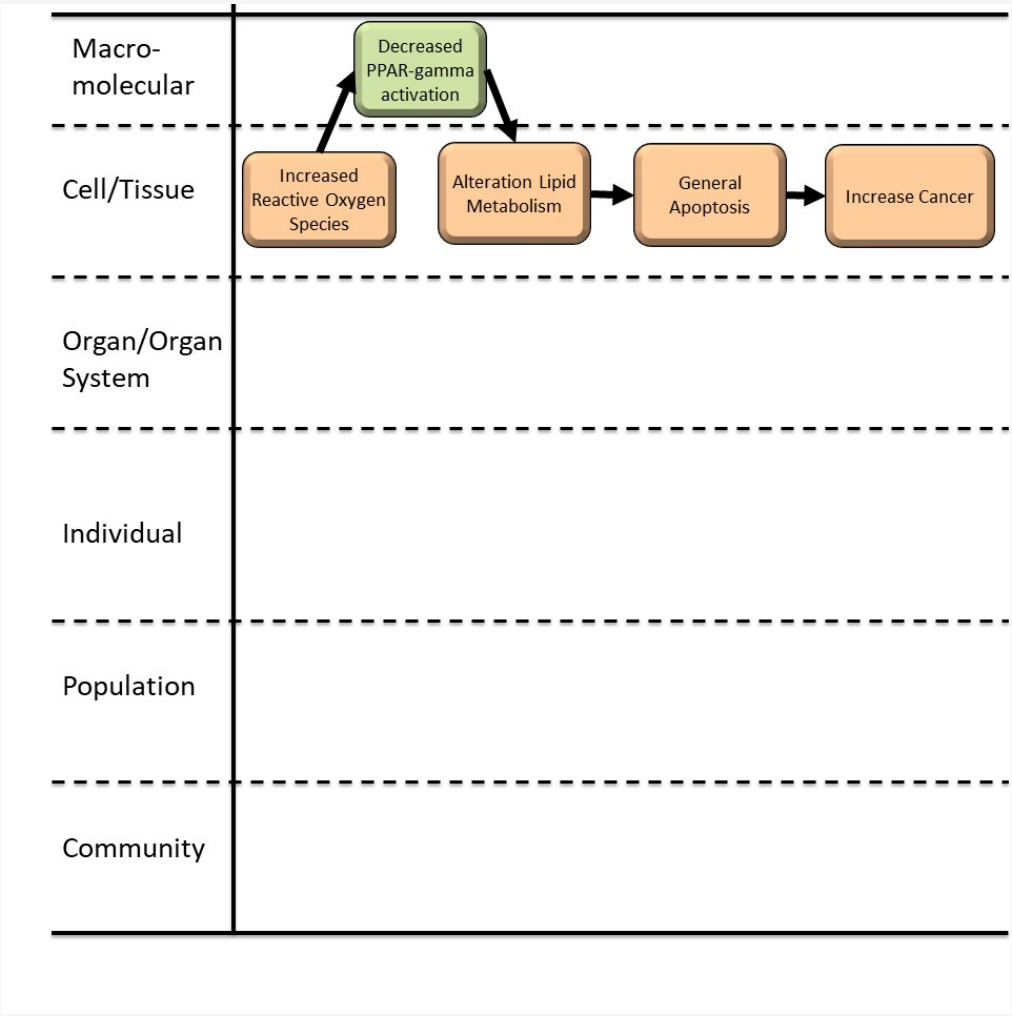
AOP ID and Title:

AOP 513: Reactive Oxygen (ROS) formation leads to cancer via Peroxisome proliferation-activated receptor (PPAR) pathway
Short Title: ROS formation leads to cancer via PPAR pathway

Graphical Representation



Authors

Of the originating work:

Jaesong Jeong and Jinhee Choi, School of Environmental Engineering, University of Seoul, Seoul, Republic of Korea

Of the content populated in the AOP-Wiki:

Daniel L. Villeneuve, US Environmental Protection Agency, Great Lakes Toxicology and Ecology Division, Duluth, MN

Travis Karschnik and John R. Frisch, General Dynamics Information Technology, Duluth, Minnesota

Status

Author status	OECD status	OECD project	SAAOP status
Under development: Not open for comment. Do not cite			

Abstract

Reactive oxygen species (ROS) are derived from oxygen molecules and can occur as free radicals (ex. superoxide, hydroxyl, peroxyl) or non-radicals (ex. ozone, singlet oxygen). ROS production occurs via a variety of normal cellular process; however, in stress situations (ex. exposure to radiation, chemical or biological stressors) reactive oxygen species levels dramatically increase and cause damage to cellular components. In this Adverse Outcome Pathway (AOP) we focus on the Peroxisome proliferation-

activated receptor (PPAR) response to increases in oxidative stress. Changes in activation rate of Peroxisome proliferation-activated receptors alter lipid metabolism, and decrease suppression of apoptosis. In this AOP we focus on the apoptosis response to cellular damage. Pathways leading to apoptosis, or single cell death, have traditionally been studied as both independent and simultaneous from pathways leading to necrosis, or tissue-wide cell death, with both overlap and distinct mechanisms (Elmore 2007). For the purposes of this AOP, we are characterizing cancer due to widespread cell-death, and recognize the complications in separating the related apoptosis and necrosis pathways.

