apoptosis (Bertram, 2001; Vogelstein & Kinzler, 2004; Sanders & Albitar, 2010). If these genes are silenced by CAs, this may remove cell cycle checkpoints, thus allowing for unhindered cellular proliferation and decreased apoptosis (Bertram, 2001; Vogelstein & Kinzler, 2004). Common TSGs are *TP53* and *RB* (Hanahan & Weinberg, 2011). Lastly, caretaker/stability genes are those involved in the prevention and detection of DNA damage, and the instigation and completion of the required DNA repair (Vogelstein & Kinzler, 2004; Hanahan & Weinberg, 2011). If the function of these caretaker/stability genes is affected by CAs, this may result in genome-wide inadequate DNA repair, which in turn may result in genetic damage to TSGs or proto-oncogenes (Vogelstein & Kinzler, 2004). Genes involved in mismatch repair (MMR), nucleotide-excision repair (NER) and base-excision repair (BER) are all examples of caretaker/stability genes (Vogelstein & Kinzler, 2004).

There are also other CAs commonly associated with cancer. In prostate cancer, truncated TSGs such as *TP53, PTEN, BRCA1*, and *BRCA2* are a result of chromosomal rearrangements (Mao et al., 2011). Similarly, chromosomal inversions were found to be responsible for just over half of the *RET* gene fusions associated with lung adenocarcinoma samples (Mizukami et al., 2014).

Empirical Evidence

There is moderate empirical evidence supporting the relationship between CAs and the cellular proliferation. The evidence presented below is summarized in table 8, here (click link). There are some available reviews that provide evidence for this relationship in the context of carcinogenesis, as high levels of cellular proliferation is one of the hallmarks of cancer (Hanahan & Weinberg, 2011). Many of these reviews focus especially on the structure and function of specific cancer-associated CAs (Mes-Masson & Witte, 1987; Ghazavi et al., 2015; Kang et al., 2016). Another interesting review discusses transgenic mouse models that have contributed to our understanding of how oncogenes and TSGs promote carcinogenesis in a variety of tissues (Fowlis & Balmain, 1992). Overall, however, there is a lack of empirical evidence available supporting dose and incidence concordance, little empirical evidence supporting temporal concordance, but strong empirical evidence supporting essentiality for this KER.

Dose and Incidence Concordance

Not identified.

Temporal Concordance

There were no studies identified that directly assessed the temporal concordance between CA and increasing rates of cellular proliferation. In a study examining MN frequency and cell proliferation in estrogen-responsive cancer cells treated with estradiol, both MN levels and proliferation rates were higher in estradiol-treated cells relative to controls at 140 and 216 hours post-treatment (Stopper et al., 2003). This suggests that both events are increased at the same time points in response to the estradiol. More work is required, however, to directly assess the temporal concordance between CA frequency and cell proliferation rates.

Essentiality

Much of the evidence for essentiality stems from studies of gene fusions produced by chromosomal translocations and the corresponding impact on cellular proliferation rates. One such gene fusion, *JAFZ1-JJAZ1*, has been identified in endometrial stromal sarcomas. The role of this relatively unknown translocation was evaluated using knock-down and knock-in experiments. When wild-type *JJAZ1* was disabled by siRNA, HEK 293 cells expressing the *JAFZ1-JJAZ1* fusion were found to have an increased rate of cellular proliferation (Li et al., 2007). Similarly, the role of the *EML4-ALK* fusion gene was examined in IL-3 dependent BA/F3 cells. These cells were transfected with a plasmid carrying only *CD8*, or *CD8* in combination with *ALK, EML4-ALK*, or mutant *EML4-ALK* (which contained a lysine to methionine mutation in the kinase domain). In all cases, cell proliferation was found to increase linearly over 7 days in the presence of IL-3; in the absence of IL-3, all cells died by day 3 of culture, with the exception, however, of cells carrying *EML4-ALK*. Only cells with *EML4-ALK* were able to maintain a positive, linear growth in both the presence and absence of IL-3. Addition of a JAK2 inhibitor to these *EML4-ALK* cells resulted in a dose-dependent decline in cellular proliferation, such that at a dose of 10 um of inhibitor, cells numbers declined steadily until death at day 5. This is in contrast to the *CD8*-expressing cells exposed to the same inhibitor doses, in which there was only a very slight decline in cellular proliferation rates (Soda et al. 2007). Both of these studies provide evidence that translocations increase proliferation rates in cells.

In addition to causing gene fusions, translocations may also lead to the production of circular RNA fusion products (f-CircRNA), which can be studied to further understand the link between CAs and cellular proliferation. For example, f-CircPR has been associated with the *PML-RARα* translocation, f-CircM9 has been associated with the *MLL-AF9* translocation, and expressions of f-CircPR or f-CircM9 were both found to increase cell proliferation rates in mouse embryonic fibroblasts. Inhibition of these f-CircRNAs, either through addition of silencing shRNA or by using a mutant non-circularizing f-CircRNA, resulted in decreased rates of cell proliferation (Guarnerio et al., 2016). These results again indicate that there is a relationship between CAs and increased cellular proliferation.

Other experiments provide evidence that CAs can increase cellular proliferation using cancer cells. Using two human Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph^+ALL) cell lines (both of which contain the *BCR-ABL* translocation), cellular proliferation was studied by cell counting and by analyzing levels of phosphorylated ErbB2. ErbB2 is a member of the ERB receptor tyrosine kinase family that is involved in pro-proliferative signalling, and it is known to be expressed in cells from ALL patients. Cell proliferation rates were found to decline in a dose-dependent fashion when treated with either an ErbB family tyrosine kinase inhibitor, or a more specific ErbB1/ErbB2 tyrosine kinase inhibitor. Furthermore, treatment with the ErbB family inhibitor also resulted in significant decreases in phosphorylated ErbB2 (Irwin et al., 2013). In another set of experiments using estrogen receptor-positive human ovarian cancer cells, treatment of cells with estrogen were found to have significantly increased levels of MN and significantly increased proliferation rates relative to vehicle-treated control cells; furthermore, there were more cells in S-phase and fewer in the G2/M phases of the cell cycle relative to controls. These results were specific to estrogen-response cells, as treatment of estrogen receptor-negative human ovarian cancer cells with estrogen did not result in any changes to MN or cell proliferation. Furthermore, addition of an estrogen antagonist to estrogen-responsive cells maintained MN frequencies and cell proliferation at control levels (Stopper et al., 2003).

Human Epidemiology Association and Genetic Studies

Very often, CAs result in gene fusions. A gene fusion occurs when two genes not normally in close proximity to each other are juxtaposed; this may result in altered expression of one or both genes, or an altered gene product (Mitelman, 2005). There are several well-known gene fusions implicated in carcinogenesis that are associated with increased cellular proliferation. One well-characterized gene fusion is the Philadelphia chromosome, also known as the *BCR-ABL1* fusion. This gene fusion is formed by a translocation between chromosome 9 and 22, and is commonly found in chronic myelogenous leukemia (CML) (Mes-Masson and Witte 1987; Kang et al. 2016), as well as acute lymphoblastic leukemia (ALL) (Ghazavi et al., 2015). The protein created from *BCR-ABL1* has elevated tyrosine kinase activity, and was shown to increase activation of cellular proliferation pathways (Ghazavi et al., 2015; Kang et al., 2016) including JAK2/STAT, PI3K-AKT, and MAPK/ERK (Kang et al., 2016). Another common gene fusion partner is *ALK*, which is a receptor tyrosine kinase involved in the PI3K-AKT signalling pathway. Very often *ALK* gene fusions result in upregulated *ALK* expression, and a resulting increase in pro-proliferative signalling in the PI3K-AKT pathway. In non-small cell lung cancer, the *ALK-EML4* gene fusion is particularly common (Sanders & Albitar, 2010). Similarly, *ETV6-RUNX1* is the most common fusion gene in B-cell precursor acute lymphoblastic leukemia (BCP-ALL), and is thought to initiate leukemogenesis (Ghazavi et al., 2015).

Uncertainties and Inconsistencies

Uncertainties in this KER are as follows:

1. A study using peripheral blood lymphocytes isolated from head and neck cancer patients found significantly increased CAs (including chromosome-type aberrations, chromatid-type aberrations, dicentric chromosomes, aneuploidy, MN, NPBs and NBUDs) relative to healthy controls. In the lymphocytes from these same cancer patients, however, the cell proliferation rates were significantly decreased (George et al., 2014).
2. Characterization of 20 different ameloblastomas, which are benign tumours associated with the jaw, found low CAs frequencies and low rates of cellular proliferation (Jääskeläinen et al., 2002).

Quantitative Understanding of the Linkage

Quantitative understanding has not been well-established for this KER. There were no studies identified that documented a response-response relationship between CA frequency and cell proliferation rates, and a severe lack of time scale-oriented studies. Overall, more research is required to establish a quantitative understanding of this KER.

Response-response relationship

Not established.

Time-scale

Studies that directly assessed the time scale between CAs and cellular proliferation were not identified. However, differences in cellular proliferation rates for cells with different CA-related manipulations or treatments were evident within the first 3 days of culture (Stopper et al., 2003; Li et al., 2007; Soda et al., 2007; Irwin et al., 2013; Guarnerio et al., 2016). More studies are required, however, to formulate a detailed time scale relating these two events.

Known modulating factors

Not established.

Known Feedforward/Feedback loops influencing this KER

Not established.

References

Bertram, J.S. (2001), "The molecular biology of cancer.", Mol. Aspects. Med. 21:166–223. doi:10.1016/S0098-2997(00)00007-8.

Bignold, L.P. (2009), "Mechanisms of clastogen-induced chromosomal aberrations: A critical review and description of a model based on failures of tethering of DNA strand ends to strand-breaking enzymes.", Mutat. Res., 681(2-3):271–298. doi:10.1016/j.mrrev.2008.11.004.

Danford, N. (2012), "The Interpretation and Analysis of Cytogenetic Data.", Methods Mol. Biol., 817:93-120. doi:10.1007/978-1-61779-421-6.

Doherty, A., S.M. Bryce & J.C. Bemis (2016), "The In Vitro Micronucleus Assay. Methods in molecular biology", (Clifton, N.J.). 817:121-41. doi: 10.1007/978-1-61779-421-6_7.

Fenech, M. & A.T. Natarajan (2011), "Molecular mechanisms of micronucleus, nucleoplasmic bridge and nuclear bud formation in mammalian and human cells.", Mutagenesis 26(1):125–132. doi:10.1093/mutage/geq052.

Ferguson, D.O. & F.W. Alt (2001), "DNA double strand break repair and chromosomal translocation: Lessons from animal models.", Oncogene 20(40):5572–5579.

Fowlis, D.J. & A. Balmain (1993), "Oncogenes and Tumour Suppressor Genes in Transgenic Mouse Models of Neoplasia.", Eur. J. of Cancer 29A(4):638-45. doi: 10.1016/S0959-8049(05)80170-4.

van Gent D.C., J.H.J. Hoeijmakers & R. Kanaar (2001), "Chromosomal stability and the DNA double-stranded break connection.", Nat. Rev. Genet. 2(3):196–206. doi:10.1038/35056049. http://www.ncbi.nlm.nih.gov/pubmed/11256071.

George, A., R. Dey & V.B. Dqhumhh (2014), "Nuclear Anomalies, Chromosomal Aberrations and Proliferation Rates in Cultured Lymphocytes of Head and Neck Cancer Patients.", Asian Pacific journal of cancer prevention. 15(3):1119-1123. doi:10.7314/APJCP.2014.15.3.1119.

Ghazavi, F. et al. (2015), "Molecular basis and clinical significance of genetic aberrations in B-cell precursor acute lymphoblastic leukemia.", Exp Hematol. 43(8):640–653. doi:10.1016/j.exphem.2015.05.015.

Gronroos, E. (2018), "Tolerance of Chromosomal Instability in Cancer: Mechanisms and Therapeutic Opportunities.", Cancer Res. 78(23):6529-6535, doi:10.1158/0008-5472.CAN-18-1958.

Guarnerio, J. et al. (2016), "Oncogenic Role of Fusion-circRNAs Derived from Article Oncogenic Role of Fusion-circRNAs Derived from Cancer-Associated Chromosomal Translocations.", Cell. 165(2):289–302. doi:10.1016/j.cell.2016.03.020.

Hanahan, D. & R.A. Weinberg (2011), "Review Hallmarks of Cancer: The Next Generation.", Cell. 144(5):646–674. doi:10.1016/j.cell.2011.02.013.

Irwin, M.E. et al. (2013), "Small Molecule ErbB Inhibitors Decrease Proliferative Signaling and Promote Apoptosis in Philadelphia Chromosome – Positive Acute Lymphoblastic Leukemia.", PLoS One, 8(8):1–10. doi:10.1371/journal.pone.0070608.

Jääskeläinen, K. et al. (2002), "Cell proliferation and chromosomal changes in human ameloblastoma.", Cancer Genetics and Cytogenetics. 136(1):31-7. doi: 10.1016/S0165-4608(02)00512-5.

Jackson, S. P., and& Bartek, J. (2009),. “The DNA-damage response in human biology and disease.” Nature, 461(7267), 1071–1078. https://doi.org/10.1038/nature08467

Kang, Z.J. et al. (2016), "The Philadelphia chromosome in leukemogenesis.", Chin J Cancer.:1–15. doi:10.1186/s40880-016-0108-0.

Lepage, C.C. et al. (2019), "Detecting Chromosome Instability in Cancer: Approaches to Resolve Cell-to-Cell Heterogeneity.", Cancers (Basel), 11(2): pii: E226. doi:10.3390/cancers11020226.

Levine, M.S. & A.J. Holland (2018), "The impact of mitotic errors on cell proliferation and tumorigenesis.", Genes Dev., 32(9-10):620–638. doi:10.1101/gad.314351.118.620.

Li, H. et al. (2007), "Effects of rearrangement and allelic exclusion of JJAZ1 / SUZ12 on cell proliferation and survival.", PNAS, 104(50):20001–20006.

Mao, X. et al. (2011), "Chromosome rearrangement associated inactivation of tumour suppressor genes in prostate cancer.", American Journal of Cancer Research. 1(5):604-17.

Mes-Masson, A.-M. & O.N. Witte (1987), "Role of The abl Oncogene in Chronic Myelogenous Leukemia.", Advances in Cancer Research. 49:53-74. doi: 10.1016/S0065-230X(08)60794-0.

Mitelman, F. (2005), "Deep Insight Section: Cancer cytogenetics update", Atlas of Genetic and Cytogenetics in Oncology and Haematology, 9(2):188–190. doi:10.4267/2042/38202.

Mizukami, T. et al. (2014), "Molecular Mechanisms Underlying Oncogenic RET Fusion in lung adenocarcinoma.", J Thorac Oncol. 9(5):622–630. doi:10.1097/JTO.0000000000000135.

Povirk, L.F. (2006), "Biochemical mechanisms of chromosomal translocations resulting from DNA double-strand breaks.", DNA Repair (Amst). 5(9-10):1199–1212. doi:10.1016/j.dnarep.2006.05.016.

Pucci, B., M. Kasten & A. Giordano (2000), "Cell Cycle and Apoptosis 1", Neoplasia, 2(4):291–299.doi: 10.1038/sj.neo.7900101

Registre, M., R. Proudlock & N. Carolina (2016), "The In Vitro Chromosome Aberration Test.", Genetic Toxicology Testing. 207-267. doi: 10.1016/B978-0-12-800764-8.00007-0.

Rode, A. et al. (2016), "Chromothripsis in cancer cells: An update.", Int. J. Cancer, 138(10):2322–2333. doi:10.1002/ijc.29888.

Russo, A. et al. (2015), "Review Article Genomic Instability: Crossing Pathways at the Origin of Structural and Numerical Chromosome Changes.", Envrion. Mol. Mutagen. 56(7):563-580. doi:10.1002/em.

Sanders, H.R. & M. Albitar (2010), "Somatic mutations of signaling genes in non-small-cell lung cancer.", Cancer Genet Cytogenet. 203(1):7–15. doi:10.1016/j.cancergencyto.2010.07.134.

Schipler, A. & G. Iliakis (2013), "DNA double-strand – break complexity levels and their possible contributions to the probability for error-prone processing and repair pathway choice.", Nucleic Acids Res., 41(16):7589–7605. doi:10.1093/nar/gkt556.

Soda, M. et al. (2007), "Identification of the transforming EML4 – ALK fusion gene in non-small-cell lung cancer.", Nature, 448(7153):561-566. doi:10.1038/nature05945.

Stopper, H. et al. (2003), "Increased cell proliferation is associated with genomic instability: elevated micronuclei frequencies in estradiol-treated human ovarian cancer cells.", Mutagenesis 18(3):243-247. doi:10.1093/mutage/18.3.243.

Targa, A. & G. Rancati (2018), "Cancer: a CINful evolution.", Curr Opin Cell Biol. 2018 Jun;52:136-144., doi:10.1016/j.ceb.2018.03.007

Thompson, L.L. et al. (2017), "Evolving Therapeutic Strategies to Exploit Chromosome Instability in Cancer.", Cancers (Basel), 9(11): pii: E151 doi:10.3390/cancers9110151.

Vicente-Duenas, C. et al. (2013), "Function of oncogenes in cancer development: a changing paradigm", EMBO J., 32(11):1502–1513. doi:10.1038/emboj.2013.97.

Vodicka, P. et al. (2018), "Genetic variation of acquired structural chromosomal aberrations.", Mutat Res Gen Tox En, 836(May):13–21. doi:10.1016/j.mrgentox.2018.05.014.

Vogelstein, B. & K.W. Kinzler (2004), "Cancer genes and the pathways they control.", Nat. Med, 10(8):789–799. doi:10.1038/nm1087.

Relationship: 2816: Modified Proteins leads to Cataracts

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Deposition of energy leading to occurrence of cataracts	adjacent	Moderate	Low

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	Moderate	NCBI
mouse	Mus musculus	High	NCBI
rat	Rattus norvegicus	Moderate	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Female	Moderate
Male	Moderate

This KER is plausible in all life stages, sexes, and organisms that have a clear lens for vision. The majority of the evidence is from in vivo studies (adult mice, and rats) and human cohorts. No in vitro evidence was found to support the relationship.

Key Event Relationship Description

The maintenance of the correct structure and location of lens proteins is crucial for the proper refraction of light in the eye. Any modifications to the proteins of the lens can result in a reduction in lens transparency and cataract formation through the mechanism of protein aggregation (Zhao et al., 2015). Cataracts are a progressive condition in which the lens of the eye develops opacities and becomes cloudy, resulting in blurred vision as well as glare and haloes around lights (National Eye Institute, 2022). For this AOP, a cataract is defined when over 5% of the lens is opacified. Under normal conditions, lens proteins work to support the eye through chaperones, gap junctional, and structural functions (Ghosh & Chauhan, 2019; NCRP, 2016). Light enters the eye and passes through the crystallin proteins of the lens, which are responsible for 90% of the proteins in a mature lens. These proteins are carefully arranged as to limit their interference with the light, and the lens cells remove their organelles once they are mature to reduces light-scattering (Moreau & King, 2012; Toyama & Hetzer, 2013). Proteins play other roles in the creation of a transparent medium. Beta- and γ-crystallins are structural proteins that ensure the proper inter-protein interactions occur for the maintenance of nuclear transparency, and alpha crystallin proteins chaperone other proteins, including beta- and γ-crystallins, around the lens (Ghosh & Chauhan, 2019; Toyama & Hetzer, 2013). Lens epithelial cells (LEC) rely on proteins, such as connexin43, to act as phenotypic markers to help organize the cells within the lens following proliferation, preventing the cells from improperly layering within the eye. LECs are packed with crystallin proteins. If the connexin43 proteins are altered, that would impair their ability to help organize the LECs properly, resulting in all the proteins found within those LECs to be disoriented compared to the proteins of neighbouring cells (Berthoud et al., 2014). This improper layering of the cells leads to modified transparency in the lens as a result of the disorganization of the many crystallin proteins within the LEC. Connexin proteins typically join chaperone proteins in a complex and repair misfolded proteins (NCRP, 2016). Proteins can be modified from exposure to stressors, and depending on the type of protein, the alteration will also differ. Following modification, proteins will be unable to correctly perform their roles within the lens, such as preventing aggregation via proper chaperone and structural actions. (Ghosh & Chauhan, 2019; Toyama & Hetzer, 2013; NCRP, 2016). Protein aggregation occurs, which is worsened by the inability of the proteins to form complexes to repair themselves, and this leads to reduced lens transparency and increased cataract incidence.

Evidence Supporting this KER

Overall Weight of Evidence: Moderate

Biological Plausibility

There is strong biological plausibility to support the link between modified proteins and cataracts. A review focusing on modified proteins and cataracts is particularly relevant as it discusses different types of protein modifications, and the resulting effect on increased human lens opacity (Truscott, 2005). Several other studies discuss multiple types of protein alterations that can cause increased cataracts/lens opacity, often attributed to improper protein function (Hamada et al., 2014; NCRP, 2016; Ghosh & Chauhan, 2019). Currently, the majority of the empirical evidence to support this relationship is derived from studies conducted in adult male subjects, therefore there is limited opportunity to comment on sex or age effects on this relationship (Fujii et al., 1986; Menard et al., 1986). There is also limited information for taxonomic comparisons of rats, humans, and mice related to modified proteins leading to cataracts, with only one paper listed for each species. However, it is evident that all three species have evidence to support the causal connectivity of this relationship (Menard et al., 1986; Truscott, 2005; Fujii et al., 1986).

Once lens fiber cells are damaged, and the intracellular proteins are modified, these modifications are permanent, as the cells lack the organelles needed to undergo protein turnover (Toyama & Hetzer, 2013). These protein modifications can in turn cause protein aggregation, which are high molecular weight proteins, and these can modify the multi-layering of cells (Bron et al., 2000; Moreau & King, 2012; NCRP, 2016). Among the different types of amino acids, tryptophan, histidine, and cysteine are all at risk for modifications from oxidative processes (Balasubramanian, 2000). Oxidized proteins can have modified water-solubility (Hamada et al., 2014; Moreau & King, 2012). The protein content in the lens of the eye needs to be optimal to ensure that the lens transparency can appropriately contribute to the refractive medium of the lens, meaning that the water solubility of proteins is crucial and can dictate the development of lens opacities. Proteins can become water-insoluble when they undergo post-translational modifications, shifting the solubility fraction of the lens proteins (Hamada et al., 2014). They are unable to aggregate while in their natural water-soluble state and must first undergo modifications to decrease solubility and generate a build-up of proteins (Moreau & King, 2012). Alpha-crystalline proteins, when modified, are unable to chaperone other proteins to the correct locations, aggravating the protein aggregation in the eye (Blakely et al., 2010; Uwineza et al., 2019). The binding of these chaperone proteins to the molten globule region of other proteins leads to the formation of high molecular weight proteins, a common protein type seen in cataract patients (NCRP, 2016; Truscott, 2005; Moreau & King, 2012). High-molecular-weight crystalline aggregation causes light to scatter at a higher rate than normal, increasing lens opacity (Uwineza et al., 2019; Bron et al., 2000; Toyama & Hetzer, 2013). Furthermore, when connexin proteins, which form intercellular channels between LECs and lens fiber cells (Tjahjono et al., 2020), are unable to perform their function, the LECs will improperly layer. Modifications to these proteins has been linked to human cataract development (NCRP, 2016).

Empirical Evidence

This relationship is poorly supported. However, there is some empirical evidence from studies using stressors such as γ- and X-rays that cause protein modifications resulting in lens opacification and cataract development. These studies are derived from in vivo mouse and rat models using whole lenses (Fujii et al., 1986; Menard et al., 1986).

Incidence Concordance

There is low evidence to support an incidence concordance relationship between modified proteins and cataract development. Following the exposure of the lenses in vivo to 15.3 Gy γ-rays, the level of soluble proteins dropped to 0.07x control levels and were associated with observed opacities being larger than those in control lenses (Menard et al., 1986). Ample evidence has shown that protein modifications, particularly phosphorylation, may be associated with cataracts. These studies used human and animal models with pre-existing cataracts, and showed the presence of phosphorylated crystallin, MDM2 and tyrosine proteins (Wang et al. 2020; Hui-Ju et al. 2013; Chandrasekher et al. 2004).

Time Concordance

There is low evidence in the literature to support time concordance between modified proteins and increased lens opacity/cataract development. High dose (>2 Gy) in vivo studies have shown that cataracts first appeared 6 ½ weeks post-modification. Modified D/L amino acid conformation ratio of lens proteins was observed in vivo in whole lenses as early as 11 days post 15 Gy X-irradiation, while lens opacities were shown to occur as early as eight weeks post-irradiation. (Fujii et al., 1986).

Essentiality

Modified proteins been found to increase cataracts above background levels. Therefore, although radiation is not essential for the development of cataracts, it is essential for promoting it above this normal level (Menard et al., 1986). There is low evidence in the literature in the form of knock-out and knock-in studies to support the essentiality of protein aggregation in the development of opacities. The return of the lens protein solubility ratio to near control levels resulted in the opacity level of the lens more closely resembling the control lens than the unshielded treatment lens, following in vivo 15.3 Gy γ-irradiation on whole lenses (Menard et al., 1986).

Uncertainties and Inconsistencies

N/A

Quantitative Understanding of the Linkage

The following tables provide representative examples of the relationship, unless otherwise indicated, all data is statistically significant.

Incidence Concordance

Reference	Experiment Description	Result
Menard et al., 1986	In vivo, rats received head-only exposure to 15.3 Gy γ-rays, proteins detected with Lowry assay and size-exclusion liquid chromatography, lens opacity assessed by slit-lamp eye examinations.	In rats exposed in vivo to 15.3 Gy γ-rays, the water-soluble protein make-up in the lens decreased 13.6x (indicating increased levels of modified proteins) and dense cataracts were observed, while controls developed minimal opacification.

Time Concordance

Reference	Experiment Description	Result
Fujii et al., 1986	In vivo, mice received whole-body exposure to 15 Gy X-rays D/L ratio of proteins was determined with gas-liquid chromatography and cataracts determined by the observation of lens opacification.	In mice exposed in vivo to 15 Gy X-rays the ratio of D/L conformation lens proteins increased 1.5x 60 days post-irradiation. Lens opacity increased at the same point in time.

Known modulating factors

Modulating Factor (MF)	MF Specification	Effect(s) on the KER	Reference(s)
Age	≥ 40 years old (human) has higher incidence of lens opacity	Proteins naturally change and degrade over time however they do not get removed from within the lens' center. This leads to a higher level of modified protein accumulation within the lens in older individuals. Protein accumulation/aggregation is linked to light scattering and cataracts.	Hains & Truscott, 2010; NCRP, 2016

Modulating Factor (MF)	MF Specification	Effect(s) on the KER	Reference(s)
5-cholesten-3b,25-diol (VP1-001)	Administration of compound	VP1-001 reversed α-crystallin aggregation in vivo, resulting in decreased lens opacity.	Molnar et al., 2019; Wang et al., 2022

Known Feedforward/Feedback loops influencing this KER

N/A

References

Balasubramanian, D. (2000), “Ultraviolet Radiation and Cataract”, Journal of Ocular Pharmacology and Therapeutics, Vol.16/3, Mary Ann Liebert Inc, Larchmont, https://doi.org/10.1089/JOP.2000.16.285.

Berthoud, V. et al. (2014), “Roles and regulation of lens epithelial cell connexins”, FEBS Letters, Vol. 588/8, Elsevier, England, https://doi.org/10.1016/j.febslet.2013.12.024

Blakely, E. et al. (2010), “Radiation Cataractogenesis: Epidemiology and Biology”, Radiation Research, Vol.173/5, Radiation Research Society, Indianapolis, https://doi.org/10.1667/RRXX19.1.

Bron, A. et al. (2000), “The Ageing Lens”, Ophthalmologica, Vol.214, Karger Publishers, Basel, https://doi.org/10.1159/000027475.

Chandrasekher, G., and& Sailaja, D. (2004),. “Alterations in lens protein tyrosine phosphorylation and phosphatidylinositol 3-kinase signaling during selenite cataract formation”,., Current eye research, Vol.28(2),. https://doi.org/10.1076/ceyr.28.2.135.26232

Fujii, N. et al. (1986), “D-Amino Acid in Irradiated and Aged Mouse”, Journal of Radiation Research, Vol.27, Oxford University Press, Oxford, https://doi.org/10.1269/JRR.27.183.

Ghosh, K. and P. Chauhan. (2019), “Crystallins and Their Complexes” in Macromolecular Protein Complexes II: Structure and Function, Subcellular Biochemistry, Vol.93, Springer Nature Switzerland AG, Handel, https://doi.org/10.1007/978-3-030-28151-9.

Hamada, N. et al. (2014), “Emerging issues in radiogenic cataracts and cardiovascular disease”, Journal of Radiation Research, Vol.55/5, Oxford University Press, Oxford, https://doi.org/10.1093/jrr/rru036.

Hains, B. and R. Truscott. (2010), “Age-dependent deamidation of lifelong proteins in the human lens”, Investigative Ophthalmology and Visual Science, Vol.51/6, Association for Research in Vision and Ophthalmology, Rockville, https://doi.org/10.1167/iovs.09-4308.

Kozbenko, T. et al. (2022), “Deploying elements of scoping review methods for adverse outcome pathway development: a space travel case example”, International Journal of Radiation Biology, 1–12. https://doi.org/10.1080/09553002.2022.2110306

Lin, H., et al. (2013), “An Increase in Phosphorylation and Truncation of Crystallin With the Progression of Cataracts”, Current Therapeutic Research, Vol. 74, https://doi.org/10.1016/j.curtheres.2012.10.003.

Menard, T. et al. (1986), “Radioprotection against cataract formation by WR-77913 in gamma-irradiated rats”, International Journal of Radiation Oncology Biology Physics, Vol.12, Elsevier, Amsterdam, https://doi.org/10.1016/0360-3016(86)90199-9.

Molnar, K. S. et al. (2019), "Mechanism of action of VP1-001 in cryAB(R120G)-associated and age-related cataracts”, Investigative Ophthalmology & Visual Science, Vol. 60/10, The Association for Research in Vision and Ophthalmology, United States, https://doi.org/ 10.1167/iovs.18-25647

Moreau, K. and J. King. (2012), “Protein misfolding and aggregation in cataract disease and prospects for prevention”, Trends in Molecular Medicine, Vol.18/5, Elsevier Ltd, London, https://doi.org/10.1016/j.molmed.2012.03.005.

National Eye Institute (2022), Cataracts, https://www.nei.nih.gov/learn-about-eye-health/eye-conditions-and-diseases/cataracts (accessed November 29, 2022).

NCRP (2016), “Guidance on radiation dose limits for the lens of the eye”, NCRP Commentary, Vol.26, National Council on Radiation Protection and Measurements Publications, Bethesda.

Tjahjono, N. et al. (2020), “Connexin 50-R205G mutation perturbs lens epithelial cell proliferation and differentiation”, Investigative Ophthalmology & Visual Science, Vol. 61/3, The Association for Research in Vision and Ophthalmology, United States, https://doi.org/10.1167/iovs.61.3.25

Toyama, B. and M. Hetzer. (2013), “Protein homeostasis: Live long, won't prosper”, Nature Reviews Molecular Cell Biology, Vol.14/1, Nature Portfolio, Berlin, https://doi.org/10.1038/nrm3496.

Truscott, R. (2005), “Age-related nuclear cataract - Oxidation is the key”, Experimental Eye Research, Vol.80/5,

Academic Press Inc, Cambridge, https://doi.org/10.1016/J.EXER.2004.12.007.

Uwineza, A. et al. (2019), “Cataractogenic load – A concept to study the contribution of ionizing radiation to accelerated aging in the eye lens”, Mutation Research-Reviews in Mutation Research, Vol.779, Elsevier, Amsterdam, https://doi.org/10.1016/J.MRREV.2019.02.004.

Wang, K. et al. (2022), “Oxysterol compounds in mouse mutant αA and αB-crystallin lenses ca improve the optical properties of the lens”, Investigative Ophthalmology & Visual Science, Vol. 63/5, The Association for Research in Vision and Ophthalmology, United States, https://doi.org/10.1167/iovs.63.5.15

Zhao, L. et al. (2015), “Lanosterol reverses protein aggregation in cataracts”, Nature, Vol.523, Nature Portfolio, Berlin, https://doi.org/10.1038/nature14650.

Relationship: 2819: Increase, Cell Proliferation leads to Cataracts

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Deposition of energy leading to occurrence of cataracts	adjacent	Moderate	Low

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
mouse	Mus musculus	Moderate	NCBI
rat	Rattus norvegicus	Moderate	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	Moderate

Sex Applicability

Sex	Evidence
Unspecific	Moderate
Mixed	Moderate
Female	Moderate

This KER is plausible in all life stages, sexes, and organisms requiring a clear lens for vision. The majority of the evidence is from in vivo mice and rats of all ages and does not specify sex. No in vitro evidence was found to support the relationship.

Key Event Relationship Description

Throughout their life, cells replicate their organelles and genetic information before dividing to form two new daughter cells, in a process known as cellular proliferation. This is regulated by the cell cycle, which is subdivided into various stages notably, G1, S, G2, and M in mammals. Progression through the cycle is dependent on sufficient nutrient availability to provide optimal nucleic acid, protein, and lipid levels, as well as sufficient cell mass. If conditions are ideal for division, cells will express genes used for duplicating centrosomes and DNA, eventually leading to cell proliferation (Cuyàs et al., 2014). Various protein complexes, known as cyclins, cyclin-dependent kinases (CDKs), and cyclin-dependent kinase inhibitors (CKIs) regulate passage through each phase of the cell cycle.

Cyclins will activate specific CDKs, which will phosphorylate and inactive proteins that control passage through the cell cycle. One example is the retinoblastoma protein, which controls passage from G1 to S. Conversely, the CKIs inhibit CDKs, preventing passage through the cell cycle (Lovicu et al., 2014). Disruption of cell cycle mechanisms can lead to uncontrolled cell proliferation. If this occurs in lens epithelial cells (LECs), then cataracts can develop. Of note, not all cells of the lens are capable of proliferation (West-Mays et al., 2009) Cataracts are a progressive condition in which the lens of the eye develops opacities and becomes cloudy, resulting in blurred vision as well as glare and haloes around lights (National Eye Institute, 2022). For this AOP, a cataract is defined when over 5% of the lens is opacified. The lens is a transparent, biconvex tissue located at the front of the eye. It is responsible for focusing light onto the retina thus, producing a clear image. However, during increased cell proliferation, the LECs will not differentiate completely, forming lens fiber cells (LFCs) that retain certain organelles. Normal LFCs contain no organelles, rendering them transparent and, as a result, the incompletely differentiated LFCs form small opacities in

the lens (Wride, 2011). As the lens has low metabolic and mitotic activity, there is very little tissue turnover. Therefore, opacities are not removed and accumulate with time (Hamada, 2017).

Evidence Supporting this KER

Overall Weight of Evidence: Moderate

Biological Plausibility

The biological plausibility of increased cell proliferation leading to cataracts has been reviewed in several articles (Lett et al., 1994; Kleiman et al., 2007; Hamada, 2017; Wride, 2011).

This KER is specific to lens cells. The germinative zone (GZ) is the only area of the lens where cells are undergoing mitosis. After replication, the LECs migrate away from LECs, becoming terminally differentiated LFCs. However, if there is excessive cell proliferation, then the LECs will be pushed out of the GZ and forced to become LFCs before they are completely differentiated. This results in LFCs that have not lost all of their organelles, therefore compromising the organelle free zone necessary to retain lens transparency. This process, combined with others such as accumulation of damaged macromolecules throughout life, increases lens opacity (Holsclaw et al., 1994; Lett et al., 1994; Pendergrass et al., 2010; Wiley et al., 2011; Wride, 2011; Fujimichi and Hamada, 2014; Saika et al., 2014; Markiewicz et al., 2015; Ainsbury et al., 2016; Hamada, 2017, McCarron et al., 2022). This process can also be initiated by a decrease in LEC, the remaining cells must therefore replicate more than normally to compensate. As a result, not all differentiation processes proceed properly, increasing the likelihood of cataracts (Ainsbury et al., 2016).

After the TZ, LECs migrate to the meridional rows, an area below the lens equator, as they are beginning to differentiate into LFCs. In situations with excessive cell proliferation the LFCs that are normally organized in a precise manner will become disorganized. The degree of disorganization also affects lens opacity and can be used as a measure of cataract severity (Holsclaw et al., 1994; Fujimichi and Hamada, 2014; Markiewicz et al., 2015; Hamada, 2017).

Furthermore, as the lens is a closed system, the damaged cells and macromolecules are not removed and continually contribute to lens opacity, and eventually cataracts (Fujimichi and Hamada et al., 2014; Ainsbury et al., 2016).

Empirical Evidence

There is limited empirical evidence supporting a relationship between increased cell proliferation and cataracts.

Dose Concordance

No studies were found that demonstrated increased cell proliferation at lower doses than cataracts. However, De Stefano et al. (2021) showed that in mice predisposed to increased cell proliferation, 2 Gy of γ-rays exacerbated the effects on cataract formation.

Time Concordance

Pendergrass et al. (2010) found that the amount of LECs drops immediately after irradiation for a period of four months (1.6x decrease) before beginning to increase. This was accompanied by a continual increase in slit-lamp grade (cataract severity) beginning one month after cell proliferation starts to increase. However, samples were harvested once a month (11 Gy X-rays at 2 Gy/min in adult female C57BL/6 mice). Additionally, Hanna and O'Brien (1963) found a 50% increase in the number of LECs compared to the control to correspond to stage II cataracts.

Essentiality

One study found that mice heterozygous for Ptch1 have lower lens opacity than wild-type mice. The Ptch1 gene helps to prevent uncontrolled cell proliferation, therefore this relationship suggests that increased cell proliferation leads to increased lens opacity and a greater risk of cataracts (0.5, 1, and 2 Gy ^{60}Co γ-rays at 0.063 and 0.3 Gy/min) (McCarron et al., 2021). Furthermore, De Stefano et al. (2021) found that mice lacking one Ptch1 allele, have increased cell proliferation which correlated to a maximum lens opacity that was 3.9 to 5.3 times higher than mice exhibiting normal cell proliferation.

Uncertainties and Inconsistencies

N/A

Quantitative Understanding of the Linkage

Increases in cell proliferation leads to increased lens opacity, which leads to cataracts. The following tables provide representative examples of the relationship, unless otherwise indicated, all data is significantly significant.

Dose Concordance

Reference	Experiment Description	Result
De Stefano et al., 2021	In vivo. Ptch1+/- /CD1, CD1, Ptch1+/- /C57Bl/6, and C57Bl/6 mice were exposed to 2 Gy ^{60}Co γ-rays at a rate of either 0.3 or 0.063 Gy/min. Ptch1+/- mice have increased cell proliferation. Lens opacity was measured using Scheimpflug analysis.	Mice genetically predisposed towards increased cell proliferation had a maximum lens opacity 2.8x that of typical mice following 2 Gy irradiation.

Incidence Concordance

No studies found

Time Concordance

Reference	Experiment Description	Result
Pendergrass et al., 2010	In vivo. 3-month-old, female, C57BL/6 mice received head-only exposure to 11 Gy X-rays at 2 Gy/min. This initiated cellular proliferation, which was measured by staining and counting nuclei with the vital dye Hoechst 33342. Cataracts were determined through slit lamp analysis.	In mice exposed to 11 Gy X-rays, cellular proliferation began to increase 4 months post-exposure. The mean slit lamp grade (cataract measurement) began to increase at the same time and reached 3.3x control seven months later (Pendergrass et al., 2010).
Hanna & O'Brien, 1963	In vivo. Adult and weanling rats (24 to 29 days old) as well and adult mice were irradiated with to 2400 r of ^{60}Co γ-rays at 40 r/min to the left eye. Cell proliferation was detected using thymidine-tritium labelling.	Cells were labelled with thymidine-tritium before the adult animal's death. This resulted in an increase of about 50% in the number of LECs undergoing DNA synthesis after one month. This was observed 7 to 14 days after irradiation and corresponded to stage I cataract formation. 6 to 12 weeks after irradiation there were almost twice as many labelled cells and the lenses were in stage II cataracts. These experiments were repeated with rats 24 to 29 days old. The same results were found, but more cells were labelled initially, and cataracts progressed more quickly.

Known modulating factors

Modulating Factor (MF)	MF Specification	Effect(s) on the KER	Reference(s)
Anti-proliferative agents	Mitomycin C, octreotide, 5-fluorouracil, doxorubicin, FGF receptor-1 antagonist SU5402, colchicines, and duanomycin	The presence of these compounds can reduce the replication rate of LECs and therefore reduce the risk of cataracts.	Raj et al., 2009
Electric currents	Presence of the currents	The lens of the eye has electric currents flowing from the equator to the posterior and anterior poles. These electric fields help to reduce cell growth. Specifically, they increase the cyclin-Cdk complex inhibitor p27kip1 and decrease the G1-specific cell cycle protein cyclin E. This results in a decrease in the number of cells moving from G1 to S phase in the cell cycle, causing a decrease in proliferation, and therefore a decreased cataract risk.	Wang et al., 2005; Raj et al., 2009

Known Feedforward/Feedback loops influencing this KER

N/A

References

Ainsbury, E. A. et al. (2016), "Ionizing radiation induced cataracts: recent biological and mechanistic developments and perspectives for future research", Mutation research. Reviews in mutation research, Vol. 770, Elsevier B.V., https://doi.org/10.1016/j.mrrev.2016.07.010

Bassnett, S. (2014), “Cell biology of lens epithelial cells”, in Lens epithelium and posterior capsular opacification, Springer, Tokyo, https://doi.org/10.1007/978-4-431-54300-8_2

Cuyàs E, et al. (2014), Cell cycle regulation by the nutrient-sensing mammalian target of rapamycin (mTOR) pathway, Methods in Molecular Biology, Humana Press, New York, https://doi.org/10.1007/978-1-4939-0888-2_7.