Background

This Adverse Outcome Pathway focuses on the key pathways in which an established molecular disruption, increased levels of reactive oxygen species (ROS), leads to increased cancer.

Summary of the AOP

Events

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

Sequence	Type	Event ID	Title	Short name
	MIE	1115	Increased, Reactive oxygen species	Increased, Reactive oxygen species
	KE	233	Decreased, PPAR-gamma activation	Decreased, PPAR-gamma activation
	KE	1060	Alteration, lipid metabolism	Alteration, lipid metabolism
	KE	1513	General Apoptosis	General Apoptosis
	AO	885	Increase, Cancer	Increase, Cancer

Key Event Relationships

Upstream Event	Relationship Type	Downstream Event	Evidence	Quantitative Understanding
Increased, Reactive oxygen species	adjacent	Decreased, PPAR-gamma activation	High	Low
Decreased, PPAR-gamma activation	adjacent	Alteration, lipid metabolism	High	Low
Alteration, lipid metabolism	adjacent	General Apoptosis	High	Low
General Apoptosis	adjacent	Increase, Cancer	High	Low

Overall Assessment of the AOP

1. Support for Biological Plausibility of Key Event Relationships: Is there a mechanistic relationship between KEup and KEdown consistent with established biological knowledge?	
Key Event Relationship (KER)	Evidence Strong = Extensive understanding of the KER based on extensive previous documentation and broad acceptance.
Relationship 3092: Increased, Reactive oxygen species leads to Decreased, PPAR-gamma activation	**Strong support**. Increases in reactive oxygen species (ROS) have been shown to cause a variety of cellular responses including decreased PPARgamma gene expression.
Relationship 3093: Decreased, PPAR-gamma activation leads to Alteration, lipid metabolism	**Strong support**. Decreased PPAR gene expression have been shown to cause an alteration of lipid metabolism. PPAR-gamma acts as a nuclear signaling element that controls the transcription of a variety of genes involved in lipid catabolism and energy production pathways.

Relationship 3094: Alteration, lipid metabolism leads to General Apoptosis	**Strong support**. Alteration of lipid metabolism have been shown to results in abnormal cell function and activity, leading to apoptosis. Alteration of lipid metabolism leads to changes in cell lipid levels, structural changes in membranes as lipids are key components, and changes in signaling pathways affecting gene and protein expression. Loss of plasma membrane integrity due to disruptions to lipid metabolism results in cellular processes identifying cells as damaged, which acts as a signal for apoptosis.
Relationship 2977: General Apoptosis leads to Increase, Cancer	**Strong support.** The relationship between failure of apoptosis pathways to initiate cell death pathways and increases in cancer is broadly accepted and consistently supported across taxa.
Overall	**Strong support.** Extensive understanding of the relationships between events from empirical studies from a variety of taxa.

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

Sex Applicability

Sex	Evidence
Unspecific	High

Life Stage: The life stage applicable is all life stages.

Sex: Applies to both males and females.

Taxonomic: Appears to be present broadly, with representative studies including mammals (humans, lab mice, lab rats), telost fish, and invertebrates (cladocerans, mussels).

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Essentiality of the Key Events

Support for the essentiality of the key events can be obtained from a wide diversity of taxonomic groups, with mammals (lab ice, lab rats, human cell lines), telost fish, and invertebrates (cladocerans and mussels) particularly well-studied.

2. Essentiality of Key Events: Are downstream KEs and/or the AO prevented if an upstream KE is blocked?	
Key Event (KE)	Evidence Strong = Direct evidence from specifically designed experimental studies illustrating essentiality and direct relationship between key events. Moderate = Indirect evidence from experimental studies inferring essentiality of relationship between key events due to difficulty in directly measuring at least one of key events.
MIE 1115: Increased, Reactive oxygen species	**Strong support.** Increased Reactive oxygen species

	Strong support (ROS) levels are a primary cause of decreases in PPARgamma gene expression. Evidence is available from studies of stressor exposure and resulting changes in gene expression and protein/enzyme levels.
KE 233: Decreased, PPAR-gamma activation	**Strong support.** The PPARgamma gene family is important in controlling rate of lipid metabolism. Evidence is available from studies of stressor exposure and resulting changes in gene expression and protein/enzyme levels.
KE 1060: Alteration, lipid metabolism	**Strong support.** Altered lipid metabolism, particularly resulting loss of plasma membrane integrity is a cause of apoptosis. Evidence is available from studies of stressor exposure and resulting changes in gene expression and protein/enzyme levels.
KE 1513: General Apoptosis	**Moderate support.** Failure of apoptosis allows cancer cells to proliferate. Evidence is available from studies of stressor exposure and resulting changes in gene expression, protein/enzyme levels, and histology.
AO 885: Increase, Cancer	**Strong support.** Cancer proliferates due to a variety of stressors and breakdown of multiple celluar processes. Evidence is available from studies of stressor exposure and resulting changes in gene expression, protein/enzyme levels, and histology.
Overall	**Moderate to strong support.** Direct evidence from empirical studies for most key events, with more inferential evidence rather than direct evidence for apoptosis.

Weight of Evidence Summary

Path	Support
Increased, Reactive oxygen species leads to Decreased, PPAR-gamma activation	Biological plausibility is high. Representative studies have been done with mammals (El Midaoui et al. 2006; Blanquicett et al. 2010; Lu et al. 2018; Jeong and Choi 2020) fish (Wang et al. 2022).
Decreased, Decreased, PPAR-gamma activation leads to Alteration, lipid metabolism	Biological plausibility is high. Representative studies have been done with mammals (Chamorro-Garcia et al. 2018; Jeong and Choi 2020); fish (Venezia et al. 2021). For review (Tickner et al. 2001; Berger and Moller 2002; Luquet et al. 2005; Den Broeder et al. 2015).
Alteration, lipid metabolism leads to General Apoptosis	Biological plausibility is high. Representative studies have been done with mammals (Cadet et al. 2010, Gao et al. 2020); invertebrates (Avio et al. 2015). For review (Huang and Freter 2015).
General Apoptosis leads to Increase, Cancer	Biological plausibility is high. Representative studies have been done with mammals (Pavet et al. 2014; Jeong and Choi 2020). For review (Heinlein and Chang 2004; Vihervaara and Sistonen 2014).

3. Empirical Support for Key Event Relationship: Does empirical evidence support that a change in KEup leads to an appropriate change in KEdown?	
Key Event Relationship (KER)	Evidence

	Strong = Experimental evidence from exposure to toxicant shows consistent change in both events across taxa and study conditions.
Relationship 3092: Increased, Reactive oxygen species leads to Decreased, PPAR-gamma activation	**Strong support**. Increases in ROS leads to decreases in PPAR gamma gene expression, primarily by examining gene expression levels.
Relationship 3093: Decreased, PPAR-gamma activation leads to Alteration, lipid metabolism	**Strong support.** Decreases in PPAR gamma expression leads to alteration of lipid metabolism, primarily by assessing lipid content and levels of energy metabolites.
Relationship 3094: Alteration, lipid metabolism leads to General Apoptosis	**Strong support.** Altered lipid metabolism leads to apoptosis; problems with lipid metabolism lead to abnormal cells, triggering apoptosis pathways.
Relationship 2977: General Apoptosis leads to Increase, Cancer	**Strong support.** Mechanistic studies show that failure for apoptosis to eliminate cancer cells allows increases in cancer proliferation.
Overall	**Strong support.** Exposure from empirical studies shows consistent change in both events from a variety of taxa.

For overview of the biological mechanisms involved in this AOP, see Liu et al. (2015) and Jeong and Choi (2020); their studies analyzed ToxCast in vitro assays of mammalian acute toxicity data to identify correlations between toxicity pathways and chemical stressors, providing support for the key event relationships represented here.