De Stefano, I. et al. (2021), “Contribution of genetic background to the radiation risk for cancer and non-cancer diseases in Ptch1+/- mice”, Radiation Research, Vol. 197/1, Radiation Research Society, https://doi.org/10.1667/RADE-20-00247.1

Fujimichi, Y. and N. Hamada (2014), “Ionizing irradiation not only inactivates clonogenic potential in primary normal human diploid lens epithelial cells but also stimulates cell proliferation in a subset of this population”, PloS one, Vol. 9/5, Public Library of Science, United States, https://doi.org/10.1371/journal.pone.0098154

Hamada, N. (2017), “Ionizing radiation sensitivity of the ocular lens and its dose rate dependence”, International journal of radiation biology, Vol. 93/10, Taylor & Francis, England, https://doi.org/10.1080/09553002.2016.1266407

Hanna, C. and J. E. O’Brien (1963), “Lens epithelial cell proliferation and migration in radiation cataracts”, Radiation research, Academic Press, Inc, United States, https://doi.org/10.2307/3571405

Holsclaw, D. S. et al. (1994), “Modulating radiation cataractogenesis by hormonally manipulating lenticular growth kinetics”, Experimental eye research, Vol. 59/3, Elsevier Ltd, London, https://doi.org/10.1006/exer.1994.1110

Kleiman, N. J. et al. (2007), “Mrad9 and Atm haplinsufficiency enhance spontaneous and X-ray-induced cataractogenesis in mice”, Radiation research, Vol. 168/5, Radiation Research Society, United States, https://doi.org/10.1667/rr1122.1

Kozbenko, T. et al. (2022), “Deploying elements of scoping review methods for adverse outcome pathway development: a space travel case example”, International Journal of Radiation Biology, 1–12. https://doi.org/10.1080/09553002.2022.2110306

Lett. J. T., A. C. Lee and A. B. Cox (1994), “Risks of radiation cataracts from interplanetary space missions”, Acta astronautica, Vol. 32/11, Elsevier Ltd, England, https://doi.org/10.1016/0094-5765(94)90169-4

Liu, Y. et al. (2017), “Cataracts”, The Lancet (British edition), Vol. 390/10094, Elsevier Ltd, England, https://doi.org/10.1016/S0140-6736(17)30544-5

Lovicu, F.J. et al. (2014), “Lens Epithelial Cell Proliferation”, In: Saika, S., Werner, L., Lovicu, F. (eds) Lens Epithelium and Posterior Capsular Opacification. Springer, Tokyo. https://doi.org/10.1007/978-4-431-54300-8_4

Markiewicz, E. et al. (2015), “Nonlinear ionizing radiation-induced changes in eye lens cell proliferation, cyclin K1 expression and lens shape”, Open biology, Vol. 5/4, The Royal Society, England, https://doi.org/10.1098/rsob.150011

McCarron, R. A. et al. (2022), “Radiation-induced lens opacity and cataractogenesis: a lifetime study using mice of varying genetic backgrounds”, Radiation research, Vol. 197/1, Radiation Research Society, United States, https://doi.org/10.1667/RADE-20-00266.1

National Eye Institute (2022), Cataracts, https://www.nei.nih.gov/learn-about-eye-health/eye-conditions-and-diseases/cataracts (accessed November 29, 2022).

Pendergrass, W. et al. (2010), “X-ray induced cataract is preceded by LEC loss, and coincident with accumulation of cortical DNA, and ROS; similarities with age-related cataracts”, Molecular vision, Vol. 16, Molecular Vision, United States, pp. 1496-1513

Raj et al. (2009), “Post-operative capsular opacification”, Nepalese journal of ophthalmology, Nepal, https://doi.org/10.3126/nepjoph.v1i1.3673

Saika, S. et al. (2014), Lens epithelium and posterior capsular opacification, Springer, Tokyo.

Vigneux, G. et al. (2022), “Radiation-induced alterations in proliferation, migration, and adhesion in lens epithelial cells and implications for cataract development”, Bioengineering, MDPI AG, Switzerland, https://doi.org/10.3390/bioengineering9010029

Wang, E. et al. (2005), “Electrical inhibition of lens epithelial cell proliferation: an additional factor in secondary cataract”, The FASEB journal, Vol. 19/7, Wiley, Hoboken, https://doi.org/10.1096/fj.04-2733fje

West-Mays, J. A., Pino, G., & Lovicu, F. J. (2010). “Development and use of the lens epithelial explant system to study lens differentiation and cataractogenesis”,. Progress in retinal and eye research, 29(2), 135–143. https://doi.org/10.1016/j.preteyeres.2009.12.001

Wiley, L. A. et al. (2011), “The tumor suppressor gene Trp53 protects the mouse lens against posterior subcapsular cataracts and the BMP receptor Acvr1 acts as a tumor suppressor in the lens”, Disease models & mechanisms, Vol. 4/4, The Company of Biologists Limited, England, https://doi.org/10.1242/dmm.006593

Wride, M. A. (2011), “Lens fibre cell differentiation and organelle loss: many paths lead to clarity”, Philosophical transactions. Biological sciences, Vol. 366/1568, The Royal Society, England, https://doi.org/10.1098/rstb.2010.0324

Relationship: 1913: Increase, Oxidative DNA damage leads to Increase, DNA strand breaks

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Oxidative DNA damage leading to chromosomal aberrations and mutations	non-adjacent	Moderate	Low
Deposition of energy leading to occurrence of cataracts	adjacent	Low	Low

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	Moderate	NCBI
mice	Mus sp.		NCBI
rat	Rattus norvegicus	Low	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	Moderate

Sex Applicability

Sex	Evidence
Unspecific	Moderate
Male	Low

This KER is plausible in all life stages, sexes, and organisms with DNA. The majority of the evidence is from in vivo male rats and human male adolescent in vitro models.

Key Event Relationship Description

The repair of oxidative DNA lesions produced by exposure to reactive oxygen species (ROS) involves excision repair, where damaged base is removed by glycosylases, a strand break is generated 5’ to the apurinic/apyrimidinic (AP) site by lyases and endonucleases, and finally, a new strand is synthesized across the break. Although these strand breaks are mostly transient under normal conditions, elevated levels of oxidative DNA lesions can increase the early AP lyase activities generating a higher number of SSBs that can be more persistent (Yang et al., 2004; Yang et al., 2006). These SSBs can exacerbate the DNA damage by interfering with the replication fork causing it to collapse, and ultimately becoming double strand breaks (DSBs). Additionally, SSBs in close proximity can become complex lesions to form DSBs (Caldecott, 2024).

Evidence Supporting this KER

Overall Weight of Evidence: Low

Biological Plausibility

The mechanism of repair of oxidative DNA damage in humans is well-established and numerous literature reviews are available on this topic (Berquist and Wilson III, 2012; Cadet and Wagner, 2013) Oxidative DNA damage is mostly repaired via base excision repair (BER) and via nucleotide excision repair (NER) to a lesser extent. With an increase in oxidative DNA lesions, the more glycosylase and lyase activities occur, introducing SSBs at a higher rate than at homeostasis. It is highly plausible that an increase in SSBs also increases the risk for DSBs, which are more difficult to repair accurately. Previous studies have reported thresholded dose-response curves in oxidative DNA damage and attributed these observations to failed repair at the inflection point on the curve, *thus allowing strand breaks to accumulate* (Gagne et al., 2012; Seager et al., 2012) When DNA bases sustain oxidative damage via ROS through base oxidation or deletion, this creates small nicks in the DNA strand (Cannan & Pederson, 2016). The bases guanine and adenine are most vulnerable to oxidative damage due to their low oxidation potentials (Fong, 2016). The mechanism of repair, BER, will work to fix these SSBs. If there are multiple SSBs close together in space and time, there will be many sites of BER occurring close together that can cause strain on the strand and result in the conversion of the SSBs to DSBs prior to completion of repair (Cannan & Pederson, 2016).

Empirical Evidence

The studies collected frequently address both dose and temporal concordance within a single study. Thus, we have not split out these types of empirical data by sub-headings. Instead, we indicate what evidence is available both in vitro and in vivo.

In vitro studies

- Concentration concordance in the formation of oxidative DNA lesions and strand breaks in HepG2 cells treated with nodularin (ROS-inducing substance (Bouaicha and Maatouk, 2004)) (Lankoff et al., 2006):
  - A concentration-dependent increase in oxidative lesions and strand breaks was observed after 6, 12, and 24h of treatment using Fpg-modified and regular comet assays, respectively.
    - At 6h, the increase in oxidative lesions was significant at 2.5, 5, and 10 µg/mL, while the increase strand breaks was significant at 5 and 10 µg/mL.
    - At 12 and 24 h, the increase in lesions was significant from 1 µg/mL and above, while significant increase in strand breaks occurred from 2.5 µg/mL and above.
  - At all time points, significant increase in oxidative DNA lesions occurred at a lower concentration than DNA strand breaks.
  - These results demonstrate the concentration concordance in the formation of oxidative DNA lesions and DNA strand breaks.
- Concentration and temporal concordance in human glioblastoma LN-229 cells treated with artesunate, a ROS inducing agent (Berdelle et al., 2011).
  - Concentration and time dependent increases in oxidative lesions were observed using the +Fpg comet test and immunofluorescence staining of 8-oxo-dG.
    - Significant increases in oxidative lesions were observed in cells treated with 25 µg/ml after 6 and 24 hours of treatment, but not 2 and 4 hours, using the + Fpg comet. No increases were observed using -Fpg comet.
    - Concentration-dependent increases in oxidative lesions were observed at the 24 hour timepoint using the +Fpg comet (50 and 75 µg/ml).
    - Oxidative lesions were also measured using immunofluorescence staining of 8-OxodG. Significant increases in oxidative lesions were observed at 6 and 8 hours of continuous treatment with 15 ug/ml artesunate, but not 1 and 4 hours.
      - Upon removal of test chemical, 8-OxodG levels decreased, returning to negative control level after 6 hours.
  - Significant increases in strand breaks as measured by ɣH2AX were observed 2 and 10 hours after treatment (15 µg/ml).
- Deferme et al. (2013) exposed HepG2 cells to 100 µM menadione, 200 µM tert butylhydroperoxide, and 50 µM hydrogen peroxide for increasing durations (30 min, 1, 2, 4, 6, 8, 24 h). The temporal profiles of strand breaks and oxidative lesions were analyzed. The results shown below demonstrate incidence and temporal concordance in oxidative lesion formation and strand breaks (Deferme et al., 2013).
  - Strand breaks were measured by alkaline comet assay.
  - Oxidative DNA lesions were measured by Fpg-modified comet assay
  - Menadione: strand breaks and oxidative lesions increased in a time-dependent manner from 30 min to 4h, when both reached their maximum. The tail moment values of fpg-digested comets were significantly higher than those of no-fpg comets at 1, 2, and 4h, indicating that the induction of oxidative lesions was significant at these time points. After 4h, both strand breaks and oxidative lesions gradually decreased.
  - Tert butylhydroperoxide: From 30 min to 1h, both strand breaks and oxidative lesions increased and gradually decreased from 2 to 24h. Oxidative lesion induction was significant at both 30min and 1h.
  - Hydrogen peroxide: The highest amount of strand breaks and oxidative lesions occurred at 30 min. From 1h onward, the levels of both decreased. Notably, the induction of oxidative lesions was significant at 30min and also at 1h, despite the decrease from 30min.
- Rat alveolar epithelial type II cells (AECII) were isolated from neonatal Wistar rats within 24h of birth and cultured. Cells were then incubated under either normorxic conditions (21% O_2 and 5% CO_2) or hyperoxic conditions (90% O_2 and 5% CO_2) for 12, 24, 48 or 72h (Jin et al., 2015).
  - Time-dependent increases in 8-oxodG were detected by ELISA under hyperoxic conditions;**the level of 8-oxodG at 24h was significantly higher than at 12h (p-value <0.05), and the level had further increased significantly when measured at 48h (p-value <0.05) and remained constant until 72h.**
  - At all time points, the level of 8-oxodG in hyperoxic cells was signficantly higher than in normoxic cells.
  - Time-dependent increases in DNA strand breaks were also observed in hyperoxic cells in the alkaline comet assay. The Olive tail moment in hyperoxic cells was significantly higher than in normoxic cells at all time points. **However, the time-dependent increase in strands breaks in hyperoxic cells was statistically significant only at 72h (p-value <0.01).**
  - No change was observed in the level of DNA strands breaks or 8-oxodG in normoxic cells across all time points.

In vivo studies

- Concentration concordance in Wistar rats orally exposed to ochratoxin A (OTA) and fumonisin B1 (FB1), ROS inducing agents (Domijan et al., 2006).
  - Kidney cells of male Wistar rats were examined using the comet assay +/- Fpg after oral exposure to OTA for 15 days (5ng, 0.05 mg, 0.5 mg/kg b.w.) or FB1 for 5 days (200 ng, 0.05 mg, 0.5 mg/kg b.w.).
    - Significant increases in oxidative lesions were observed using +Fpg comet at all concentrations tested

of both OTA and FB1
    - Significant increases were observed in strand breaks using the standard comet assay at all concentrations of both OTA and FB1.

Uncertainties and Inconsistencies

As demonstrated by the Domijan et al paper, results can be complicated by mixed MOA's. The comet results were positive with and without Fpg suggesting oxidative stress is not the only mechanism.

Quantitative Understanding of the Linkage

A limited number of studies explored the quantitative correlation between oxidative DNA lesions and DNA strand breaks. There are computational models availabe that describe this relationship. Spassova et al. (2015) developed a simulated kinetic model of $KBrO_3$-induced oxidative DNA damage based on Michaelis-Menten enzyme kinetics to study the effect of BER on the shape of the dose-response curve of 8-oxo-dG lesions and strand breaks (Spassova et al., 2015).

- Both time and concentration dependence of the responses were explored.
- The time course simulation of a sustained exposure at various concentrations produced a sharp increase in 8-oxo-dG immediately following exposure.
    - The authors attributed this accumulation to lagged, inefficient repair.
- This increase was later followed by a steep decrease in 8-oxo-dG lesions, accompanied by a linear increase in SSBs.
    - The repair of adducts by BER, both successful and failed, are responsible for the decrease of 8-oxo-dG; the SSBs are generated as a result of repair failure.
- Moreover, the concentration-response model of 8-oxo-dG showed a thresholded curve, where no DNA damage was observed at low concentrations due to effective repair up to a certain concentration of $KBrO_3$ indicating insufficient repair at the inflection point.

Known Feedforward/Feedback loops influencing this KER

N/A

References

Berdelle, N., Nikolova, T., Quiros, S., Efferth, T., Kaina, B. (2011), Artesunate Induces Oxidative DNA Damage, Sustained DNA Double-Strand Breaks, and the ATM/ATR Damage Response in Cancer Cells, Mol Cancer Ther, 10:2224-2233.

Berquist, B., Wilson III, D. (2012), Pathways for Repairing and Tolerating the Spectrum of Oxidative DNA Lesions, Cancer Lett, 327:61-72.

Bouaicha, N., Maatouk, I. (2004), Microcystin-LR and nodularin induce intracellular glutathione alteration, reactive oxygene species production and lipid peroxidation in primary cultured rat hepatocytes, Toxicol Lett, 148:53-63.

Cadet, J., Wagner, J.R. (2013), DNA Base Damage by Reactive Oxygen Species, Oxidizing Agents, and UV Radiation, Cold Spring Harb Perspect Biol, 5:a012559.

Cannan, W. and D. Pederson. (2016), "Mechanisms and consequences of double-strand DNA break formation in chromatin", Journal of Cell Physiology, Vol.231/1, Wiley, Hoboken, https://doi.org/10.1002/jcp.25048.

Deferme, L., Briede, J.J., Claessen, S.M., Jennen, D.G., Cavill, R., Kleinjans, J.C. (2013), Time series analysis of oxidative stress response patterns in HepG2: A toxicogenomics approach , Toxicol, 306:24-34.

Domijan, A., Zeljezic, D., Kopjar, D., Peraica, M. (2006), Standard and Fpg-modified comet assay in kidney cells of ochratoxin A- and fumonisin B(1)-treated rats, Toxciol, 222:53-59.

Fong, C.W. (2016), "Platinum anti-cancer drugs: Free radical mechanism of Pt-DNA adduct formation and anti-neoplastic effect", Free Radical Biology and Medicine, Vol.95/June 2016, Elsevier, Amsterdam, https://doi.org/10.1016/j.freeradbiomed.2016.03.006.

Gagne, J., Rouleau, M., Poirier, G. (2012), PARP-1 Activation— Bringing the Pieces Together, Science, 336:678-279.

Jin, L., Yang, H., Fu, J., Xue, X., Yao, L., Qiao, L. (2015), Association between oxidative DNA damage and the expression of 8-oxoguanine DNA glycosylase 1 in lung epithelial cells of neonatal rats exposed to hyperoxia, Mol Med Rep, 11: 4079-4086.

Kozbenko, T. et al. (2022), "Deploying elements of scoping review methods for adverse outcome pathway development: a space travel case example", International Journal of Radiation Biology, 1–12. https://doi.org/10.1080/09553002.2022.2110306

Lankoff, A., Wojcik, A., Fessard, V., Meriluoto, J. (2006), Nodularin-induced genotoxicity following oxidative DNA damage and aneuploidy in HepG2 cells, Toxicol Lett, 164:239-248.

Seager, A., Shah, U., Mikhail, J., Nelson, B., Marquis, B., Doak, S., Johnson, G., Griffiths, S., Carmichael, P., Scott, S., Scott, A., Jenkins, G. (2012), Pro-oxidant Induced DNA Damage in Human Lymphoblastoid Cells: Homeostatic Mechanisms of Genotoxic Tolerance, Toxicol Sci, 128:387-397.

Spassova, M., Miller, D., Nikolov, A. (2015), Kinetic Modeling Reveals the Roles of Reactive Oxygen Species Scavenging and DNA Repair Processes in Shaping the Dose-Response Curve of KBrO3-Induced DNA Damage, Oxid Med Cell Longev, 2015:764375.

Yang, N., Chaudry, A., Wallace, S. (2006), Base excision repair by hNTH1 and hOGG1: A two edged sword in the processing of DNA damage in gamma-irradiated human cells, DNA Repair, 5:43-51.

Yang, N., Galick, H., Wallace, S. (2004), Attempted base excision repair of ionizing radiation damage in human lymphoblastoid cells produces lethal and mutagenic double strand breaks, DNA Repair, 3:1323-1334.

List of Non Adjacent Key Event Relationships

Relationship: 2813: Energy Deposition leads to Increase, Oxidative DNA damage

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Deposition of energy leading to occurrence of cataracts	non-adjacent	Moderate	Moderate

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	Low	NCBI
mouse	Mus musculus	Moderate	NCBI
rabbit	Oryctolagus cuniculus	Moderate	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	Low

Sex Applicability

Sex	Evidence
Unspecific	Moderate

This KER is plausible in all life stages, sexes, and organisms with DNA. The majority of the evidence is from in vivo female mice and rabbits, and female human and mice in vitro models.

Key Event Relationship Description

Energy can be deposited onto biomolecules stochastically from various forms of radiation. As radiation passes through an organism, it loses energy; potentially causing direct and indirect molecular-level damage in the process. The extent of damage occurs at various levels depending on ionization and non-ionization events (excitation of molecules). Reaction with water molecules can produce reactive oxygen species (ROS). Additionally, enzymes involved in reactive oxygen and nitrogen species (RONS) production can be directly upregulated (de Jager, Cockrell & Plessis, 2017). When one ROS interacts with the DNA, it produces DNA-protein cross-links, inter and intra-strand links, and tandem base lesions. When at least two ROS associate with DNA it produces oxidatively generated clustered DNA lesions (OCDLs), more complex damage. This can include single and double strand breaks, abasic sites, and oxidized bases (Cadet et al., 2012), which can lead to chromosomal aberrations, cytotoxicity, and oncogenic transformations (Stohs, 1995) as well as structural changes to the DNA, blocking polymerases (Zhang et al., 2010). Cells contain DNA repair mechanisms that help lessen the damage, but they are not perfect and can lead to insufficient repair , resulting in sustained damage (Eaton, 1995; Ainsbury et al., 2016; Markkanen, 2017).

Evidence Supporting this KER

Overall Weight of Evidence: Moderate

Biological Plausibility

As energy is deposited in an organism, it produces ROS (Pendergrass et al., 2010; Cheng, 2019). As their formation is highly regulated, any changes can be undesirable, inducing a state of oxidative stress where cellular defense mechanisms, such as antioxidants, are overwhelmed by ROS levels (Brennan & Kantorow, 2009). A low level of DNA damage constantly exists in healthy cells, with cells acquiring an estimated 70 000 lesions per day, mostly due to ROS produced during normal metabolism and base hydrolysis (Amente et al., 2019). This number increases under oxidative stress (Lee et al., 2004). If cells replicate, any damage to their DNA that is not correctly repaired is passed on to their descendants (Wolf et al., 2008). Furthermore, these mechanisms and outcomes may vary dependent on the stressor. Different stressors may interact and produce a greater than additive effect (Di Girolamo, 2010). For example, singlet oxygen plays an important role in activating mitogen-activated protein kinases (MAPKs), which act as signal transducers to initiate DNA damage.

Throughout this process, DNA repair pathways are also activated. These include the nucleotide excision repair (NER) pathway (Mesa, 2013), and the base excision repair (BER) pathway (Cheng et al., 2019). They can repair certain amounts of damage but may become overwhelmed when faced with large numbers of DNA lesions (Lee et al., 2004). Different lesions are also repaired at different rates or with different amounts of fidelity, which can affect the amount of residual damage. For example, SSBs are usually repaired quickly (Collins, 2014), while DSBs are more complex and are therefore, less likely to be repaired correctly (Schoenfeld et al., 2012; Markkanen 2017). However, some SSBs can lead to complex lesions resulting in DSBs (Caldecott, 2024). For example, DNA single strand breaks are usually repaired quickly (Collins, 2014), while double strand breaks are more complex and are therefore less likely to be repaired correctly (Schoenfeld et al., 2012; Markkanen 2017). The efficiency and effectiveness of the repair pathways will influence the amount of residual oxidative DNA damage.

Empirical Evidence

The empirical evidence supporting this KER mostly measures different indicators of oxidative DNA damage, namely 8-OH-DG, 8-OH G, cyclobutane pyrimidine dimers, and multiple chromophores such as NADH. Research was conducted primarily in human cells and mice, mostly using UV or X-rays as a stressor. It is widely accepted that the deposition of energy, results in immediate ionization or non-ionization events leading to oxidative stress and damage to DNA molecules.

Dose Concordance

There is a limited amount of data supporting dose concordance. One study found that human LECs exposed to 0 – 5 Gy X-rays displayed a gradual increase in 8-OH-DG concentration, reaching 2.25x control at the maximum dose (Bahia et al., 2018).

Time Concordance

There are limited studies to demonstrate a consistent trend, this is due to the differences in the oxidative DNA damage marker being measured across studies. However, one study by Pendergrass et al. (2010) found that in mice exposed to 11 Gy X-rays at 2 Gy/min, the amount of 8-OH G positive DNA fragments increased from a total number of 5 to 60 from the control at 9.5 and 11 months (female, adult C57BL/6 mouse)

Essentiality

Radiation exposure has been found to increase levels of oxidative DNA damage above background levels. Bahia et al. (2018) found that human LECs exposed to 5 Gy X-rays had a level of 8-hydroxyguanosine, an oxidative DNA damage indicator, that was twice as high as in sham-irradiated cells. Similarly, Pendergrass et al. (2010) found that mice exposed to 11 Gy X-rays had 3.6 times as many 8-OH G positive DNA fragments as unirradiated mice. In both cases, radiation exposure resulted in significant increase in markers of oxidative DNA damage above baseline levels. This indirectly highlights that radiation promotes an environment of oxidative stress that can then lead to downstream modification to the DNA molecules and, in the absence of radiation, there is no further initiation of oxidative stress induced DNA damage. This is also supported by studies where a dose-response relationship is assessed; it is noted that at lower levels of deposited energy there is less oxidative DNA damage (Bahia et al., 2018). Since deposited energy initiates events immediately, the removal of deposited energy, a physical stressor, also supports the essentiality of the key event. Studies that do not deposit energy are observed to have no downstream effects.

Uncertainties and Inconsistencies

There are several uncertainties for this KER.

- Some of the data indicates that oxidative DNA damage increases as the time since exposure (Pendergrass et al., 2010; Mesa and Bassnett, 2013). However, other data found a very slight decrease (Mesa and Bassnett, 2013).
- Certain studies found that doses less than 0.5 Gy decrease ROS levels in a non-significant manner. This is thought to be due to radio-tolerance, where low doses induce defense mechanisms, such as glutathione or superoxide dismutase. As the dose is low, these defenses can overcome the effects of radiation, but as doses increase, they become overwhelmed, leading to increases in ROS levels (Bahia et al., 2018). These changes subsequently cause a similar pattern in DNA oxidative damage that dips between 0 and 0.5 Gy, where it begins to slowly increase (Bahia et al., 2018; Cheng et al., 2019).

Quantitative Understanding of the Linkage

The following tables provide representative examples of the relationship, unless otherwise indicated, all data is statistically significant.

Dose Concordance

Reference	Experiment Description	Result
Bahia et al., 2018	In vitro. Human lens epithelial cells were exposed to X rays at either 1.62 cGy/min or 38.2 cGy/min. 20 min HPLC-CoulArray analysis was used to determine 8-hydroxy deoxyguanosine (8-OH-DG) concentration.	Human LECs exposed to 0 – 5 Gy X-rays displayed a gradual increase in 8-OH-DG concentration, reaching 2.25x control at the maximum dose.
Cheng et al., 2019	In vitro. Human SRA01/04 lens epithelial cells exposed to UVB (312 nm peak, 280-320 nm range), the 8-OHdG ELISA assay was used to measure the amount of 8-OHdG present.	In cells exposed to UVB, the addition of siRNA2, a type of H19-siRNA, caused a 1.2x increase in 8-OHdG relative to control. Similarly, the addition of H19 into a pcDNA vector caused a 1.25x decrease in 8-OHdG relative to control.

Incidence Concordance

No data found.

Time Concordance

Reference	Experiment Description	Result
Pendergrass et al., 2010	In vivo. Female, adult, C57BL/6 mouse lenses received whole-body irradiation with 11 Gy X-rays at 2 Gy/min. Immunofluorescence was then used to determine the number of 8-OH-dG positive cortical nuclear fragments beneath the central zone.	In mice lenses immediately exposed in vivo to 11 Gy X-rays the level of 8-OH G positive DNA fragments increased to 3.5x control 9.5 months post-irradiation.

Response-response relationship

As the time since irradiation increases, damage levels slowly increase during the first few months, but begin to rise more quickly as time passes (Pendergrass et al., 2010; Mesa and Bassnett, 2013).

Known modulating factors

Modulating Factor (MF)	MF Specification	Effect(s) on the KER	Reference(s)
Antioxidants	Increased concentration, examples of antioxidants studied include glutathione and superoxide dismutase	Antioxidants scavenge ROS, resulting in a decrease in oxidative DNA damage.	Pendergrass et al., 2010; Bahia et al., 2018
UV absorbing contact lenses	Examples include senofilcon A	Helps to protect the eye against high doses of UVA, therefore decreasing oxidative DNA damage.	Giblin et al., 2012
Xeroderma pigmentosum	Presence of the genetic condition	Increases sensitivity to UV-induced oxidative DNA damage by affecting the nucleotide excision repair system.	Di Girolamo, 2010
lncRNA H19	Knockdown of lncRNA H19	Increases sensitivity to UVB-induced oxidative DNA damage by affecting the nucleotide excision repair system.	Cheng et al., 2019
Low radiation doses	Radiotolerance	Cells may display radio-tolerance by activating ROS scavenger defense mechanisms at low doses, resulting in a decrease in ROS levels and therefore a decrease in oxidative DNA damage, compared to the control. However, at higher doses these defenses are overwhelmed, and ROS levels rise.	Bahia et al., 2018
Replication rate	Increased replication	Cells that are actively replicating have increased rates of photolesion repair, and therefore, lower rates of oxidative DNA damage, as opposed to quiescent cells.	Mesa & Bassnett, 2013

Known Feedforward/Feedback loops influencing this KER

N/A

References

Ainsbury, E. A. et al. (2021), “Radiation-induced lens opacities: Epidemiological, clinical and experimental evidence, methodological issues, research gaps and strategy”, Environment international, Vol. 146, Elsevier Ltd, Netherlands, https://doi.org/10.1016/j.envint.2020.106213

Amente, S. et al. (2019), “Genome-wide mapping of 8-oxo-7,8-dihydro-2’-deoxyguanosine reveals accumulation of oxidatively-generated damage at DNA replication origins within transcribed long genes of mammalian cells”, Nucleic Acids Research 2019, Vol. 47/1, Oxford University Press, England, https://doi.org/10.1093/nar/gky1152

Bahia, S. et al. (2018), “Oxidative and nitrative stress-related changes in human lens epithelial cells following exposure to X-rays”, International journal of radiation biology, Vol. 94/4, England, https://doi.org/10.1080/09553002.2018.1439194

Brennan, L. A. and M. Kantorow (2009), “Mitochondrial function and redox control in the aging eye: Role of MsrA and other repair systems in cataract and macular degernerations”, Experimental Eye Research, Vol. 88/2, Elsevier Ltd, England, https://doi.org/10.1016/j.exer.2008.05.018

Cadet, J. et al. (2012), “Oxidatively generated complex DNA damage: tandem and clustered lesions”, Cancer letters, Vol. 327/1, Elsevier Ireland Ltd, Ireland, https://doi.org/10.1016/j.canlet.2012.04.005

Caldecott K. W. (2024). “Causes and consequences of DNA single-strand breaks”,. Trends in biochemical sciences, Vol.49/1(1), 68–78. https://doi.org/10.1016/j.tibs.2023.11.001.

Cheng, T. et al. (2019), “lncRNA H19 contributes to oxidative damage repair in the early age-related cataract by regulating miR-29a/TDG axis”, Journal of cellular and molecular medicine, Vol. 23/9, Wiley Subscription Services, Inc. England, https://doi.org/10.1111/jcmm.14489

Collins, A. R. (2014), “Measuring oxidative damage to DNA and its repair with the comet assay”, Biochimica et biophysica acta. General subjects, Vol. 1840/2, Elsevier B.V., https://doi.org/10.1016/j.bbagen.2013.04.022

de Jager, T.L., Cockrell, A.E., Du Plessis, S.S. (2017), “Ultraviolet Light Induced Generation of Reactive Oxygen Species”, in Ultraviolet Light in Human Health, Diseases and Environment. Advances in Experimental Medicine and Biology, Springer, Cham, https://doi.org/10.1007/978-3-319-56017-5_2

Di Girolamo, N. (2010), “Signalling pathways activated by ultraviolet radiation: role in ocular and cutaneous health”, Current pharmaceutical Design, Vol. 16/12, Benthem Science Publishers Ltd, https://doi.org/10.2174/1381-6128/10

Eaton, J. W. (1995), “UV-mediated cataractogenesis: a radical perspective”, Documenta ophthalmologica, Vol. 88/3-4, Springer, Dordrecht, https://doi.org/10.1007/BF01203677

Giblin, F. J. et al. (2012), “A class I UV-blocking (senofilcon A) soft contact lens prevents UVA-induced yellow fluorescence and NADH loss in the rabbit lens nucleus in vivo”, Experimental eye research, Vol. 102, Elsevier Ltd, England, https://doi.org/10.1016/j.exer.2012.06.007

Kozbenko, T. et al. (2022), “Deploying elements of scoping review methods for adverse outcome pathway development: a space travel case example”, International Journal of Radiation Biology, 1–12. https://doi.org/10.1080/09553002.2022.2110306

Lee, J. et al. (2004), “Reactive oxygen species, aging, and antioxidative nutraceuticals”, Comprehensive reviews in food science and food safety, Vol. 3/1, Blackwell Publishing Ltd, Oxford, https://doi.org/10.1111/j.1541-4337.2004.tb00058.x

Markkanen, E. (2017), “Not breathing is not an option: How to deal with oxidative DNA damage”, DNA repair, Vol. 59, Elsevier B.V., Netherlands, https://doi.org/10.1016/j.dnarep.2017.09.007

Mesa, R. and S. Bassnett (2013), “UV-B induced DNA damage and repair in the mouse lens”, Investigative ophthalmology & visual science, Vol. 54/10, the Association for Research in Vision and Ophthalmology, United States, https://doi.org/10.1167/iovs.13-12644

Pendergrass, W. et al. (2010), “X-ray induced cataract is preceded by LEC loss, and coincident with accumulation of cortical DNA, and ROS; similarities with age-related cataracts”, Molecular vision, Vol. 16, United States, pp. 1496-1513

Schoenfeld, M. P. et al. (2012), “A hypothesis on biological protection from space radiation through the use of new therapeutic gases as medical counter measures”, Medical gas research, Vol. 2/1, BioMed Central Ltd, India, https://doi.org/10.1186/2045-9912-2-8

Stohs, S. J. (1995), “The role of free radicals in toxicity and disease”, Journal of Basic and Clinical Physiology and Pharmacology, Vol. 6/3-4, https://doi.org/10.1515/JBCPP.1995.6.3-4.205

Wolf, N. et al. (2008), “Radiation cataracts: mechanisms involved in their long delayed occurrence but then rapid progression”, Molecular vision, Molecular Vision, United States, pp. 274-285

Zhang, Y. et al. (2010), “Oxygen-induced changes in mitochondrial DNA and DNA repair enzymes in aging rat lens”, Mechanisms of ageing and development, Vol. 131/11, Elsevier Ireland Ltd, Clare, https://doi.org/10.1016/j.mad.2010.09.003

Relationship: 1981: Energy Deposition leads to Increase, Mutations

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Deposition of energy leading to lung cancer	non-adjacent	High	High
Deposition of energy leading to occurrence of cataracts	non-adjacent	High	High

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	High	NCBI
rat	Rattus norvegicus	High	NCBI
mouse	Mus musculus	High	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Unspecific	High

The domain of applicability applies to single-celled organisms such as bacteria and yeast, eukaryotic cells, and multi-cellular organisms such as fish, mice and humans.

Key Event Relationship Description

Energy can be deposited on biomolecules from various forms of radiation. Radiation with high linear energy transfer (LET) tends to produce more complex, dense structural damage than low LET radiation; both, however, can lead to detrimental damage within a cell (Hada & Georgakilas, 2008; Okayasu, 2012; Lorat et al., 2015; Nikitaki et al., 2016). The DNA is particularly susceptible to damage which can be in the form of DNA strand breaks and the inadequate repair of these lesions can lead to mutations. DNA damage can be caused by direct and indirect mechanisms. Indirect involves formation of free radicals from the breakage of water molecules that can oxidize DNA and direct involves action on the DNA leading to strand breaks and complex lesions (Cannan & Pederson, 2016). Mutations may occur in germ cells or somatic cells; mutations in germ stem and progenitor cells are often of the greatest concern, as they may persist and be propagated to offspring. Regardless of the cell type, there are several different categories of mutations including: missense, nonsense, insertion, deletion, duplication, and frame-shift mutations. These mutations can present with different downstream effects which are not predictable but can potentially initiate a path to carcinogenesis.

Evidence Supporting this KER

Biological Plausibility

The biological rationale for linking direct deposition of energy by ionizing radiation to mutation induction is strong. The structural and functional relationships in this KER contribute sufficiently to the overall biological plausibility.

There are numerous studies that demonstrate, using various model systems, an increase in mutation frequency in response to radiation exposure (Russell et al., 1957; Winegar et al., 1994; Gossen et al., 1995; Suzuki & Hei 1996; Albertini et al., 1997; Dubrova et al., 1998; Kraemer et al., 2000; Dubrova, Plumb, et al., 2000; Canova et al., 2002; Dubrova et al., 2002; Dubrova & Plumb, 2002; Masumura et al., 2002; Somers et al., 2004; Burr et al., 2007; Ali et al., 2012; Bolsunovsky et al., 2016; Mcmahon et al., 2016; Matuo et al., 2018; Nagashima et al., 2018; Wu et al., 1999; Hei et al., 1997; Nagasawa and Little, 1999; Barnhart and Cox, 1979; Thacker et al., 1982; Zhu et al., 1982; Metting et al., 1992; Schwartz et al., 1991; Chen et al., 1984; Albertini et al., 1997). The process of mutation induction by radiation is initiated when cells are exposed to ionizing radiation. These high-energy waves or particles interact with the genetic material in the nucleus, damaging the DNA and triggering a cascade of signalling events and activities aimed at repairing the damage. It has been shown that various dose rates of radiation exposure can lead to distinct types of damage. High dose-rate radiation has been observed to generate a higher number of DNA strand breaks, resulting in a variety of mutations, including small base changes and deletions. Moreover, the likelihood of insufficient repair is elevated, contributing to an overall increase in mutation frequency. In contrast, low dose-rate radiation has been found to have a significantly lower mutation frequency, particularly in deletions and rearrangements (Brooks et al., 2016; Sankaranarayanan & Nikjoo, 2015). Of note, radiation is not likely to impact only one gene; more often than not, the random nature of energy deposition by radiation results in mutations to many genes and genomic sites clustered in the same area (Sankaranarayanan & Nikjoo, 2015;

Adewoye et al., 2015). Many of the radiation-induced mutations have been documented as deletions (Gossen et al., 1995; Behjati et al., 2016), often of differing sizes in a number of different genes (Sankaranarayanan & Nikjoo, 2015). The mechanism for radiation-induced mutations is thought to be similar to the process for spontaneously-occurring mutations, as the structure of radiation-induced mutations examined at expanded simple tandem repeat (ESTR) loci was not found to differ from the structure of spontaneous mutations (Dubrova, 2005). Moreover, exposure to radiation may produce specific mutational signatures. Two ionizing radiation-specific mutational signatures were found when 12 radiation-induced secondary tumours across 4 different tumour types underwent whole-genome sequencing and bioinformatics processing. In particular, these radiation-exposed tumours were significantly enriched in small deletions and balanced inversions. These results were validated when the same mutational signatures were observed in radiation-exposed but not radiation-naïve prostate tumours from a previously-published dataset (Behjati et al., 2016). Similarly, another study examining mutations present in radiation-induced tumours of *Nf1* heterozygous and wild-type mice revealed three distinctive mutational signatures. Interestingly, these signatures were found in all of the tumours regardless of its histology or of the animal's genotype. Moreover, these signatures were still present after removal of the 33 most mutated samples from the analysis, after analysis of only the non-synonymous substitutions, and after analysis of only the synonymous substitutions (though the third mutational signature could not be extracted in this last analysis group) (Sherborne et al. 2015). There were also common cellular pathways that were found to be frequently mutated in the tumours of these mice. In sarcomas from mice of both genetic backgrounds (*Nf1* heterozygous and wild-type), the top two pathways harbouring mutations were those influencing cellular assembly and organization, and those involved in cellular function and maintenance. Additionally, Ras pathways were commonly mutated in tumours from both genetic backgrounds. Specific to wild-type sarcomas, mutations were also found in cell cycle and cell signalling pathways (Sherborne et al., 2015). Supporting the finding that different genetic backgrounds in mice do not affect mutational signatures in tumours (Sherborne et al., 2015), there also does not appear to be strain-specific differences in ESTR mutational frequencies in response to radiation. One study examined five different strains of male mice that were irradiated and mated to unirradiated females at least 4 weeks post-irradiation. Although there was a difference in doubling doses between strains, the ESTR mutations themselves were not significantly different. Furthermore, there were no significant differences found between strains in terms of germline mutation induction (Dubrova, 2005).

Germline mutations have been further interrogated in studies examining the effects of radiation exposure on germ cells. There is evidence from mouse studies suggesting that the germ cells of radiation-exposed males have elevated ESTR mutations and that the offspring of these irradiated males inherit more ESTR mutations as a result of the germline mutations (Dubrova et al., 1998; Dubrova, Bersimbaev, et al., 2000; Dubrova & Plumb, 2002; Somers et al., 2004; Barber et al., 2009; Ali et al., 2012; T.E. Wilson et al., 2015). This was reviewed by Somers et al. (2006). Interestingly, *in utero* irradiation of embryos at day 12 resulted in increased ESTR mutations across several tissue types in males and females; however, only the offspring of the irradiated males showed an elevated ESTR mutation rate (Barber et al., 2009). On a genome-wide scale, the offspring of irradiated males were found to have significantly more clustered single nucleotide variants (SNVs) and insertion/deletion events compared to offspring from unirradiated fathers (Adewoye et al., 2015).

Human studies have also shown correlations in radiation exposure and increased germline mutations. This relationship was assessed in families exposed accidently to high doses of ionizing radiation after the Chernobyl accident in Ukraine, and in families living in close proximity to the Semipalatinsk nuclear test site in Kazakhstan. In both cases, germline mutations were evaluated using eight hypervariable minisatellite probes. In the Chernobyl study, the paternal mutation rate in the exposed group was significantly increased by 1.6-fold relative to an unexposed control group; there was, however, no significant difference in the maternal germline mutation rates between the exposed group and the unexposed control group (Dubrova et al., 2002C). In the Semipalatinsk study, analysis of families living in the affected region over three generations found that germline mutations in the first and second generation were significantly increased relative to unexposed families living in a low-radiation area. Overall, the germline mutation rate in the families exposed to radiation from this test site was doubled (Dubrova, Bersimbaev, et al., 2000).