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Appendix 1

List of MIEs in this AOP

Event: 1115: Increased, Reactive oxygen species

Short Name: Increased, Reactive oxygen species

Key Event Component

Process	Object	Action
reactive oxygen species biosynthetic process	reactive oxygen species	increased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:186 - unknown MIE leading to renal failure and mortality	KeyEvent
Aop:213 - Inhibition of fatty acid beta oxidation leading to nonalcoholic steatohepatitis (NASH)	KeyEvent
Aop:303 - Frustrated phagocytosis-induced lung cancer	KeyEvent
Aop:383 - Inhibition of Angiotensin-converting enzyme 2 leading to liver fibrosis	KeyEvent
Aop:382 - Angiotensin II type 1 receptor (AT1R) agonism leading to lung fibrosis	KeyEvent
Aop:384 - Hyperactivation of ACE/Ang-II/AT1R axis leading to chronic kidney disease	KeyEvent
Aop:396 - Deposition of ionizing energy leads to population decline via impaired meiosis	KeyEvent
Aop:409 - Frustrated phagocytosis leads to malignant mesothelioma	KeyEvent
Aop:413 - Oxidation and antagonism of reduced glutathione leading to mortality via acute renal failure	KeyEvent
Aop:416 - Aryl hydrocarbon receptor activation leading to lung cancer through IL-6 toxicity pathway	KeyEvent
Aop:418 - Aryl hydrocarbon receptor activation leading to impaired lung function through AHR-ARNT toxicity pathway	KeyEvent
Aop:386 - Deposition of ionizing energy leading to population decline via inhibition of photosynthesis	KeyEvent

AOP ID and Name	Event Type
Aop:387 - Deposition of ionising energy leading to population decline via mitochondrial dysfunction	KeyEvent
Aop:319 - Binding to ACE2 leading to lung fibrosis	KeyEvent
Aop:451 - Interaction with lung resident cell membrane components leads to lung cancer	KeyEvent
Aop:476 - Adverse Outcome Pathways diagram related to PBDEs associated male reproductive toxicity	MolecularInitiatingEvent
Aop:492 - Glutathione conjugation leading to reproductive dysfunction via oxidative stress	KeyEvent
Aop:497 - ERa inactivation alters mitochondrial functions and insulin signalling in skeletal muscle and leads to insulin resistance and metabolic syndrome	KeyEvent
Aop:500 - Activation of MEK-ERK1/2 leads to deficits in learning and cognition via ROS and apoptosis	KeyEvent
Aop:505 - Reactive Oxygen Species (ROS) formation leads to cancer via inflammation pathway	MolecularInitiatingEvent
Aop:513 - Reactive Oxygen (ROS) formation leads to cancer via Peroxisome proliferation-activated receptor (PPAR) pathway	MolecularInitiatingEvent
Aop:521 - Essential element imbalance leads to reproductive failure via oxidative stress	KeyEvent

Biological Context

Level of Biological Organization

Cellular

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Vertebrates	Vertebrates	High	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Unspecific	High

ROS is a normal constituent found in all organisms.

Key Event Description

Biological State: increased reactive oxygen species (ROS)

Biological compartment: an entire cell -- may be cytosolic, may also enter organelles.

Reactive oxygen species (ROS) are O2- derived molecules that can be both free radicals (e.g. superoxide, hydroxyl, peroxyl, alcoxyl) and non-radicals (hypochlorous acid, ozone and singlet oxygen) (Bedard and Krause 2007; Ozcan and Ogun 2015). ROS production occurs naturally in all kinds of tissues inside various cellular compartments, such as mitochondria and peroxisomes (Drew and Leeuwenburgh 2002; Ozcan and Ogun 2015). Furthermore, these molecules have an important function in the regulation of several biological processes – they might act as antimicrobial agents or triggers of animal gamete activation and capacitation (Goud et al. 2008; Parrish 2010; Bisht et al. 2017).
However, in environmental stress situations (exposure to radiation, chemicals, high temperatures) these molecules have its levels drastically increased, and overly interact with macromolecules, namely nucleic acids, proteins, carbohydrates and lipids, causing cell and tissue damage (Brieger et al. 2012; Ozcan and Ogun 2015).

How it is Measured or Detected

Photocolorimetric assays (Sharma et al. 2017; Griendling et al. 2016) or through commercial kits purchased from specialized companies.

Yuan, Yan, et al., (2013) described ROS monitoring by using H_2-DCF-DA, a redox-sensitive fluorescent dye. Briefly, the harvested cells were incubated with H_2-DCF-DA (50 µmol/L final concentration) for 30 min in the dark at 37°C. After treatment, cells were

immediately washed twice, re-suspended in PBS, and analyzed on a BD-FACS Aria flow cytometry. ROS generation was based on fluorescent intensity which was recorded by excitation at 504 nm and emission at 529 nm.

Lipid peroxidation (LPO) can be measured as an indicator of oxidative stress damage Yen, Cheng Chien, et al., (2013).

Chattopadhyay, Sukumar, et al. (2002) assayed the generation of free radicals within the cells and their extracellular release in the medium by addition of yellow NBT salt solution (Park et al., 1968). Extracellular release of ROS converted NBT to a purple colored formazan. The cells were incubated with 100 ml of 1 mg/ml NBT solution for 1 h at 37 °C and the product formed was assayed at 550 nm in an Anthos 2001 plate reader. The observations of the 'cell-free system' were confirmed by cytological examination of parallel set of explants stained with chromogenic reactions for NO and ROS.