Empirical Evidence

Overall, there is strong supporting evidence that direct deposition of energy increases the frequency of mutations.The evidence presented below is summarized in table 2, here (click link). In general, exposure to ionizing radiation has been documented to elevate mutation frequencies in a number of different studies spanning different models and cell types (Russell et al., 1957; Winegar et al., 1994; Gossen et al., 1995; Suzuki & Hei, 1996; Albertini et al., 1997; Kraemer et al., 2000; Canova et al., 2002; Dubrova & Plumb, 2002; Masumura et al., 2002; Bolsunovsky et al., 2016; Mcmahon et al., 2016; Matuo et al., 2018; Nagashima et al., 2018; Wu et al., 1999; Hei et al., 1997; Nagasawa and Little, 1999; Barnhart and Cox, 1979; Thacker at al., 1982; Zhu et al., 1982; Metting et al., 1992; Schwartz et al., 1991; Chen et al., 1984; Albertini et al., 1997) . Furthermore, several reviews outline evidence of the relationship specifically between radon gas exposure and mutation frequency (Jostes, 1996; Robertson et al., 2013; ICRP, 2005). At low doses (<1 Gy) the induction of mutations in cells has been observed for high-LET radiation such as alpha particles (Wu et al., 1999; Hei et al., 1997; Nagasawa and Little, 1999; Barnhart and Cox, 1979; Thacker at al., 1982; Zhu et al., 1982; Metting et al., 1992; Schwartz et al., 1991; Chen et al., 1984; Albertini et al., 1997).

Figure 1: *Plot of example studies (y-axis) against equivalent dose (Sv) used to determine the empircal link between direct deposition of energy and increased cell mutation rates. The z-axis denotes the equivalent dose rate used in each study. The y-axis is ordered from low LET to high LET from top to bottom.*

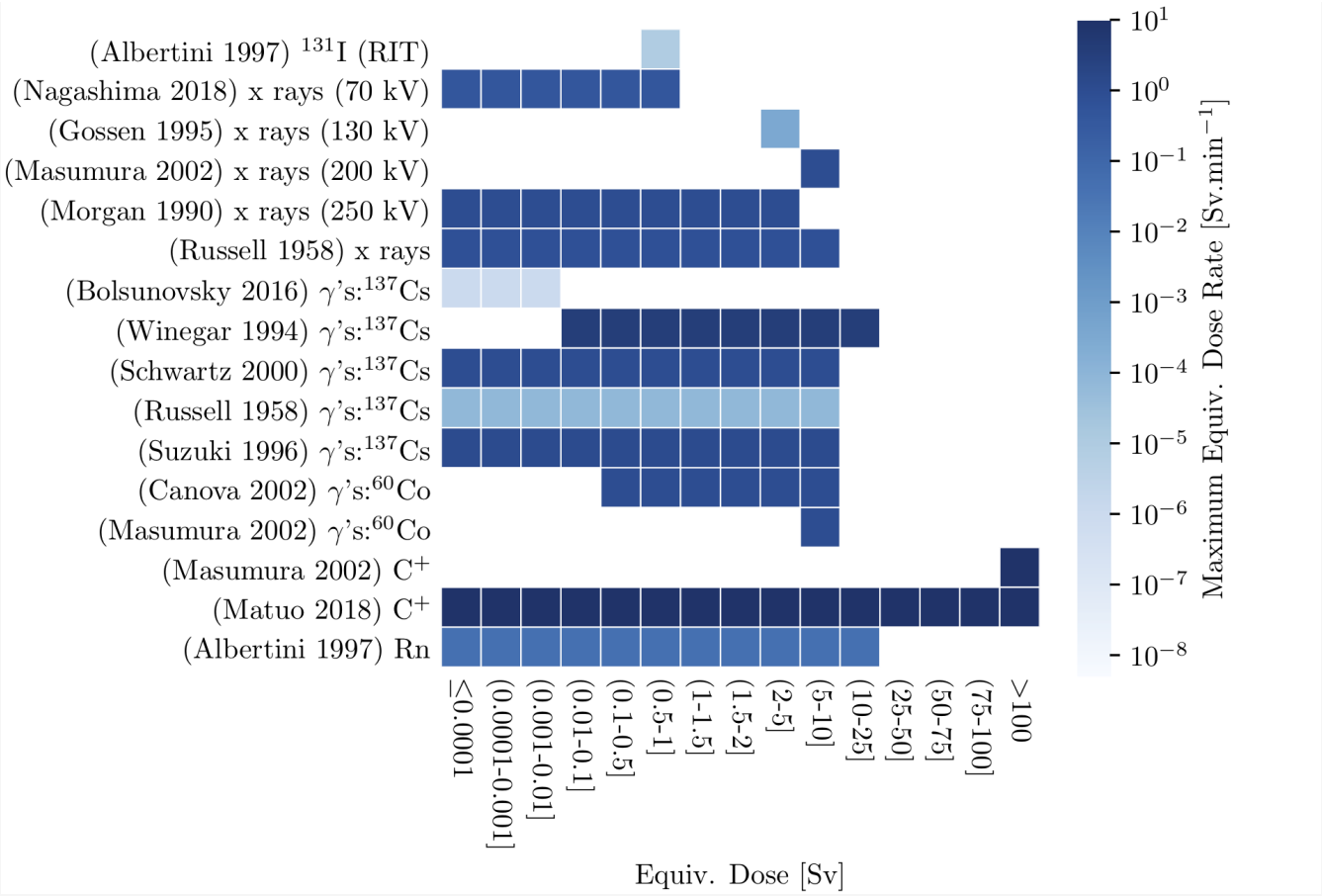
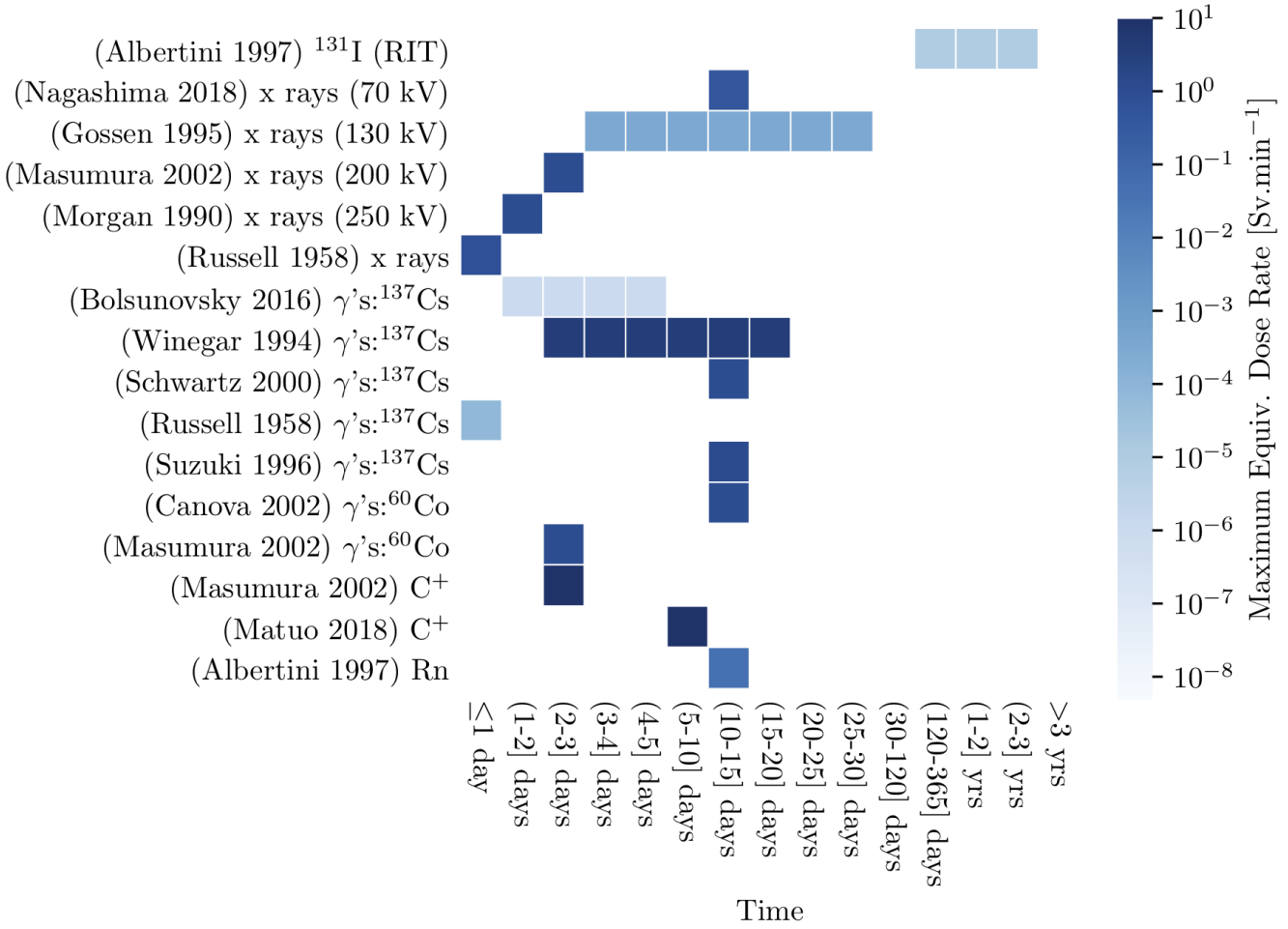


Figure 2: *Plot of example studies (y-axis) against time scales used to determine the empircal link between direct deposition of energy and and increased cell mutation rates. The z-axis denotes the equivalent dose rate used in each study. The y-axis is ordered from low LET to high LET from top to bottom.*



Dose and Incidence Concordance

It is clear that increasing doses of ionizing radiation is concordant with increased incidence of mutations (see table under ***Quantitative Understanding of the Linkage***). Extensive evidence from *in vitro* studies using human cells (Suzuki & Hei 1996; Canova et al., 2002), animal cells (Schmidt and Kiefer, 1998; Canova et al., 2002; Mcmahon et al., 2016; Nagashima et al., 2018), hybrid cell lines (Kraemer et al., 2000), yeast cells (Matuo et al., 2018), and bacteria (Bolsunovsky et al., 2016) demonstrates this concordance. *In vivo* studies using mice have also found a dose-dependent increase in mutations across several different types of radiation (Russell et al., 1957; Dubrova & Plumb 2002).

This relationship between radiation exposure and mutation incidence is impacted by several different factors. Higher LET radiation, such as high LET carbon ions and neutrons, were found to induce more mutations in comparison to radiation of a lower LET, including low LET carbon ions, gamma-rays and X-rays (Dubrova & Plumb, 2002; Matuo et al., 2018). Similarly, more mutations were present in the gametes of mice exposed to acute X-rays compared to those exposed to chronic gamma-rays (Russell et al., 1957). The tissue being irradiated may also have a role in determining mutant frequency, as whole body irradiation of mice led to a significant increase in mutations (mostly deletions) of the spleen, liver, lung and kidneys (Gossen et al., 1995; Masumura et al., 2002), but not the testis (Masumura et al., 2002). Furthermore, the specific kind of mutation may be dependent on the type of radiation. In one study, irradiation of the liver with carbon ions resulted in a significant increase in deletion mutations, while irradiation with X-rays or gamma-rays resulted in a significant increase in point mutations (Masumura et al., 2002).

Temporal Concordance

Temporal concordance is well established. As described above, energy deposition happens immediately upon radiation exposure, with an increased incidence of mutations documented days or weeks after irradiation (Russell et al., 1957; Winegar et al., 1994; Gossen et al., 1995; Albertini et al., 1997; Canova et al., 2002; Dubrova & Plumb, 2002; Masumura et al., 2002; Matuo et al., 2018; Nagashima et al., 2018).

Essentiality

Not identified.

Uncertainties and Inconsistencies

Uncertainties and inconsistencies in this KER are as follows:

1. In a review paper describing the role ionizing radiation plays in elevating mutation frequency in the germline and therefore genetic risk, Sankaranarayanan & Nikjoo (2015) stated that most radiation-induced mutations tended to be deletions. In contrast, an examination of ESTR loci mutations in offspring and their irradiated fathers found that the ESTR mutations tended to be gains more often than losses (Dubrova

,2005). This may, however, highlight a characteristic specific to ESTR mutations rather than mutations in general.

2. In a study examining the long-term of effects of *in utero* radiation exposure, males irradiated at embryonic day 12 showed significant increases in both somatic and germline ESTR mutations as adults, and produced offspring with significantly elevated ESTR mutations in their sperm (Barber et al., 2009). In contrast, male mice exposed to radiation during their neonatal days (6 - 8 days old) or pubertal stage (18 - 25 days) did not have increased mutations in adult spermatozoa, as mutant frequencies that were present in spermatogenesis stages immediately after radiation returned to normal levels later in the spermatogenesis process (Xu et al., 2008).
3. Factors such as dose, dose-rate, tissue type and radiation quality can influence mutation rate induction (Hooker et al., 2004; Rydberg et al., 2005; Day et al., 2007; Okudaira et al., 2010; Brooks et al., 2016).
4. Difference in measurements of mutational frequency can affect the interpretation of the data.

Quantitative Understanding of the Linkage

Below are representative examples of the mutation frequency rates across different studies. Overall, a quantitative understanding of this linkage suggests that mutation rates can be predicted and are dependent on the type and dose of radiation exposure.

Reference	Summary
Matuo et al., 2018	Study of impact of high and low LET radiation (high LET: carbon ions, 25 keV/um, low LET: carbon ions, 13 keV/um) in the dose range of 0 - 200 Gy incident on Saccharomyces cerevisiae (yeast cells). Found a 24-fold increase over baseline of mutations from high LET radiation and an 11-fold increase for low LET radiation.
Nagashima et al, 2018	Study of X-rays incident on GM06318-10 hamster cells in the dose range of 0-1 Gy. Found a calculated mutation rate of 19.0 ± 6.1 mutants per 10^4 survivors per Gy.
Albertini et al., 1997	Study of T-lyphocytes from human peripheral blood exposed to low LET gamma-rays and high LET radon gas. Doses in the range 0.5 - 5 Gy (gamma-rays) and 0 - 1 Gy (radon gas). The calculated mutation rate was as follows: gamma-rays (0-2 Gy): $7.0x10^{-6}$ mutants / Gy, gamma-rays (2-4 Gy): $54.0x10^{-6}$ mutants / Gy, radon gas (0-1 Gy): $63.0x10^{6}$ mutants / Gy.
Dubrova and Plumb 2002	Study of paternal ESTR mutation rates in CBA/H mice. Mice exposed to acute low LET X-rays, chronic low LET gamma-rays and chronic high LET neutrons. X-rays in the 0 - 1 Gy dose (D) range, gamma-rays: 0 - 1 Gy & neutrons: 0 - 0.5 Gy. Calculated mutation rate (y) (of the form y = a + bD) as follows: X-rays (a, b := 0.111, 0.338), gamma-rays (a,b := 0.110, 0.373 ± 0.082), neutrons (a, b := 0.136, 1.135 ± 0.202).
McMahon et al., 2016	Study across various studies of the HPRT gene in chinese hamster cells exposed to doses in the range of 1 - 6 Gy. Found 0.2 mutations in HPRT gene per 10^4 cells and 0.1 point mutations per 10^4 cells (1 Gy). At higher doses (6 Gy) observed 1.5 mutations per 10^4 cells and 0.4 point mutations per 10^4 cells.

Response-response relationship

There is evidence of a positive response-response relationship between the radiation dose and the frequency of mutations (Russell et al., 1957; Suzuki & Hei, 1996; Albertini et al., 1997; Kraemer et al., 2000; Canova et al., 2002; Dubrova & Plumb, 2002; J.W. Wilson et al., 2015; Bolsunovsky et al., 2016; Mcmahon et al., 2016; Nagashima et al., 2018) . Most studies found that the response-response relationship was linear (Russell et al., 1957; Albertini et al., 1997; Canova et al., 2002; Dubrova et al., 2002; Nagashima et al., 2018). There were however, two exceptions. In a study using normal human bronchial epithelial cells irradiated with 1 - 6 Gy of gamma-rays, the relationship between the number of induced *HPRT* mutants and the radiation dose was described as non-linear (Suzuki & Hei, 1996) Similarly, in a study examining *HPRT* mutations in isolated peripheral blood T-lymphocytes irradiated with low LET gamma-rays, the slope of the line from 0 - 2 Gy differed from the slope at the 2 - 4 Gy interval; thus this was described as two different linear relationships or an overall linear-quadratic relationship (Albertini et al., 1997). In a study with V79 Chinese hamster cells, a curvilinear response was also seen as a result of x-ray response while a linear response was seen for Am-241 alpha-particle exposure (Schmidt and Keifer, 1998).

Time-scale

The time scale relationship between radiation exposure and the frequency of mutations is not well defined. Most studies look for manifestation of mutations days or weeks after irradiation, making it particularly difficult to pinpoint exactly when the mutations first occur. Analysis of various organs from mice after *in vivo* radiation found that mutations were present at 2 days (Winegar et al., 1994; Masumura et al., 2002) and 3 days (Gossen et al., 1995)(Gossen, 1995) post-exposure. Mutations were still present at 7 days and 14 days (Winegar et al., 1994), and 10 days and 21 days (Gossen, 1995) following irradiation. One study documented a doubling in the number of mutations from 7 to 14 days (Winegar et al., 1994) while the other reported a two-fold decrease from 3 to 21 days (Gossen et al., 1995).

An attempt to better define this time scale relationship was made in a study using *Salmonella typhimurium* bacteria. This study was designed to determine how mutation frequency was affected by constant cesium-137 gamma-ray radiation exposure at defined dose rates of 67.8 uGy/hour, 3.2 uGy/hour, and 0.6 uGy/hour; these mutation frequencies were compared to a control group exposed to background radiation levels (0.09 uGy/hour). Mutation frequencies were evaluated after 24, 48, 72 and 96 hours of constant exposure. At 24 hours, the 67.8 uGy/hour, 3.2 uGy/hour and 0.6 uGy/hour mutant frequencies were significantly higher than background exposure controls. Interestingly, however, these levels were decreased at 48 hours and continued to decline gradually towards control frequencies over time. This decline was proposed to be due to an elimination of the highly mutated cells, leaving behind an increasing number of cells that had adapted to the radiation and were thus more equipped for survival (Bolsunovsky et al., 2016). Other studies are required to build a more complete understanding of this timeline.

Known modulating factors

There are several factors that have been documented to affect the relationship between direct deposition of energy and increased mutation frequency. The sex, age, and use of adaptive dosing have been demonstrated to affect the radiation-induced mutations present in offspring. In contrast to male mice, female mice that were irradiated in utero (Barber et al., 2009) or as adults (Ali et al., 2012)(Ali, 2012) did not produce offspring with increased ESTR mutations. This suggests that radiation-induced mutations are only heritable through the paternal line. As such, the age of the father may affect the mutant frequency in the offspring, as increased mutations were present in spermatogenic cells of older male mice relative to younger males both at baseline levels and post-irradiation (Xu et al., 2012). Lastly, the use of 'adaptive' radiation dosing, or giving a very small dose 24 hours prior to the full radiation dose, may also affect offspring's mutational frequency. In male mice who received adaptive dosing relative to males who received only the full radiation dose, there were significant decreases in germline mutation frequencies and in the rate of paternal mutations in their offspring (Somers et al., 2004) .

The radiation-mutation relationship may also be impacted by the genetics of the organism, as the genotype appears to play an important role in determining how the biological system responds to radiation. In yeast with inactivated *rad50* or *rad52*, the radiation-induced mutation frequency was significantly increased relative to wild-type yeast (Matuo et al., 2018). *Msh2* knock-out mice (Burr et al., 2007) and medaka fish (Otozai et al., 2014) both had significantly increased baseline mutation frequencies relative to wild-type animals. Irradiation, however, did not change this mutation rate from baseline for these *Msh2* knock-out animals (Burr et al., 2007; Otozai et al., 2014). Similarly, *BRCA2* knock-out embryos had significantly elevated baseline mutation rates relative to wild-type littermates; however, *in utero* radiation was found to increase the mutation rate of all genotypes. Thus irradiated *BRCA2* knock-out embryos also had a significantly increased mutation frequency relative to wild-type embryos by approximately three-fold (Tutt et al., 2002). Finally, baseline mutation levels in *p53* knock-out medaka fish did not differ from wild-types; however, *p53* knock-out fish exposed to radiation were found to have a 24-fold increase in mutation frequency relative to unirradiated *p53* knock-out fish (Otozai et al., 2014). Construction of a dose response curve found the following mutation rates for wild-type, *Msh2* knock-out, *p53* knockout, and *Msh2/p53* double knock-out medaka fish, respectively: $1.1x10^{-4}$ mutations/allele/Gy, $1.1x10^{-4}$ mutations/allele/Gy, $4.3x10^{-4}$ mutations/allele/Gy, and $5.6x10^{-4}$ mutations/allele/Gy (Otozai et al., 2014).

Finally, factors such as dose, dose-rate, tissue type and radiation quality can influence mutation rate induction (Suzuki & Hei ,1996; Hooker et al., 2004; Rydberg et al., 2005; Day et al., 2007; Okudaira et al., 2010; Brooks et al., 2016).

Known Feedforward/Feedback loops influencing this KER

Not identified.

References

Adewoye, A.B. et al. (2015), "Mutation induction in the mammalian germline.", Nature Comm. 6:(6684), doi:10.1038/ncomms7684.

Albertini, R.J. et al. (1997), "Radiation Quality Affects the Efficiency of Induction and the Molecular Spectrum of HPRT Mutations in Human T Cells", Radiat Res. 148(5 Suppl):S76-86

Ali, A.H.E., R.C. Barber & Y.E. Dubrova (2012), "The effects of maternal irradiation during adulthood on mutation induction and transgenerational instability in mice.", Mutat Res. 732:21–25. doi:10.1016/j.mrfmmm.2012.01.003.

Barber, R.C. et al. (2009), "The effects of in utero irradiation on mutation induction and transgenerational instability in mice.", Mutat Res. 664:6–12. doi:10.1016/j.mrfmmm.2009.01.011.

Barnhart BJ and SH Cox. 1979. Mutagenicity and Cytotoxicity of 4.4-MeV alpha-particles Emitted by Plutonium-238. Radiat Res. 80:542-548.

Behjati, S. et al. (2016), "Mutational signatures of ionzing radiation in second malignancies". 7:12605, doi:10.1038/ncomms12605.

Bolsunovsky, A. et al. (2016), "Low doses of gamma-radiation induce SOS response and increase mutation frequency in Escherichia coli and Salmonella typhimurium cells.", Ecotoxicol Environ Saf. 134:233–238. doi:10.1016/j.ecoenv.2016.09.009.

Brooks, A.L., D.G. Hoel & R.J. Preston (2016), "The role of dose rate in radiation cancer risk: evaluating the effect of dose rate at the molecular, cellular and tissue levels using key events in critical pathways following exposure to low LET radiation.", Int. J. Radiat. Biol. 92(8):405–426. doi:10.1080/09553002.2016.1186301.

Burr, K.L. et al. (2007), "The effects of MSH2 deficiency on spontaneous and radiation-induced mutation rates in the mouse germline.", 617(1-2):147–151. doi:10.1016/j.mrfmmm.2007.01.010.

Canova, S. et al. (2002), "Minisatellite and HPRT Mutations in V79 And Human Cells Irradiated with Gamma Rays.", Radiat Prot. Dosimetry, 99:207–209. doi: 10.1093/oxfordjournals.rpd.a006763

Chen DJ, Striniste GF, Tokita N. 1984. The Genotoxicity of Alpha Particles in Human Embryonic Skin Fibroblasts. Radiat Res. 100:321-327.

Day, T.K. et al. (2007), "Adaptive Response for Chromosomal Inversions in pKZ1 Mouse Prostate Induced by Low Doses of X Radiation Delivered after a High Dose.", Radiat Res. 167(6):682–692. doi:10.1667/rr0764.1.

Dubrova, Y.E. (2005), "Radiation-Induced Mutation at Tandem Repeat DNA Loci in the Mouse Germline: Spectra and Doubling Doses", Radiat Res., 163(2):200-207 doi: 10.1667/RR3296.

Dubrova, Y.E. et al. (2002), "Nuclear Weapons Tests and Human Germline Mutation Rate.", Science, 295(5557):1037, doi:10.1126/science.1068102.

Dubrova, Y.E. et al. (2002), "Elevated Minisatellite Mutation Rate in the Post-Chernobyl Families from Ukraine.", Am. J. Hum. Genet. 74(4):801-809, doi: 10.1086/342729.

Dubrova, Y.E. et al. (2000), "Induction of minisatellite mutations in the mouse germline by low-dose chronic exposure to Y -radiation and fission neutrons.", Mutat Res. 453(1):17–24. doi: 10.1016/s0027-5107(00)00068-3.

Dubrova. Y.E. et al. (1998), "Stage specificity, dose response, and doubling dose for mouse minisatellite germ-line mutation induced by acute radiation.", Proc. natl. Acad. Sci. 95(11):6251–6255. doi: 10.1073/pnas.95.11.6251.

Dubrova, Y.E. & M.A. Plumb (2002), "Ionising radiation and mutation induction at mouse minisatellite loci The story of the two generations.", Mutat Res., 499(2):143–150. doi: 10.1016/s0027-5107(01)00284-6.

Gossen, J.A. et al. (1995), "Spontaneous and X-ray-induced deletion mutations in a LacZ plasmid-based transgenic mouse model.", Mutat Res., 331(1):89–97.

Hada, M. & A.G. Georgakilas (2008), "Formation of Clustered DNA Damage after High-LET Irradiation: A Review.", J. Radiat. Res., 49(3):203–210. doi:10.1269/jrr.07123.

Hei TK, Wu LJ, Liu SX, Vannais D, Waldren CA, Randers-Pehrson G. 1997. Mutagenic effects of a single and an exact number of alpha particles in mammalian cells. Proc Natl Acad Sci USA. 94:1765-3770.

Hooker, A.M. et al. (2004), "Cancer-associated genes can affect somatic intrachromosomal recombination early in carcinogenesis.", Mutat Res. - Fundam Mol Mech Mutagen. 550(1–2):1–10. doi:10.1016/j.mrfmmm.2004.01.003.

Valentin J. (2005), "Low-dose Extrapolation of Radiation-related Cancer Risk.", Ann. ICRP, 35(4):1-140

Jostes, R.F. (1996), "Genetic, cytogenetic, and carcinogenic effects of radon: a review.", Mutat. Res. / Rev. in Genet. Toxicol. 340(2-3):125–139. doi: 10.1016/s0165-1110(96)90044-5.

Kraemer, S.M. et al., (2000), Measuring the Spectrum of Mutation Induced by Nitrogen Ions and Protons in the Human-Hamster Hybrid Cell Line ALC., Rad Res., 153:743-751. doi: 10.1667/0033-7587(2000)153[0743:mtsomi]2.0.co;2.

Lorat. Y. et al. (2015), "Nanoscale analysis of clustered DNA damage after high-LET irradiation by quantitative electron microscopy – The heavy burden to repair.", DNA Repair (Amst). 28:93–106. doi:10.1016/j.dnarep.2015.01.007.

Masumura, K. et al. (2002), "Heavy-Ion-Induced Mutations in the gpt Delta Transgenic Mouse : Comparison of Mutation Spectra Induced by Heavy-Ion , X-Ray , and - Y-Ray Radiation.", Envrion. Mol. Mutagen, 40(3):207–215. doi:10.1002/em.10108.

Matuo, Y. et al. (2018), "Biological effects of carbon ion beams with various LETs on budding yeast Saccharomyces cerevisiae.", Mutat Res Fund Mol Mech Mutagen. 810(November 2017):45–51. doi:10.1016/j.mrfmmm.2017.10.003.

McMahon, S.J. et al. (2016), "Mechanistic Modelling of DNA Repair and Cellular Survival Following Radiation-Induced DNA Damage.", Nat. Publ. Gr. (April):1–14. doi:10.1038/srep33290.

Metting NF, Palayoor ST, Macklis RM, Atcher RW, Liber HL, Little JB. 1992. Induction of Mutations by Bismuth-212 Alpha Particles at Two Genetic Loci in Human B-Lymphoblasts. Radiat Res. 132:339-345.

Nagasawa H, Robertson J, Little JB. 1990b. Induction of chromosomal aberrations and sister chromatid exchanges by alpha particles in density-inhibited cultures of mouse 10T1/2 and 3T3 cells. Int J Radiat Biol. 57(1):35-44.

Nagashima, H. et al. (2018), "Induction of somatic mutations by low-dose X-rays : the challenge in recognizing radiation-induced events.", J. Radiat. Res., 59(October 2017):11–17. doi:10.1093/jrr/rrx053.

Nikitaki, Z. et al. (2016), "Measurement of complex DNA damage induction and repair in human cellular systems after exposure to ionizing radiations of varying linear energy transfer (LET).", Free Radic Res., 5762. doi:10.1080/10715762.2016.1232484.

Okayasu, R. (2012), "Heavy ions — a mini review.", 1000:991–1000. doi:10.1002/ijc.26445.

Okudaira, N. et al. (2010), "Radiation Dose-Rate Effect on Mutation Induction in Spleen and Liver of gpt delta Mice.", Radiat Res. 173(2):138–147. doi:10.1667/rr1932.1.

Otozai, S. et al. (2014), "p53 -Dependent suppression of genome instability in germ cells.", Mutat. Res. 760:24–32. doi: 10.1016/j.mrfmmm.2013.12.004.

Robertson, A. et al. (2013), "The Cellular and Molecular Carcinogenic Effects of Radon Exposure: A Review.", Int. J. Mol. Sci., doi: 10.3390/ijms140714024.

Russell, W.L. et al. (1957), "Radiation Dose Rate and Mutation Frequency.", Science, 128(3338):1546-50. doi: 10.1126/science.128.3338.1546.

Rydberg, B. et al. (2005), "Dose-Dependent Misrejoining of Radiation-Induced DNA Double-Strand Breaks in Human Fibroblasts: Experimental and Theoretical Study for High- and Low-LET Radiation.", Radiat. Res. 163(5):526–534. doi:10.1667/RR3346.

Sankaranarayanan, K. & H. Nikjoo (2015), "Genome-based, mechanism-driven computational modeling of risks of ionizing radiation: The next frontier in genetic risk estimation?", Mutat Res. 764:1–15. doi:10.1016/j.mrrev.2014.12.003.

Schmidt, P. and J. Kiefer, (1998), Deletion-pattern analysis of a-particle and x-ray induced mutations at the HPRT locus of V79 Chinese hamster cells., Mutat Res. 412:149-161. doi: 10.1016/s0027-5107(98)00159-6.

Schwartz JL, Ashman CR, Atcher RW, Sedita BA, Shadley JD, Tang J, Whitlock JL, Rotmensch J. 1991. Differential locus sensitivity to mutation induction by ionizing radiations of different LETs in Chinese hamster ovary K1 cells. Carcinog. 12(9):1721-1726.

Sherborne, A.L. et al. (2015), "Mutational Analysis of Ionizing Radiation Induced Article Mutational Analysis of Ionizing Radiation Induced Neoplasms.", Cell Reports. 12(11):1915–1926. doi:10.1016/j.celrep.2015.08.015.

Somers, C.M. (2006), "Expanded simple tandem repeat (ESTR) mutation induction in the male germline: Lessons learned from lab mice.", Mutat Res., 598(1-2):35-49 doi:10.1016/j.mrfmmm.2006.01.018.

Somers, C.M. et al. (2004), "Gamma radiation-induced heritable mutations at repetitive DNA loci in out-bred mice.", Mutat. Res., 568(1):69–78. doi:10.1016/j.mrfmmm.2004.06.047.

Suzuki, K. & T.K. Hei (1996), "Mutation induction in gamma-irradiated primary human bronchial epithelial cells and molecular analysis of the HPRT-mutants.", Mutat Res., 349(1):33-41. doi: 10.1016/0027-5107(95)00123-9.

Thacker J, Stretch A, Goodhead DT. 1982. The Mutagenicity of Alpha-Particle from Plutonium-238. Radiat Res. 92:343-352.

Tutt, A.N.J. et al. (2002), "Disruption of Brca2 increases the spontaneous mutation rate in vivo : synergism with ionizing radiation.", EMBO Rep., 3(3):255–260. doi: 10.1093/embo-reports/kvf037.

Wilson, J.W. et al. (2015), "The effects of extremely low frequency magnetic fields on mutation induction in mice.", Mutat Res - Fundam Mol Mech Mutagen. 773:22–26. doi:10.1016/j.mrfmmm.2015.01.014.

Wilson, T.E. et al. (2015), "Large transcription units unify copy number variants and common fragile sites arising under replication stress.", Genome Res. 25(2):189–200. doi:10.1101/gr.177121.114.

Winegar, R.A. et al. (1994), "Radiation-induced point mutations, deletions and micronuclei in lacI transgenic mice.", Mutat Res., 307(2):479–487. doi: 10.1016/0027-5107(94)90258-5.

Wu LJ, Randers-Pehrson G, Xu A, Waldren CA, Geard CR, Yu ZL, Hei TK. 1999. Targeted cytoplasmic irradiation with alpha particles induces mutations in mammalian cells. Proc Natl Acad Sci USA. 96:4959-4964.

Xu, G. et al. (2008), "Recovery of a low mutant frequency after ionizing radiation-induced mutagenesis during spermatogenesis.", Mutat Res., 654(2):150–157. doi:10.1016/j.mrgentox.2008.05.012.

Xu, G. et al. (2012), "Ionizing radiation-induced mutant frequencies increase transiently in male germ cells of older mice.", Mutat Res., 744(2):135–139. doi:10.1016/j.mrgentox.2012.01.003.

Zhu LX, Waldren CA, Vannais D, Hei TK. 1996. Cellular and Molecular Analysis of Mutagenesis Induced by Charged Particles of Defined Linear Energy Transfer. Radiat Res. 145:251-259.

Relationship: 1982: Energy Deposition leads to Increase, Chromosomal aberrations

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Deposition of energy leading to lung cancer	non-adjacent	High	High
Deposition of energy leading to occurrence of cataracts	non-adjacent	High	High

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	High	NCBI
mouse	Mus musculus	High	NCBI
rat	Rattus norvegicus	High	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Unspecific	High

This KER is plausible in all life stages, sexes, and organisms with chromosomes. The majority of the evidence is from in vivo adult mice and human, and bovine in vitro models.

Key Event Relationship Description

Energy can be deposited on biomolecules from various forms of radiation in a randomized manner. Radiation with high linear energy transfer (LET) tends to produce more complex, dense structural damage than low LET radiation; both, however, can lead to detrimental damage within a cell (Bauchinger and Schmid 1998; Evans et al., 2001; Hada and Georgakilas 2008; Okayasu 2012; Lorat et al. 2015; Nikitaki et al. 2016)The DNA is particularly susceptible to damage in the form of DNA strand breaks. This damaged DNA can lead to aberrations/rearrangements in chromosomes and chromatids. Examples of chromosome-type aberrations include chromosome-type breaks, ring chromosomes, and dicentric chromosomes, while chromatid-type aberrations refer to chromatid-type breaks and chromatid exchanges (Hagmar et al. 2004; Bonassi et al. 2008). Other types of CAs that may occur in response to radiation include micronuclei (MN), nucleoplasmic bridges (NPBs), and copy number variants (CNVs). CAs may also be classified as stable aberrations (translocations, inversions, insertions and deletions) and unstable aberrations (dicentric chromosomes, acentric fragments, centric rings and MN) (Hunter and Muirhead 2009; Zölzer et al. 2013; Qian et al. 2016).

Evidence Supporting this KER

Overall Weight of Evidence: High

Biological Plausibility

The biological plausibility for this KER is strong, as there is a broad mechanistic understanding of the process CA induction from deposited energy in the form of radiation, which is widely accepted. This has especially been demonstrated in humans and adult mammals. Reviews have been published that provide details regarding the relationships between radiation of different LETs and the relative effectiveness of CA induction (Hunter and Muirhead 2009), ionizing radiation and genomic instability (Smith et al. 2003), and low dose ionizing radiation and chromosomal translocations (Tucker 2008). When ionizing radiation comes into contact with a cell, it is able to deposit energy through ionization and excitation of molecules, which results in the freeing of electrons. These electrons have enough energy to break chemical bonds; thus if the high-energy electrons come into contact with DNA, they may break DNA bonds and cause damage in the form of double-strand breaks, single-strand breaks, base damage, or the crosslinking of DNA to other molecules. Direct damage to DNA occurs when radiation directly interacts with the DNA molecule, causing structural alterations such as breaks or cross-links. In contrast, indirect damage results from radiation interacting with nearby molecules, producing reactive species like free radicals, which can then indirectly affect the DNA by causing chemical modifications and impairing its integrity (Chatterjee et al, 2017). This damage should trigger DNA repair. If the enzymatic repair, however, is incorrect or incomplete, this could push the cell towards apoptotic pathways. However, the repair processes may lead to asymmetrical exchanges in the chromosomes that are not removed from the cell and can propagate in the form of aberrations. Radiation-damaged cells display accumulated CAs in the form of chromosomal rearrangements, genetic amplifications and/or MN (Smith et al. 2003; Christensen 2014; Sage and Shikazono 2017).

CNVs may also be generated through deposition of energy by ionizing radiation. Due to the structural similarities between CNVs that are radiation-induced, chemically-induced, and spontaneously-occurring, all CNVs are likely produced by a similar mechanism. The chemicals, aphidicolin and hydroxyurea, are known inducers of DNA replication stress. This suggests that radiation-induced CNVs are also formed through a similar replication-dependent mechanism(Arlt et al. 2014). Additionally, CNVs may affect germline cells. In fact, there was a significant 8-fold increase in de novo CNVs in the progeny of irradiated male mice, regardless of whether the radiation affected post-meiotic sperm or pre-meiotic sperm. The majority of these CNVs were found to be large deletions, often more than 1000 kB (Adewoye et al. 2015).

Empirical Evidence

Evidence supporting the formation of CAs from the direct deposition of energy in the form of ionizing radiation is strong. Of this data, humans are the most common organisms, adults are the most common life stage, and α particles and X rays are the most common stressors studied. There is also more in vivo data compared to in vitro, however most of the human studies are in vitro (Worgul et al., 1989; Tao et al., 1993; Loucas and Geard, 1994; Tao et al., 1994; Bauchinger and Schmid 1998; Durante et al., 1998; Williams et al., 1999; Belkacémi et al., 2000; Belkacémi et al., 2001; Evans et al., 2001; Schmid et al. 2002; Hande et al., 2003; Thomas et al. 2003; Maffei et al. 2004; Tucker et al., 2004; Hande et al., 2005; Tucker et al. 2005a; Tucker et al. 2005b; Wolf et al., 2008; George et al. 2009; George et al., 2010; Blakely, 2012; Balajee et al. 2014; George et al. 2014; Han et al. 2014; Vellingiri et al. 2014; Suto et al. 2015; Adewoye et al. 2015; Cheki et al. 2016; Foray et al., 2016; McMahon et al. 2016; Morishita et al. 2016; Qian et al. 2016; Basheerudeen et al. 2017; Meenakshi et al. 2017; Abe et al. 2018; Dalke et al., 2018; Bains et al., 2019; Jang et al. 2019; Udroiu et al., 2020; Puig et al., 2016; Barquinero et al., 2004; Curwen et al., 2012; Testa et al., 2018; Franken et al., 2012; Cornforth et al., 2002; Loucas et al., 2013; Nagasawa et al., 1990a; Nagasawa et al., 1990b; Edwards et al., 1980; Themis et al., 2013; Schmid et al., 1996; Mestres et al., 2004; Bilbao et al., 1989; Mill et al., 1996; Brooks, 1975; Tawn and Thierens, 2009; Durante et al., 1992; Hamza and Mohankumar, 2009; Takatsuji and Sasaki, 1984; Moquet et al., 2001; Purrott et al., 1980; duFrain et al., 1979).