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Yen, Cheng Chien, et al. "Inorganic arsenic causes cell apoptosis in mouse cerebrum through an oxidative stress-regulated signaling pathway." Archives of toxicology 85 (2011): 565-575.

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List of Key Events in the AOP

Event: 233: Decreased, PPAR-gamma activation

Short Name: Decreased, PPAR-gamma activation

Key Event Component

Process	Object	Action
peroxisome proliferator activated receptor signaling pathway	peroxisome proliferator-activated receptor gamma	decreased

Process	Object	Action

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:36 - Peroxisomal Fatty Acid Beta-Oxidation Inhibition Leading to Steatosis	MolecularInitiatingEvent
Aop:513 - Reactive Oxygen (ROS) formation leads to cancer via Peroxisome proliferation-activated receptor (PPAR) pathway	KeyEvent

Biological Context

Level of Biological Organization

Molecular

Cell term

Cell term

hepatocyte

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	Not Specified	NCBI
mouse	Mus musculus	Not Specified	NCBI
rat	Rattus norvegicus	Not Specified	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	Not Specified

Sex Applicability

Sex	Evidence
Unspecific	Not Specified

Life Stage: All life stages.

Sex: Applies to both males and females.

Taxonomic: Appears to be present broadly, with representative studies in mammals.

Key Event Description

The Peroxisome Proliferator-Activated Receptors (PPAR) family of genes involved in regulation of lipid metabolism and energy pathways (Desvergne and Wahli 1999, Hihi et al. 2002, Ahmed et al. 2007). Fatty acids stimulate the expression of PPAR genes, which initiate a variety of cellular responses focused on lipid metabolism, but also inflammation and apoptosis pathways. Decreases in PPAR-gamma expression are associated with disruption of adipocyte differentiation and glucose homeostasis.

How it is Measured or Detected

Peroxisome proliferation-activated receptors are investigated by changes in gene expression and protein levels. X-ray crystallography can be used to determine molecular structure. Effects of PPAR gamma on expression of downstream genes can be investigating using metabolomics and RT-qPCR approaches.

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Event: 1060: Alteration, lipid metabolism

Short Name: Alteration, lipid metabolism

Key Event Component

Process	Object	Action
lipid metabolic process		abnormal

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:166 - PPARalpha activation leading to pancreatic acinar tumors in the rat and mouse	KeyEvent
Aop:513 - Reactive Oxygen (ROS) formation leads to cancer via Peroxisome proliferation-activated receptor (PPAR) pathway	KeyEvent

Biological Context

Level of Biological Organization

Cellular

Cell term

Cell term
eukaryotic cell

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	Not Specified	NCBI
mouse	Mus musculus	Not Specified	NCBI
rat	Rattus norvegicus	Not Specified	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	Not Specified

Sex Applicability

Sex	Evidence
Unspecific	Not Specified

Life Stage: All life stages.

Sex: Applies to both males and females.

Taxonomic: Appears to be present broadly, with representative studies in mammals.

Key Event Description

Lipids are important molecules for efficient energy storage, in addition to roles as signaling molecules and basic building blocks in organisms. In addition to energy release, lipid metabolism affects the amount of stored fat. Alteration of lipid metabolism reflects a disruption of normal function, as evidenced by changes in gene expression, enzyme levels, break-down products, or fat content. Peroxisome proliferation-activated receptors pathways (and associated genes and proteins) are commonly monitored for downstream effects on lipid metabolism (Luquet et al. 2005; Den Broeder et al. 2015; Chamorro-Garcia et al. 2018; Venezia et al. 2021).

How it is Measured or Detected

Changes in lipid metabolism can be detected by examining organism fat content, or by examination of organs (ex. stomach, liver, intestines) for break-down products (ex. proteins) or changes in gene expression.

References

Chamorro-Garcia, R., Shoucri, B.M., Willner, S., Kach, H., Janesick, A., and Blumberg, B. 2018. Effect of perinatal exposure to dibutyltin chloride on fat and glucose metabolism in mice, and molecular mechanisms, in vitro. Environmental Health Perspectives 126: 057006.

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Event: 1513: General Apoptosis

Short Name: General Apoptosis

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:260 - CYP2E1 activation and formation of protein adducts leading to neurodegeneration	KeyEvent
Aop:505 - Reactive Oxygen Species (ROS) formation leads to cancer via inflammation pathway	KeyEvent
Aop:513 - Reactive Oxygen (ROS) formation leads to cancer via Peroxisome proliferation-activated receptor (PPAR) pathway	KeyEvent

Biological Context

Level of Biological Organization

Cellular

Domain of Applicability

Taxonomic Applicability

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

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Unspecific	High

Taxonomic: appears to be present broadly among multicellular organisms.