Figure 1: *Plot of example studies (y-axis) against equivalent dose (Sv) used to determine the empircal link between direct deposition of energy and increased rates of chromosomal aberrations. The y-axis is ordered from low LET to high LET from top to bottom.*

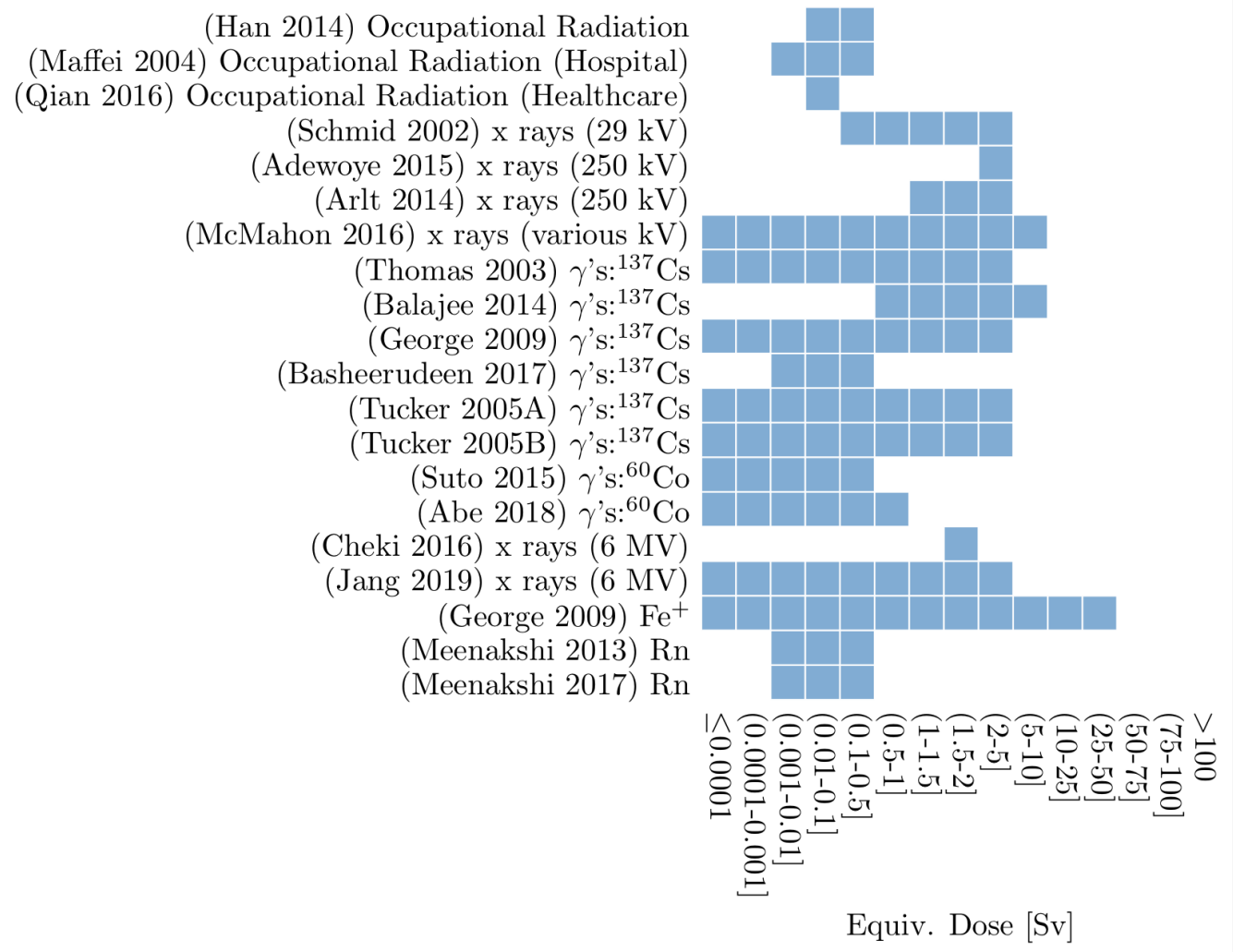
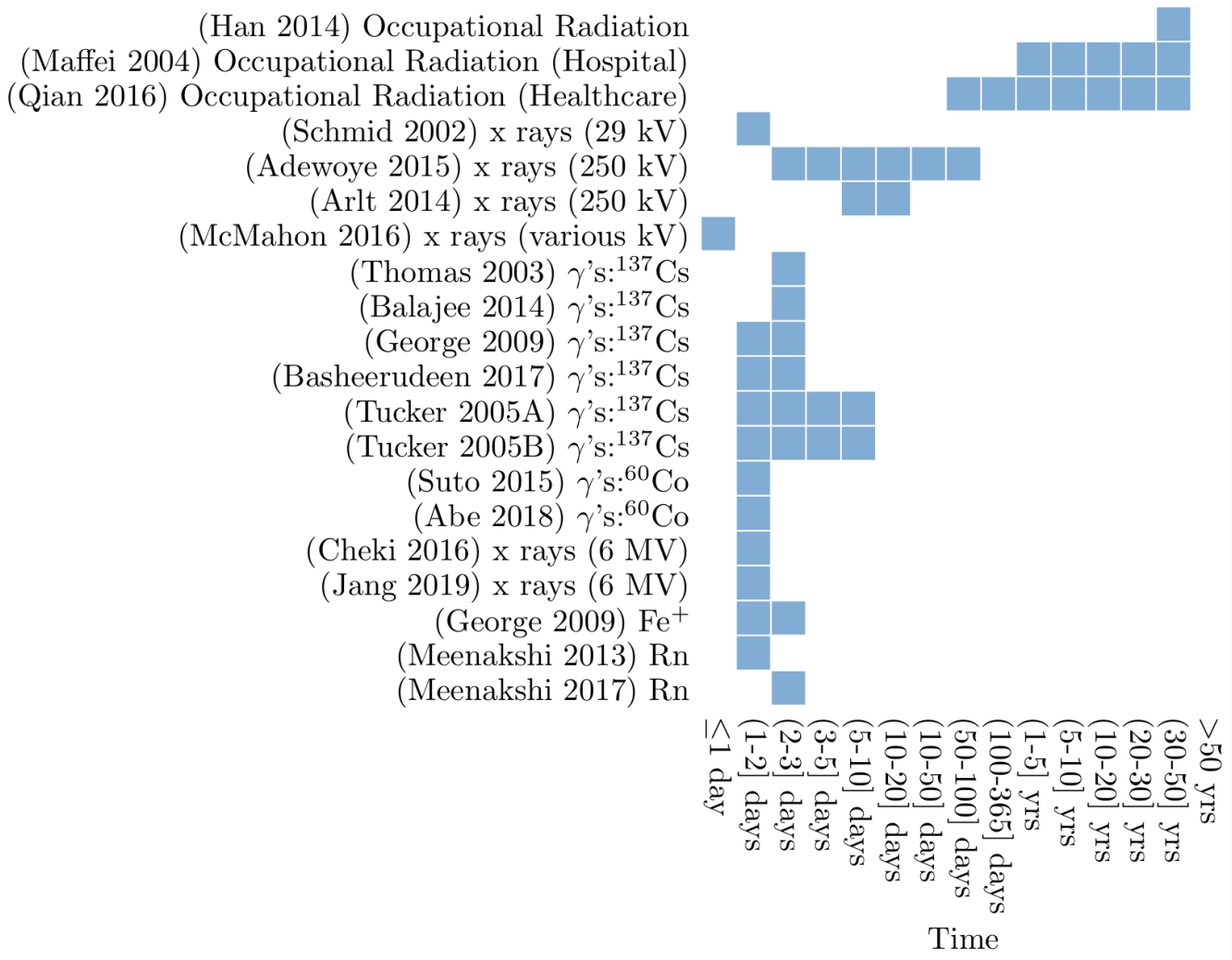


Figure 2: *Plot of example studies (y-axis) against time over which studies were conducted for a temporal response used to determine the empircal link between direct deposition of energy and increased rates of chromosomal aberrations. The y-axis is ordered from low LET to high LET from top to bottom.*



Human relevance

Several human epidemiological studies have provided evidence of both dose/incidence and temporal concordance in terms of deposition of energy by ionizing radiation and resultant CAs. In a study involving 34 health professionals occupationally exposed to radiation, there was a significant increase in the number of chromosome breaks and aberrant cells relative to a group of 35 unexposed professionals from the same hospital. Furthermore, when the exposed group was broken into two groups based on the levels of radiation exposure (those with an effective dose of ≤50 mSv and those with an effective dose of >50 mSv), there was a dose-dependent increase in aberrant cells, chromosome breaks and chromatid breaks such that the higher exposure group had significantly elevated aberrations relative to controls for all three parameters scored (Maffei et al. 2004). In a similar study involving 1,392 radiation healthcare workers in the city of Tangshan in 2010, there was a significant increase in CA and MN in exposed workers relative to unexposed healthy controls. Furthermore, there were significant, dose-dependent increases in the CA rate and the MN rate when the exposed workers were split into groups according to effective dose, ranging from <10 mSv up to >50 mSv. There was also a time-dependent increase in CA and MN rate, such that workers with longer exposure times had significantly increased CAs and MNs. Exposure times ranged from less than 10 years to greater than 20 years (Qian et al. 2016). A final study with 31 plutonium and reactor workers found that those exposed to high doses of Pu had a frequency of complex translocations that was 2.9% higher and a frequency of simple translocations that was 5.1% higher compared to unexposed workers. They also found that workers exposed to γ-rays had a frequency of complex translocations that was only 0.21% higher than that of controls, and a frequency of simple translocations that was 4% higher than controls (Hande et al., 2005).

Dose Concordance

There is a clear correlation between radiation dose (i.e., increasing amounts of energy deposition) and different clastogenic endpoints including dose-dependent increases in: dicentric aberrations(Schmid et al. 2002; Thomas et al. 2003; Tucker et al. 2005A; Suto et al. 2015; Mcmahon et al. 2016; Abe et al. 2018; Jang et al. 2019), centric rings (Tucker et al. 2005a) (Schmid et al. 2002; Thomas et al. 2003; Tucker et al. 2005A), acentric fragments (Loucas and Geard, 1994; Schmid et al. 2002; Thomas et al. 2003) , translocations (Hande et al., 2005; Tucker et al. 2005A; Tucker et al. 2005B; Suto et al. 2015; Abe et al. 2018; Jang et al. 2019), CNVs (Arlt et al. 2014), large deletions (Mcmahon et al. 2016), NPBs (Thomas et al. 2003), MNs (Tao et al., 1993; Tao et al., 1994; Thomas et al. 2003; Balajee et al. 2014), fragmented nuclei (Tao et al., 1993), strand breaks (Durante et al., 1998) and CAs in general (Williams et al., 1999; Evans et al., 2001; Hande et al., 2003; George et al., 2009; Mcmahon et al. 2016) (George et al. 2009).

Interestingly, MN structural complexity was likewise demonstrated to be dose-dependent between 1 and 10 Gy. MN were found to contain fragments from two or more different chromosomes at and above 2 Gy; between 5 and 10 Gy, MN contained material from 3 - 5 different chromosomes. These results suggest that MN formation appears to become increasingly more complex with higher doses of radiation due to the increasing number of acentric fragments and the resultant fusion of these fragments (Balajee et al. 2014). Of note, the photon energy of the radiation has an effect on the relationship between direct deposition of energy and the resulting CAs. Specifically, dicentric aberration frequency in human peripheral blood lymphocytes was observed to change with voltage of the ionizing radiation. As the X-ray voltage decreased from 60 kV to 10 kV, there was an increase in the number of dicentric aberrations (Schmid et al. 2002).

Time Concordance

Temporal concordance is well established. Energy deposition happens immediately upon radiation exposure, with an increased incidence of CAs documented minutes, hours or days after irradiation (Loucas and Geard, 1994; Durante et al., 1998; Schmid et al. 2002; Thomas et al. 2003; Tucker et

al. 2005A; Tucker et al. 2005B; George et al. 2009; Meenakshi and Mohankumar 2013; Arlt et al. 2014; Balajee et al. 2014; Suto et al. 2015; Cheki et al. 2016; Mcmahon et al. 2016; Basheerudeen et al. 2017; Meenakshi et al. 2017; Abe et al. 2018; Jang et al. 2019).

Essentiality

Radiation exposure has been found to increase levels of CAs above background levels and lead to more complex damage, highlighting the essentiality of deposition of energy (Tao et al., 1994; Durante et al., 1998; Williams et al., 1999; Evans et al., 2001; Schmid et al., 2002; Hande et al., 2005; Wolf et al., 2008; George et al., 2009; Suto et al., 2015; Abe et al., 2018; Dalke et al., 2018; Bains et al., 2019; Jang et al., 2019; Udroiu et al., 2020). In the absence of radiation, there are minimal amounts of CAs. For example, abnormal karyotypes are half as likely in non-irradiated cells compared to irradiated cells (Wolf et al., 2008). Since deposited energy initiates events immediately, the removal of deposited energy, a physical stressor, also supports the essentiality of the key event. Studies that do not deposit energy are observed to have no downstream effects.

Uncertainties and Inconsistencies

Uncertainties and inconsistencies in this KER are as follows:

- An individual's response to radiation can be affected by a large variety of factors. Many of them cannot be controlled in a study, therefore leading to inconsistencies in results (Bender et al., 1988).
- When an organism is exposed to an initial low radiation dose followed by a higher dose, it can initiate an adaptive response, therefore decreasing the resulting damage. Day et al. also found this to be applicable when a low radiation dose was followed by an even lower dose (2007).

Quantitative Understanding of the Linkage

Quantitative understanding of this linkage suggests that CA frequency can be predicted from the radiation, as per the representative examples provided below. When predicting this relationship, the characteristics of the radiation and the model system should be taken into account (Smith et al. 2003; Hunter and Muirhead 2009). It is widely accepted that the deposition of energy, at all doses, results in immediate ionization events, followed by downstream events. The following tables provide representative examples of the relationship, unless otherwise indicated, all data is significantly significant.

Dose Concordance

Reference	Experiment Description	Result
Suto et al., 2015	In vitro. Human peripheral blood lymphocytes were exposed to 60Co γ-rays at 0 – 300 mGy and 5 mGy/sec. The number of dicentrics and translocations in chromosomes 1, 2, and 4 were determined using three differentially colored chromosome painting probes.	Study of human peripheral blood lymphocytes from a healthy donor subjected to γ-ray radiation in the dose (D) range of 0 - 300 mGy found a calculated CA rate (y) of dicentrics, translocations and dicentrics+translocations (of the quadratic form, y = a + bD + bD^2) found - dicentrics + translocations (a,b,c := 0.0023 ± 0.0003, 0.0015 ± 0.0058, 0.0819 ± 0.0225), dicentrics (a,b,c := 0.0004 ± 0.0001, 0.0008 ± 0.0028, 0.0398 ± 0.0117), translocations (a,b,c := 0.0019 ± 0.0003, 0.0008 ± 0.0028, 0.0398 ± 0.0117).
Abe et al., 2018	In vitro. Human mononuclear blood cells from five donors (four males aged 23, 35, 44, and 55 years old, and a 33-year-old female) were exposed to 60Co γ-rays at 0 – 1000 mGy. The dose rate in the irradiator was 26.6 mGy/min with an additional 6.42 mGy to the sample while entering and leaving the irradiation source. None of the subjects had a history of radiotherapy, smoking, or chemotherapy. The number of dicentrics and translocations in chromosomes 1, 2, and 4 were determined using Giemsa staining and Centromere-FISH staining.	Study of human mononuclear blood cells from healthy donors; analyzed for dicentric chromosomes. Exposure to γ-ray doses (D) in the 0 - 1000 mGy range. Quadratic form fit for the CA rate in Giemsa staining and Centromere-FISH staining cases (y) (of the form y = a + bD + cD^2) found to be: Giemsa staining: (a,b,c := 0.0013 ± 0.0005, 0.0067 ± 0.0071, 0.0313 ± 0.0091), Centromere-FISH staining (a,b,c := 0.0010 ± 0.0004, 0.0186 ± 0.0081, 0.0329 ± 0.0104). Study of mononuclear blood cells from healthy donors; analyzed for translocations. Exposure to γ-ray doses (D) in the 0 - 1000 mGy range. Quadratic form fit for the CA rate (y) before and after donor age adjustment (of the form y = a + bD + cD^2) found to be: before donor age adjustment: (a,b,c := 0.0053 ± 0.0009, 0.259 ± 0.0127, 0.0826 ± 0.0161), after donor age adjustment (a,b,c := 0.0015 ± 0.0009, 0.0049 ± 0.0155, 0.1033 ± 0.0223).
Jang et al., 2019	In vitro. Human peripheral blood lymphocytes from four different donors (two males, 36 and 22 years old, and two females, 28 and 24 years old) were exposed to 6 MV X-rays at 0-5 Gy and 0.5 Gy/min. None of the subjects had a history of chemotherapy, smoking, or radiotherapy. The number of dicentrics and translocations in chromosomes 1, 2, and 4 were determined using Giemsa staining and chromosome painting, respectively.	Human peripheral blood lymphocytes studied from healthy donors. Lymphocytes irradiated with X-rays in a dose (D) range 0 - 5 Gy. Calculated CA rate from dicentrics or translocations (y) (of the form y = a + bD + cD^2). Dicentrics, (a,b,c := 0.0011 ± 0.0004, 0.0119 ± 0.0032, 0.0617 ± 0.0019). Translocations, (a,b,c := 0.0015 ± 0.0004, 0.0048 ± 0.0024, 0.0237 ± 0.0014).
Schmid et al., 2002	In vitro. Human peripheral blood lymphocytes from one male donor were exposed to 29 kV X-rays at 0.115 – 2.194 Gy and at 0.009 – 0.140 Gy/min. CAs were determined with fluorescence plus Giemsa staining.	Study of various X- and γ-ray types irradiating peripheral human blood lymphocytes, analyzed dicentrics and acentrics (10, 29, 60, 220 kV X-rays & Cs-137, Co-60 γ-rays). See Schmid et al. (2002) for details on equations.
George et al., 2009	In vitro. HF19 normal primary lung fibroblasts, AT primary fibroblasts, NSB1-deficient primary fibroblasts, M059K glioblastoma cells, and M059J glioblastoma cells were exposed to iron nuclei (1000 MeV/nucleon, 0.2 – 0.5 Gy/min, average LET of 151 keV/um), and 137Cs γ-rays (0.2 – 1 Gy/min) at 0 – 3 Gy. CAs in chromosomes 1, 2, 4, and 11 were determined using FISH staining.	γ-rays and iron nuclei irradiating HF19 normal primary lung fibroblasts; Ataxia telangiectasia (AT) primary fibroblasts; NSB1-deficient primary fibroblasts (Nijmegen breakage syndrome); M059K glioblastoma cells & M059J glioblastoma cells (lack DNA-dependent protein kinase activity). Dose range of 0 - 3 Gy. See Table 5 & 6 of George et al. (2009) for details on equations.

Wolf et al. 2008	In vivo. Female, C57BL/6 mice received head-only exposure to 11 Gy soft X-rays. Mice were either 2 or 26 months-old at irradiation. 6 were irradiated and 6 were non-irradiated controls. Chromosomal aberrations were detected using Wright-trypsin G-banding.	Irradiation of eight-week-old mice with 11 Gy of X-rays increased the number of abnormal chromosomes from 15 (control) to 27. Irradiation of 26-month-old mice with 11 Gy of X-rays increased the number of abnormal chromosomes from 0 (control) to 30). However, the significance of these results was not indicated.
Williams et al., 1999	In vitro. Human lymphocyte cells were exposed to Fe ions (0 – 1 Gy, 1 GeV incident energy), 137Cs photons (0 – 7.5 Gy, 83 cGy/min), and protons (5 Gy, 250 keV incident energy). Chromosomal aberrations were identified using Leishman's stain.	CAs rose from 0 to 0.001, 0.004, 0.014, and 0.017 after exposure to 1 Gy 137Cs, 1 Gy Fe, 5 Gy 137Cs, and 5 Gy energetic photons respectively.
Evans et al., 2001	In vitro. TK6 and WTK1 human lymphoblastoid cells were exposed to 56Fe ions (0 – 2.25 Gy, 1087 MeV/nucleon) and 137Cs γ-rays (0 – 4 Gy, 0.87 – 0.92 Gy/min). CAs were assessed using Giemsa staining.	Exposure to higher doses resulted in increased amounts of aberrant cells. For example, in TK6 cells after 56Fe irradiation, 0% of cells were aberrant in the control, 19% were aberrant after 0.63 Gy, and 66% were aberrant after 2.25 Gy. Additionally, 56Fe ions induced increased numbers of aberrant cells when compared to 137Cs γ-rays.
Hande et al., 2005	In vivo. Lymphocytes from 31 Russian reactor and plutonium workers were exposed to plutonium ions and γ-rays. Highly exposed plutonium workers received 1.1 Gy of Pu and 1.5 Gy of γ-rays. Moderately exposed plutonium workers received 0.19 Gy of Pu and 0.19 Gy of γ-rays. Reactor workers received only 2.3 Gy of γ-rays. Values are averages. CAs were detected using the mFISH assay.	The amount of interchromosomal complex translocations were 2.9, 0.23, and 0.21% higher than controls in workers highly exposed, moderately exposed, and exposed to only γ-rays, respectively. The amount of interchromosomal simple translocations were 5.1, 1.5, and 4% higher than controls in workers highly exposed, moderately exposed, and exposed to only γ-rays, respectively.
Durante et al., 1998	In vitro. Human male lymphocyte cells were exposed to carbon ions (5 Gy, 290 meV/nucleon, 42 keV/µm), neon ions (7 Gy, 400 MeV/nucleon, 31 keV/µm), hydrogen ions (1, 3, 5, 7 Gy, 0.4 keV/µm), and iron ions (0.5, 0.75, 1, 2, Gy, 1000 MeV/nucleon, 140 keV/µm). Chromosomal aberrations were detected using DAPI-counterstaining the PAINT classification.	As the dose increased from 0 to 7 Gy, the number of DNA breaks per human male lymphocyte cell also increased from 0 to 1.2. This included data using iron ions, hydrogen ions, carbon, and neon.
Tao et al., 1994	In vivo. 90 – 110-day old B6CF1/Anl mice received irradiation to the anterior 2/3 of the body with 60Co γ-rays (10 – 40 cGy), 20Ne (670 MeV/amu, 25 keV/um), 56Fe (600 MeV/amu, 193 keV/um), 93Nb (600 MeV/amu, 464 keV/um), 139La (593 MeV/um, 953 keV/um). All stressors except 60Co delivered 10, 20, 40, 80, 160, and 320 cGy. Doses were delivered to the anterior 2/3 of the body. CAs were detected using a modified Feulgen method 64 weeks post-exposure.	Mouse lenses exposed to all radiation types showed increased MN number per whole mount at increasing doses from 10 to 160 cGy. For example, irradiation from 56Fe particles led to a MN number of 10 after 10 cGy and an MN number of 100 after 160 cGy.
Bains et al., 2019	In vitro. Human LECs were exposed to X-rays at 0, 0.001, 0.01, 0.02, 0.1, 1, and 2 Gy. Doses of 0.1, 1.0, and 2.0 had a dose rate of 0.58 Gy/min. 0.001, 0.01 and 0.02 Gy at 0.022 Gy/min. The γ-H2AX assay was used to determine the number of telomere dysfunction induced foci (TIF).	At 30 min after irradiation, the number of TIF/human lens epithelial cell remained almost 0 after exposure to 0, 0.001, and 0.01 Gy. It then rose quickly to 4.75 TIF/cell at 2 Gy.
Udroiu et al., 2020	In vitro. Human LECs were exposed to X-rays at 25, 51.25, 135, 235, and 300 mGy at 0.51, 0.15, and 0.228 Gy/min. The micronuclei frequency was measured with a cytokinesis-blocked micronucleus assay.	In human LECs exposed to 25 – 300 mGy the micronuclei frequency increased steadily, reaching 2.4x control at the maximum dose.
Dalke et al., 2018	In vivo. 10-week-old, mixed sex B6C3F1 and B6RCF1 hybrid mice received whole-body exposure to 0.063, 0.125, and 0.5 Gy 60Co γ-rays at 0.063 Gy/min. CAs were assessed using Giemsa staining.	In heterozygous Ercc2 mutants, 12 months after irradiation, the number of aberrations/cell rose from 0.1 (control) to 0.82 (0.5 Gy). There was also a slightly smaller increase after 18 months where the number of chromosomal aberrations/cell rose from 0.1 (control) to 0.3 (0.5 Gy). In wild type mice the largest increase occurred after 12 months where the number of aberrations/cell rose from 0.12 (control) to 0.32 (0.5 Gy).

Time Concordance

Reference	Experiment Description	Result
Tucker et al., 2004	In vivo. 7-week-old female C57BL/6 mice transgenic for lacZ received whole-body exposure to 1 Gy of 26Fe ions at 1 Gy/min. Aberrations on chromosomes 1, 2, 3, and 8 were determined with a FISH assay.	Lymphocytes from mice exposed immediately to 1 Gy of iron ions had 19 translocations per 100 cells, 14 acentric fragments per 100 cells, and 10 dicentric chromosomes per 100 cells at 1 week post-irradiation.
Tao et al., 1994	In vivo. 90 – 110-day old B6CF1/Anl mice received irradiation to the anterior 2/3 of the body with 60Co γ-rays (10 – 40 cGy), 20Ne (670 MeV/amu, 25 keV/um), 56Fe (600 MeV/amu, 193 keV/um), 93Nb (600 MeV/amu, 464 keV/um), 139La (593 MeV/um, 953 keV/um). All stressors except 60Co delivered 10, 20, 40, 80, 160, and 320 cGy. Doses were delivered to the anterior 2/3 of the body. CAs were detected using a modified Feulgen method.	Mouse lenses exposed immediately to all radiation types showed increased MN number per whole mount at increasing doses from 10 to 160 cGy at 64 weeks post-irradiation. For example, irradiation from 56Fe particles led to a MN number of 10 after 10 cGy and an MN number of 100 after 160 cGy at 64 weeks post-irradiation.

Tao et al., 1993	In vivo. 90 – 110-day old female B6CF mice received irradiation to the anterior 2/3 of the body with 10, 20, 40, 80, 160, and 320 cGy of protons: 250 MeV, LET 0.4 keV/um. 20Ne: 670 MeV/amu, LET 25 keV/um. 56Fe: 600 MeV/amu, LET 183 keV/um. 56Fe: 350 MeV/amu, LET 219 keV/um. 93Nb: 600 MeV/amu, LET 464 keV/um. 139La: 593 MeV/amu, 953 keV/amu. Fragmented nuclei (FN) and MN were detected using a modified Feulgen staining method or haematoxylin and eosin staining.	In mice immediately irradiated with iron ions, the number of FN in the whole mount of lens cells increased from 1.5 FN (control) to 11 FN at 160 cGy after 64 weeks. The number of FN in the meridional rows increased from 0 (control) to 6 FN at 160 cGy after 64 weeks as well. The number of MN on the whole mount also increased from 0 to 100 at 160 cGy after 64 weeks. Similar changes were also observed with the other radiation types.
Belkacémi et al., 2001	In vitro. Bovine lens cells were exposed to 10 Gy at 2 Gy/min from a linear accelerator. The Hoechst 33342 fluorescence was used to measure chromosomal aberrations.	In lens cells immediately irradiated with X-rays, Hoechst 33342 fluorescence increased 13%, 25%, and 32% above controls at 24, 72, and 96 h post-irradiation, respectively.
Bains et al., 2019	In vitro. Human LECs were exposed to X-rays at 0, 0.001, 0.01, 0.02, 0.1, 1, and 2 Gy. Doses of 0.1, 1.0, and 2.0 had a dose rate of 0.58 Gy/min. 0.001, 0.01 and 0.02 Gy at 0.022 Gy/min.	In human LECs immediately exposed in vitro to 0.02 Gy, the number of telomere dysfunction-induced foci (TIF)/cell increased to 3x control 30 min post-irradiation.
Dalke et al., 2018	In vivo. 10-week-old, mixed sex B6C3F1 and B6RCF1 hybrid mice received whole-body exposure to 0.063, 0.125, and 0.5 Gy 60Co γ-rays at 0.063 Gy/min. CAs were assessed using Giemsa staining.	In mice immediately exposed to 0.125-0.5 Gy of γ-rays, CAs were observed after 12 months, increasing to a maximum of 0.82 CAs/cell at 0.5 Gy.

Response-response relationship

There is evidence of a positive response-response relationship between the radiation dose and the frequency of CAs(Tao et al., 1993; Tao et al., 1994; Durante et al., 1998; Williams et al., 1999; Belkacémi et al., 2001; Evans et al., 2001; Schmid et al. 2002; Thomas et al. 2003;Tucker et al., 2004; Hande et al., 2005; Tucker et al. 2005a; Tucker et al. 2005b; Wolf et al., 2008; George et al. 2009; Arlt et al. 2014; Balajee et al. 2014; Suto et al. 2015; Mcmahon et al. 2016; Abe et al. 2018; Dalke et al., 2018; Bains et al., 2019; Jang et al. 2019; Udroiu et al., 2020). Most studies found that the response-response relationship was linear-quadratic (Schmid et al. 2002; Suto et al. 2015; Foray et al., 2016; Abe et al. 2018; Jang et al. 2019) One study, however, reported different results when CAs were examined across five cell lines that had been irradiated with either iron nuclei or γ-rays. For complex aberrations in three types of fibroblasts (two of which were deficient in DNA repair), the best fit was a quadratic relationship for both γ-rays and iron ions; for simple aberrations induced by iron ions in these cells, there was a linear relationship found. In two tumor cell lines, a linear response was defined for simple aberrations for both types of radiation, while the response for complex aberrations was not well-defined by the models that were evaluated (George et al. 2009).

Time-scale

The time scale relationship between radiation exposure and the frequency of CAs has been examined. Most studies search for CAs hours, days, weeks, or even years after exposure to radiation (Loucas and Geard, 1994; Durante et al., 1998; Schmid et al. 2002; Thomas et al. 2003; Tucker et al., 2004; Tucker et al. 2005a; Tucker et al. 2005b; George et al. 2009; Meenakshi and Mohankumar 2013; Arlt et al. 2014; Balajee et al. 2014; Han et al. 2014; Suto et al. 2015; Cheki et al. 2016; Mcmahon et al. 2016; Basheerudeen et al. 2017; Meenakshi et al. 2017; Abe et al. 2018; Jang et al. 2019) ; this makes it particularly difficult to identify CA induction in relation to the deposition of energy by ionizing radiation. There is an account, however, of CAs appearing within 20 minutes of irradiation, with levels peaking at 40 min and plateauing for the remainder of the experiment (up to 100 min) (Mcmahon et al. 2016). CAs have also been documented 2 - 3 h after radiation exposure, with frequency being shown to increase slightly at 24 h (Basheerudeen et al. 2017). CA frequency begins to decrease after exposure, but not all aberrations are repaired (Loucas and Geard, 1994; Durante et al., 1998; Tucker et al., 2004). This process also appears to depend on LET, with strand breaks induced by radiation with a lower LET able to be repaired quicker than those induced by a higher LET (Durante et al., 1998). Furthermore, a study examining CAs in human blood samples for 2 - 7 days following irradiation with γ-rays found that CAs were present at the 2-day mark, but had declined by day 7 (Tucker et al. 2005a; Tucker et al. 2005b) to suspected asymptotic minimum levels (Tucker et al. 2005b). For translocations specifically, the relationship between time and translocation frequency was found to be linear at low doses (0 - 0.5 Gy) and linear quadratic at higher doses (0.5 - 4 Gy) (Tucker et al. 2005b). The sharpest decline over the 7 days was found in dicentrics, acentric fragments, and ring chromosomes (Tucker et al. 2005a).

Interestingly, in vivo radiation exposure has been shown to induce long-lasting CAs in a relatively short time-frame. When lymphocytes from patients undergoing an interventional radiology procedure were compared pre-procedure and 2-3 h post-procedure, there were significant increases in chromatid-type aberrations, chromosome-type aberrations, dicentrics and MN in post-procedure lymphocytes)(Basheerudeen et al. 2017). Similarly, lymphocytes from subjects exposed to radiation 32-41 years prior to blood collection were found to have significantly increased chromosome-type aberrations (acentric fragments, dicentrics and translocations) and MN relative to unexposed controls (Han et al. 2014). Taken together, the results from these two studies suggest that CAs are not only induced within mere h of radiation exposure, but that these radiation-induced CAs may also endure for several decades.

Known modulating factors

Modulating Factor	Details	Effects on the KER	References
Sex	Females	Females were found to have increased aberrant cells and chromosome breaks relative to males.	Maffei et al., 2004
Age	Increased or decreased age	Increases in age were associated with increased CAs. However, it has also been found that young organisms are more sensitive to radiation. One possible explanation for this is that dividing cells are more radiosensitive than those that are quiescent.	Blakely, 2012; Santovito et al., 2013; Vellingiri et al., 2014
Smoking	Smoking status	Smoking was found to increase chromosomal damage. Chromosome breaks were found to be significantly increased in smokers relative to non-smokers. Likewise, blood samples from smokers that were exposed to radon gas had lymphocytes with significantly increased dicentric aberrations, acentric fragments, chromatid breaks, MN, and NPBs relative to lymphocytes from non-smokers also exposed to radon gas.	Maffei et al., 2004; Meenakshi and Mohankumar 2013; Meenakshi et al., 2017
Hyperthermia	Increased temperature	In cells exposed to hyperthermic conditions (41°C for 1 h) followed by radiation (4 Gy), there were significant increases in chromosomal translocations and chromosomal fragments at 1 and at 24 h post-exposure, respectively, as compared to cells exposed only to radiation.	Bergs et al., 2016

DNA ligase IV	Presence	DNA ligase IV helps prevent DNA degradation and increase accurate DNA rejoining, therefore decreasing chromosome breaks and radiation-induced MN.	Smith et al., 2003; Foray et al., 2016
Genetic syndromes	Cockayne syndrome, AT-like disorder, Nijmegen breakage syndrome, Bloom's syndrome, xeroderma pigmentosum, Fanconi anemia, and ataxia telangiectasia	The presence of one of these conditions can increase the number of CAs.	Bender et al., 1988; Foray et al., 2016
Antioxidants or antigenotoxic agents	Increased concentration, examples include dimethyl sulfoxide (DMSO)	The compounds can help decrease the frequency of CAs after irradiation.	Yang, 1999; Kim and Lee, 2007

Known Feedforward/Feedback loops influencing this KER

Not identified.

References

Abe, Y. et al. (2018), "Dose–response curves for analyzing of dicentric chromosomes and chromosome translocations following doses of 1000 mGy or less, based on irradiated peripheral blood samples from fi ve healthy individuals.", J. Radiat. Res., 59(1):35–42. doi:10.1093/jrr/rrx052.

Adewoye, A.B. et al. (2015), "Mutation induction in the mammalian germline.", Nature Comm. 6:(6684), doi:10.1038/ncomms7684.

Arlt, M.F. et al. (2014), "Copy number variants are produced in response to low-dose ionizing radiation in cultured cells", Envrion. and Mol. Mutagen, 55(2):103–113. doi:10.1002/em.21840.

Bains, S. K. et al. (2019), "Effects of ionizing radiation on telomere length and telomerase activity in cultured human lens epithelium cells", International journal of radiation biology, Vol. 95/1, Taylor & Francis, Abingdon, https://doi.org/10.1080/09553002.2018.1466066

Balajee, A.S. et al. (2014), "Multicolour FISH analysis of ionising radiation induced micronucleus formation in human lymphocytes.", Mutagenesis, 29(6):447–455. doi:10.1093/mutage/geu041.

Barquinero JF, Stephan G and Schmid E. 2004. Effect of americium-241 alpha-particles on the dose-response of chromosome aberations in human lymphocytes analysed by fluorescence in situ hybridization. Int J Radiat Biol. 80(2):155-164.

Basheerudeen, S.S. et al. (2017), "Entrance surface dose and induced DNA damage in blood lymphocytes of patients exposed to low-dose and low-dose-rate X-irradiation during diagnostic and therapeutic interventional radiology procedures.", Mutat Res Gen Tox En. 818(April):1–6., doi:10.1016/j.mrgentox.2017.04.001.

Bauchinger, M. and E. Schmid, (1998), LET dependence of yield ratios of radiation-induced intra- and interchromosomal aberrations in human lymphocytes., Int J Radiat Biol., Jul;74(1):17-25. doi: 10.1080/095530098141681

Bauchinger, M. et al., (1994), Chromosome aberrations in peripheral lymphocytes from occupants of houses with elevated indoor radon concentrations., Mutat Res., Oct 1;310(1):135-42

Belkacémi, Y. et al. (2000), "Ionizing radiation-induced death in bovine lens epithelial cells: Mechanisms and influence of irradiation dose rate", International journal of cancer, Vol. 90/3, John Wiley & Sons, Inc, New York, https://doi.org/10.1002/1097-0215(20000620)90:3<138::AID-IJC3>3.0.CO

Belkacémi, Y. et al. (2001), "Lens epithelial cell protection by aminothiol WR-1065 and anetholedithiolethione from ionizing radiation", International journal of cancer, Vol. 96/S1, Wiley, New York, https://doi.org/10.1002/ijc.10346

Bender, M.A. et al. (1988), "Current status of cytogenetic procedures to detect and quantify previous exposures to radiation.", Mutat Res Genet Toxicol. 196(2):103–159. doi:10.1016/0165-1110(88)90017-6.

Bergs, J.W. et al. (2016), "Dynamics of chromosomal aberrations, induction of apoptosis, BRCA2 degradation and sensitization to radiation by hyperthermia.", Int. J. Mol. Med., 38(1):243–250. doi:10.3892/ijmm.2016.2611.

Bilbao A, Prosser JS, Edwards AA, Moody JC, Lloyd DC. 1989. The Induction of Micronuclei in Human Lymphocytes by in vitro Irradiation with Alpha Particles from Plutonium-239. Int J Radiat Biol. 56(3):287-292.

Blakely, E. A. (2012), "Lauriston S. Taylor lecture on radiation protection and measurements: what makes particle radiation so effective?", Health physics, Vol. 103/5, Health Physics Society, United States, https://doi.org/10.1097/HP.0b013e31826a5b85

Bonassi, S. et al. (2008), "Chromosomal aberration frequency in lymphocytes predicts the risk of cancer: results from a pooled cohort study of 22,358 subjects in 11 countries.", Carcinogenesis, 29(6):1178–1183. doi:10.1093/carcin/bgn075.

Brooks AL. 1975. Chromosome damage in liver cells from low dose rate alpha, beta, and gamma irradiation: derivation of RBE. Sci. 190(4219):1090-1092.

Chatterjee, N., & Walker, G. C. (2017),. "Mechanisms of DNA damage, repair, and mutagenesis",. Environmental and molecular mutagenesis, 58(5), 235–263,. https://doi.org/10.1002/em.22087

Cheki, M. et al. (2016), "The radioprotective effect of metformin against cytotoxicity and genotoxicity induced by ionizing radiation in cultured human blood lymphocytes.", Mutat Res - Genet Toxicol Environ Mutagen. 809:24–32. doi:10.1016/j.mrgentox.2016.09.001.

Christensen, D.M. (2014), "Management of Ionizing Radiation Injuries and Illnesses, Part 3: Radiobiology and Health Effects of Ionizing Radiation.", J. Am. Osteopath Assoc., 114(7):556–565. doi:10.7556/jaoa.2014.109.

Cornforth MN, Bailey SM, Goodwin EH. 2002. Dose Responses for Chromsome Aberrations Produced in Noncycling Primary Human Fibroblasts by Alpha Particles, and by Gamma Rays Delivered at Sublimating Low Dose Rates. Radiat Res. 158:43-53.

Curwen GB, Tawn EJ, Cadwell KK, Guyatt L, Thompson J, Hill MA. 2012. mFISH Analysis of Chromsome Aberrations Induced In Vitro by Alpha-Particle Radiation: Examination of Dose-Response Relationships. Radiat Res. 178:414-424.

Dalke, C. et al. (2018), “Lifetime study in mice after acute low-dose ionizing radiation: a multifactorial study with special focus on cataract risk”, Radiation and environmental biophysics, Vol. 57/2, Springer, Berlin/Heidelberg, https://doi.org/10.1007/s00411-017-0728-z

Day, T.K. et al. (2007), "Adaptive Response for Chromosomal Inversions in pKZ1 Mouse Prostate Induced by Low Doses of X Radiation Delivered after a High Dose.", Radiat Res. 167(6):682–692. doi:10.1667/rr0764.1.

Durante M, Grossi GF, Napolitano M, Pugliese M, Gialanella G. 1992. Chromsome damage induced by high-LET alpha-particles in plateau-phase C3H 10T1/2 cells. Int J Radiat Biol. 62(5):571-580.

Durante et al. (1998), “Rejoining and misrejoining of radiation-induced chromatin breaks. IV. Charged particles”, in Radiation Research, Radiation Research Society, Oak Brook. DOI: 10.2307/3579784

Edwards AA, Purrott RJ, Prosser JS, Lloyd DC. 1980. The induction of chromosome aberrations in human lymphocytes by alpha-radiation. Int J Radiat Biol. 38(1):83-91.

Evans, H. H. et al. (2001), “Genotoxic effects of high-energy iron particles in human lymphoblasts differing in radiation sensitivity”, Radiation research, Vol. 156/2, Radiation Research Society, Oak Brook, https://doi.org/10.1667/0033-7587(2001)156[0186:GEOHEI]2.0.CO

Foray, N. et al. (2016), “Individual response to ionizing radiation”, Mutation research, Elsevier B.V, Amsterdam, https://doi.org/10.1016/j.mrrev.2016.09.001

duFrain RJ, Littlefield G, Joiner EE, Frome EL. 1979. Human Cytogenetic Dosimetry: A Dose-Response Relationship for Alpha Particle Radiation from ^{241}Am. Health Phys. 37:279-289.

Franken NAP, Hovingh S, Cate RT, Krawczyk P, Stap J, Hoebe R, Aten J, Barendsen GW. 2012. Relative biological effectiveness of high linear energy transfer alpha-particles for the induction of DNA-double-strand breaks, chromosome aberrations and reproductive cell death in SW-1573 lung tumour cells. Oncol reports. 27:769-774.

George, A., R. Dey & V.B. Dqhumhh (2014), "Nuclear Anomalies, Chromosomal Aberrations and Proliferation Rates in Cultured Lymphocytes of Head and Neck Cancer Patients.", Asian Pacific journal of cancer prevention. 15(3):1119-1123. doi:10.7314/APJCP.2014.15.3.1119.

George, K.A. et al. (2009), "Dose Response of γ Rays and Iron Nuclei for Induction of Chromosomal Aberrations in Normal and Repair-Deficient Cell Lines Dose Response of c Rays and Iron Nuclei for Induction of Chromosomal Aberrations in Normal and Repair-Deficient Cell Lines.", Radiat. Res., 171(6):752–763.doi: 10.1667/RR1680.1.

George, K. et al. (2010), “Persistence of space radiation induced cytogenetic damage in the blood lymphocytes of astronauts”, Mutation Research, Vol. 701/1, Elsevier, Amsterdam, https://doi.org/10.1016/j.mrgentox.2010.02.007

Guerrero-Carbajal, C., A.A. Edwards & D.C. Lloyd (2003), "Induction of chromosome aberration in human lymphocytes and its dependence on X ray energy. Radiat Prot Dosimetry.", Radiat. Prot. Dosimetry 106(2):131–135. doi:10.1093/oxfordjournals.rpd.a006342.

Hada, M. & A.G. Georgakilas (2008), "Formation of Clustered DNA Damage after High-LET Irradiation: A Review.", J. Radiat. Res., 49(3):203–210. doi:10.1269/jrr.07123.

Hagmar, L. et al. (2004), "Impact of Types of Lymphocyte Chromosomal Aberrations on Human Cancer Risk : Results from Nordic and Italian Cohorts.", Cancer Res., 64(6):2258–2263. doi: 10.1158/0008-5472.CAN-03-3360.

Hamza VZ and Mohankumar MN. 2009. Cytogenetic damage in human blood lymphocytes exposed in vitro to radon. Mutat Res. 661(1-2):1-9.

Han. L. et al. (2014), "Cytogenetic analysis of peripheral blood lymphocytes, many years after exposure of workers to low-dose ionizing radiation.", Mutat. Res. Genet. Toxicol. Environ. Mutagen. 1(771):1–5, doi: 10.1016/j.mrgentox.2014.06.003

Hande, M.P. et al., (2003), Past Exposure to Densely Ionizing Radiation Leaves a Unique Permanent Signature in the Genome., Am J Hum Genet. 72:1162-1170. doi: 10.1086/375041.

Hande, M. P. et al. (2005), “Complex chromosome aberrations persist in individuals many years after occupational exposure to densely ionizing radiation: an mFISH study”, Genes chromosomes & cancer, Vol. 44/1, Wiley, Hoboken, https://doi.org/10.1002/gcc.20217

Hunter, N. & C.R. Muirhead (2009), "Review of relative biological effectiveness dependence on linear energy transfer for low-LET radiations Review of relative biological effectiveness dependence.", J. Radiol. Prot. 29(1):5-21, doi:10.1088/0952-4746/29/1/R01.

Jang, M. et al. (2019), "Dose Estimation Curves Following In Vitro X-ray Irradiation Using Blood From Four Healthy Korean Individuals.", Ann. Lab. Med. 39(1):91–95, doi: 10.3343/alm.2019.39.1.91.

Karthik K, Rajan V, Pandey BN, Sivasubramanian K, Paul SFD, Venkatachalam P, 2019. Direct and bystander effects in human blood lymphocytes exposed to ^{241}Am alpha particles and the relative biological effectiveness using chromosomal aberration and micronucleus assay. Int J Radiat Biol. 95(6):725-736.

Kim, Jeoum Nam and B. M. Lee (2007), “Risk factors, health risks, and risk management for aircraft personnel and frequent flyers”, Journal of toxicology and environmental health, Taylor & Francis Group, England, https://doi.org/10.1080/10937400600882103

Kozbenko, T. et al. (2022), “Deploying elements of scoping review methods for adverse outcome pathway development: a space travel case example”, International Journal of Radiation Biology, 1–12. https://doi.org/10.1080/09553002.2022.2110306

Li, W. A. Lee & P.K. Gregersen (2009), "Copy-number-variation and copy-number-alteration region detection by cumulative plots.", BMC Bioinformatics, 10(Suppl. 1):S67, doi:10.1186/1471-2105-10-S1-S67.

Lorat. Y. et al. (2015), "Nanoscale analysis of clustered DNA damage after high-LET irradiation by quantitative electron microscopy – The heavy burden to repair.", DNA Repair (Amst). 28:93–106. doi:10.1016/j.dnarep.2015.01.007.

Loucas BD, Durante M, Bailey SM, Cornforth MN. 2013. Chromosome Damage in Human Cells by Gamma Rays, Alpha Particles and Heavy Ions: Track Interactions in Basic Dose-Response Relationships. Radiat Res. 179(1):9-20.

Loucas, B. D and C. R. Geard (1994), “Kinetics of chromosome rejoining in normal human fibroblasts after exposure to low- and high-LET radiations”, Radiation research, Vol. 138/3, Radiation Research Society, Oak Brook, https://doi.org/10.2307/3578683

Maffei, F. et al. (2004), "Spectrum of chromosomal aberrations in peripheral lymphocytes of hospital workers occupationally exposed to low doses of ionizing radiation.", Mutat Res., 547(1-2):91–99. doi:10.1016/j.mrfmmm.2003.12.003.

McMahon, S.J. et al. (2016), "Mechanistic Modelling of DNA Repair and Cellular Survival Following Radiation-Induced DNA Damage.", Nat. Publ. Gr. (April):1–14. doi:10.1038/srep33290.

Meenakshi, C. & M.N. Mohankumar (2013), "Synergistic effect of radon in blood cells of smokers - An in vitro study.", Mutat. Res., 757(1):79–82. doi: 10.1016/j.mrgentox.2013.06.018.

Meenakshi, C., K. Sivasubramanian & B. Venkatraman (2017), "Nucleoplasmic bridges as a biomarker of DNA damage exposed to radon.", Mutat Res - Genet Toxicol Environ Mutagen. 814:22–28. doi:10.1016/j.mrgentox.2016.12.004.

Mestres M, Caballin MR, Schmid E, Stephan E, Stephan G, Sachs R, Barrios L, Barquinero JF. 2004. Analysis of alpha-particle induced chromosome aberrations in human lymphocytes, using pan-centromeric and pan-telomeric probes. 80(10):737-744.

Mill AJ, Wells J, Hall SC, Butler A. 1996. Micronucleus Induction in Human Lymphocytes: Comparative Effects of X Rays, Alpha Particles, Beta Particles and Neutrons and Implications for Biological Dosimetry. Radiat Res. 145:575-585.

Moquet JE, Fernandex JL, Edwards AA, Lloyd DC. 2001. Lymphocytes Chromsomal Aberrations and Their Complexity Induced In Vitro by Plutonium-239 Alpha-Particles and Detected by FISH. Cell Mol Biol. 47(3):549-556.

Morishita, M. et al. (2016), "Chromothripsis-like chromosomal rearrangements induced by ionizing radiation using proton microbeam irradiation system.", Oncotarget, 7(9):10182-10192, doi:10.18632/oncotarget.7186.

Nagasawa H, Little JB, Inkret WC, Carpenter S, Thompson K, Raju MR, Chen DJ, Strniste GF. 1990a. Cytogenetic effects of extremely low doses of plutonium-238 alpha-particle irradiation in CHO K-1 cells. Mutat Res. 244:233-238.

Nagasawa H, Robertson J, Little JB. 1990b. Induction of chromosomal aberrations and sister chromatid exchanges by alpha particles in density-inhibited cultures of mouse 10T1/2 and 3T3 cells. Int J Radiat Biol. 57(1):35-44.

Nikitaki, Z. et al. (2016), "Measurement of complex DNA damage induction and repair in human cellular systems after exposure to ionizing radiations of varying linear energy transfer (LET).", Free Radic. Res., 50(Sup1):S64-S78, doi:10.1080/10715762.2016.1232484.

Okayasu, R. (2012), "Heavy ions — a mini review.", 1000:991–1000. doi:10.1002/ijc.26445.

Puig R, Pujol M, Barrios L, Caballin MR, Barquinero J-F. 2016. Analysis of alpha-particle-induced chromosomal aberrations by chemically-induced PCC. Elaboration of dose-effect curves. Int J Radiat Biol. 92(9):493-501.

Purrott RJ, Edwards AA, Lloyd DC, Stather JW. 1980. The induction of chromosome aberrations in human lymphocytes by in vitro irradiation with alpha-particles from plutonium-239. Int J Radiat Biol. 38(3):277-284.

Qian, Q.Z. et al. (2016), "Effects of Ionising Radiation on Micronucleus Formation and Chromosomal Aberrations in Chinese Radiation Workers.", Radiat. Prot. Dosimetry, 168(2):197–203. doi: 10.1093/rpd/ncv290.

Sage, E. & N. Shikazono (2017), "Radiation Induced clustered DNA lesions : Repair and mutagenesis.", Free Radic Biol Med. 107(December 2016):125–135. doi:10.1016/j.freeradbiomed.2016.12.008.

Santovito, A., P. Cervella & M. Delpero (2013), "Increased frequency of chromosomal aberrations and sister chromatid exchanges in peripheral lymphocytes of radiology technicians chronically exposed to low levels of ionizing radiations.", Environ Toxicol Pharmacol. 37(1):396–403. doi:10.1016/j.etap.2013.12.009.

Schmid E, Hiever L, Heinzmann U, Roos H, Kellerer AM. 1996. Analysis of chromosome aberrations in human peripheral lymphocytes induced by in vitro alpha-particle irradiation. Radiat Environ Biophys. 35:179-184.

Schmid, E. et al. (2002), "The Effect of 29 kV X Rays on the Dose Response of Chromosome Aberrations in The Effect of 29 kV X Rays on the Dose Response of Chromosome Aberrations in Human Lymphocytes.", Radiat Res. 158(6):771–777. doi: 10.1667/0033-7587(2002)158[0771:TEOKXR]2.0.CO;2.

Smith, J. et al. (2003), "Impact of DNA ligase IV on the delity of end joining in human cells.", Nucleic Acids Res., 31(8). doi:10.1093/nar/gkg317.

Suto, Y. et al. (2015), "Construction of a cytogenetic dose – response curve for low-dose range gamma-irradiation in human peripheral blood lymphocytes using three-color FISH.", Mutat. Res. Genet. Toxicol. Environ. Mutagen, 794:32–38. doi: 10.1016/j.mrgentox.2015.10.002.

Suzuki, K. & T.K. Hei (1996), "Mutation induction in gamma-irradiated primary human bronchial epithelial cells and molecular analysis of the HPRT-mutants.", Mutat Res., 349(1):33-41. doi: 10.1016/0027-5107(95)00123-9.

Tao, F. et al. (1993), “Accelerated heavy ions and the lens. IX. Late effects of LET and dose on cellular parameters in the murine lens”, International journal of radiation biology, Vol. 64/1, Informa UK Ltd, London, https://doi.org/10.1080/09553009314551151

Tao, F. et al. (1994), “Radiation effects on late cytopathological parameters in the murine lens relative to particle fluence”, Advances in space research, Vol. 14/10, Elsevier Ltd, England, https://doi.org/10.1016/0273-1177(94)90503-7

Tawn EJ and Thierens H. 2009. Dose Response Relationships for Chromsome Aberrations Induced by Low Doses of Alpha-Particle Radiation. Radiat Prot Dosim. 135(4):268-271.

Testa A, Ballarini F, Giesen U, Gil OM, Carante MP, Tello J, Langner F, Rabus H, Palma V, Pinto M, Patrono C. 2018. Analysis of Radiation-Induced Chromsomal Aberrations on a Cell-by-Cell Basis after Alpha-Particle Microbeam Irradiation: Experimental Data and Simulations. Radiat Res. 189:597-604.

Themis M, Garimberti E, Hill MA, Anderson RM. 2013. Reduced chromosome aberration complexity in normal human bronchial epithelial cells exposed to low-LET gamma-rays and high-LET alpha-particles. Int J Radiat Biol. 89(11):934-943.

Thomas, P., K. Umegaki & M. Fenech (2003), "Nucleoplasmic bridges are a sensitive measure of chromosome rearrangement in the cytokinesis-block micronucleus assay Nucleoplasmic bridges are a sensitive measure of chromosome rearrangement in the cytokinesis-block micronucleus assay.", Mutagenesis, 18(2):187-194, doi:10.1093/mutage/18.2.187.

Tucker, J. D. et al. (2004), “Persistence of chromosome aberrations in mice acutely exposed to 56Fe + 26 ions”, Radiation research, United States, https://doi.org/10.1667/RR3177

Tucker, J.D. (2008), "Low-dose ionizing radiation and chromosome translocations : A review of the major considerations for human biological dosimetry.", Mutat. Res., 659(3):211–220. doi:10.1016/j.mrrev.2008.04.001.

Tucker, J.D. et al. (2005A), "Persistence of Chromosome Aberrations Following Acute Radiation: I, PAINT Translocations, Dicentrics, Rings, Fragments, and Insertions.", Environ. Mol. Mutagen. 45(2-3):229-248, doi:10.1002/em.20090.

Tucker, J.D. et al. (2005B), "Persistence of Chromosome Aberrations Following Acute Radiation: II , Does It Matter How Translocations Are Scored?", Environ. Mol. Mutagen., 45(2-3):249-257, doi:10.1002/em.20089.

Udroiu, I. et al. (2020), “DNA damage in lens epithelial cells exposed to occupationally-relevant X-ray doses and role in cataract formation”, Scientific reports, Vol. 10/1, Nature Research, Berlin, https://doi.org/10.1038/s41598-020-78383-2

Vellingiri, B. et al. (2014), "Ecotoxicology and Environmental Safety Cytogenetic endpoints and Xenobiotic gene polymorphism in lymphocytes of hospital workers chronically exposed to ionizing radiation in Cardiology, Radiology and Orthopedic Laboratories.", Ecotoxicol. Environ. Saf. 100:266–274, doi:10.1016/j.ecoenv.2013.09.036.

Weissenborn, U. & C. Streffer (1988), "Analysis of structural and numericalchromosomal anomalies at the first, second, and thirdmitosis after irradiation of one-cell mouse embryos with X-rays or neutrons.", Int. J. Radiat. Biol., 54(3):381–394, doi: 10.1080/09553008814551771

Williams, J. R. et al. (1999), “Predicting cancer rates in astronauts from animal carcinogenesis studies and cellular markers”, Fundamental and Molecular Mechanisms of Mutagenesis, Vol. 430/2, Elsevier, https://doi.org/10.1016/S0027-5107(99)00137-2

Worgul, B. V. et al. (1989), “Accelerated heavy ions and the lens. IV: Biomicroscopic and cytopathological analyses of the lenses of mice irradiated with 600 MeV/amu 56Fe ions”, Radiation research, Vol. 120/2, Radiation Research Society, Oak Brook, pp. 280-293

Wolf, N. et al. (2008), “Radiation cataracts: Mechanisms involved in their long delayed occurrence but then rapid progression”, Molecular vision, Vol. 14, Molecular Vision, Atlanta, pp. 274-285

Yang, T. C. (1999), “Proton radiobiology and uncertainties”, Radiation measurements, Vol. 30/3, Elsevier Ltd, Oxford, https://doi.org/10.1016/S1350-4487(99)00209-7

Zölzer, F. et al., (2013), Micronuclei in lymphocytes from radon spa personnel in the Czech Republic, Int Arch Occup Environ Health., Aug;86(6):629-33. doi: 10.1007/s00420- 012-0795-z. Epub 2012 Jul

Relationship: 2814: Energy Deposition leads to Increase, Cell Proliferation

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Deposition of energy leading to occurrence of cataracts	non-adjacent	Moderate	Moderate

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	Low	NCBI
mouse	Mus musculus	High	NCBI
rat	Rattus norvegicus	Moderate	NCBI
rabbit	Oryctolagus cuniculus	Moderate	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Unspecific	High

This KER is plausible in all life stages, sexes, and organisms. The majority of the evidence is from in vivo adult mice and rats with no specificity on sex, as well as adult human in vitro models that do not specify sex.

Key Event Relationship Description

Energy can be deposited onto biomolecules stochastically from various forms of radiation (both ionizing and non-ionizing). As radiation passes through an organism, it loses energy; in the process it can potentially cause direct and indirect molecular-level damage. The extent of damage occurs at various levels depending on ionization and non-ionization events (excitation of molecules). Energy deposition onto cells causes an alteration to a variety of cellular functions (BEIR, 1990). Under homeostatic conditions, cells duplicate at a rate set by the speed of the cell cycle. Any disruption in regulators of the cell cycle can result in cellular transformation (Lee & Muller, 2010). Cell proliferation rates can be altered via deposited energy-induced genetic alterations, signaling pathway activation, and increased

production of growth factors (Reynolds & Schecker, 1995; Liang et al., 2016; Vigneux et al., 2022).

Proliferative rates increase for cells when genes that regulate this activity are altered in such a way that they are either encouraging or unable to discourage replication. Oncogenes promote abnormal proliferation and can be turned on by genetic mutations. These types of mutations are known to occur when cells are exposed to ionizing radiation (Reynolds & Schecker, 1995). Tumor suppressor genes operate to slow unregulated cell proliferation (Lee & Muller, 2010). The suppressor protein p53 is associated with delays in cell cycle progression at G1, reducing the speed of cell proliferation (Khan & Wang, 2022). These genes can also be prevented from performing their function via radiation-induced alterations. When p53 is inactivated, this can cause a cell to pass through the G1 checkpoint, even when elements within the cell are damaged (Reynolds & Schecker, 1995). Other cell cycle checkpoints can also be activated by energy deposition via ionizing radiation, including G2/M and intra S stages. Transient arrests are linked with low dose exposures, though high doses can make the change permanent (Khan & Wang, 2022).

Evidence Supporting this KER

Overall Weight of Evidence: Moderate

Biological Plausibility

The biological plausibility of the relationship between deposited energy leading to increased proliferation of cells is moderately supported by the literature. The deposition of energy, such as that from ionizing radiation, starting at doses of <0.5 Gy for in vivo and >2 Gy for in vitro has been shown to induce changes in cell proliferation (Uwineza et al., 2019; Hamada & Sato, 2016; Hamada, 2017a; Ainsbury et al., 2016). Rabbit, human, and rat models and many cell types have been used to support this connection. Increased cell proliferation is induced via energy deposition through a poorly understood mechanism of signalling changes and gene expression alterations. The evidence supporting the connection spans multiple life stages and sexes, though no observable differences can be delineated between the groups (Markiewicz et al., 2015; Fujimichi & Hamada, 2014; Worgul et al., 1986; Richards, 1966; Barnard et al., 2022; Riley et al., 1989; von Sallmann, 1952; Soderberg et al., 1986; Ramsell & Berry, 1966; Treton & Courtois, 1981).

One of the main cell-related changes that can occur as a result of energy deposition is changes in gene expression. This is an event that then causes cell proliferation to increase. Energy deposition via stressors, such as ionizing radiation, increase cell proliferation through the inactivation of tumor suppressor genes and oncogene activation. Activated oncogenes can increase cell proliferation while the inactive suppressor genes are unable to regulate this change. Cyclin-D1, an oncogene protein, has been linked to shortened times between G1 and S stages of the cell cycle when found in excess in the cell (Reynolds & Schecker, 1995).

Other events also occur within the organism that contribute to increased proliferation following energy deposition. Alterations in cell growth factor genes such as MAPK1, as a result of deposited energy, can also cause increased proliferation rates in LECs (Vigneux et al., 2022). The mitogen-activated protein kinases/extracellular signal-regulated kinase (MAPK/ERK) and phosphatidylinositol 30-kinase (PI3K/AKT) signalling pathways are anti-apoptotic, with the former being essential for G2 checkpoint arrest (Hein et al., 2014). PI3K/AKT signalling is altered by growth factors and hindered by tumor suppressors (Dillon et al., 2007). MAPK/ERK and PI3K/AKT signalling pathways are both activated by energy deposition, such as ionizing radiation, and this has been shown to increase cell proliferation in human lung fibroblast cells. (Liang et al., 2016). PI3K is turned on by the increased growth factor levels and suppressor gene downregulation (Lee & Muller, 2010). This then signals down the pathway until it reaches AKT. AKT turns on the MAPK/ERK pathway, which induces increased cell proliferation, though the exact mechanism by which this happens is still uncertain (Liang et al., 2016).

Empirical Evidence

The empirical evidence relating to this KER strongly supports the relationship between the deposition of energy and increased cell proliferation. A variety of cell growth changes have been used to measure this relationship, including mitotic activity and the presence of proliferative markers. The data comes from a mix of in vivo and in vitro studies on mice, rats, rabbits, and human, with space-related radiation types such as γ-rays, UV, or X-rays (Markiewicz et al., 2015; Bahia et al., 2018; Fujimichi & Hamada, 2014; Worgul et al., 1986; Richards, 1966; Barnard et al., 2022; Riley et al., 1989; von Sallmann, 1952; Soderberg et al., 1986; Andley et al., 1994; Ramsell & Berry, 1966; Treton & Courtois, 1981).

Dose Concordance

High evidence exists to support dose response between energy deposition and increased cell proliferation. There is much available evidence to support this relationship using the lens of the eye.

Mitotic activity changes can be observed in vivo starting at 2 Gy neutron and 3 Gy X-ray exposures. With X-ray doses up to 1 Gy, no mitotic change in lens epithelial cells is observed. At 3 Gy, there is a ~0.5%/day change in mitosis, and this increases linearly to ~2.25% at 10 Gy (Riley et al., 1989). With a 3.5 Gy and 10 Gy dose, mitotic lens cells increased to about ~35% and ~45% relative to control treatment groups, respectively. Similarly, lens cells irradiated with 1 Gy argon ions had ~30% of cells undergoing mitosis (Worgul et al., 1986). In this study, mitotic activity in lens epithelial cells reached a peak at ~3 fold of the normal range after single 2 Gy neutron irradiation, in contrast to ~2-fold after single 4 Gy X-ray irradiation. When doses are administered in fractions, mitotic activity reached its peak at 2x normal range after neutron exposure and 3x after X-ray exposure. Whole lenses irradiated with neutrons showed a 6-fold increase in proliferating cells compared to X-irradiated cells. Irradiation of smaller areas wthin lens cells

resulted in a smaller increase in mitotic activities (Richards, 1966). In another study, it was shown that lens epithelium were displaying 69% higher levels of proliferating cells than the control following an in vivo 10 Gy X-ray exposure (Ramsell & Berry, 1966). Unscheduled synthesis, as measured by the number of grains in non-S phase cells and defined as an excess in the normal duplication of the genome prior to mitosis, increased in the lens epithelium to 8.3x control following in vivo exposure to 6 kJ/m2 of UV (Soderberg et al., 1986). In vitro [3H]-thymidine incorporation in lens epithelial cells following 250 J/m2 UV was 6.46x control levels (Andley et al., 1994). 72 weeks post 50 J/m2 UV exposure, the epithelio-distal region lens cells reached 5.3x control's grains/nuclei and the epithelio-central region cells reached 11x control using an in vitro model. The treatment group for the mitotic zone reached 2.4x control (Treton & Courtois, 1981).

An increase in the in vivo incorporation of the cell proliferation marker EdU was observed in lens cells following X-ray exposure of 0.05 Gy or higher. This then decreased following exposure to 1 Gy. Cells in the lens transition zone had 2x more EdU+ cells following 0.05 Gy, compared to the control. This increased linearly to 13x control levels following 0.25 Gy. Lens cells in the germination zone increased 5x when exposed to 0.25 Gy. Cells in both zones returned to control levels at doses of 1 Gy and above (Markiewicz et al., 2015).

Indirect assessment of cell proliferation through measurement of cell number, following exposure of immortalized human lens epithelial cells to X-rays from 0.01- 5 Gy showed the number of cells increased at 0.01 Gy with levels 1.5x above the control. At 0.5 Gy and above, the in vitro lens epithelial cell number decreased towards control levels (Bahia et al., 2018). Another in vitro study examined the amount of the epithelium area covered in the lens, finding that it remained consistent with control groups up to 2 Gy, and increased significantly at doses ≥2 Gy, remaining above 1.5x control. The number of colonies considered large (mean control area + 2 standard deviations) also increased at doses above 2 Gy after remaining near control levels at lower doses (Fujimichi & Hamada, 2014).

Time Concordance

High evidence exists to support time response between energy deposition and increased cell proliferation. Most studies show increased proliferation occurring at varied time points following the initial incidence of energy deposition, with most evidence being from high (≥2 Gy) doses of ionizing radiation exposure.

In examining, mitotic activities within in vivo lens cells irradiated with 1 Gy 40-Ar and 3.5 Gy X-ray, a suppression of proliferating cells was observed at 1-day post-irradiation, followed by an overshoot from control levels as early as day 3 and then a decline ~3 weeks post-irradiation (Worgul et al., 1986). Mitotic activity in another in vivo study reached peak at ~ 13 days post following irradiation from a single 2 Gy neutron source and 7 days after a single 4 Gy X-irradiation for lens epithelial cells. Activity reached normal or below normal range 14 days to ~1-month post-exposure. When administered in fractioned doses, activity peaked at 5-7 days post neutron exposure and 3-5 days post X-irradiation (Richards, 1966). Lens cell mitotic levels at 29 days post in vivo irradiation were ~15% higher than seen at time zero, following 1.25 Gy neutron exposure (Riley et al., 1989). After in vivo exposure to 2 Gy neutron radiation, there was an increase in mitotic activity starting within 4 hrs post exposure and occurring in up to ~45% of the lens cell population. Cells exposed to 10 Gy X-rays showed a similar trend, but only in up to ~27.5% of cell population (Riley et al., 1988). There was no response to the in vitro 15 Gy X-ray exposure up until 6 days post-irradiation, when the mitotic response reached 150 mitoses/lens epithelial cell. 12 days post-irradiation, the mean score was about 345 mitoses/cell while control remained at 0 mitoses/cell for the whole testing period (von Sallmann, 1952).

Essentiality

Moderate evidence exists to support the essentiality of energy deposition in the induction of cell proliferation. Since deposited energy initiates events immediately, its removal, also supports the essentiality of the key event. Studies that do not deposit energy are observed to have no downstream effects. in vivo No energy deposition, as seen in the control of experiments of many studies does not lead to proliferating cells (Worgul et al., 1986; von Sallmann, 1952; Richards, 1966). Similarly, shielding 40 – 60% of the lens from X-irradiation shows a decreased response, starting 1-2 weeks after in vivo exposure to 1400 r X-rays (Pirie & Drance, 1959).

Proliferation markers are also not incorporated into the cells at the same rate when no energy is deposited in cells. For example, within the lens transitional zone, 11 Edu+ cells/0.045mm2 were seen when exposed to 0.05 Gy X-rays, but only 5 EdU+cells/0.045 mm2 were detected in control groups. Similarly, the germinative zone of the lens displayed 28 Edu+cells/0.045 mm2 when exposed to 0.25 Gy, 4x the number EdU+ cells for control (Markiewicz et al., 2015).

Cell numbers are not significantly increased when energy deposition does not occur. Control lens epithelium samples had 2.8x105 cells/ml, 0.8x the 3.5x105 cells/ml seen when exposed in vitro to low dose rate 0.01 Gy X-rays (Bahia et al., 2018). Lens epithelium cell area coverage reached 27 mm2 after 2 Gy of in vitro X-rays, but only 18 mm2 for control (Fujimichi & Hamada, 2014).

Uncertainties and Inconsistencies

- Exposure to radiation has been associated with the arrest of the cell cycle (Khan & Wang, 2022; Hein et al., 2014; Wang et al., 2018; Turesson et al., 2003). The cell cycle function is associated with the cell's ability to undergo mitosis and generate additional cells (Khan & Wang, 2022; Reynolds & Schecker, 1995). Radiation turns on cell cycle checkpoints, causing cycle arrest (Wang et al., 2018; Turesson et al. 2003). When the cycle is

arrested, cells are unable to progress to the next stage, meaning that any cells not in the mitotic phase would then be unable to proliferate (Hein et al., 2014; Khan & Wang, 2022). Several studies show doses as low as 10 mGy (of alpha particle irradiation on human fibroblast cells) leading to less proliferation than control groups (Khan & Wang, 2022). Other studies found that proliferation was either increased or decreased based on the time since irradiation. In the earlier stages, 4 to 7 days post-irradiation, there was a decrease in cell proliferation (von Sallmann et al., 1955; Barnard et al., 2022). During this time, larger radiation doses led to a larger decrease. After this point, cell proliferation began to increase and larger radiation doses led to increased proliferation (rabbits, 125, 250, 500, 1000, 2000 rep) (von Sallmann et al., 1955). Pirie and Drance also found a similar effect, but they noted a continued decrease in proliferation after the increase seen by von Sallmann et al. (1959).

- Furthermore, LECs also see inconsistent results in radiation effects, with some radiation exposed cells forming colonies through excessive proliferation and others becoming inactivated or dead. This inactivation involves a long-term cell cycle arrest that is nonpermanent but does prevent proliferation from occurring (Fujimichi & Hamada, 2014). However, a subpopulation of LECs demonstrated increased sensitivity to radiation induced premature senescence and therefore, a cessation of proliferation for any cells not in mitosis (Hamada, 2017b).

Quantitative Understanding of the Linkage

The following tables provide representative examples of the relationship, unless otherwise indicated, all data is significantly significant. It is widely accepted that the deposition of energy, at all doses, results in immediate ionization events, followed by downstream events.

Dose Concordance

Reference	Experimental Description	Results
Worgul et al., 1986	In vivo, rats received head-only exposure to 3.5-10 Gy X-rays or 1 Gy 40Ar ions with nucleotide analog incorporation of [3H]-TdR for mitotic activity assay.	In the germinative zone of the rat lens epithelium, all radiation types resulted in an initial decrease in mitotic activity followed by an increase in mitosis by 1-week post-irradiation. However, X-ray radiation from 3.5-10 Gy did not consistently produce greater mitosis at higher doses, with the largest increase in mitosis (2.2x above control) occurring at 6 Gy after 3 days. At 5 days after 1 Gy irradiation with argon, mitosis was 1.6x above the control.
Richards, 1966	In vivo, mice received head-only exposure to 1 or 2 Gy neutron or 4 or 8 Gy X-ray with Lilly's hematoxylin and Feulgen staining to detect mitosis. Fractionated doses were also given with each radiation type.	Mitotic activity in lens epithelia initially decreased after both radiation types and all doses but were increased by 1 week. X-ray irradiation increased mitosis 2.2x above the control after 4 Gy and 1.8x after 8 Gy. However, fractionated X-ray doses at 8 or 9 Gy showed higher mitotic activity than the 4 Gy dose. Neutron irradiation increased mitosis 2.7x above the control after 1 Gy and 3.1x after 2 Gy. However, fractionated neutron doses at 2.25 Gy resulted in lower increases than the 1 Gy dose.
Markiewicz et al., 2015	In vivo, mice received whole-body exposure to 50-2000 mGy X-rays with an EdU incorporation assay to determine proliferative activity.	Mice lenses exposed in vivo to 0-2000 mGy X-rays showed an approximately linear increase in EdU-positive cells (indicative of increased cell proliferation) which peaked at a dose of 250 mGy, 13x control.
Fujimichi & Hamada, 2014	In vitro, human lens epithelial cells exposed to 0-6 Gy X-rays with stereomicroscopy to determine colony size, increased size considered proliferative.	Human LECs exposed to 0-6 Gy X-rays showed a gradual increase in mean colony size that began to plateau after 4 Gy, reaching 2.4x control at the maximum dose.
Bahia et al., 2018	In vitro, human lens epithelial cells exposed to 0.01-5 Gy X-rays with trypan blue exclusion assay for cell counting. Dose rates of either 1.62 cGy/min or 38.2 cGy/min were used.	There was a ~1.5-fold increase in cell number after 0.01, 0.02 and 0.25 Gy X-ray exposure at both the high and low dose rates, with 0.02 Gy being the peak number of cells. The cell numbers were relatively similar to the unexposed cells after exposed to 0.5, 2 and 5 Gy.
Riley et al., 1989	In vivo, rats received head-only exposure to 0-10 Gy X-rays or head-and-tail exposure to 1.25-2 Gy neutrons with stained and counted cells to determine mitotic activity. Wounding was performed at 28-36h post-irradiation to stimulate mitogenesis.	Immediately following irradiation, a large decrease in mitotic activity occurs. Subsequently, X-ray exposure up to 1 Gy shows no change but at 3 Gy there is a ~0.5%/day increase in mitosis. This increases to ~2.25% at 10 Gy. This is a linear increase with dose.
Treton & Courtois, 1981	In vitro, rat lens epithelial cells exposed to 50 J/m2 UV with [3H]-Thymidine incorporation as proliferation assay.	72 weeks following 50 J/m2 UV exposure the epithelio-distal region treatment group has 1.33 grains/nuclei, 5.3x control's 0.25 grains/nuclei. The epithelio-central treatment group has 11x control. The treatment group in the mitotic zone has 2.4x control.

Andley et al., 1994	In vitro, rabbit lens epithelial cells exposed to 250 J/m2 UVB with [3H]-Thymidine incorporation into newly synthesized cells marking proliferation.	There is a 6.46x control increase in [3H]-Thymidine labelled cells when treated with 250 J/m2 UVB.
Ramsell & Berry, 1966	In vivo, rabbit lenses were exposed to 10 Gy X-rays with Feulgen staining to detect mitosis.	The 10 Gy X-ray irradiated lens epithelium had a mitosis level that is 169% that of non-irradiated control levels.
Soderberg et al., 1986	In vivo, rat eyes were exposed to 6 kJ/m2 UV with the mean number of grains per non-S-phase nucleus in a section used as a proliferation assay.	There is an 8.3x increase of nuclei with unscheduled synthesis in 6 kJ/m2 UV treated cell compared to control.

Time Concordance

Reference	Experimental Description	Results
Riley et al., 1988	In vivo, mice received head-only exposure to 2 Gy neutrons or 10 Gy X-rays with nucleotide analog incorporation of [3H]-TdR for mitotic activity assay. Lenses were mechanically wounded at various times post-irradiation to stimulate mitogenesis.	In mice immediately exposed to radiation, wounding occurred between 1 h and 4 weeks post-irradiation. After each radiation type and dose, mitosis was first shown increased about 16 weeks post-wounding. For example, exposure to a single 2 Gy dose of neutrons increased the percent of labelled cells from ~12% (control) to ~45% at 24 weeks post-wounding in the central zone when wounding was done 4 weeks post-exposure. Similarly for 10 Gy of X-rays, the first peak in mitosis occurred 24 weeks post-wounding in the central zone, as mitosis increased from 12% (control) to 27% when wounding was done 4 weeks post-exposure.
Worgul et al., 1986	In vivo, rats received head-only exposure to 3.5-10 Gy X-rays or 1 Gy 40Ar ions with nucleotide analog incorporation of [3H]-TdR for mitotic activity assay.	In rats immediately exposed in vivo to 1 Gy 40Ar, mitotic activity began to increase one day post-irradiation, reaching a peak seven days post-irradiation at 1.6x control.
Richards, 1966	In vivo, mice received head-only exposure to 1 or 2 Gy neutron or 4 or 8 Gy X-ray with Lilly's hematoxylin and Feulgen staining to detect mitosis. Fractionated radiation was also given at similar doses.	In mice immediately exposed to radiation, mitotic activity reached peak at 13 days post-single 2 Gy neutron irradiation (3.1x above control), in contrast to 7 days after single 4 Gy X-irradiation (2.2x above control). Both types of radiation increased mitotic activity above the control as early as 3 days post-irradiation.
Riley et al., 1989	In vivo, rats received head-only exposure to 0-10 Gy X-rays or 1.25-2 Gy neutrons with stained and counted cells to determine mitotic activity. Wounding was performed at 28-36h post-irradiation to stimulate mitogenesis.	Immediately following irradiation, rats showed an initial decrease in mitosis down to less than 10% of the control after 10 Gy. However, after 28 days the levels of mitosis had partially recovered after all X-ray doses and after 1.25 Gy of neutrons.
von Sallmann et al., 1955	In vivo, 2- to 3-month-old male chinchilla rabbits had their ocular lenses irradiated with X-ray doses of 125, 250, 500, 1000, or 2000 r. Cell proliferation was determined by the mitoses in % of control eyes.	An initial decrease in mitosis was observed in rabbits immediately irradiated with X-rays. By 4-10 days mitosis was increased above the control, reaching a peak at 14 days post-irradiation of a 150% increase above the control at 2000 r.
von Sallmann, 1952	In vitro, rabbit lens epithelium exposed to 1500 r of X-rays with Feulgen staining to detect mitosis.	In rabbits immediately irradiated with X-rays, there is no response to the 1500 r X-ray exposure up until 6 days post-irradiation. After this time, the mitotic response continues to increase linearly until 20 days post-exposure. At 12 days post-irradiation, the mean score is about 345 mitoses/cell. Control stays within the normal range of mitoses for the whole measurement period.

Pirie & Drance, 1959	In vivo, 6-12-week-old Dutch rabbits had their right eyes exposed to 1400 r, with a dose rate of either 67 or 72 r/min. Mitosis was detected either through phase-contrast microscopy, or microscopy without phase contrast, depending on the specimen.	In rabbits immediately irradiated with X-rays, mitosis was completely reduced after 1 week. Mitosis subsequently increased up to 2.5x the control after 2- and 4-weeks post-irradiation. However, after 8 weeks, mitosis decreased to below control levels and continued to decrease until 36 weeks.

Known modulating factors

N/A

Modulating Factor (MF)	MF Specification	Effect(s) on the KER	Reference(s)

Known Feedforward/Feedback loops influencing this KER

N/A

References

Ainsbury, E. A. et al. (2016), “Ionizing radiation induced cataracts: recent biological and mechanistic developments and perspectives for future research”, Mutation research. Reviews in mutation research, Vol. 770, Elsevier B.V., https://doi.org/10.1016/j.mrrev.2016.07.010

Andley, U. et al. (1994), “Modulation of lens epithelial cell proliferation by enhanced prostaglandin synthesis after UVB exposure”, Investigative Ophthalmology and Visual Science, Vol.35/2, Association for Research in Vision and Ophthalmology, Rockville, pp.374-381.

Bahia, S. et al. (2018), “Oxidative and Nitrative Stress-Related Changes in Human Lens Epithelial Cells Following Exposure to X-Rays", International Journal of Radiation Biology, Vol.94/4, Informa, London, https://doi.org/10.1080/09553002.2018.1439194.

Barnard, S. et al. (2022), “Lens epithelial cell proliferation in response to ionizing radiation”, Radiation Research, Vol. 197/1, Radiation Research Society, https://doi.org/10.1667/RADE-20-00294.1

BEIR V. (1990), “Health effects of exposure to low levels of ionising radiation”, National Research Council (US) Committee on Biological Effects of Ionizing Radiation (BEIR V), National Academic Press, Washington, https://doi.org/10.17226/1224.

Dillon, R., D. White and W. Muller. (2007), “The phosphatidyl inositol 3-kinase signaling network: implications for human breast cancer”, Oncogene, Vol.26, Nature, Portfolio, London, https://doi.org/10.1038/sj.onc.1210202.

Fujimichi, Y. and N. Hamada. (2014), “Ionizing irradiation not only inactivates clonogenic potential in primary normal human diploid lens epithelial cells but also stimulates cell proliferation in a subset of this population”, PLoS ONE, Vol.9/5, PLOS, San Francisco, https://doi.org/ 10.1371/journal.pone.0098154.

Hamada, N. (2017a), “Ionizing radiation sensitivity of the ocular lens and its dose rate dependence”, International Journal of Radiation Biology, Vol.93/10, Informa, London, https://doi.org/10.1080/09553002.2016.1266407.

Hamada, N. and T. Sato. (2016), “Cataractogenesis following high-LET radiation exposure”, Mutation Research - Reviews in Mutation Research, Vol.770/Pt B, Elsevier, Amsterdam, https://doi.org/10.1016/j.mrrev.2016.08.005.

Hein, A.L., M.M. Ouellette and Y. Yan. (2014), “Radiation-induced signaling pathways that promote cancer cell survival (Review)”, International Journal of Oncology, Vol.45, Spandidos Publications, Athens, https://doi.org/10.3892/ijo.2014.2614.

Khan, M.G.M. and Y. Wang. (2022), “Advances in the Current Understanding of How Low-Dose Radiation Affects the Cell Cycle”, Cells, Vol.11/356, MDPI, Basel, https://doi.org/10.3390/cells11030356.

Kozbenko, T. et al. (2022), “Deploying elements of scoping review methods for adverse outcome pathway development: a space travel case example”, International Journal of Radiation Biology, 1–12. https://doi.org/10.1080/09553002.2022.2110306

Lee, E. Y. and W.J. Muller. (2010), “Oncogenes and tumor suppressor genes”, Cold Spring Harbor perspectives in biology, Vol.2/10, Cold Spring Harbor Laboratory Press, Long Island, https://doi.org/10.1101/cshperspect.a003236.

Liang, X. et al. (2016), “Low-Dose Radiation Induces Cell Proliferation in Human Embryonic Lung Fibroblasts but not in Lung Cancer Cells: Importance of ERK1/2 and AKT Signaling Pathways”, Dose-Response, Vol. January-March, SAGE Publications, Thousand Oaks, https://doi.org/10.1177/1559325815622174.

Markiewicz, E. et al. (2015), “Nonlinear ionizing radiation-induced changes in eye lens cell proliferation, cyclin D1 expression and lens shape”, Open Biology, Vol.5/4, Royal Society, London, https://doi.org/10.1098/rsob.150011.

Pirie, A. and S. M. Drance (1959), “Modification of X-ray damage to the lens by partial shielding”, International Journal of Radiation Biology and Related Studies in Physics, Chemistry and Medicine, Vol. 1/3, Taylor & Francis, England, https://doi.org/10.1080/09553005914550391

Ramsell, T. and R. Berry. (1966), “Recovery from x-ray damage to the lens. The effects of fractionated x-ray doses observed in rabbit lens epithelium irradiated in vivo”, The British journal of radiology, Vol.39/467, British Institute of Radiology, London, https://doi.org/10.1259/0007-1285-39-467-853.

Reynolds, RJ. and J.A. Schecker. (1995), “Radiation, Cell Cycle, and Cancer”, Los Alamos Science, Vol.23, Los Alamos National Laboratory, Los Alamos, pp.51-89.

Richards, R. (1966), “Changes in lens epithelium after x-ray or neutron irradiation (mouse and rabbit)”, Transactions of the American Ophthalmological Society, Vol.64, American Ophthalmological Society, Rochester, pp.700-34.

Riley, E. et al. (1989), “Comparison of Recovery from Potential Mitotic Abnormality in Mitotically Quiescent Lens Cells after X, Neutron, and ^{56}Fe Irradiations”, Radiation Research, Vol.119/2, Radiation Research Society, Indianapolis, https://doi.org/10.2307/3577616.

Riley, E., R. Miller and A. Lindgren. (1988), “Recovery of Murine Lens Epithelial Cells from Single and Fractionated Doses of X Rays and Neutrons”, Radiation Research, Vol.114, Radiation Research Society, Indianapolis, https://doi.org/10.2307/3577127.

Söderberg, P., B. Philipson and B. Lindström. (1986), “Unscheduled DNA synthesis in lens epithelium after in vivo exposure to UV radiation in the 300 nm wavelength region”, Acta Ophthalmologica, Vol.64/2, Wiley, Hoboken, https://doi.org/10.1111/j.1755-3768.1986.tb06894.x.

Turesson, I. et al. (2003), “Biological Response to Radiation Therapy”, Acta Oncologica, Vol.42/2, Taylor & Francis, Oxfordshire, https://doi.org/10.1080/02841860310004959.