Key Event Description

Apoptosis is the programmed cell death in general. This process is well regulated with a sequence of events before cell fragmentation occurs. Changes in the nucleus of a cell are the first step in apoptosis. Before that, other factors such as stress, inflammation, cell damage can induce expression or activation of signal proteins which will activate the pathway for apoptosis. Examples of proteins which are involved in apoptosis are the proteins p53, Bcl-2, JNK, and several caspases. When the first step is taken in the apoptosis process the cell will end in membrane-bounded apoptotic bodies. These bodies are cleared by macrophages or other cells where the degradation process starts within heteorphagosomes.

How it is Measured or Detected

There are several possibilities to measure and detect apoptosis, some common techniques are:

- The detection of Lactate dehydrogenase (LDH) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) substances which are released from cells which undergo apoptosis.
- An older but effective technique it the annexin V – affinity assay. The principle of this assay is the high affinity binding between annexin V and phosphatidylserine. In a vital cell there is a membrane lipid asymmetry where phosphatidylserine molecules are facing the cytosol. During apoptosis the membrane lipid asymmetry is lost, and the phosphatidylserine molecules are expressed in the outer membrane. When annexin-V is present in combination with Ca^{2+} it binds with high affinity to phosphatidylserine. With a hapten label at the annexin-V this process can be detected.
- Another technique is the detection of cleaved caspase-3, which could be done with western blot or enzyme-linked immunosorbent assays.
- Cytochrome c is also a protein which is released in an early stage of apoptosis. Detection of cytochrome c can be done with metal nanoclusters which have a fluorescent probe in addition to western blot assay.

References

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List of Adverse Outcomes in this AOP

Event: 885: Increase, Cancer

Short Name: Increase, Cancer

Key Event Component

Process	Object	Action
	Neoplasms	increased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:141 - Alkylation of DNA leading to cancer 2	AdverseOutcome
Aop:139 - Alkylation of DNA leading to cancer 1	AdverseOutcome
Aop:505 - Reactive Oxygen Species (ROS) formation leads to cancer via inflammation pathway	AdverseOutcome
Aop:513 - Reactive Oxygen (ROS) formation leads to cancer via Peroxisome proliferation-activated receptor (PPAR) pathway	AdverseOutcome

Biological Context

Level of Biological Organization

Tissue

Domain of Applicability

Taxonomic Applicability

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

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Unspecific	High

Life Stage: All life stages. Older individuals are more likely to manifest this key event (adults > juveniles > embryos).

Sex: Applies to both males and females.

Taxonomic: Appears to be present broadly, with representative studies including mammals (humans, lab mice, lab rats), teleost fish, and invertebrates (cladocerans, mussels).

Key Event Description

Cancer is a general key event for related diseases each exhibiting uncontrolled proliferation of abnormal cells (for review see Hanahan and Weinberg 2011). A cancer often is initially associated with a specific organ, with malignant tumors developing ability to metastasize, or travel to other areas of the body. Most cancers develop from genetic mutations in normal cells, although a minority of cancers are hereditary. Exposure to chemical stressors, radiation, tobacco smoke, or viruses can increase the likelihood that cancer will develop.

Cancer cells proliferate due to capabilities summarized by Hanahan and Weinberg (2011):

1. Sustained proliferation signaling – by deregulating normal cell signals, cancer cells can sustain chronic proliferation.
2. Evading growth suppressors – by evading activities of tumor suppressor genes, cancer cells continue to proliferate.
3. Activating invasion and metastasis – by altering shape and attachment to cells in the extracellular matrix, cancer cells gain ability to move to other locations.
4. Enabling replicative immortality – by disabling senescence pathways, cancer cells have extended lifespans.
5. Inducing angiogenesis – by enabling neovasculature, cancer cells receive nutrients and oxygen and get rid of waste products.
6. Resisting cell death – by evading apotosis and necrosis defense pathways, cancer cells avoid elimination.

How it is Measured or Detected

Most carcinogenicity studies are conducted with rodents (see OECD 2018; Zhou et al. 2023 for methods) or in-vitro with mammalian cell lines (see OECD 2023 for methods). Cancer is usually detected by biopsy or histopathological examination of

tissue. Gene expression levels can also be assessed, as increased transcription of known genes have been associated with specific cancers (ex. Tumor Necrosis Factor (Pavet et al. 2014); Heat Shock Factors (Vihervaara and Sistonen 2014; Androgen Receptor (Heinlein and Chang 2004)).