Treton, J. and Y. Courtois. (1981), “Evolution of the distribution, proliferation and ultraviolet repair capacity of rat lens epithelial cells as a function of maturation and aging”, Mechanisms of Ageing and Development, Vol.15, Elsevier Ireland, Dublin, https://doi.org/10.1016/0047-6374(81)90134-2.

Uwineza, A. et al. (2019), “Cataractogenic load – A concept to study the contribution of ionizing radiation to accelerated aging in the eye lens”, Mutation Research - Reviews in Mutation Research, Vol.779, Elsevier, Amsterdam, https://doi.org/10.1016/j.mrrev.2019.02.004.

Vigneux, G. et al. (2022), “Radiation-Induced Alterations in Proliferation, Migration, and Adhesion in Lens Epithelial Cells and Implications for Cataract Development”, Bioengineering, Vol.9/29, MDPI, Basel, https://doi.org/10.3390/bioengineering9010029.

von Sallmann, L. (1952), “Experimental studies on early lens changes after roentgen irradiation III. Effect of X-radiation on mitotic activity and nuclear fragmentation of lens epithelium in normal and cysteine-treated rabbits”, Transactions of the American Ophthalmological Society, Vol.8, American Ophthalmological Society, Rochester, https://doi.org/10.1001/archopht.1952.01700030313005.

von Sallmann, L. et al. (1955), “Effects of high-energy particles, X-rays, and aging on lens epithelium”, A.M.A. Archives of Ophthalmology, Vol. 54/4, American Medical Association, United States, https://doi.org/10.1001/archopht.1955.00930020495003

Wang, J. S., H.J. Wang and H.L. Qian. (2018), “Biological effects of radiation on cancer cells”, Military Medical Research, Vol.5/1, Springer Nature, Berlin, https://doi.org/10.1186/s40779-018-0167-4.

Worgul, B., G. Merriam and C. Medvedovsky. (1986), “Accelerated heavy particles and the lens II. Cytopathological changes”, Investigative Ophthalmology and Visual Science, Vol.27/1, Association for Research in Vision and Ophthalmology, Rockville, pp.108-114.

Relationship: 2815: Energy Deposition leads to Cataracts

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Deposition of energy leading to occurrence of cataracts	non-adjacent	High	High

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links

Term	Scientific Term	Evidence	Links
human	Homo sapiens	High	NCBI
mouse	Mus musculus	High	NCBI
rat	Rattus norvegicus	High	NCBI
rhesus monkeys	Macaca mulatta	Moderate	NCBI
rabbit	Oryctolagus cuniculus	Moderate	NCBI
guinea pig	Cavia porcellus	Moderate	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Female	High
Male	High

This KER is supported by a large body of literature and is plausible in all life stages, sexes, and organisms that have a clear lens for vision. Due to the large volume of studies, only in vivo studies were examined. The majority of the evidence supports adult humans, mice, and rats, and is not sex specific however, there is evidence supporting all ages, and sexes, as well as rabbits, voles, monkeys, guinea pigs, and rainbow trout.

Key Event Relationship Description

Energy can be deposited onto biomolecules stochastically from various forms of radiation (both ionizing and non-ionizing). As radiation passes through an organism, it loses energy; in the process it can potentially cause direct and indirect molecular-level damage. The extent of damage occurs at various levels depending on ionization and non-ionization events (excitation of molecules). The resulting particle-radiation interactions produce a cascade of negative biological consequences, if this occurs in the lens of the eye, it can lead to the formation of lens opacification, leading to the formation of cataracts. This multistep process is initiated by the deposition of radiation energy onto the DNA molecules or crystallin proteins within lens cells. As a result, DNA damage is incurred, frequently as double-strand breaks of the DNA helix. Inadequate repair of DNA damage can lead to mutations and chromosomal aberrations. Accumulation of such genetic damage in critical genes involved in cell-cycle checkpoints can promote uncontrolled cellular proliferation (Hamada 2017; Hamada et al., 2020). An abnormally high rate of cell proliferation ultimately disrupts normal lens development, which is dependent on precise spatiotemporal regulation to maintain lens transparency. The lens is a closed system and has a limited turnover of macromolecule components (Uwineza et al., 2019), therefore the buildup of damaged components, including lens crystallin proteins, contributes to opacification of the lens known as cataracts. Cataracts are a progressive condition in which the lens of the eye develops opacities and becomes cloudy, resulting in blurred vision as well as glare and haloes around lights (National Eye Institute, 2022). For this AOP, a cataract is defined when over 5% of the lens is opacified.

Evidence Supporting this KER

Overall Weight of Evidence: High

Biological Plausibility

There is strong biological plausibility for the association between direct deposition of energy by ionizing radiation and cataract incidence. It is a well established relationship that has been described by many reviews and research articles (Ainsbury et al., 2021; NCRP, 2016; Hamada and Sato, 2016; Uwineza et al., 2019; Kleiman, 2012; Shore et al., 2010; ICRP, 2012; Little et al., 2021).

Cataracts are an eye condition in which the clear lens of the eye becomes opaque, resulting in reduced vision. Although cataracts are typically associated with aging, exposure of the lens to ionizing radiation is a known risk factor for the acceleration/induction of opacification within human lenses (Ainsbury et al., 2021; Dauer 2018). The majority of evidence supporting radiation-induced cataracts is drawn from primary research using experimental animals that have been exposed to varying qualities of radiation and from epidemiological investigations of health care professionals, flight personnel, astronauts, the Chernobyl cleanup workers, and atomic bomb survivors (NCRP, 2016; Bouffler et al., 2012; Hamada 2017; ICRP, 2012; Hamada & Sato, 2016).

Ionizing radiation can be in the form of high energy particles (such as alpha particles, beta particles or heavy ions) or high energy waves (such as γ-rays or X-rays). Due to a thin layering of tissue covering the lens, radiation particles can reach sensitive areas in the lens and target cellular components that are biologically active (Ainsbury et al., 2021; Hamada, 2017). Primary targets of radiation in the lens are DNA molecules, crystallin proteins, and lens epithelial

cells (LECs) in the germinative zone (Hamada et al., 2014; Ainsbury et al., 2016; ICRP, 2012). Crystallin proteins comprise 90% of the total protein content in lens fiber cells (Moreau and King, 2012), which form the bulk of the eye lens, making them a frequent target of radiation energy. Lens tissue is normally kept in a relatively low oxygen environment; hence it is prone to oxidative stress (Truscott, 2005). Radiation exposure can induce oxidative stress through interaction with surrounding molecules, such as water, to generate reactive oxygen species. Such reactive molecules distribute throughout the lens to initiate early destructive molecular events (Blakely et al., 2012). As well, deposition of energy can directly upregulate enzymes involved in reactive oxygen and nitrogen species (RONS) production (de Jager, Cockrell and Du Plessis, 2017).

Cataracts can result from two main mechanisms: an abnormal increase in LEC proliferation, and changes in crystallin protein conformation. The uncontrolled cell proliferation is possibly the consequence of overwhelming genetic and chromosomal instability incurred in critical genes that regulate cell cycle checkpoints (Uwineza et al., 2019; Hamada et al., 2020). Radiation-stimulated LEC proliferation has been reported in in vitro human lens cells (Bahia et al., 2018.), in vitro animal lens cells (von Sallmann, 1951) and in vivo experimental animals (Worgul et al., 1986; Richards, 1966; Markiewicz et al., 2015; Riley et al., 1989; Ramsell and Berry, 1966; Barnard et al., 2022). When the rate of division in mitotically-active LECs becomes too high, they become incapable of differentiating into typical elongated, organelle-free lens fiber cells (Wride 2011; Ainsbury et al., 2016). When there are changes in the structural properties of the highly soluble lens crystallin proteins, they denature and become insoluble, and thus tend to aggregate (Uwineza et al., 2019; Moreau and King, 2012). Radiation-stimulated lens crystallin protein alterations have been reported by many authors (Abdelkawi, 2012; Bahia et al., 2018; Kim et al., 2015; Shang et al., 1994). Both of these mechanisms produce opaqueness in the lens, reducing the ability of the lens to focus light onto the retina and produce sharp vision.

The literature contains large amounts of epidemiological data, focused primarily on atomic bomb survivors, cancer survivors, and radiation workers. The overall consensus is that cataract risk increases with radiation dose as a stochastic effect, as measured based on various forms of cataracts, cataract surgery, or general opacities (Hall et al. 1999; Neriishi et al. 2012; Chodick et al. 2016; Su et al. 2020; Yamada et al. 2004; Jacobson, 2005).

Empirical Evidence

Dose Concordance

There is strong evidence in the literature to support a dose-response for the development of cataracts following the deposition of energy. Several studies by NASA and others have investigated the exposure of flight personnel and astronauts to cosmic radiation and cataract development. Chylack et al. (2009) found that there was an exponential radiation dose-response in cataract prevalence in astronauts following their space travels. The prevalence of cataracts was more than twice as high in astronauts exposed to higher doses of ionizing and UV radiation than those exposed to relatively lower doses (Chylack et al., 2009; Chylack et al., 2012). The development of cataracts can be observed from space radiation doses as low as 8 mSv (Cucinotta et al., 2001). Commercial pilots with career radiation doses up to 21 mSv were also at risk of developing cataracts (Rafnsson et al., 2005), although the risk was not as high as that typically seen in astronauts (Jones et al., 2007).

There was an exponential dose-response in prevalence of cataract development in individuals following the exposure to radiation released from the atomic bomb dropped on Hiroshima (Minamoto et al., 2004; Nakashima et al., 2006; Otake and Schull, 1990). The odds ratio remained ~1 when exposed to 1 Sv of radiation, but the odds ratio increased to 4 when exposed to up to 4 Sv (Minamoto et al., 2004; Nakashima et al., 2006). Cataract development can be detected as low as 0.01 Gy in 1% of individuals (Otake and Schull, 1990). Exposed at a higher dose of 2 Gy, the prevalence increased to ~7%, but this prevalence further increased to 42% when exposed to 6 Gy (Otake and Schull 1990; Nefzger et al., 1969; Choshi et al., 1983). Individuals exposed to nuclear radiation at Chernobyl in the Worgul et al. (2007) study showed a similar result where <600 mGy exposure resulted in an odds ratio of ~1 and increased to ~1.7 when the exposure increased to 1 Gy. It has been reported there is a significantly increased risk of cataracts below 100 mGy (but not below 50 mGy) in occupational technologists exposed to radiation (Little et al., 2018; Little et al., 2020). Patients with head and neck cancer showed a rise in the percentage of lens opacity three and six months following radiotherapy (Arefpour et al., 2021).

Several animal studies have investigated the low dose effect of high energy particles on cataract development. These particles include fast neutrons, ^{56}Fe, ^{40}Ar, ^{20}Ne, ^{12}C, protons and ^{4}He. There was a linear dose-response in cataract prevalence resulting from exposure to the following radiation: 0.5-4 Gy 4He and 0.03-0.5 Gy 12C (Fedorenko et al., 1995), and 0.01-0.3 Gy fast neutrons (Bateman et al., 1963). The rate of cataract formation increased linearly with dose when exposed to ^{56}Fe (Brenner et al., 1993; Worgul et al., 1993). Cataract severity plateaus after 0.6 Gy X-rays, but not after exposure to the same dose of ^{40}Ar, ^{20}Ne or ^{12}C (Cox et al., 1983; Ainsworth et al., 1981). Within each of the studies, dose-responses were more profound when observed at later, rather than earlier, post-irradiation time points. The dose-response for cataract development also depends on the type of radiation and its energy level or linear energy transfer (LET). When exposed to the same dose ranges, ^{40}Ar (LET of 100 keV/µm) resulted in more severe cataract formations compared to that of radiation with lower LET, such as ^{20}Ne (LET of 30 keV/µm) and ^{12}C (LET of 10 keV/µm) (Cox et al., 1983; Ainsworth et al., 1981). When examined 40 weeks post-irradiation, lenses exposed to a range of 1-4 Gy of 645 MeV protons showed an apparent linear dose-response, compared the dose-response to 9 GeV protons over the same dose range that was linear until 2 Gy and then reached a plateau (Fedorenko et al., 1995). Additionally, it was observed that cataracts were more prevalent in lenses exposed to 9 GeV protons than in lenses exposed to 645 MeV protons. In another study, various mouse species exposed to 6060Co γ-irradiation atmultiple doses (0.5, 1 and 2 Gy) using two dose rates (0.063 and 0.3 Gy min-1) revealed that the average lens density rose was elevated with dose and dose rate when Ercc2 and Ptch1 mutations were present

(McCarron et al., 2022).

Time-Concordance

High support exists for a time-response relationship between the deposition of energy and cataracts. There is an exponential time-response in cataract development to space radiation exposure. The shape of the exponential time-response curve was greatly influenced by radiation dose. Astronauts exposed to <8 mSv had a similarly shaped time-response curve for cataract prevalence, although at a lower prevalence level, compared to astronauts exposed to >8 mSv (Cucinotta et al., 2001). Large opacifications were not detected in the astronauts exposed to <8 mSv until 25 years after their first space travel. This observation greatly differs from astronauts exposed to >8 mSv, where opacification was detected in <5 years.

Epidemiological studies in humans investigating the time-response of cataract development from low doses of high energy particles is limited. High doses of high energy particles are used commonly in radiation therapy for uveal melanoma patients. Gragoudas et al. (1995), Meecham et al. (1994), and Char et al. (1998) showed that there was an increase cataract prevalence with time after exposure to radiation used in therapies. The change in cataract prevalence over time was greatly influenced by radiation dose and percent of the lens exposed. Thirty-six months after proton beam therapy with doses up to 70 cobalt Gy-equivalents (GyE), cataract prevalence ranged from 20~75%, depending on the risk group (based on the dose that the lens received and tumor height) (Gragoudas et al., 1995). Twelve years after helium ion therapy using a dose range of 48 to 80 GyE, cataracts were found in 20~100 % of those treated, depending on the proportional area of the lens that was irradiated (Meecham et al., 1994). In a similar helium ion therapy, Char et al. (1998) found that 20~90% of patients developed cataracts ten years after treatments using a dose range of 50~80 GyE. Reports from radiotherapy patients and cancer survivors also serve as evidence for radiation induced cataracts. In a study by Chodick et al. (2016) 3.5% of subjects experienceds a case of cataracts during the first 5 years after cancer diagnosis, with prevalence increasing as the dose of radiotherapy increased.

Several authors have investigated the time-response of cataract development in animal models exposed to low dose radiation released from high energy particles. The majority of the studies showed a linear time-response in cataract development to radiation exposures. The results were similar across different animal species. A time-response was evident for cataract severity by exposing animals to 90-180 reps (ca 0.9–1.8 Gy) cyclotron (Upton et al., 1956), 45-180 reps (ca 0.45-1.8 Gy) fission neutrons (Upton et al., 1956), 1.3-37.3 reps (ca 0.013-0.373 Gy) PO-B neutrons (Upton et al., 1956), 1-200 cGy ^{56}Fe (Riley et al., 1991; Wu et al., 1994; Worgul et al., 1993; Worgul et al., 1995), 0.01-1 Gy ^{40}Ar (Merriam et al., 1984) and 0.25-1.25 Gy protons (Cox et al., 1992; Cleary et al., 1972). The steepness of the linear response curve was greatly influenced by dose. Exposure to higher doses was likely to result in an immediate linear time-response in cataract prevalence, while an initial lag phase was present prior to entering the linear phase after exposures to relative low doses (Worgul et al., 1996; Worgul et al., 1993; Medvedovsky et al., 1994; Brenner et al., 1993). It is uncertain whether the results of some of these studies using low dose exposures would continue their linear increase to the 100% maximum over time, or if they would reach a plateau without attaining the 100% maximum. A longer post-irradiation time of observation would be required to determine this. A sigmoidal time-response curve for cataract severity was observed after radiation exposures within a range of 0.05-2 Gy ^{56}Fe (Lett et al., 1986) and 0.25-1.00 Gy protons (Cleary et al., 1972). Merriam et al. (1984) demonstrated exposure to 0.01-0.25 Gy did not produce a significant effect on cataract severity until >40 week post-irradiation, while a significant effect appeared relatively earlier (at 10 week post-irradiation) after an exposure of 1 Gy ^{40}Ar. McCarron et al. exposed different mouse species to 60Co γ-irradiation at doses of 0.5, 1, and 2 Gy, employing two dose rates (0.063 and 0.3 Gy min-1). Lens opacity was evaluated from 0-18 months post irradiation. The results indicate that with the passage of time, there is a gradual rise in lens opacity (McCarron et al., 2022).

Essentiality

Radiation exposure has been found to increase cataracts above background levels, studies using various radiation types demonstrate this relationship (Bateman et al., 1963; Cleary et al., 1972; Cox et l., 1983; Lett et al., 1986; Orake & Schull, 1990; Riley et al., 1991; Worgul et al., 1993; Jones et al., 2007; Kocer et al., 2007).

Uncertainties and Inconsistencies

Definition of cataracts and measurement methodology

Cataracts are synonymously associated with lens opacification, yet there was no consistency in how cataracts were defined between studies. Such inconsistency was also apparent in the use of different methodologies to measure cataracts across different studies (Hammer et al., 2013; NCRP, 2016). Many of the scoring systems used to measure opacification were subjective, making it difficult to compare the risks observed between different studies (Jacob et al., 2011; Hamada et al., 2014). Although opacification is a direct measurement for cataracts, visual impairment was suggested to be the ultimate endpoint for radiation-induced cataracts, namely vision-impairing cataracts (VIC) (NCRP, 2016). Studies using visual acuity to measure cataracts pose challenges as the test is not specific for cataracts, even though the measurement is an indicative test for the ultimate function of the lens.

Latency effect

The time lapse for detection of cataracts varies roughly inversely with dose. The risk for cataracts caused by low doses of high energy particles may be underestimated in many studies due to length of the observation period used.

More cataract development might have been seen with longer periods of observation. The extended latency period for cataract development posed challenges in the determination of causality, given the additional and complex confounding factors associated with aging (Ainsbury et al., 2021; Sakashita et al, 2019; Dauer et al., 2017). Whether opacification remains constant or progresses depends (in part, at least) on the level of radiation dose received; whether minor opacifications will transition to major visual impairments is also not entirely certain (Hamada et al., 2014; Shore et al., 2016; Hamada 2017; Hamada et al., 2020; Ainsbury et al., 2021).

Partial lens irradiation

There is evidence that non-irradiated sections of a lens can develop opacities following the irradiated sections receiving a dose of 10 or 50 mGy of X-rays. The irradiated section of the lens has a reduced number of opacities compared to lenses that were fully irradiated by the same dose, indicating a protective effect by the non-irradiated section (Worgul et al. 2005b). A second study found that opacities of partially irradiated lens are not long lasting and do not worsen over time into cataracts (Leinfelder & Riley, 1956). This suggests that some radiation-induced opacities are unable to manifest into cataracts, despite the deposition of energy initiating the relationship (Hamada & Fujimichi, 2015).

Challenged study

A study by Lehmann et al., 2016, showed cataracts in approximately 70% of voles from within contaminated Chernobyl zones. The study identified a positive relationship between cataracts and radiation doses of 20 – 80,000 µSv. However, the work is disputed as these cataracts detected may be due to the conditions under which the voles were preserved rather than radiation exposure (Smith et al., 2020; Laskowski et al., 2022). Furthermore, other studies did not find higher cataract rates when compared to non-exposed voles captured in the same geographical area as those in the Lehmann study (Williams, 2019).

Stochastic vs. Deterministic Effects

The overall consensus is that cataract risk increases with radiation dose as a stochastic effect due to the linkage of cataracts to genotoxic effects (Seals et al., 2016). This is reinforced through cataract occurrence in animals with genetic mutations relating to DNA repair and cell division; the stochasticity is apparent because damage to singular cells is transmitted to successive cells, resulting in cataract formation (Seals et al., 2016). However, there is also controversy on whether there is a threshold dose below which tissue reactions (deterministic effects) do not occur (Thome et al., 2018; Hamada, 2023).

Quantitative Understanding of the Linkage

The following tables provide representative examples of the relationship, unless otherwise indicated, all data is significantly significant.

Dose Concordance

Reference	Experiment Description	Result
Chylack et al., 2009	In vivo, mixed sex humans exposed to median dose 12.9 mSv from UVF and space travel with LOCSIII grading of lens opacities.	Astronauts exposed to various doses of radiation from 0 to ~200 mSv showed a general trend of exposure to higher doses leading to the largest posterior subcapsular opacity. The odds ratio for increased risk of high opacity was 2.23 for astronauts exposed to higher space radiation doses.
Chylack et al., 2012	In vivo, mixed sex humans exposed to median dose of 12.9 mSv from space travel with % opaque area measuring opacity severity.	In astronauts exposed to doses from ~10-275 mSv, the progression rate of cortical opacification was estimated to be (0.25 ± 0.13) % lens area/Sv/year.
Rafnsson et al., 2005	In vivo, male humans exposed to 1-48 mSv from space travel with the World Health Organization simplified grading system to measure cataracts.	Humans exposed to radiation from 1-48 mSv showed a greater risk of cataracts at higher doses. Compared to the unexposed group, the odds ratio of nuclear cataract risk was 2.48-2.82 for pilots exposed to cosmic radiation of 1-21 mSv. Those who had the most exposure (22-48 mSv) had the highest odds ratio of 4.19.

Cucinotta et al., 2001	In vivo, mixed sex humans exposed to 0.2-91 mSv radiation from space travel with slit lamp microscopy to grade lens opacification.	Astronauts exposed to radiation from 0.2-91 mSv showed that the probability of developing any type of cataracts in astronauts exposed to high dose radiation (>8 mSv) was up to ~2.5-fold higher than those exposed to low dose radiation (<8 mSv). In comparison, the same study showed the probability of developing non-trace cataracts (loss of vision) can increase up to 3-fold due to space radiation.
Jones et al., 2007	In vivo, male humans exposed to space with slit lamp microscopy to grade opacity severity.	At the age of 70, the prevalence of cataracts in astronauts exposed to high doses was 1.2-fold higher than that seen in astronauts exposed to low doses, 2.8-fold higher than that seen in commercial pilots, and 8.5-fold higher than that seen in healthy US males.
Minamoto et al., 2004	In vivo, mixed sex humans exposed to atomic bomb irradiation at doses of 0.005-3 Sv with slit lamp and LOCSII grading of opacification.	Humans exposed to doses of 0.005-3 Sv after an atomic bomb showed a gradual increase in the odds ratio for posterior subcapsular opacities (indicative of cataract risk) with the maximum dose displaying a 3x increase compared to control.
Nakashima et al., 2006	In vivo, mixed sex humans exposed to 0.005-4 Gy of radiation from an atomic bomb with LOCSII grading of opacities.	Atomic bomb survivors exposed to doses of 0.005-4 Gy showed an increased risk of cataracts at higher doses. The odds ratio for cataract development increased from 1 to 3-4 with increasing radiation doses up to 4 Gy.
Nefzger et al., 1969	In vivo, mixed sex humans exposed to 0-2 Gy of irradiation from an atomic bomb with slit lamp examination to determine level of opacification.	Atomic bomb survivors exposed to doses of 0-2 Gy showed an increased risk of cataracts at higher doses. Individuals exposed to higher dose (>0.2 Gy) and lower dose (<0.2 Gy) radiation had ~28% and ~7% increases in cataract incidence, respectively, compared to those not exposed.
Choshi et al., 1983	In vivo, mixed sex humans exposed to 0-1.00+ Gy irradiation from an atomic bomb with slit lamp examination for lens opacities.	Atomic bomb survivors exposed to doses of 0 to over 1 Gy showed an increased risk of cataracts at higher doses. The prevalence of cataracts was increased by ~5-10 percentage points for individuals exposed to >1.00 Gy, compared to those exposed to 0.01-0.99 Gy.
Otake & Schull, 1990	In vivo, humans exposed to up to 6 Gy of irradiation from an atomic bomb with slit lamp biomicroscopy observation of opacities.	Atomic bomb survivors exposed to doses of <0.01-6 Gy showed an increased risk of cataracts at higher doses. One percent of individuals developed cataracts after exposure to <0.01 Gy. This prevalence increased to 42.3% for individuals exposed to 4-5.99 Gy.
Worgul et al., 2007	In vivo, mixed sex humans exposed to up to 1000 mGy of radiation from Chernobyl with modified Merriam-Focht opacity grading.	Humans exposed to Chernobyl radiation from <100-1000 mGy showed increased cataracts risk at increasing doses, increasing from 1 with low doses (<100 mGy) to 1.77 when exposed to greater than 800 mGy.
Little et al., 2018	In vivo, 67,246 mixed sex US radiologic technologists who held a certification during at least two years from 1926 to 1982 completed four questionnaires over the course of 31 years to determine information on their cataract history as well as potential modulating factors.	Radiologic technologists occupationally exposed to <10.0 - 499.9 mGy showed a gradual increase in hazard ratios for cataract risk, eventually increasing to 1.76x control at the maximum dose.
Hall et al., 1999	In vivo, mixed sex human children were exposed to β, γ or X-rays with a mean dose of 0.4 Gy with a range of 0 – 8.4 Gy, and an average dose rate of 0.13 Gy/h, given over an average of 2.1 treatments. Cataracts were measured using LOCS I.	Children who received a dose to the lens of 1 Gy were 35% more likely to develop a cortical opacity (95% confidence interval (CI) 1.07-1.69, LOCS ≥ 1.0), and 50% more likely to develop a posterior subcapsular opacity than unexposed children (95% CI 1.10-2.05, LOCS ≥ 1.0).

Neriishi et al., 2012	In vivo, Japanese atomic bomb survivors that were exposed to a mean dose of 0.50 Gy with a range of 0.0 – 5.14 Gy, the mean age at exposure was 20.4 years. Cataracts were measured based on surgical removal.	Atomic bomb survivors demonstrated that the incidence of cataracts increased as the dose increased. The estimated excess cases were 33 per 10,000 people/year/Gy.
Chodick et al., 2016	In vivo, mixed sex childhood cancer survivors were exposed to a mean dose of 2.2 Gy with a range of 0 – 66 Gy during radiotherapy. After an average of 21.4 years post-exposure, their cataract history was measured via questionnaire.	In childhood cancer survivors exposed to 0-66 Gy radiation there was a linear dose-response relationship between lens dose and cataracts (excess odds ratio per Gy = 0.92; 95% CI 0.65-1.20). Furthermore, doses greater than 0.5 Gy had an increased odds ratio (OR) compared to doses less than 0.5 Gy (OR = 2.2; 95% CI 1.3-3.7).
Su et al., 2020	In vivo, mixed sex humans ≥45 years that were exposed to a cumulative lens dose of 189.5 ± 36.5 mGy and range of 0.0221 – 0.3104 Gy, after residing in a high natural background radiation area in Yangjian City, had the presence of cataracts determined using the LOCS III system.	In humans exposed to high background radiation, the estimated dose threshold for cortical opacities was 140 mGy (90% CI 110-160 mGy). Furthermore, the odds ratios for cortical, nuclear, and posterior subcapsular opacities at 100 mGy were 1.26 (95% CI 1.00-1.60), 0.81 (95 CI 0.64-1.01), and 1.73 (95% CI 1.05-285).
Yamada et al. 2004	In vivo, mixed sex atomic bomb survivors exposed to 0-3+ Sv of radiation with cataractogenesis determined by biennial health examinations.	In atomic bomb survivors, there was a positive linear dose-response relationship (risk ratio of 1.06 at 1 Sv, P=0.026) between radiation dose and cataracts. However, there was only a significant relationship between the two KEs for those under 60 years of age (risk ratio of 1.16 at 1 Sv, P=0.009).
Jacobson, 2005	In vivo, mixed sex retired radiation workers (median age of 76) with transuranic body burdens from three DOE-supported installations received a lifetime occupational exposure to actinide (0-600 mSv) with ophthalmologist-reported diagnoses of cataracts.	The authors predicted an increase in the odds ratio for posterior subcapsular cataracts in radiation workers exposed to 0-600 mSv of 40.5% per additional 100 mSv (logistic regression coefficient of 0.0034 ± 0.0016 mSv-1. Furthermore, workers with lifetime doses over 201 mSv were significantly more likely to develop posterior subcapsular cataract compared to those with lifetime doses under 201 mSv.
Bateman et al., 1963	In vivo, female mice received partial-body exposure to 0.01-0.30 Gy of neutrons (0.43 Mev, no dose rate available) or 0.5-9.75 Gy of X-rays with opacification graded based on % of area covered.	In mice, lens opacification increased as the dose of neutron irradiation increased. For example, after 26 weeks post-irradiation with 1.8 MeV neutrons, 0.01 Gy resulted in a 2x increase to lens opacity, while 0.3 Gy resulted in an 11x increase to lens opacity. Identical trends were observed using 0.43 MeV neutrons (0.01-0.3 Gy) and 250 kVp X-rays (0.5-3.5 Gy).
Worgul et al., 1993	In vivo, rats received head-only exposure to 1-50 cGy iron ions with Merriam-Focht scoring of opacities.	In rats exposed to ^{56}Fe at 450 MeV/amu, the cumulative cataract rate increased ~2-fold when the dose increased from 2 to 5 cGy. The rate further increased 4-fold following an exposure to 25 cGy.
Brenner et al., 1993	In vivo, rats received head-only exposure to 0.01, 0.02, 0.05, 0.25, 0.5 Gy of iron ions with slit lamp and Merriam-Focht scoring of cataracts.	In rats exposed to ^{56}Fe at 450 MeV/amu, the cumulative cataract rate increased 3x in a log-log plot when the dose increased from 0.01 to 0.02 Gy. A similar increase was observed between 0.02 and 0.04 Gy. The ^{56}Fe had LET of 192 keV/um.

Cox et al., 1983; Ainsworth et al., 1981	In vivo, mice received whole-body exposure to 0.05-0.9 Gy heavy ions with opacification grading based on % of area affected. The dose rate was 0.5-2 Gy/min. ^{40}Ar with 570 MeV/amu had a LET of 100 keV/um. ^{20}Ne with 425 MeV/amu had a LET of 30 keV/um. ^{12}C with 400 MeV/amu had a LET of 10 keV/um.	Cox et al. (1983) showed that in mice exposed to 0.05-0.9 Gy of ^{40}Ar, ^{20}Ne, and ^{12}C, higher doses resulted in relatively more severe cataracts, with a maximum opacity score of ~2.6 at 0.9 Gy. The opacity score in the control was ~0. There was a relatively larger difference in severity between 0.05 and 0.9 Gy for the ^{40}Ar exposure than for the ^{20}Ne, with less still for ^{12}C. Ainsworth et al. (1981) conducted similar experiments showing similar data but using ^{20}Ne with 470 MeV/amu.
Fedorenko et al., 1995	In vivo, mixed sex mice received either head-only or whole-body exposure to 0.03-4 Gy of heavy ions and 1-6 Gy of protons with electrophtalmoscope and opacification grading. The 4He with 5 GeV/nucleon had a LET of 0.82 keV/um, and was administered at a dose rate of 1.5 cGy/sec. The ^{12}C with 300 MeV/nucleon had an LET of 12 keV/um, and was administered at a dose rate of 0.004 cGy/sec. The protons with 645 MeV were administered at a dose rate of 6.3 cGy/sec and had an LET of 0.25 keV/um. The protons with 9 GeV were administered at a dose rate of 2 cGy/sec and had a LET of 0.23 keV/um.	Mice exposed to 0.5 Gy vs. 2 Gy of 4He showed a nearly 60% increase in cataract prevalence. Prevalence also increased with increased post-irradiation time. Exposure to 0.5 Gy of ^{12}C resulted in an ~50% increase in cataract prevalence compared to exposure to 0.03 Gy. Mice exposed to 1 Gy vs 4 Gy of 9 GeV protons showed a 30% increase in cataract prevalence, while mice exposed to the same doses of 645 MeV protons showed a 65% increase in cataract prevalence.
Rastegar et al., 2002	In vivo, human lenses exposed to 2-373 days in space with digital Scheimpflug imaging to determine opacification.	Aged from 40 to 70 years old, cataract severity increased ~4-fold for astronauts compared to ~1.3-fold for the non-astronaut reference group of the same age range who received negligible doses of radiation.
Riley et al., 1991	In vivo, male rats received head-only exposure to 0, 0.1, 0.5, 1, 2 Gy of ^{56}Fe with subjective opacification grading. The 56Fe had an energy of 600 MeV/A and an LET of 190 keV/µm.	In rats exposed to 0.1-2 Gy of ^{56}Fe, the cataracts severity increased with increasing doses, with 2 Gy resulting in stage 3.5 cataracts while lower doses resulting in stage 3 or less.
Wu et al., 1994	In vivo, rat lenses exposed to 25-50 cGy of ^{56}Fe with Merriam-Focht scoring of opacification. The ^{56}Fe had an energy of 450 keV/amu.	In rats irradiated immediately with 25 and 50 cGy from ^{56}Fe, cataracts had reached stage 2 and 2.5, respectively.
Lett et al., 1986; Cox et al., 1992	In vivo, rabbit lenses exposed to 0.05, 0.1, 0.25, 0.5, 0.75, 1, 2, 3, 4 Gy of ^{56}Fe with slit lamp microscopy and opacification grading. The ^{56}Fe had an LET of 223 keV/µm.	In rabbits irradiated with 0.5~2 Gy ^{56}Fe, 2 Gy resulted in stage 1 cataracts, while lower doses resulted in stage <1. The study was continued by Cox et al. (1992). Six years post-irradiation, these authors found the rabbits that had been exposed to 1 Gy showed a slight increase to stage >1 cataract severity, while rabbits exposed to 0.5 Gy remained at stage <1.
Merriam et al., 1984	In vivo, rats received head-only exposure to 0.01, 0.05, 0.25, 1, 3.5 Gy of argon (570 MeV/amu). Merriam-Focht grading following slit lamp examination.	In rats irradiated with ^{40}Ar, 1 Gy resulted in stage 3-3.5 cataracts, while the results of lower doses were cataracts of stage <2.5.
Cleary et al., 1972	In vivo, rabbit lenses were locally exposed to 0.25-10 Gy of protons (100 MeV) with slit lamp observation and opacity grading.	In rabbits irradiated with 25-100 rad of protons, cataracts severity reached stage 5, 3.5, and 2 after exposure to 100, 50, and 25 rad, respectively.
Arefpour et al., 2021	Humans (both sexes) with head and neck cancer were exposed to radiation therapy ranging from 0-22 Gy) for treatment. Lens opacity was measured in 3 and 6 months after radiation therapy.	the analysis of the data derived from radiotherapy patients exposed to doses of radiation using a linear accelerator ranging from 0-22 Gy showed an exponential dose response relationship with maximum lens opacity observed after 3 months post-exposure.

McCarron et al., 2022	In vivo, mixed sex mouse models of lenses were exposed to 0.5, 1, 2 Gy of 60Co γ-irradiation with a dose-rate of 0.063 and 0.3 Gy min-1 and the maximum opacification were measured 1-18 months post-irradiation.	Mice irradiated to 0.5, 1, 2 Gy 60Co γ-rays at a dose-rate of 0.063 and 0.3 Gy min-1 resulted in an increased incidence of lens opacity in a dose response manner.

Time Concordance

Reference	Experiment Description	Result
Cucinotta et al., 2001	In vivo, mixed sex human lenses exposed to averages of 45 mGy or 3.6-4.7 mGy of radiation from space travel with slit lamp microscopy to grade opacity severity.	In astronauts immediately occupationally exposed to space radiation (average lens dose of 3.6 mSv) cataract probability slowly increased with time since exposure, with a particularly large increase to 25% 16 years after the average exposure.
Gragoudas et al., 1995	In vivo, mixed sex lenses of uveal melanoma patients that had undergone fractionated radiotherapy exposed to 70 cobalt gray equivalent (CGE) of protons with subjective cataract grading based on opacity and lens changes.	In humans exposed immediately to 70 CGE of protons, cataracts were detected ~2 months post-irradiation. By 36 months post-irradiation, there was a 20% increase in cataract prevalence detected in patients with <50% area of lens exposed to radiation. In patients with >50% area of lens exposed, there was an 80% prevalence of cataracts.
Meecham et al., 1994	In vivo, lenses of uveal melanoma patients that had undergone fractionated radiotherapy exposed to 48-80 GyE of helium ions with subjective lens grading.	In humans exposed immediately 48-80 GyE, cataracts were detected <2 years post-irradiation. By 4 years post-irradiation, nearly 100% of the patients with 76~100% irradiated lens area had cataracts. In comparison, the prevalence was ~10% for the patients with 0~25% irradiated lens area. This prevalence increased to ~20% by 14 years post-irradiation.
Char et al., 1998	In vivo, mixed sex lenses of uveal melanoma patients that had undergone fractionated radiotherapy exposed to 50-80 GyE of helium ions with subjective lens grading.	In humans exposed immediately 50-80 GyE, cataracts were detected <2 years post-irradiation. By 4 years post-irradiation, 90% of the patients with 75~100% irradiated lens area had cataracts. In comparison, the prevalence was ~10% for the patients with 0~24% irradiated lens area. This prevalence increased to ~70% by 16 years post-irradiation.
Upton et al., 1956	In vivo, mixed sex mice, rats, and guinea pigs exposed to various doses of neutrons with opacity grading system. The cyclotron fast neutrons had dose rate of 60-125 rep/min. Fast neutrons from a Po-B source had energies of 2-3 MeV and a dose rate of 1-4 rep/h.	In mice, rats, and guinea pigs that were immediately administered 90-180 reps (equivalent dose to 0.837-1.674 Gy) of cyclotron fast neutrons, cataracts were detected in <5 months post-irradiation. Rats irradiated with 180 reps reached the highest severity of cataracts in <20 months, but not so for guinea pigs exposed to the same dosage. In mice irradiated with either 0.45 or 1.8 Gy of fission neutrons or 1.3-37.3 reps Po-B neutrons, cataracts were detected in <5 months.
Worgul et al., 1996	In vivo, mixed sex rat eyes exposed to 2-250 mGy of neutrons with modified Merriam-Focht opacity grading. The irradiating neutrons had an energy of 440 keV and a dose rate of 8 mGy/min.	In rats immediately irradiated with 2-250 mGy neutrons, cataracts were detected (0.5 grade) within 20 weeks. Rats exposed to 2 mGy showed a maximum cataract severity within 60 weeks, while rats exposed to 250 mGy showed a maximum cataract severity within 20 weeks.
Riley et al., 1991	In vivo, male rats received head-only exposure to 0, 0.1, 0.5, 1, 2 Gy of ^{56}Fe with subjective opacification grading. The ^{56}Fe had an energy of 600 MeV/A and an LET of 190 keV/µm.	In rats treated immediately with 0.1-2 Gy ^{56}Fe, cataracts were detected within 20 weeks. Rats exposed to 2 Gy had stage 3.5 cataracts at 80 weeks post-irradiation.
Wu et al., 1994	In vivo, rat lenses exposed to 25-50 cGy of ^{56}Fe with Merriam-Focht scoring of opacification. The ^{56}Fe had an energy of 450 keV/amu.	In rats irradiated immediately with 25 and 50 cGy from ^{56}Fe, cataracts were detected within 10 weeks. By 40 weeks post-irradiation, the cataracts had reached stage 2 and stage 2.5, respectively, from the two different doses.
Worgul et al., 1993; Worgul et al., 1995	In vivo, rats received head-only exposure to 1, 2, 5, 25, and 50 cGy of ^{56}Fe with an energy of 450 keV/amu and an LET of 190 keV/µm. Merriam-Focht scoring was used to quantify opacities.	In rats exposed immediately to 10-50 cGy ^{56}Fe, cataracts were detected within 10 weeks. By 110 weeks post-irradiation, cataracts reached stage 3 or higher from exposure to 50 cGy.

Lett et al., 1986; Cox et al., 1992	In vivo, rabbit lenses exposed to 0.05, 0.1, 0.25, 0.5, 0.75, 1, 2, 3, 4 Gy of ^{56}Fe with slit lamp microscopy and opacification grading. The ^{56}Fe had an LET of 223 keV/µm.	In rabbits irradiated immediately with 0.5-2 Gy ^{56}Fe, stage 1 cataracts were detected at ~150 days post-irradiation (Lett et al., 1986) Severity remained steady at 150~600 days post-irradiation. The study was continued by Cox et al. (1992), who observed cataracts greater than stage 1 in the rabbits six years post-irradiation.
Medvedovsky et al., 1994	In vivo, male mice exposed to 5, 10, 20, 40, 150, 360, 504 cGy of ^{56}Fe with modified Merriam-Focht grading. The ^{56}Fe had energy of 600 MeV/amu and an LET of 175 keV/µm.	Mice immediately irradiated with 5-40 cGy ^{56}Fe showed cataract formation in ~40 weeks. Mice treated with all doses reached 100% cataract prevalence prior to 120 weeks post-irradiation.
Brenner et al., 1993	In vivo, rats received head-only exposure to 0.01, 0.02, 0.05, 0.25, 0.5 Gy of ^{56}Fe with slit lamp examination and Merriam-Focht grading.	Rats were immediately exposed to 0.01-0.5 Gy ^{56}Fe. Cataracts were detected at ~10 weeks post-irradiation for doses of 0.05-0.5 Gy and ~50 weeks for doses of 0.01 Gy. All doses reached 100% cataract prevalence prior to 80 weeks post-irradiation.
Merriam et al., 1984	In vivo, rats received head-only exposure to 0.01, 0.05, 0.25, 1, 3.5 Gy of argon (570 MeV/amu). Merriam-Focht grading following slit lamp examination.	In rats immediately irradiated with ^{40}Ar, mild stage cataracts were detected ~10 weeks post-irradiation for all doses. Cataracts were observed up to stage 3.5 by ~50 weeks post-irradiation.
Cox et al., 1992	In vivo, monkeys were exposed to 1.25, 2.5, 5, 7.5 Gy of protons (55 MeV) with slit lamp examination and subjective opacification grading.	In rhesus monkeys immediately exposed to proton doses of 1.25 Gy, cataracts were detected 20-22 years post-irradiation, and the severity increased slightly to stage ~1 after 25 years.
Cleary et al., 1972	In vivo, rabbit lenses were locally exposed to 0.25-10 Gy of protons (100 MeV) with slit lamp observation and opacity grading.	In rabbits immediately irradiated with 25-100 rad of protons, cataracts were detected <0.5 years post-irradiation. By 1 year post-irradiation, severity increased to stage 5 at the highest. At 1.5 years post-irradiation, cataract severity remained constant.
McCarron et al., 2022	In vivo. Female and male 8–12-week-old Ptch1+/-/CD1 and CD1 mice received whole-body exposure to ^{60}Co γ-rays with doses of 05, 1, and 2 Gy, and dose rates of 0.063 and 0.3 Gy/min. Lens opacification was measured via Scheimpflug imaging.	In mice immediately exposed to 1 Gy, the two largest maximum opacification values were 35 and 27%, detected in female Ptch1+/-/CD1 mice 15 and 16 months after exposure. Generally, the maximum opacification increased as time post-irradiation increased.
Arefpour et al., 2021	Humans (both sexes) with head and neck cancer were exposed to radiation therapy ranging from 0-22 Gy) for treatment. Lens opacity was measured in 3 and 6 months after radiation therapy.	The analysis of the data derived from radiotherapy patients exposed to doses of radiation using a linear accelerator ranging from 0-22 Gy showed a time response relationship with maximum lens opacity observed after 3 months post-exposure.