Regulatory Significance of the AO

Cancer is a critical endpoint in human health risk assessment. It is embedded in regulatory frameworks for human health protection in many countries (see OSHA 2023 for examples of US regulations and European Parliament 2022 for examples of regulations in Europe).

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Appendix 2

List of Key Event Relationships in the AOP

List of Adjacent Key Event Relationships

Relationship: 3092: Increased, Reactive oxygen species leads to Decreased, PPAR-gamma activation

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Reactive Oxygen (ROS) formation leads to cancer via Peroxisome proliferation-activated receptor (PPAR) pathway	adjacent	High	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
Unspecific	High

Life Stage: The life stage applicable to this key event relationship is all life stages.

Sex: This key event relationship applies to both males and females.

Taxonomic: This key event relationship appears to be present broadly, with representative studies including mammals (humans, lab mice, lab rats) and teleost fish.

Key Event Relationship Description

Oxidative stress occurs due to the accumulation of reactive oxygen species (ROS). ROS can damage DNA, lipids, and proteins (Shields et al. 2021). Superoxide dismutase is an enzyme in a common cellular defense pathway, in which superoxide dismutase converts superoxide radicals to hydrogen peroxide. When cellular defense mechanisms are unable to mitigate ROS formation from mitochondrial respiration and stressors (biological, chemical, radiation), one established pathway that is disrupted involves Peroxisome proliferation-activated receptors.

Evidence Supporting this KER

Biological Plausibility

The biological plausibility linking decreases in Peroxisome proliferation-activated receptors to reactive oxygen species (ROS) is strong. Reactive oxygen species (ROS) are produced by many normal cellular processes (ex. cellular respiration, mitochondrial electron transport, specialized enzyme reactions) and occur in multiple chemical forms (ex. superoxide anion, hydroxyl radical, hydrogen peroxide). Antioxidant enzymes play a major role in reducing reactive oxygen species (ROS) levels in cells (Ray et al. 2012) to prevent cellular damage to lipids, proteins, and DNA (Juan et al. 2021). This Key Event Relationship focuses on the disruption of Peroxisome proliferation-activated receptors gene expression due to increases in Reactive oxygen species (ROS) level.

Empirical Evidence

Species	Duration	Dose	Increased ROS?	Decreased PPAR?	Summary	Citation
Lab rats (*Rattus norvegicus*)	4 weeks	Diet exposure of 10% D-glucose, with 1000 mg/kg feed alpha-lipoic acid supplement evaluated to mitigate D-glucose effects	Yes	Yes	Male rats showed increased superoxide levels in glucose treatment but not glucose plus alpha-lipoic acid treatment, and corresponding patterns in PPAR-gamma gene expression in the treatments.	El Midaoui et al. (2006)
Human (*Homo sapiens*) and cow (*Bos taurus*)	72 hours	In vitro exposure of 1-1000 uM hydrogen peroxide	Assumed	Yes	Human umbilical vein endothelial cells and bovine aortic endothelial cells showed increased dose-dependent	Blanquicett et al. (2010)

					cytotoxicity when was assumed to correlated with higher reactive oxygen species (ROS) levels, PPARgamma gene expression levels showed corresponding decreases.	
Lab mice (*Mus musculus*)	5 weeks	Diet exposure of 100, 1000 ug/L of 0.5, 50 um polystyrene microplastics	Assumed	Yes	Study selected stressor(s) known to elevate reactive oxygen species (ROS) levels. Male mice showed decreased gene expression of Peroxisome proliferation-activated receptor (PPAR-gamma) in blood.	Lu et al. (2018)
Zebrafish (*Danio rerio*)	4 weeks	Diet exposure of rosiglitazone, mitigation with N-acetylcysteine, L-carnitine, cold and heat stress, fish with PPAR-gamma mutations	Yes	Yes	Male and female fish had increased ROS levels and corresponding decreases in PPAR-gamma expression levels	Wang et al. (2022)

1 Assumed: study selected stressor(s) known to elevate reactive oxygen species (ROS) levels, endpoints verified increased oxidative stress and disrupted pathway.

References

Blanquicett, C., Kang, B-Y., Ritzenthaler, J.D. Jones, D.P., and Hart, C.M. 2010. Oxidative stress modulates PPARγ in vascular endothelial cells. Free Radical Biology and Medicine 48: 1618-1625.

El Midaoui, A., Wu, L., Wang, R., and de Champlain, J. 2006. Modulation of cardiac and aortic peroxisome proliferator-activated receptor-gamma expression by oxidative stress in chronically glucose-fed rats. American Journal of Hypertension 19: 407-412.