Time-scale

The lag time of cataract development is inversely related to radiation dose in humans. At high doses, lens opacities or cataracts can develop within months of radiation administration (Hamada, 2017). Based on an acute exposure of ~0.5 Gy, it takes >20 years to develop cataracts that impairs vision (ICRP, 2012). Mathematical modelling by Sakashita et al. (2019) estimated a latency period of 5 years to produce cataracts.

Known modulating factors

Modulating Factor (MF)	MF Specification	Effect(s) on the KER	Reference(s)
Exposure regime	Fractionated dose exposures of high charge and low LET radiation types	Fractionated exposures of high charge particles were either effective at causing cataracts or made no difference compared with acute exposures. Results were demonstrated using ^{40}Ar, ^{12}C, neutrons, and ^{56}Fe. These results were found to be in contrast to those produced with low-LET radiation, where fractionation exposures produced a tissue-sparing effect in cataract development.	Worgul et al., 1989; Ainsworth 1986; Worgul et al., 1996; Bateman et al., 1963; Worgul et al., 1993; Medvedovsky et al., 1994; Abdelkawi, 2012; Hamada, 2017

Modulating Factor (MF)	MF Specification	Effect(s) on the KER	Reference(s)
Sex	Females and estrogen treated rats	Females among the atomic bomb survivors had a higher odds ratio of developing cataracts than males. Investigation following the radiation exposure from the Chernobyl nuclear plant also found a positive dose-response in female voles. Upon estrogen treatment, ^{56}Fe-exposed rats had a higher and earlier onset cataract incidence than untreated animals of both sexes. ^{56}Fe-exposed rats treated with estrogen also had a higher and earlier onset cataract incidence than ovariectomized females without the treatment under the same exposure.	Choshi et al., 1983; Dynlacht et al., 2006; Nakashima et al., 2006; Chodick et al., 2008; Bigsby et al., 2009; Garrett et al., 2020; Henderson et al., 2010; Dynlacht et al., 2011; Azizova et al., 2018; Little et al., 2018; Azizova et al., 2019
Sex	Males	Contrary to the row above, males have also been found to have increased cataract incidence compared to females.	Henderson et al., 2009; Pawliczek et al., 2021
Age	People below 20 (& 70) years of age	Exposure to radiation at a younger age appeared to increase the risk of developing cataracts, compared to similar exposures in older individuals. Epidemiological studies showed that the risk of developing cataracts was highly significant for those younger than 70 years of age, particularly those under 20 years of age, following exposure to the radiation released from an atomic bomb. Adults over 20 years old are less sensitive to radiation. The estimated latency period for the onset radiation-induced cataracts at five years. However, the onset time became smaller and less dose-dependent as age at exposure increased. The incidence of age-related cataracts increased at age over 50 years and became indistinguishable from radiation-induced cataracts. Results in an animal study were consistent with the results from human trials.	Choshi et al., 1983; Nakashima et al., 2006; Neriishi et al., 2012; Sakashita et al., 2019; Cox et al., 1983
Genetics	Genes *ATM, BRCA1, Ptch1, p53, Ercc2* and *RAD9*	Individuals who are sensitive to radiation exposure are likely to have mutations in genes associated with DNA repair. Several studies have observed early onset radiation-induced cataracts in Atm-deficient animals. See Hamada & Fujimichi (2015) for a more in-depth list of genotypes potentially increasing the risk of cataracts.	Worgul et al., 2002; Worgul et al., 2005a; Hall et al., 2006. Kleiman et al., 2007; Blakely et al., 2010; De Stefano et al., 2014; De Stefano et al., 2016; McCarron et al., 2021; Worgul et al., 2002; Hamada & Fujimichi, 2015; Barnard & Hamada, 2022; McCarron et al., 2022; Tanno et al., 2022
Body Mass	BMI > or < “normal” range of 18.5-24.9 kg/m^2	The BMI group most at risk to cataracts following irradiation is those with a BMI above or equal to 30 kg/m^2 (Hazard ratio (HR) of 1.26 compared to “normal” BMI of 18.5-24.9 kg/m^2). Other BMI cohorts also have elevated risk compared to the “normal” group; 0-18.4 kg/m^2 people have an HR of 1.10 and 25-29.9 kg/m^2 have an HR of 1.08.	Little et al., 2018
Pre-existing Conditions	Diabetes	Individuals with diabetes are 2.18x more likely to develop cataracts following occupational radiation exposure than those without the condition.	Little et al., 2018
Substance Use	History of cigarette use	People with a history of cigarette use have a higher risk of developing cataracts following occupational radiation exposure than people who have never smoked. Former smokers have an HR of 1.04 compared to non-smokers, which is still less than current smokers’ HR of 1.18.	Little et al., 2018
Race	White People	White people have an elevated risk of developing radiation-induced cataracts (HR of 1) compared to Black or Other racial groups (HRs of 0.82 and 0.74).	Little et al., 2018

Modulating Factor (MF)	MF Specification	Effect(s) on the KER	Reference(s)
Chemical modulators	Nigella sativa oil (NSO), zinc, L-carnitine, thymoquinone (TG), WR-77913, and propolis	Supplementation with antioxidants, particularly NSO, has led to decreased cataract formation following radiation-exposure. Other radioprotective agents, such as WR-77913, have led to similar results.	Menard et al., 1986; Taysi et al., 2022

Known Feedforward/Feedback loops influencing this KER

N/A

References

Abdelkawi, S. (2012), “Lens crystallin response to whole body irradiation with single and fractionated doses of gamma radiation”, International Journal of Radiation Biology, Vol.88/8, Informa UK Ltd, London, https://doi.org/10.3109/09553002.2012.695097.

Ainsbury, E. et al. (2021), “Radiation-induced lens opacities: Epidemiological, clinical and experimental evidence, methodological issues, research gaps and strategy”, Environment International, Vol.146, Elsevier Ltd, London, https://doi.org/10.1016/j.envint.2020.106213.

Ainsbury, E. et al. (2016), “Ionizing Radiation Induced Cataracts: Recent Biological and Mechanistic Developments and Perspectives for Future Research”, Mutation Research - Reviews in Mutation Research, Vol.770, Elsevier, Amsterdam, https//doi.org/10.1016/j.mrrev.2016.07.010.

Ainsworth, E. J. (1986), “Early and Late Mammalian Responses to Heavy Charged Particles”, Advances in Space Research, Vol.6/11, Elsevier, Amsterdam, https://doi.org/10.1016/0273-1177(86)90288-7.

Ainsworth, E. J. et al. (1981), “Cataract Production in Mice by Heavy Charged Particles”, US Department of Energy, Berkeley, https//doi.org/10.2172/6395022.

Arefpour, A. M. et al. (2021),. “Evaluating Dose-response of Cataract Induction in Radiotherapy of Head and Neck Cancers Patients”,. Journal of biomedical physics & engineering, Vol.11/1, https://doi.org/10.31661/jbpe.v0i0.834.

Azizova, T. V. et al. (2018), “Risk of various types of cataracts in a cohort of Mayak workers following chronic occupational exposure to ionizing radiation”, European Journal of Epidemiology, Vol. 33/12, Springer, https://doi.org/10.1007/s10654-018-0450-4

Azizova, T. V. et al. (2019), “Risk of cataract removal surgery in Mayak PA workers occupationally exposed to ionizing radiation over prolonged periods”, Radiation and Environmental Biophysics, Vol. 58, Springer, Germany, https://doi.org/10.1007/s00411-019-00787-0

Bahia, S. et al. (2018), “Oxidative and Nitrative Stress-Related Changes in Human Lens Epithelial Cells Following Exposure to X-Rays", International Journal of Radiation Biology, Vol.94/4, Informa, London, https://doi.org/10.1080/09553002.2018.1439194.

Barnard, S. et al. (2022), “Lens Epithelial Cell Proliferation in Response to Ionizing Radiation”, Radiation Research, Vol.197, Radiation Research Society, Indianapolis, https://doi.org/10.1667/RADE-20-00294.1.

Bateman, J.L., V.P. Bond, and H.H. Rossi. (1963), “Lens Opacification in Mice Exposed to Monoenergetic Fast Neutrons”, US Department of Energy, Upton, https://doi.org/10.2172/4163234.

Bigsby, R. M. et al. (2009), “Ovarian hormone modulation of radiation-induced cataractogenesis: dose-response studies”, Investigative Ophthalmological Visual Sciences, Vol. 50/7, https://doi.org/10.1167/iovs.08-3262

Blakely, E. (2012), “Lauriston S. Taylor lecture on radiation protection and measurements: What makes particle radiation so effective?”, Health Physics, Vol.103/5, Lippincott, Williams and Wilkins Ltd, Philadelphia, https://doi.org/10.1097/HP.0b013e31826a5b85.

Blakely, E. A. et al. (2010), “Radiation cataractogenesis: epidemiology and biology”, Radiation Research, Vol. 173/5, The Radiation Research Society, United States, https://doi.org/10.1667/RRXX19.1

Bouffler, S. et al. (2012), “Radiation-Induced Cataracts: The Health Protection Agency’s Response to the ICRP Statement on Tissue Reactions and Recommendation on the Dose Limit for the Eye Lens”, Journal of radiological protection : official journal of the Society for Radiological Protection, Vol.32/4, IOP Publishing, Bristol, https://doi.org/10.1088/0952-4746/32/4/479.

Brenner, D.J. et al. (1993), “Accelerated Heavy Particles and the Lens. VIII. Comparisons between the Effects of Acute Low Doses of Iron Ions (190 KeV/Microns) and Argon Ions (88 KeV/Microns)”, Radiation Research, Vol.133/2, Radiation

Research Society, Indianapolis, https://doi.org/10.2307/3578357.

Char, D.H., S.M. Kroll, and J. Castro. (1998), “Ten-Year Follow-Up of Helium Ion Therapy for Uveal Melanoma”, American Journal of Ophthalmology, Vol.125/1, Elsevier, Amsterdam, https://doi.org/10.1016/S0002-9394(99)80238-4.

Chodick, G. et al. (2016), “The risk of cataract among survivors of childhood and adolescent cancer: a report from the childhood cancer survivor study”, Radiation Research, Vol. 185/4, Radiation Research Society, Indianapolis, https://doi.org/10.1667/RR14276.1.

Chodick, G. et al. (2008), “Risk of cataract after exposure to low doses of ionizing radiation: a 20-year prospective cohort study among US radiologic technologists”, American Journal of Epidemiology, Vol. 168/6, Oxford University Press, https://doi.org/10.1093/aje/kwn171

Choshi, K. et al. (1983), “Ophthalmologic Changes Related to Radiation Exposure and Age in Adult Health Study Sample, Hiroshima and Nagasaki”, Radiation Research, Vol.96/3, Radiation Research Society, Indianapolis, https://doi.org/10.2307/3576122.

Chylack, L.T. Jr et al. (2012), “NASCA Report 2: Longitudinal Study of Relationship of Exposure to Space Radiation and Risk of Lens Opacity”, Radiation Research, Vol.178/1, Radiation Research Society, Indianapolis, https://doi.org/10.1667/RR2876.1.

Chylack, L.T. Jr et al. (2009), “NASA Study of Cataract in Astronauts (NASCA). Report 1: Cross-Sectional Study of the Relationship of Exposure to Space Radiation and Risk of Lens Opacity”, Radiation Research, Vol.172/1, Radiation Research Society, Indianapolis, https://doi.org/10.1667/RR1580.1.

Cleary, S.F. et al. (1972), “X-Ray and Proton Induced Lens Changes in the Rabbit”, Health Physics, Vol.23/4, Lippincott Williams & Wilkins, Philadelphia, https://doi.org/10.1097/00004032-197210000-00001.

Cox, A.B. et al. (1992), “Late Cataractogenesis in Primates and Lagomorphs after Exposure to Particulate Radiations”, Advances in Space Research, Vol.12/2–3, Elsevier, Amsterdam, https://doi.org/10.1016/0273-1177(92)90133-I.

Cox, A.B. et al. (1983), “Cataractogenesis from High-LET Radiation and the Casarett Model”, Advances in Space Research, Vol.3/8, Elsevier, Amsterdam, https://doi.org/10.1016/0273-1177(83)90191-6.

Cucinotta, F.A. et al. (2001), “Space Radiation and Cataracts in Astronauts”, Radiation Research, Vol.156/5 Pt 1, Radiation Research Society, Indianapolis, https://doi.org/10.1667/0033-7587(2001)156[0460:SRACIA]2.0.CO;2.

Dauer, L.T. (2018), “Seeing through a glass darkly and taking the next right steps”, European Journal of Epidemiology, Vol.33/12, Springer Science + Business Media, Berlin, https://doi.org/10.1007/s10654-018-0458-9.

Dauer, L.T. et al. (2017), “Guidance on Radiation Dose Limits for the Lens of the Eye: Overview of the Recommendations in NCRP Commentary No. 26”, International Journal of Radiation Biology, Vol.93/10, Informa, London, https://doi.org/10.1080/09553002.2017.1304669.

de Jager, T.L., Cockrell, A.E., Du Plessis, S.S. (2017), “Ultraviolet Light Induced Generation of Reactive Oxygen Species”, in Ultraviolet Light in Human Health, Diseases and Environment. Advances in Experimental Medicine and Biology, Springer, Cham, https://doi.org/10.1007/978-3-319-56017-5_2

De Stefano, I. et al. (2021), “Contribution of genetic background to the radiation risk for cancer and non-cancer diseases in Ptch1+/- mice”, Radiation Research, Vol. 197/1, Radiation Research Society, https://doi.org/10.1667/RADE-20-00247.1

De Stefano, I. et al. (2016), “Nonlinear radiation-induced cataract using the radiosensitive Ptch1+/- mouse model”, Radiation Research, Vol. 186/3, The Radiation Research Society, United States, https://doi.org/10.1667/RR14440.1

De Stefano, I. et al. (2014), “The patched 1 tumor-suppressor gene protects the mouse lens from spontaneous and radiation-induced cataract”, The American Journal of Pathology, Vol. 185/1, American Society for Investigative Pathology, https://doi.org/10.1016/j.ajpath.2014.09.019

Dynlacht, J. R. et al. (2006), “Effect of estrogen on radiation-induced cataractogenesis”, Radiation Research, Vol. 165/1, United States, https://doi.org/10.1667/RR3481.1

Dynlacht, J.R. et al. (2011), “Age and Hormonal Status as Determinants of Cataractogenesis Induced by Ionizing Radiation. I. Densely Ionizing (High-LET) Radiation”, Radiation Research, Vol.175/1, Radiation Research Society, Indianapolis, https://doi.org/10.1667/RR2319.1.

Fedorenko, B.S., A.N. Abrosimova and O.A. Smirnova. (1995), “The Effect of High-Energy Accelerated Particles on the Crystalline Lens of Laboratory Animals”, Nutrition Reviews, Vol.26/5, Pleiades Publishing, Warrensburg, pp.573–88.

Garrett, J. et al. (2020), “The Protective Effect of Estrogen Against Radiation Cataractogenesis Is Dependent Upon the Type of Radiation”, Radiation Research, Vol.194/5, Radiation Research Society, Indianapolis, https://doi.org/10.1667/RADE-20-00015.1.

Gragoudas, E.S. et al. (1995), “Lens Changes after Proton Beam Irradiation for Uveal Melanoma”, American Journal of

Ophthalmology, Vol.119/2, Elsevier, Amsterdam, https://doi.org/10.1016/S0002-9394(14)73868-1.

Hall, E. et al. (2006), “The relative biological effectiveness of densely ionizing heavy-ion radiation for inducing ocular cataracts in wild type versus mice heterozygous for the ATM gene”, Radiation and Environmental Biophysics, Vol.45/2, Springer, New York, https://doi.org/10.1007/S00411-006-0052-5.

Hall, P. et al. (1999), “Lenticular opacities in individuals exposed to ionizing radiation in infancy”, Radiation Research, Vol. 152/2, Radiation Research Society, Oak Brook, https://doi.org/10.2307/3580093.

Hamada N. (2023), “Noncancer Effects of Ionizing Radiation Exposure on the Eye, the Circulatory System and beyond: Developments made since the 2011 ICRP Statement on Tissue Reactions”,. Radiation research, Vol. 200/2(2), 188–216. https://doi.org/10.1667/RADE-23-00030.1

Hamada, N., T.V. Azizova and M.P. Little. (2020), “An Update on Effects of Ionizing Radiation Exposure on the Eye”, British Institute of Radiobiology, Vol.93/1115, British Institute of Radiology, London, https://doi.org/10.1259/bjr.20190829.

Hamada, N. (2017), “Ionizing radiation sensitivity of the ocular lens and its dose rate dependence”, International Journal of Radiation Biology, Vol.93/10, Informa, London, https://doi.org/10.1080/09553002.2016.1266407.

Hamada, N. and T. Sato. (2016), “Cataractogenesis following high-LET radiation exposure”, Mutation Research - Reviews in Mutation Research, Vol.770/Pt B, Elsevier, Amsterdam, https://doi.org/10.1016/j.mrrev.2016.08.005.

Hamada, N. and Y. Fujimichi. (2015), “Role of carcinogenesis related mechanisms in cataractogenesis and its implications for ionizing radiation cataractogenesis”, Cancer Letters, Vol.368/2, Elsevier, Amsterdam, https://doi.org/10.1016/j.canlet.2015.02.017.

Hamada, N. et al. (2014), “Emerging issues in radiogenic cataracts and cardiovascular disease”, Journal of Radiation Research, Vol.55/5, Oxford University Press, Oxford, https://doi.org/10.1093/jrr/rru036.

Hamada, N. (2014), “What Are the Intracellular Targets and Intratissue Target Cells for Radiation Effects?”, Radiation Research, Vol.181/1, Radiation Research Society, Indianapolis, https://doi.org/10.1667/RR13505.1.

Hamada, N. & Y. Fujimichi (2015), “Role of carcinogenesis related mechanisms in cataractogenesis and its implications for ionizing radiation cataractogenesis”, Cancer Letters, Vol. 368/2, Elsevier, Ireland, https://doi.org/10.1016/j.canlet.2015.02.017

Hammer, G.P. et al. (2013), “Occupational Exposure to Low Doses of Ionizing Radiation and Cataract Development: A Systematic Literature Review and Perspectives on Future Studies”, Radiation and Environmental Biophysics, Vol.52/3, Springer, Berlin, https://doi.org/10.1007/s00411-013-0477-6.

Henderson, M.A. et al. (2010), “Effects of Estrogen and Gender on Cataractogenesis Induced by High-LET Radiation”, Radiation Research, Vol.173/2, Radiation Research Society, Indianapolis, https://doi.org/10.1667/RR1917.1.

Henderson, M. A. et al. (2009), “Effect of gender on radiation-induced cataractogenesis”, Radiation Research, Viol. 172/1, Radiation Research Society, pp. 129-133

ICRP. (2012), “ICRP Publication #118: ICRP Statement on Tissue Reactions and Early and Late Effects of Radiation in Normal Tissues and Organs - Threshold Doses for Tissue Reactions in a Radiation Protection Context”, Annals of the ICRP, Vol.41/1-2, Elsevier, Amsterdam, https://doi.org/10.1016/j.icrp.2012.02.001.

Jacob, S. et al. (2011), “Ionizing Radiation as a Risk Factor for Cataract: What about Low-Dose Effects?” Journal of Clinical & Experimental Ophthalmology, Suppl.1/005, OMICS International, Hyderabad, https://doi.org/10.4172/2155-9570.S1-005.

Jacobson, B.S. (2005), “Cataracts in retired actinide-exposed radiation workers”, Radiation Protection Dosimetry, Vol.113, Oxford University Press, Oxford, https://doi.org/10.1093/RPD/NCH427.

Jones, J.A. et al. (2007), “Cataract Formation Mechanisms and Risk in Aviation and Space Crews”, Aviation, Space and Environmental Medicine, Vol.78/4 Suppl, Aerospace Medical Association, A56-66.

Kim, I. et al. (2015), “Site specific oxidation of amino acid residues in rat lens γ-crystallin induced by low-dose γ-irradiation”, Biochemical and Biophysical Research Communications, Vol.466/4, Elsevier, Amsterdam, https://doi.org/10.1016/j.bbrc.2015.09.075.

Kleiman, N.J. (2012), “Radiation cataract”, Annals of the ICRP, Vol.41/3-4, Elsevier, Amsterdam, https:/doi.org/10.1016/j.icrp.2012.06.018.

Kleiman, N.J. et al. (2007), “Mrad9 and Atm Haploinsufficiency Enhance Spontaneous and X-Ray-Induced Cataractogenesis in Mice”, Radiation Research, Vol.168/5, Radiation Research Society, Indianapolis, https://doi.org/10.1667/rr1122.1. Leinfelder, P.J. and E.F. Riley. (1956), “Further studies of effects of X-radiation on partially shielded lens of rabbit”, Archives of Ophthalmology, Vol.55/1, American Medical Association, Chicago, https://doi.org/10.1001/ARCHOPHT.1956.00930030086012.

Kozbenko, T. et al. (2022), “Deploying elements of scoping review methods for adverse outcome pathway

development: a space travel case example", International Journal of Radiation Biology, 1–12. https://doi.org/10.1080/09553002.2022.2110306

Laskowski, L. et al. (2022), "Environmental and industrial developments in radiation cataractogenesis", International Journal of Radiation Biology, Vol. 98/6, Taylor & Francis, England, https://doi.org/10.1080/09553002.2020.1767820

Lehmann, P. et al. (2016), "Fitness costs of increased cataract frequency and cumulative radiation dose in natural mammalian populations from Chernobyl", Scientific Reports, Vol. 6/1, Nature Publishing Group, England, https://doi.org/10.1038/srep19974

Lett, J.T., A.B. Cox and A.C. Lee. (1986), "Cataractogenic Potential of Ionizing Radiations in Animal Models That Simulate Man", Advances in Space Research, Vol.6/11, Nature, Berlin, https://doi.org/ 10.1016/0273-1177(86)90305-4.

Little, M. P. et al. (2021), "Low- and moderate-dose non-cancer effects of ionizing radiation in directly exposed individuals, especially circulatory and ocular diseases: a review of the epidemiology", International Journal of Radiation Biology, Vol. 97/6, Taylor & Francis, England, https://doi.org/10.1080/09553002.2021.1876955.

Little, M. P. et al. (2018), "Occupational radiation exposure and risk of cataract incidence in a cohort of US radiologic technologists", European Journal of Epidemiology, Vol. 33/12, Springer, https://doi.org/10.1007/s10654-018-0435-3.

Markiewicz, E. et al. (2015), "Nonlinear ionizing radiation-induced changes in eye lens cell proliferation, cyclin D1 expression and lens shape", Open Biology, Vol.5/4, Royal Society, London, https://doi.org/10.1098/rsob.150011.

McCarron, R. et al. (2021), "Radiation-Induced Lens Opacity and Cataractogenesis: A Lifetime Study Using Mice of Varying Genetic Backgrounds", Radiation Research, Vol.196, Radiation Research Society, Indianapolis, https://doi.org/10.1667/RADE-20-00266.1.

Medvedovsky, C. et al. (1994), "The Influence of Dose, Dose-Rate and Particle Fragmentation on Cataract Induction by Energetic Iron Ions", Advances in Space Research, Vol.14/10, Elsevier, Amsterdam, https://doi.org/10.1016/0273-1177(94)90502-9.

Meecham, W.J. et al. (1994), "Anterior Segment Complications After Helium Ion Radiation Therapy for Uveal Melanoma", Archives of Ophthalmology, Vol.112/2, American Medical Association, Chicago, https://doi.org/10.1001/ARCHOPHT.1994.01090140073026.

Menard, T. W. et al. (1986), "Radioprotection against cataract formation by WR-77913 in γ-irradiated rats", International Journal of Radiation Oncology, Vol. 12/8, Astro, https://doi.org/10.1016/0360-3016(86)90199-9

Merriam, G.R. Jr et al. (1984), "Accelerated Heavy Particles and the Lens: I. Cataractogenic Potential", Radiation Research, Vol.98/1, Radiation Research Society, Indianapolis, https://doi.org/10.2307/3576057.

Minamoto, A. et al. (2004), "Cataract in Atomic Bomb Survivors", International Journal of Radiation Biology, Vol.80/5, Informa, London, https://doi.org/10.1080/09553000410001680332.

Moreau, K. and J. King. (2012), "Protein misfolding and aggregation in cataract disease and prospects for prevention", Trends in Molecular Medicine, Vol.18/5, Elsevier Ltd, London, https://doi.org/10.1016/j.molmed.2012.03.005.

Nakashima, E., K. Neriishi and A. Minamoto. (2006), "A Reanalysis of Atomic-Bomb Cataract Data, 2000-2002: A Threshold Analysis", Health Physics, Vol.90/2, Lippincott Williams & Wilkins, Philadelphia, https://doi.org/10.1097/01.HP.0000175442.03596.63.

National Eye Institute (2022), Cataracts, https://www.nei.nih.gov/learn-about-eye-health/eye-conditions-and-diseases/cataracts (accessed November 29, 2022).

NCRP. (2016), "Guidance on radiation dose limits for the lens of the eye", NCRP Commentary, Vol.26, National Council on Radiation Protection and Measurements Publications, Bethesda.

Nefzger, M.D., R. J. Miller and T. Fujino. (1969), "Eye Findings in Atomic Bomb Survivors of Hiroshima and Nagasaki: 1963-1964", American Journal of Epidemiology, Vol.89/2, Oxford University Press, Oxford, https://doi.org/10.1093/OXFORDJOURNALS.AJE.A120922.

Neriishi, K. et al. (2012), "Radiation dose and cataract surgery incidence in atomic bomb survivors, 1986-2005", Radiology, Vol. 265/1, Radiological Society of North America, Oak Brook, https://doi.org/10.1148/radiol.12111947.

Otake, M. and W. J. Schull. (1990), "Radiation-Related Posterior Lenticular Opacities in Hiroshima and Nagasaki Atomic Bomb Survivors Based on the DS86 Dosimetry System", Radiation Research, Vol.121/1, Radiation Research Society, Indianapolis, https://doi.org/10.2307/3577557.

Pawliczek, D. et al. (2021), "On the nature of murine radiation-induced subcapsular cataracts: optical coherence tomography-based fine classification, in vivo dynamics and impact on visual acuity", Radiation Research, Vol. 197/1, Radiation Research Society, United States, https://doi.org/10.1667/RADE-20-00163.1

Rafnsson, V. et al. (2005), "Cosmic Radiation Increases the Risk of Nuclear Cataract in Airline Pilots: A Population-Based Case-Control Study", Archives of Ophthalmology, Vol.123/8, American Medical Association, Chicago, https://doi.org/10.1001/ARCHOPHT.123.8.1102.

Ramsell, T. and R. Berry. (1966), “Recovery from x-ray damage to the lens. The effects of fractionated x-ray doses observed in rabbit lens epithelium irradiated in vivo”, The British Journal of Radiology, Vol.39/467, British Institute of Radiology, London, https://doi.org/10.1259/0007-1285-39-467-853.

Rastegar, N., P. Eckart, M. Mertz (2002), “Radiation-induced cataract in astronauts and cosmonauts”, Graefe’s Archive for Clinical and Experimental Ophthalmology, Vol. 240/7, Springer, Germany, https://doi.org/10.1007/s00417-002-0489-4

Richards, R. (1966), “Changes in lens epithelium after x-ray or neutron irradiation (mouse and rabbit)”, Transactions of the American Ophthalmological Society, Vol.64, American Ophthalmological Society, Rochester, pp.700-34.

Riley, E. et al. (1991), “Relative Cataractogenic Effects of X Rays, Fission-Spectrum Neutrons, and 56Fe Particles: A Comparison with Mitotic Effects”, Radiation Research, Vol.125/3, Radiation Research Society, Indianapolis, https://doi.org/10.2307/3578114.

Riley, E. et al. (1989), “Comparison of Recovery from Potential Mitotic Abnormality in Mitotically Quiescent Lens Cells after X, Neutron, and 56Fe Irradiations”, Radiation Research, Vol.119/2, Radiation Research Society, Indianapolis, https://doi.org/10.2307/3577616.

Sakashita, T., T. Sato and N. Hamada (2019), “A Biologically Based Mathematical Model for Spontaneous and Ionizing Radiation Cataractogenesis”, PLoS ONE, Vol.14/8, PLoS, San Francisco, https://doi.org/10.1371/journal.pone.0221579.

Seals, K. et al. (2016), “Radiation-Induced Cataractogenesis: A Critical Literature Review for the Interventional Radiologist”,. Cardiovasc Intervent Radiol, Vol.39, https://doi.org/10.1007/s00270-015-1207-z

Shang, F., L. Huang and A. Taylor. (1994), “Degradation of Native and Oxidized Beta-and Gamma-Crystallin Using Bovine Lens Epithelial Cell and Rabbit Reticulocyte Extracts”, Current Eye Research, Vol.13/6, Taylor & Francis, Oxfordshire, https://doi.org/10.3109/02713689408999870.

Shore, R.E. (2016), “Radiation and Cataract Risk: Impact of Recent Epidemiologic Studies on ICRP Judgments”, Mutation Research - Reviews in Mutation Research, Vol.770, Elsevier, Amsterdam, https://doi.org/10.1016/j.mrrev.2016.06.006.

Shore, R.E., K. Neriishi and E. Nakashima. (2010), “Epidemiological Studies of Cataract Risk at Low to Moderate Radiation Doses: (Not) Seeing Is Believing”, Radiation Research, Vol.174/6 B, Radiation Research Society, Indianapolis, https://doi.org/10.1667/RR1884.1.

Smith, J. (2020), “Field evidence of significant effects of radiation on wildlife at chronic low dose rates is weak and often misleading. A comment on ‘Is non-human species radiosensitivity in the lab a good indicator of that in the field? Making the comparison more robust’ by Beaugelin-Seiller et al. “, Journal of Environmental Radioactivity, Vol. 211, Elsevier, England, https://doi.org/10.1016/j.jenvrad.2019.01.007

Su, Yinping et al. (2021), “Lens opacity prevalence among the residents in high natural background radiation area in Yangjiang, China”, Journal of Radiation Research, Vol. 62/1, Oxford University Press, England, https://doi.org/10.1093/jrr/rraa073.

Tanno, B. et al. (2021), “miRNA-signature of irradiated Ptch1+/- mouse lens is dependent on genetic background”, Radiation Research, Vol. 197/1, Radiation Research Society, United States, https://doi.org/10.1667/RADE-20-00245.1

Thome, C. et al. (2018). “Deterministic Effects to the Lens of the Eye Following Ionizing Radiation Exposure: is There Evidence to Support a Reduction in Threshold Dose?”. Health Physics, Vol.114, https://doi.org/10.1097/HP.0000000000000810

Truscott, R. (2005), “Age-related nuclear cataract - Oxidation is the key”, Experimental Eye Research, Vol.80/5, Academic Press Inc, Cambridge, https://doi.org/10.1016/j.exer.2004.12.007.

Upton, A.C. et al. (1956), “The Relative Biological Effectiveness of Neutrons, x-Rays, and Gamma Rays for the Production of Lens Opacities: Observations on Mice, Rats, Guinea-Pigs, and Rabbits”, Radiology, Vol.67/5, Radiological Society of North America, Oak Brook, https://doi.org/10.1148/67.5.686.

Uwineza, A. et al. (2019), “Cataractogenic load- A concept to study the contribution of ionizing radiation to accelerated aging in the eye lens”, Mutation Research - Reviews in Mutation Research, Vol.779, Elsevier, Amsterdam, https://doi.org/10.1016/j.mrrev.2019.02.004.

von Sallmann, L. (1952), “Experimental studies on early lens changes after roentgen irradiation III. Effect of X-radiation on mitotic activity and nuclear fragmentation of lens epithelium in normal and cysteine-treated rabbits”, Transactions of the American Ophthalmological Society, Vol.8, American Ophthalmological Society, Rochester, https://doi.org/10.1001/archopht.1952.01700030313005.

Williams, D. (2019), “Do rodents in radioactive areas around Chernobyl Ukraine have high prevalence of cataracts?”, BSAVA Congress Proceedings, BSAVA Library, pp. 458

Worgul, B. V. et al. (2007), “Cataracts among Chernobyl Clean-up Workers: Implications Regarding Permissible Eye Exposures”, Radiation Research, Vol.167/2, Radiation Research Society, Indianapolis, https://doi.org/10.1667/RR0298.1.

Worgul, B. et al. (2005a), “Mice heterozygous for the ATM gene are more sensitive to both X-ray and heavy ion exposure than are wildtypes”, Advances in Space Research, Vol.35/2, Elsevier Ltd, London, https://doi.org/10.1016/J.ASR.2005.01.030.

Worgul, B.V., N.J. Kleiman and J. David. (2005b), “A positive and negative bystander effect influences cataract outcome in the irradiated lens”, Investigative Ophthalmology & Visual Science, Vol.46/13, Association for Research in Vision and Ophthalmology, Rockville, pp.832

Worgul, B. et al. (2002), “Atm heterozygous mice are more sensitive to radiation-induced cataracts than are their wild-type counterparts”, PNAS, Vol.99/15, National Academy of Sciences, Washington, https://doi.org/10.1073/pnas.162349699.

Worgul, B.V., C. Medvedovsky and B. Wu. (1995), “Use of Non-Subjective Analysis of Lens Transparency in Experimental Radiation Cataract Research”, Ophthalmic Research, Vol.27/Suppl 1, Karger Publishers, Basel, https://doi.org/10.1159/000267855.

Worgul, B.V. et al. (1996), “Quantitative Assessment of the Cataractogenic Potential of Very Low Doses of Neutrons”, Radiation Research, Vol.145/3, Radiation Research Society, Indianapolis, https://doi.org/10.2307/3578991.

Worgul, B.V. et al. (1993), “Accelerated Heavy Particles and the Lens VII: The Cataractogenic Potential of 450 MeV/Amu Iron Ions”, Investigative Ophthalmology and Visual Science, Vol.34/1, Association for Research in Vision and Ophthalmology, Rockville, pp.184–93.

Worgul, B.V. et al. (1989), “Accelerated Heavy Particles and the Lens : III. Cataract Enhancement by Dose Fractionation”, Radiation Research, Vol.118/1, Radiation Research Society, Indianapolis, https://doi.org/10.2307/3577425.

Worgul, B., G. Merriam and C. Medvedovsky. (1986), “Accelerated heavy particles and the lens II. Cytopathological changes”, Investigative Ophthalmology and Visual Science, Vol.27/1, Association for Research in Vision and Ophthalmology, Rockville, pp.108-114.

Wride, M.A. (2011), “Lens Fibre Cell Differentiation and Organelle Loss: Many Paths Lead to Clarity”, Philosophical Transactions of the Royal Society B: Biological Sciences, Vol.366/1568, Royal Society, London, https://doi.org/10.1098/rstb.2010.0324.

Wu, B., C. Medvedovsky and B.V. Worgul. (1994), “Non-Subjective Cataract Analysis and Its Application in Space Radiation Risk Assessment”, Advances in Space Research, Vol.14/10, Elsevier, Amsterdam, https://doi.org/10.1016/0273-1177(94)90504-5.

Yamada, M. et al. (2004), “Noncancer disease incidence in atomic bomb survivors, 1958–1998”, Radiation Research, Vol.161, Radiation Research Society, Indianapolis, https://doi.org/10.1667/RR3183.

Relationship: 2817: Inadequate DNA repair leads to Cataracts

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Deposition of energy leading to occurrence of cataracts	non-adjacent	Low	Low

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	Low	NCBI
mouse	Mus musculus	Moderate	NCBI
rat	Rattus norvegicus	Low	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	Moderate

Sex Applicability

Sex	Evidence
Unspecific	Moderate

Sex	Evidence
Mixed	Moderate

This KER is plausible in all life stages, sexes, and organisms with DNA and requiring a clear lens for vision. The majority of the evidence is from in vivo adult mice and does not specify sex and weanling mice in vitro models that do not specify sex.

Key Event Relationship Description

Inadequate repair of DNA is the inability for the cell's repair machinery to properly maintain correct DNA structure and sequences following the creation of errors (Helleday et al., 2008; Massey & Jones, 2018). DNA repair has several different pathways when functioning correctly. Pathway examples include base excision repair (BER), non-homologous end-joining (NHEJ), nucleotide-excision repair (NER), homologous recombination (HR), and single-strand break repair (SSBR). These pathways are triggered to start when their specific type of DNA lesion is detected (Helleday et al., 2008). Some of these pathways, like NHEJ, are considered to be error-prone (Chiruvella et al., 2013; Hamada & Fujimichi, 2015). The dysregulation and breakdown of these pathways results in the cell having an accumulation of DNA damage (Massey & Jones, 2018). This accumulated genomic damage can lead to improper cellular morphology and if this occurs in lens cells, it can lead to cataracts (Worgul et al., 1989). Cataracts are a progressive condition in which the lens of the eye develops opacities and becomes cloudy, resulting in blurred vision as well as glare and haloes around lights (National Eye Institute, 2022). For this AOP, a cataract is defined when over 5% of the lens is opacified.

Evidence Supporting this KER

Overall Weight of Evidence: Low

Biological Plausibility

The biological plausibility of the relationship between inadequate DNA repair leading to cataracts is moderately supported by the literature (Kleiman 2013; Hall et al. 2005; Ainsbury et al. 2021; Ainsbury et al. 2016; Hamada 2017; Hamada et al. 2015; Blakely et al. 2010; Dauer et al. 2014; Ainsbury et al. 2009; Foray et al. 2016; NCRP 2016; ICRP 2012; Kleiman 2012). Mouse models have been used to support this connection, with all listed ages below 3 months old (Worgul et al., 2002; Worgul et al., 2005; Hall et al., 2006; Kleiman et al., 2007; McCarron et al., 2022). Humans have higher levels of repair enzyme-coding gene expression than mice, and most human repair pathways are more sufficiently activated (MacRae et al., 2015).

Cataracts may be at increased risk of development following the cell's inability to properly repair DNA damage. High levels of single-strand DNA damage have been seen in the epithelial cells of cataract patients (Kleiman & Spector, 1993). Epithelial cells with DNA damage typically have elevated levels of p21, implying an inability to breakdown the nuclear envelope of the cell. This impedes lens epithelial cell differentiation into proper lens fiber cells, contributing to cataract incidence (Siddam et al., 2018; NCRP, 2016; Worgul et al., 1989). Lens fiber cells typically have a dissolved nuclear envelope and no organelles, this is because, these structures interfere with light scattering, which is essential for the proper functioning of the lens. Furthermore, when the nuclear envelope is not dissolved, as in cases of aberrant differentiation, it presents an opportunity for light to scatter, reducing visual acuity (Siddam et al., 2018; Moreau & King, 2012). This becomes problematic as lens cells are not replaced, so any damage sustained will accumulate, potentially leading to cataracts (Toyama & Hetzer, 2013). The complete understanding of this process is still needed (Worgul et al., 1991; Barnard et al., 2018). The maintenance of lens transparency involves the participation of DNA repair pathways (NER, BER, repair of DNA strand breaks, and direct reversal of DNA damage) and changes in the activity of DNA repair genes have been linked to age-related cataracts. The lens epithelium expresses a minimum of 92 genes associated with DNA repair, crucial for safeguarding the integrity of the cellular genome (Ainsbury et al., 2016). Haploinsufficiency is a large contributor to inadequate DNA repair resulting in cataract formation (Kleiman, 2007). Genes such as Mrad9, Brca1, and ATM are important for the proper functioning of DNA repair machinery s, and by acting as cell cycle checkpoints (ICRP, 2012; Foray et al., 2016; Hamada & Fujimichi, 2015; Blakely et al., 2010; Hamada, 2017; Dauer et al., 2014). When these genes are heterozygous in an organism, this raises the risk of haploinsufficiency (Kleiman, 2007). Individuals that are haploinsufficient in these genes have a higher likelihood of developing cataracts (Foray et al., 2016; Kleiman, 2007; Hamada & Fujimichi, 2015; ICRP, 2012). This is because genetic susceptibility to cataracts is partially contingent on repair deficits developing (Blakely et al., 2010; Kleiman, 2012; Ainsbury et al., 2009). The inability to adequately repair DNA damage in the lens epithelium can cause genomic damage retention, which can then lead to cataract development (ICRP, 2012). It has also been shown that the presence of heterozygosity in two genes, where one is ATM and the other is either Mrad9 or Braca1, increases the risk of cataracts more than heterozygosity in just one of the genes (Blakely et al., 2010; NCRP, 2016; ICRP, 2012). The Ercc2 gene is responsible for nucleotide excision repair (Weber et al., 1988). Ercc2 heterozygous B6C3F1 mice experience significant effects on mean and maximum opacity. Female mice have a higher risk of cataracts, as well as experiencing an estrogen-implicated increase in speed of cataract progression (McCarron et al., 2021). Furthermore, some genetic disorders that relate heavily to impaired repair function, such Cockanye syndrome and trichothiodystrophy, have cataract development as a symptom of the condition (Dollfus et al., 2003).

Empirical Evidence

This KER has moderate empirical evidence to support the relationship between inadequate repair of DNA and the development of cataracts. There is low support for time response and moderate for incidence response, though

essentiality is strongly supported. The models used to support this connection are in vivo and in vitro mice (Kleiman et al., 2007; Worgul et al., 2005; Worgul et al., 2002; Hall et al., 2006).

Dose/Incidence Concordance

No data available.

Time Concordance

There is low evidence to support time response for the relationship of inadequate DNA repair to cataracts. Following low dose (0.5 Gy) in vitro X-ray exposures, low grade cataracts appeared in ATM heterozygote lenses within 17 weeks, but wild type animals took 18 weeks. Vision-impairing cataracts developed in 9 weeks in ATM heterozygotes, 10 weeks faster than wild type animals following 4 Gy X-ray exposure. Both groups increased linearly, though the wild type mice experience several plateaus (Worgul et al., 2002). After exposure to 1 Gy of X-rays, in vivo ATM heterozygous lenses developed grade 1 cataracts 3 weeks before wild type animals, within 3 months of exposure. Once the incidences of cataracts began, both groups saw sharp increases as time passed, with heterozygous mice slightly ahead of wild type in incidence numbers (Worgul et al., 2005).

Essentiality

There is a large amount of evidence supporting the essentiality of inadequate DNA repair in cataract development. Single and double ATM and Mrad9 heterozygous mice have been found to develop less severe cataracts compared to wild types (Kleiman et al., 2007). Studies have also found that ATM mutants develop cataracts faster than wild types. For example, ATM homozygotes developed grade 1.0 cataracts 10 weeks before wild type and heterozygous ATM mutants after in vitro exposure to 0.5 Gy X-rays (Worgul et al., 2002). Similarly, ATM mutants developed grade 0.5 cataracts two weeks prior to wild type mice after in vivo exposure to 0.325 Gy ^{56}Fe or 2 Gy X-rays (Worgul et al., 2005). Another study found ATM mutants developed grade 2.0 cataracts five weeks prior to wild type mice after in vivo exposure to 0.325 Gy ^{56}Fe or 1 Gy X-rays (Hall et al., 2006). It has been observed that E2, a form of estrogen, may disrupt the pathways responsible for repairing direct DNA damage. This disruption could lead to an increased occurrence and faster progression of cataracts in groups exposed to E2, whether it is produced within the body or comes from external sources (Garrett et al., 2020).

Uncertainties and Inconsistencies

Although a higher risk of cataracts has been reported in females with an association of estrogen-implicated increase in cataract progression (McCarron et al., 2022), some studies have reported a protective effect of estrogen in ovariectomized rats (Dynlacht et al., 2006; 2008).

Quantitative Understanding of the Linkage

The following tables provide representative examples of the relationship, unless otherwise indicated, all data is significantly significant.

Dose Concordance

No evidence found.

Incidence Concordance

No evidence found.

Time Concordance

Reference	Experimental Description	Results
Worgul et al., 2002	In vitro, mice lenses exposed to 0.5-4 Gy X-rays with Merriam-Focht grading of cataracts and ATM partial knockouts for inadequate repair.	Vision-impairing cataracts appear 10 weeks earlier in ATM heterozygotes than in wild type animals following 4 Gy X-ray exposure. The heterozygotes had a linear increase, while the wild types had multiple plateaus between their linear increases. At lower dose (0.5 Gy) exposure, low grade cataracts appear in ATM heterozygotes 1 week sooner than wild type animals.
Worgul et al., 2005	In vivo, mice exposed to 1 Gy X-rays in one eye with ATM partial knockouts for inadequate repair and slit-lamp examinations and Merriam-Focht scoring for cataracts.	After 1 Gy X-ray exposure, animals that were heterozygous for ATM developed grade 1 cataracts 3 weeks sooner than wild type animals. Both groups have large increases in incidence once initiated, though both did have a slight drop in numbers early on that was quickly recovered.

Known modulating factors

Modulating Factor (MF)	MF Specification	Effect(s) on the KER	Reference(s)
Genetics	Ptch1	Heterozygosity for Ptch1 increases cataract susceptibility, particularly after exposure to higher radiation doses.	De Stefano et al., 2014; De Stefano et al., 2016; Tanno et al., 2022
Genetics	ATM	Humans carrying the A allele of ATM rs189037 had increased cataract risk.	Gao et al., 2022
Genetics	TP53	Humans carrying the C allele of TP53 had increased cataract risk.	Gao et al., 2022

Known Feedforward/Feedback loops influencing this KER

N/A

References

Ainsbury, E. et al. (2021), “Radiation-induced lens opacities: Epidemiological, clinical and experimental evidence, methodological issues, research gaps and strategy”, Environment International, Vol.146, Elsevier Ltd, London, https://doi.org/10.1016/j.envint.2020.106213.

Ainsbury, E. A. et al. (2016), “Ionizing radiation induced cataracts: recent biological and mechanistic developments and perspectives for future research”, Mutation research. Reviews in mutation research, Vol. 770, Elsevier B.V., https://doi.org/10.1016/j.mrrev.2016.07.010

Ainsbury, E. et al. (2009), “Radiation Cataractogenesis: A Review of Recent Studies”, Radiation Research, Vol.172/1, Radiation Research Society, Indianapolis, https://doi.org/10.1667/RR1688.1.

Blakely, E. et al. (2010), “Radiation Cataractogenesis: Epidemiology and Biology”, Radiation Research, Vol.173/5, Radiation Research Society, Indianapolis, https://doi.org/10.1667/RRXX19.1.

Chiruvella, K., Z. Liang and T. Wilson. (2013), “Repair of double-strand breaks by end joining”, Cold Spring Harbor Perspectives in Biology, Vol.5, Cold Spring Harbor Laboratory Press, Long Island, https://doi.org/10.1101/cshperspect.a012757.

Dauer, L. et al. (2014), “Epidemiology and Mechanistic Effects of Radiation on the Lens of the Eye: Review and Scientific Appraisal of the Literature”, EPRI Technical Report, Vol.2014, Electric Power Research Institute, Palo Alto.

De Stefano, I. et al. (2014), “The patched 1 tumor-suppressor gene protects the mouse lens from spontaneous and radiation-induced cataract”, American Society for Investigative Pathology, Vol. 185/1, Elsevier, https://doi.org/10.1016/j.ajpath.2014.09.019

De Stefano, I. et al. (2016), “Nonlinear radiation-induced cataract using the radiosensitive Ptch1(+/-) mouse model”, Radiation Research, Vol. 186/3, BioOne, https://doi.org/10.1667/RR14440.1

Dollfus, H. et al. (2003), “Ocular Manifestations in the Inherited DNA Repair Disorders”, Survey of Ophthalmology, Vol.48/1, Elsevier, Amsterdam, https://doi.org/10.1016/S0039-6257(02)00400-9.

Dynlacht, J. R. et al. (2006), “Effect of estrogen on radiation-induced cataractogenesis.” Radiation research Vol. 165/1, JSTOR, https://doi.org/10.1667/rr3481.1

Dynlacht, J. R. et al. (2008), “Estrogen protects against radiation-induced cataractogenesis.” Radiation research Vol. 170/6, Oxford University Press, Oxford, https://doi.org/10.1667/RR1416.1

Foray, N., M. Bourguignon and N. Hamada. (2016), “Individual response to ionizing radiation”, Mutation Research - Reviews in Mutation Research, Vol.770, Elsevier, Amsterdam, https://doi.org/10.1016/j.mrrev.2016.09.001.

Gao, Y. et al. (2022), “ATM and TP53 polymorphisms modified susceptibility to radiation-induced lens opacity in natural high background radiation area, China”, International Journal of Radiation Biology, Vol. 98/7, https://doi.org/10.1080/09553002.2022.2024294

Garrett, J et al. (2020), “The protective effect of estrogen against radiation cataractogenesis is dependent upon the type of radiation”, Radiation Research, Vol. 194/5, Radiation Research Society, United States, https://doi.org/10.1667/RADE-20-00015.1

Hall, E. et al. (2006), “The relative biological effectiveness of densely ionizing heavy-ion radiation for inducing ocular cataracts in wild type versus mice heterozygous for the ATM gene”, Radiation and Environmental Biophysics, Vol.45/2, Springer, New York, https://doi.org/10.1007/S00411-006-0052-5.

Hall, E. et al. (2005), “Genetic susceptibility to radiation”, Advances in Space Research, Vol.35/2, Elsevier Ltd, London, https://doi.org/10.1016/J.ASR.2004.12.032.

Hamada, N. (2017), “Ionizing radiation sensitivity of the ocular lens and its dose rate dependence”, International

Journal of Radiation Biology, Vol.93/10, Informa, London, https://doi.org/10.1080/09553002.2016.1266407.

Hamada, N. and Y. Fujimichi. (2015), “Role of carcinogenesis related mechanisms in cataractogenesis and its implications for ionizing radiation cataractogenesis”, Cancer Letters, Vol.368/2, Elsevier, Amsterdam, https://doi.org/10.1016/j.canlet.2015.02.017.

Helleday, T. et al. (2008), “DNA repair pathways as targets for cancer therapy”, Nature Reviews Cancer, Vol.8/3, Nature Portfolio, London, https://doi.org/10.1038/nrc2342.

ICRP (2012), “ICRP Publication #118: ICRP Statement on Tissue Reactions and Early and Late Effects of Radiation in Normal Tissues and Organs - Threshold Doses for Tissue Reactions in a Radiation Protection Context”, Annals of the ICRP, Vol.41/1-2, Elsevier, Amsterdam, https://doi.org/10.1016/j.icrp.2012.02.001.

Kleiman, N.J. (2013), “Low-Dose Radiation Cataract and Genetic Determinants of Radiosensitivity”, Columbia University, New York, https://doi.org/10.2172/1124670.

Kleiman, N.J. (2012), “Radiation cataract”, Annals of the ICRP, Vol.41/3-4, Elsevier, Amsterdam, https:/doi.org/10.1016/j.icrp.2012.06.018.

Kleiman, N.J. et al. (2007), “Mrad9 and Atm Haploinsufficiency Enhance Spontaneous and X-Ray-Induced Cataractogenesis in Mice”, Radiation Research, Vol.168/5, Radiation Research Society, Indianapolis, https://doi.org/10.1667/rr1122.1.

Kleiman, N.J. and A. Spector. (1993), “DNA single strand breaks in human lens epithelial cells from patients with cataract”, Current Eye Research, Vol.12, Taylor & Francis, Oxfordshire, https://doi.org/10.3109/02713689309024624.

Kozbenko, T. et al. (2022), “Deploying elements of scoping review methods for adverse outcome pathway development: a space travel case example”, International Journal of Radiation Biology, 1–12. https://doi.org/10.1080/09553002.2022.2110306

MacRae, S. L. et al. (2015), “DNA repair in species with extreme lifespan differences”, Aging, Vol.7/12, Impact Journals, New York, https://doi.org/10.18632/AGING.100866.

Massey, T.H. and L. Jones. (2018), “The central role of DNA damage and repair in CAG repeat diseases”, Disease Models and Mechanisms, Vol.11/1, The Company of Biologists, Cambridge, https://doi.org/10.1242/dmm.031930.

McCarron, R. et al. (2021), “Radiation-Induced Lens Opacity and Cataractogenesis: A Lifetime Study Using Mice of Varying Genetic Backgrounds”, Radiation Research, Vol.196, Radiation Research Society, Indianapolis, https://doi.org/10.1667/RADE-20-00266.1.

Moreau, K. and J. King. (2012), “Protein misfolding and aggregation in cataract disease and prospects for prevention”, Trends in Molecular Medicine, Vol.18/5, Elsevier Ltd, London, https://doi.org/10.1016/j.molmed.2012.03.005.

National Eye Institute (2022), Cataracts, https://www.nei.nih.gov/learn-about-eye-health/eye-conditions-and-diseases/cataracts (accessed November 29, 2022).

NCRP (2016), “Guidance on radiation dose limits for the lens of the eye”, NCRP Commentary, Vol.26, National Council on Radiation Protection and Measurements Publications, Bethesda.

Siddam, A.D. et al. (2018), “The RNA-binding protein Celff1 post-transcriptionally regulates p27Kip1 and Dnase2b to control fiber cell nuclear degradation in lens development”, PLOS Genetics, Vol.14/3, PLOS, San Francisco, https://doi.org/10.1371/journal.pgen.1007278.

Tanno, B. et al. (2022), “miRNA-signature of irradiated Ptch1+/- mouse lens is dependent on genetic background”, Radiation Research, Vol. 197/1, https://doi.org/10.1667/RADE-20-00245.1

Toyama, B. and M. Hetzer. (2013), “Protein homeostasis: Live long, won't prosper”, Nature Reviews Molecular Cell Biology, Vol.14/1, Nature Portfolio, London, https://doi.org/10.1038/nrm3496.

Weber, C.A. et al. (1988), “Molecular cloning and biological characterization of a human gene, ERCC2, that corrects the nucleotide excision repair defect in CHO UV5 cells”, Molecular and Cellular Biology, Vol.8/3, American Society for Microbiology, Washington, https://doi.org/10.1128/mcb.8.3.1137-1146.1988.

Worgul, B. et al. (2005), “Mice heterozygous for the ATM gene are more sensitive to both X-ray and heavy ion exposure than are wildtypes”, Advances in Space Research, Vol.35/2, Elsevier Ltd, London, https://doi.org/10.1016/J.ASR.2005.01.030.

Worgul, B. et al. (2002), “Atm heterozygous mice are more sensitive to radiation-induced cataracts than are their wild-type counterparts”, PNAS, Vol.99/15, National Academy of Sciences, Washington, https://doi.org/10.1073/pnas.162349699.

Worgul, B. et al. (1991), “Evidence of genotoxic damage in human cataractous lenses”, Mutagenesis, Vol.6/6, Oxford University Press, Oxford, https://doi.org/10.1093/MUTAGE/6.6.495.

Worgul, B.V., C. Medvedovsky and G.R. Merriam. (1989), “Cortical cataracts development-the expression of primary damage to the lens epithelium”, Lens Eye Toxicity Research, Vol.6, Marcel Dekker, New York, pp557-571.

Relationship: 2818: Oxidative Stress leads to Cataracts

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Deposition of energy leading to occurrence of cataracts	non-adjacent	Moderate	Low

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	Low	NCBI
mouse	Mus musculus	Moderate	NCBI
rat	Rattus norvegicus	Moderate	NCBI
Pig	Pig	Low	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	Moderate

Sex Applicability

Sex	Evidence
Mixed	Moderate
Female	Moderate
Unspecific	Low

This KER is plausible in all life stages, sexes, and organisms requiring a clear lens for vision. The majority of the evidence is from in vivo mice and rats of all ages with no specification on sex, as well as using human in vitro models that do not specify sex.

Key Event Relationship Description

Oxidative stress refers to a state in which the amount of reactive oxygen (ROS) and nitrogen (RNS) species overwhelms the cells antioxidant defense system. This loss in redox homeostasis can lead to oxidative damage to proteins, lipids, and nucleic acids (Schoenfeld et al., 2012; Tangvarasittichai & Tangvarasittichai, 2019; Turner et al., 2002). ROS are molecules with oxygen as the functional center and at least one unpaired electron in the outer orbits. Organisms contain a defense system of antioxidants to help manage ROS levels. When the antioxidant system is overwhelmed by the amount of ROS, the cell can enter a state of oxidative stress (Balasubramanian, 2000; Ganea & Harding, 2006; Karimi et al., 2017).

Increased ROS levels from different pathways of oxidative stress can damage proteins, lipids, and important cellular processes. If this occurs in the lens of the eye and damage accumulates over time, eventually the increased opacity of the lens prevents light from passing freely, leading to cataracts (Tangvarasittichai and Tangvarasittichai, 2019). Cataracts are a progressive condition in which the lens of the eye develops opacities and becomes cloudy, resulting in blurred vision as well as glare and haloes around lights (National Eye Institute, 2022). For this AOP, a cataract is defined when over 5% of the lens is opacified.

Evidence Supporting this KER

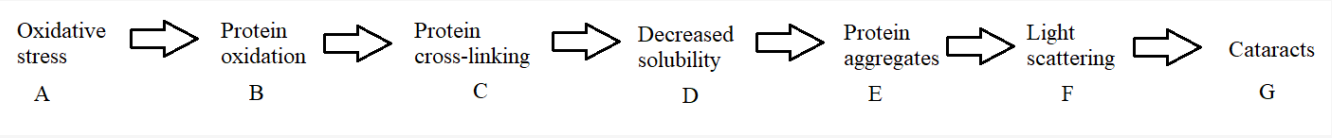
Overall Weight of Evidence: Moderate

Biological Plausibility

There are several different pathways leading from oxidative stress to lens opacity and it is the progressive accumulation of oxidative damage from several different mechanisms that causes cataracts (Babizhayev et al., 2011). These paths include protein oxidation, lipid peroxidation, increased calcium levels, DNA damage, apoptosis, and gap junction damage. As this is a non-adjacent (indirectly linked) KER, the direct KERs will provide greater detail for the individual pathways.

The best-studied route is from oxidative stress through protein oxidation, to cataracts. This occurs as ROS oxidize proteins, causing cross-linking, a decrease in solubility, the formation of protein aggregates that scatter light, lens

opacities, and finally cataracts (see figure 1 for a list of sources). In a more detailed version, crystallins are the primary lens proteins (Hamada et al., 2014), and they must maintain a specific organization to allow for transparency (Spector, 1995). ROS can oxidize these proteins, removing their sulfhydryl (-SH) groups, as a result, they form non-disulfide bonds and become cross-linked to each other. These molecules are now less water-soluble and therefore clump together, eventually forming large protein aggregates that scatter light, resulting in lens opacity, and cataracts (Ahmad and Haseeb, 2020).



A → B Van Kuijk, 1991; Liu et al., 2013; Qin et al., 2019; Ahmad & Haseeb, 2020

B → C Van Kuijk, 1991; Liu et al., 2013; Ahmad & Haseeb, 2020

C → D Qin et al., 2019; Ahmad & Haseeb, 2020

D → E Hamada et al., 2017; Qin et al., 2019; Ahmad & Haseeb, 2020

E → F Li et al., 1995; Spector, 1995; Hamada et al., 2014; Qin et al., 2019

F → G Spector, 1995; Qin et al., 2019

A → C Hamada et al., 2014; Qin et al., 2019; Tangvarasittichai & Tangvarasittichai, 2019

B → E Zhang, 2012; Qin et al., 2019

B → G Spector, 1995; Karslioğlu et al., 2005; Liu et al., 2013; Tangvarasittichai & Tangvarasittichai, 2019

C → E Hamada et al., 2014; Tangvarasittichai & Tangvarasittichai, 2019

E → G Li et al., 1995; Hamada et al., 2017; Qin et al., 2019

Figure 1. Pathway for oxidative stress to cataracts passing through protein oxidation. The bottom portion of the figure provides references supporting the various connections.

Another pathway leading from oxidative stress to cataracts is lipid peroxidation (LPO) (Van Kuijk, 1991; Babizhayev et al., 2011; Tangvarasittichai and Tangvarasittichai, 2019; Ahmad and Haseeb, 2020). This is where ROS attack polyunsaturated fatty acids, forming lipid peroxides that damage DNA, cell membranes, and cytosol regions (Babizhayev et al., 2011; Tangvarasittichai and Tangvarasittichai, 2019), this in turn can cause cataracts (Hightower, 1995; Sacca et al., 2009; Babizhayev et al., 2011; Ahmad and Haseeb, 2020). Furthermore, several studies have found increased concentration of LPO products in cataractous lenses (Spector, 1995; Sacca et al., 2009; Babizhayev et al., 2011; Ahmad and Haseeb, 2020) and aqueous-humour samples (Sacca et al., 2009; Ahmad and Haseeb, 2020) as opposed to healthy ones. LPO also has the potential to cause protein aggregates large enough to increase lens opacity. Moreover, products of LPO such as 4-hydroxynonenal (HNE) can induce the fragmentation of lens proteins, increasing lens opacity and ultimately cataracts (Ahmad and Haseeb, 2020). Finally, this process forms a particularly large contribution to the formation of cataracts because only one ROS is required to form several phospholipid hydroperoxides (Babizhayev et al., 2011).

Oxidative stress can also increase calcium levels in the lens, leading to cataracts (Hightower, 1995; Ahmad and Haseeb, 2020). ROS can change the ionic homeostasis of the lens, increasing the concentration of calcium ions, which activates calpains (Ca2+ dependent cytosolic cysteine proteases), leading to the degradation and aggregation of crystalline proteins (Li et al., 1995; Ahmad and Haseeb, 2020). From there, as shown in figure 1, the aggregation will scatter light, increasing lens opacity, and ultimately causing cataracts.

An additional mechanism leading from oxidative stress to cataracts is unrepaired DNA damage to the lens epithelial cells (Karslioğlu et al., 2005; Liu et al., 2013). Oxidative stress can also cause apoptosis, leading to the induction of cataracts (Li et al., 1995; Mok et al., 2014). Finally, oxidative stress can damage lens gap junctions, therefore causing cataracts. When ROS damage these junctions, it causes changes in intercellular communication, which Ahmad and Haseeb (2020) believe contributes to the formation of cataracts.

Also of note, the lens uses a variety of antioxidants to protect against oxidative stress. However, the concentration of these antioxidants is lower in cataractous lenses as opposed to healthy ones, which suggests that the cataractous lenses are most likely in a state of oxidative stress (Van Kuijk, 1991; Babizhayev et al., 2011; Varma et al., 2011; Zhang et al., 2012; Tangvarasittichai and Tangvarasittichai, 2019; Ahmad and Haseeb, 2020).

Empirical Evidence

There is a moderate amount of empirical evidence for this KER. The data evaluates lens opacity and cataracts, as well as indirect measurements such as light intensity, visual quality, and GSH levels. The qualitative data mostly uses H_2O_2 to induce oxidative stress, but other data uses a wider range of stressors, particularly various forms of radiation

with the assumption that they cause oxidative stress.

Dose Concordance

Studies to support the dose concordance of this relationship are minimal. One study found that irradiated samples showed a 68.8% increase in oxidative stress and a corresponding 90% increase in cataract presence compared to control (Karslioğlu et al., 2005).

Through indirect evidence from multiple independent studies, the current evidence indicates that oxidative stress can be induced by radiation doses as low as 0.1 Gy (Buonanno et al., 2011; Veeraraghan et al., 2011), 0.25 Gy (Cervelli et al., 2017; Ahmadi et al., 2021), and 0.5 Gy (Limoli et al., 2007; Yan, 2016). However, other studies have found that oxidative stress does not occur until 2 Gy (Giedzinski et al., 2005; Acharya et al., 2010; Soltani et al., 2016; Huang et al., 2018; Liu et al., 2019; Bai et al., 2019). Evidence, assembled by the International Commission on Radiological Protection (ICRP), has determined 0.5 Gy as the radiation threshold dose with 1% incidence for cataracts. Currently this threshold applies for acute, fractionated, protracted and chronic doses (Stewart et al., 2012). As doses of 0.5 Gy and below can induce oxidative stress while doses of over 0.5 Gy can cause cataracts, the data seems to indicate that oxidative stress occurs at lower radiation doses than cataracts. It should be noted however, that there is uncertainty involved in these values, which is discussed under “uncertainties & inconsistencies”.

Time Concordance

Overall, studies have found opacity to increase the longer lenses are under oxidative stress. In one study, the samples had a lens opacity that increased from 13.3 to 17.5x higher than controls when examined one and four days after exposure to H_2O_2 (Liu et al., 2013). In another study, exposure to oxidative stress induced an approximately linear 1.2x decrease in the mean gray value compared to control over the course of 20 days.

Essentiality

Studies have shown that healthy lenses have increased light intensity (Qin et al., 2019), increased visual quality (Smith et al., 2016), decreased opacity (Liu et al., 2013), increased glutathione levels (Zhang et al., 2012), and decreased cataract levels (Karslioğlu et al., 2005) when compared to lenses that underwent oxidative stress. Additionally, one study found inhibition of PARP-1, which is important for repairing oxidative damage, decreased lens opacity following oxidative stress (Smith et al., 2016). Another source notes that when protein aggregation (a consequence of oxidative stress,) is reversed, the transparency of animal lenses increases (Qin et al., 2019). Furthermore, Van Kuijk found that having high plasma levels of at least two antioxidant vitamins reduces the risk of cataracts (Van Kuijk, 1991). This is further supported by research showing that knock out mice for SOD, an antioxidant, develop spontaneous cataracts (Varma et al., 2011). Other experiments have found that in lenses exposed to markers of oxidative stress (via H_2O_2, hydroxyl radical or superoxide radical), the use of an antioxidant (glutathione peroxidase mimic, which decreases oxidative stress) prevents cataracts (Spector, 1995).

Uncertainties and Inconsistencies

There are several uncertainties and inconsistencies pertaining to this KER.

- It is typically assumed that lens glutathione reductase activity (helps protect against oxidative stress) decreases with age however, one paper contradicts this finding. As an organism ages, the mass of fiber cells, which are metabolically inactive, increases. Spector (1995) suggests that this results in an apparent decrease in glutathione reductase activity, leaving the actual activity constant.

Quantitative Understanding of the Linkage

The level of quantitative understanding for this KER is low. Studies examine the relationship between various oxidative stress inducers, such as H_2O_2 and radiation, and either lens opacity/cataracts, or indirect indicators such as visual quality. The following tables provide representative examples of the relationship, unless otherwise indicated, all data is statistically significant.

Dose Concordance

Reference	Experiment Description	Result
Karslioğlu et al., 2005	In vivo. Female, 8–12-week-old, Sprague-Dawley rats received head-only exposure to 5 Gy of ^{60}Co γ-rays at 0.59 Gy/min to induce oxidative stress, measured via the presence of malondialdehyde (MDA). Cataracts were characterised using the lens opacities classification system, version III (LOCS III).	Rats exposed in vivo to 5 Gy of ^{60}Co γ-rays displayed a 68.9% increase in malondialdehyde levels (indicative of increased oxidative stress) and a 90% increase in cataract prevalence relative to control.

Incidence Concordance

No studies found.

Time Concordance

Reference	Experiment Description	Result
Qin et al., 2019	In vitro. Human urinary cell-derived induced pluripotent stem cells were differentiated to form lentoid bodies. These were then exposed to 500 µM/day of H_2O_2 to induce oxidative stress. Cataract progression was measured via light microscopy and mean gray values (light intensity, where a lower gray value indicates increased opacity).	Exposure to oxidative stress induced an approximately linear 1.2x decrease in the mean gray value compared to control over the course of 20 days.
Liu et al., 2013	In vitro. 4 porcine lenses were exposed in vitro to 2 mM of H_2O_2, an ROS known to cause oxidative stress. Lens opacity was measured after one and four days.	In porcine lenses exposed in vitro to 2 mM of H_2O_2 (induces oxidative stress), lens opacity increased to 20x control one day post-exposure.

Known modulating factors

Modulating Factor (MF)	MF Specification	Effect(s) on the KER	Reference(s)
Antioxidants	Vitamin C, vitamin E, micronutrients, β-carotene, ascorbic acid, polyphenols, phytate, SOD, pyruvate, xanthine alkaloids, peroxiredoxin 6, anthocyanin, melatonin, N-acetylcysteine (NAC), N-acetylcysteine amide (NACA), and N-acetylcarnosine (NC)	Adding antioxidants decreases the occurrence and progression of cataracts.	Karslioğlu et al., 2005; Sacca et al., 2009; Babizhayev et al., 2011; Varma et al., 2011; Hamada et al., 2014; Mok et al., 2014; Lee & Afshari, 2023
Age	Increased age	Cataracts is due to an accumulation of small opacities in the lens, which increases with age. Furthermore, the concentration of various antioxidants such as GSH also decrease with age, increasing the lens' vulnerability to oxidative stress. Younger lenses also show better recovery after oxidative stress, possibly due to higher levels of thioltransferase and thioredoxin and increased ability to upregulate appropriate genes.	Spector, 1995; Sacca et al., 2009; Zhang et al., 2012; Ahmad and Haseeb, 2020
Genetics	Variations in the genes coding for antioxidant enzymes such as SOD, GPX, and catalase. An example includes the G/G genotype of the SOD1-251A/G polymorphism.	Mutations in critical genes can reduce cell protective capacity to handle oxidative stress, and therefore the formation of lens opacities.	Tangvarasittichai and Tangvarasittichai, 2019
Oxygen	Increased oxygen levels	Higher oxygen concentrations increase oxidative stress, and therefore the risk of cataracts.	Blakely, 2012; Hamada and Sato; 2016; Richardson, 2022
Diabetes/ hyperglycemia	Diabetes/hyperglycemia diagnosis	These conditions increase oxidative stress and therefore the risk of cataracts. They increase mitochondrial production of ROS and decreases glutathione regeneration. Additionally, these effects have been found to continue even after hyperglycemia has been returned to euglycemia in a phenomenon known as metabolic memory.	Qin et al., 2019
Lanosterol and its derivatives	Increased lanosterol levels	Lanosterol and its derivatives can depolymerize protein aggregates, which reduces lens opacity and can help to reverse cataract development. However, this has not been tested in humans.	Qin et al., 2019

Known Feedforward/Feedback loops influencing this KER

The main endogenous source of ROS production is the electron transport chain (ETC) in the mitochondria (Babizhayev et al., 2011). The mitochondrial DNA (mtDNA) responsible for the ETC is vulnerable to oxidative damage because it lacks protective proteins and histones. It is also located near the main source of endogenous ROS, the electron transport chain. Furthermore, some ROS have very short half-lives, meaning that they cannot travel very far. For example, hydroxyl radicals have half-lives in the order of 10-9 s. When mtDNA is damaged, the electron transport chain dysfunctions that create ROS become more common. This creates a feedforward loop where oxidative stress causes oxidative damage to mtDNA, which then causes the production of more ROS, increasing the oxidative stress in a vicious cycle (Lee et al., 2004; Zhang et al., 2010; Tangvarasittichai and Tangvarasittichai, 2019; Ahmad and Haseeb, 2020).

References

Acharya, M. M. et al. (2010), “Consequences of ionizing radiation-induced damage in human neural stem cells”, Free Radical Biology & Medicine, Vol. 49/12, Elsevier Inc, United States, https://doi.org/10.1016/j.freeradbiomed.2010.08.021

Ahmad, A. and H. Ahsan (2020), “Biomarkers of inflammation and oxidative stress in ophthalmic disorders”, Journal of immunoassay & immunochemistry, Vol. 41/3, Taylor & Francis, England, https://doi.org/10.1080/15321819.2020.1726774

Ahmadi, M. et al. (2021), “Early responses to low-dose ionizing radiation in cellular lens epithelial models”, Radiation Research, Vol. 197/1, Radiation Research Society, United States, https://doi.org/10.1667/RADE-20-00284.1

Babizhayev, M. A. et al. (2011), “Telomere-dependent senescent phenotype of lens epithelial cells as a biological marker of aging and cataractogenesis: the role of oxidative stress intensity and specific mechanism of phospholipid hydroperoxide toxicity in lens and aqueous”, Fundamental & clinical pharmacology, Vol. 25/2, Blackwell Publishing Ltd, Oxford, UK, https://doi.org/10.1111/j.1472-8206.2010.00829.x

Bai, J. et al. (2019), “Irradiation-induced senescence of bone marrow mesenchymal stem cells aggravates osteogenic differentiation dysfunction via paracrine signaling”, American Journal of Physiology, Vol. 318/5, Unites States, https://doi.org/10.1152/ajpcell.00520.2019

Balasubramanian, D. (2000), “Ultraviolet radiation and cataract”, Journal of Ocular Pharmacology and Therapeutics, Vol.16/3, Mary Ann Liebert Inc, Larchmont, https://doi.org/10.1089/jop.2000.16.285

Blakely, E. A. (2012), “Taylor lecture on radiation protection and measurements: what makes particle radiation so effective?”, Health Physics, Vol. 103/5, Health Physics Society, United States, https://doi.org/10.1097/HP.0b013e31826a5b85

Buonanno, M. et al. (2011), “Long-term consequences of radiation-induced bystander effects depend on radiation quality and dose and correlate with oxidative stress”, Radiation Research, Vol. 175/4, The Radiation Research Society, Kansas, https://doi.org/10.1667/RR2461.1

Cervelli, T. et al. (2017), “A new natural antioxidant mixture protects against oxidative and DNA damage in endothelial cell exposed to low-dose irradiation”, Oxidative Medicine and Cellular Longevity, Hindawi, United States, https://doi.org/10.1155/2017/9085947

Elliott, D. B. et al. (1990). “Comparing clinical tests of visual function in cataract with the patient's perceived visual disability”,. Eye (London, England), 4 (Pt 5), 712–717. https://doi.org/10.1038/eye.1990.100

Ganea, E. and J.J. Harding (2006), “Glutathione-related enzymes and the eye”, Current eye research, Vol. 31/1, Taylor & Francis, England, https://doi.org/10.1080/02713680500477347

Giedzinski, E. et al. (2005), “Efficient production of reactive oxygen species in neural precursor cells after exposure to 250 MeV protons”, Radiation Research, Vol. 164/4, Radiation Research Society, United States, https://doi.org/10.1667/RR3369.1

Hamada, N. (2017), “Ionizing radiation sensitivity of the ocular lens and its dose rate dependence”, International journal of radiation biology, Vol. 93/10, Taylor & Francis, England, https://doi.org/10.1080/09553002.2016.1266407

Hamada, N. and T. Sato (2016), “Cataractogenesis following high-LET radiation exposure”, Reviews in mutation research, Vol. 770, Elsevier B.V., https://doi.org/10.1016/j.mrrev.2016.08.005

Hamada, N. et al. (2014), “Emerging issues in radiogenic cataracts and cardiovascular disease”, Journal of radiation research, Vol. 55/5, Oxford University Press, England, https://doi.org/10.1093/jrr/rru036

Hightower, K. R. (1995), “The role of the lens epithelium in development of UV cataract”, Current eye research, Vol. 14/1, Informa UK Ltd, England, https://doi.org/10.3109/02713689508999916

Huang, B. et al. (2018), “Sema3a inhibits the differentiation of Raw264.7 cells to osteoclasts under 2 Gy radiation by reducing inflammation”, PloS one, Vol. 13/7, Public Library of Science, United States, https://doi.org/10.1371/journal.pone.0200000

Karimi, N. et al. (2017), “Radioprotective effect of hesperidin on reducing oxidative stress in the lens tissue of rats”, International Journal of Pharmaceutical Investigation, Vol. 7/3, Medknow Publications and Media Pvt. Ltd, India,

https://doi.org/10.4103/jphi.JPHI_60_17

Karslioğlu, I. et al. (2005), “Radioprotective effects of melatonin on radiation-induced cataract”, Journal of radiation research, Vol. 46/2, The Japan Radiation Research Society, England, https://doi.org/10.1269/jrr.46.277

Kozbenko, T. et al. (2022), “Deploying elements of scoping review methods for adverse outcome pathway development: a space travel case example”, International Journal of Radiation Biology, 1–12. https://doi.org/10.1080/09553002.2022.2110306

Lee, B. and Afshari, N. A. (2023), “Advances in drug therapy and delivery for cataract treatment”, Current Opinion in Ophthalmology, Vol. 34/1, Lippincott Williams & Wilkins, United States, https://doi.org/10.1097/ICU.0000000000000910

Lee, J. et al. (2004), “Reactive oxygen species, aging, and antioxidative nutraceuticals”, Comprehensive reviews in food science and food safety, Vol. 3/1, Blackwell Publishing Ltd, Oxford, http://doi.org/10.1111/j.1541-4337.2004.tb00058.x

Li, W. C. et al. (1995), “Lens epithelial cell apoptosis appears to be a common cellular basis for non-congenital cataract development in humans and animals”, The Journal of cell biology, Vol. 130/1, Rockfeller University Press, United States, https://doi.org/10.1083/jcb.130.1.169

Limoli, C. L. et al. (2007), “Redox changes induced in hippocampal precursor cells by heavy ion irradiation”, Radiation and Environmental Biophysics, Vol. 46/2, Springer, Germany, https://doi.org/10.1007/s00411-006-0077-9

Liu, H. et al. (2013), “Sulforaphane can protect lens cells against oxidative stress: implications for cataract prevention”, Investigative ophthalmology & visual science, Vol. 54/8, United States, https://doi.org/10.1167/iovs.13-11664

Liu, F. et al. (2019), “Transcriptional response of murine bone marrow cells to toal-body carbon-ion irradiation”, Mutation Research, Vol. 839, Elsevier B.V, Netherlands, https://doi.org/10.1016/j.mrgentox.2019.01.014

Mok, J. W., D. Chang and C. Joo (2014), “Antiapoptotic effects of anthocyanin from the seed coat of black soybean against oxidative damage of human lens epithelial cell induced by H_2O_2”, Current eye research, Vol. 39/11, Informal Healthcare USA, Inc, England, https://doi.org/10.3109/02713683.2014.903497

National Eye Institute (2022), Cataracts, https://www.nei.nih.gov/learn-about-eye-health/eye-conditions-and-diseases/cataracts (accessed November 29, 2022).

Qin, Z. et al. (2019), “Opacification of lentoid bodies derived from human induced pluripotent stem cells is accelerated by hydrogen peroxide and involves protein aggregation”, Journal of cellular physiology, Vol. 234/12, Wiley Subscriptions, United States, https://doi.org/10.1002/jcp.28943

Richardson, R. B. (2022), “The role of oxygen and the Goldilocks range in the development of cataracts induced by space radiation in US astronauts”, Experimental Eye Research, Vol. 223, Elsevier, https://doi.org/10.1016/j.exer.2022.109192

Sacca, S. C. et al. (2008) “Gene-environment interactions in ocular diseases”, Mutation research, https://doi.org/10.1016/j.mrfmmm.2008.11.002

Schoenfeld, M. P. et al. (2012), “A hypothesis on biological protection from space radiation through the use of new therapeutic gases as medical counter measures”, Medical gas research, Vol. 2/8, BioMed Central Ltd, London, https://doi.org/10.1186/2045-9912-2-8.

Shen, Y. et al. (2018), “Transplantation of bone marrow mesenchymal stem cells prevents radiation-induced artery injury by suppressing oxidative stress and inflammation”, Oxidative Medicine and Cellular Longevity, Vol. 2018, Hindawi, United States, https://doi.org/10.1155/2018/5942916

Smith, A. J. O. et al. (2016), “PARP-1 inhibition influences the oxidative stress response of the human lens”, Redox biology, Vol. 8/C, Elsevier, Netherlands, https://doi.org/10.1016/j.redox.2016.03.003

Soltani, B. et al. (2016), “Nanoformulation of curcumin protects HUVEC endothelial cells against ionizing radiation and suppresses their adhesion to monocytes: potential in prevention of radiation-induced atherosclerosis”, Biotechnology Letters, Vol. 38/12, Springer, Netherlands,, https://doi.org/10.1007/s10529-016-2189-x

Spector, A. (1995), “Oxidative stress-induced cataract: mechanism of action”, The FASEB Journal, Vol.9/12, Federation of American Societies for Experimental Biology, Bethesda, https://doi.org/10.1096/fasebj.9.12.7672510.

Stewart, F. A. et al. (2012), “ICRP publication 118: ICRP statement on tissue reactions and early and late effects of radiation in normal tissues and organs – threshold doses for tissue reactions in a radiation protection context”, Annals of the ICRP, Vol. 41/1-2, Elsevier Ltd, England, https://doi.org/10.1016/j.icrp.2012.02.001

Tangvarasittichai, O and S. Tangvarasittichai (2019), “Oxidative stress, ocular disease, and diabetes retinopathy”, Current Pharmaceutical Design, Vol. 24/40, Bentham Science Publishers, https://doi.org/10.2174/1381612825666190115121531

Turner, N. et al. (2002), “Opportunities for nutritional amelioration of radiation-induced cellular damage”, Nutrition,

Vol. 18/10, Elsevier Inc, New York, http://doi.org/10.1016/S0899-9007(02)00945-0

van Kuijk, F. J. (1991), “Effects of ultraviolet light on the eye: role of protective glasses”, Environmental health perspectives, Vol. 96, National Institute of Environmental Health Sciences, Unites States, https://doi.org/10.1289/ehp.9196177

Varma, S. D., S. Kovtun and K. R. Hegde (2011), “Role of ultraviolet irradiation and oxidative stress in cataract formation – medical prevention by nutritional antioxidants and metabolic agonists”, Eye & contact lens, Vol. 37/4, Lippincott Willians & Wilkins Inc, United States, https://doi.org/10.1097/ICL.0b013e31821ec4f2

Veeraraghavan, J. et al. (2011), “Low-dose γ-radiation-induced oxidative stress response in mouse brain and gut: Regulation by NFκB-MnSOD cross-signaling”, Mutation Research, Vol. 718/1, Elsevier B.V, Amsterdam, https://doi.org/10.1016/j.mrgentox.2010.10.006

Yan, J. et al. (2016), “^{56}Fe irradiation-induced cognitive deficits through oxidative stress in mice”, Toxicology Research, Vol. 5/6, Royal Society of Chemistry, https://doi.org/10.1039/c6tx00282j

Zhang, J. et al. (2012), “Ultraviolet radiation-induced cataract in mice: the effect of age and the potential biochemical mechanism”, Investigative ophthalmology & visual science, Vol. 53/11, the Association for Research in Vision and Ophthalmology, United States, https://doi.org/10.1167/iovs.12-10482