Juan, C.A., de la Lastra, J.M.P., Plou, F.J., and Lebena, E.P. 2021. The chemistry of reactive oxygen species (ROS) revisited: Outlining their role in biological macromolecules (DNA, lipids and proteins) and induced pathologies. International Journal of Molecular Sciences 22: 4642.

Lu, L., Wan, Z., Luo, T., Fu, Z., and Jin, Y. 2018. Polystyrene microplastics induce microbiota dysbiosis and hepatic lipid metabolism disorder in mice. Science of the Total Environment 631-632: 449-458.

Ray, P.D., Huang, B.-W., and Tsuji, Y. 2012. Reactive oxygen species (ROS) homeostasis and redox regulation in cellular

signalling. Cellular Signalling 24:981-990.

Relationship: 3093: Decreased, PPAR-gamma activation leads to Alteration, lipid metabolism

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Reactive Oxygen (ROS) formation leads to cancer via Peroxisome proliferation-activated receptor (PPAR) pathway	adjacent	High	Low

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

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

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Unspecific	High

Life Stage: The life stage applicable to this key event relationship is all life stages.

Sex: This key event relationship applies to both males and females.

Taxonomic: This key event relationship appears to be present broadly, with representative studies including mammals (humans, lab mice, lab rats) and teleost fish.

Key Event Relationship Description

Expression of Peroxisome proliferator-activated receptors (PPAR) family genes are closely related to different aspects of lipid metabolism, and resulting organism fat content. PPAR-alpha, PPAR-gamma, and PPAR-delta families of genes are most often discussed when considering lipid metabolism. PPAR-alpha family genes are linked to regulation of lipid metabolism, lipoprotein synthesis, and metabolism processes, while PPAR-gamma family genes are linked to the proliferation of adipose cells, and PPAR-delta family genes are linked to changes in metabolic response due to environmental change. In this Key Event Relationship, we focus on the effects of decreased expression of PPAR-gamma family genes, with altered lipid metabolism.

Evidence Supporting this KER

Biological Plausibility

The biological plausibility linking decreases in Peroxisome proliferation-activated receptors to lipid metabolism is strong. Disruption of cellular processors via stressors have been shown to decrease PPAR-gamma gene expression, with corresponding decreases in lipid metabolism and/or increases in fat content of organisms.

Empirical Evidence

For review see Berger et al. (2002), Luquet et al. (2005), Den Broder et al. (2015). Experiments cited here have been conducted with lab mammals and with fish.

Species	Duration	Dose	Decreased PPAR?	Alteration lipid metabolism?	Summary	Citation
Human (*Homo sapiens*)	2 hours – 16 weeks	In vitro exposure of 10e-10M to	Yes	Yes	In human and mouse cells, as well as lab	Chamorro-Garcia et al. (2018)

and lab mice (*Mus musculus*)		10e-5M dibutyltin and tributyltin and 500 nm rosiglitazone and diet exposure of 50, 500 nM dibutyltin and 50 nM tributyltin.			mice, increased activation of PPAR-gamma gene expression was correlated with increases in glucose levels and increased weight gain.	
Zebrafish (*Danio rerio*)	3 days	Aquatic exposure of 10 µM Rosiglitazone, T0070907, GW6471, GW590735, GSK3787, or GW501516.	Yes	Yes	Embryos exposed to PPAR antagonist compounds had decreased PPAR-gamma gene expression correlated with decreased lipid accumulations, embryos exposed to PPAR agonist compounds had increased PPAR-gamma gene expression correlated with increased lipid accumulations.	Venezia et al. (2021)
Lab mice (*Mus musculus*)	5 weeks	Diet exposure of 100, 1000 ug/L of 0.5, 50 um polystyrene microplastics	Yes	Yes	Male mice showed decreased gene expression of Peroxisome proliferation-activated receptor (PPAR-gamma) correlated with decreased glucose levels and fat content.	Lu et al. (2018)

References

Berger, J. and Moller, D. 2002. The mechanisms of action of PPARS. Annual Review of Medicine 53: 409-435.

Chamorro-Garcia, R., Shoucri, B.M., Willner, S., Kach, H., Janesick, A., and Blumberg, B. 2018. Effect of perinatal exposure to dibutyltin chloride on fat and glucose metabolism in mice, and molecular mechanisms, in vitro. Environmental Health Perspectives 126(5): 057006.

Den Broeder, M.J., Kopylova, V.A., Kamminga, L.M. Legler, J. 2015. Zebrafish as a model to study the role of peroxisome proliferating-activated receptors in adipogenesis and obesity. PPAR Research 2015: 358029.

Lu, L., Wan, Z., Luo, T., Fu, Z., and Jin, Y. 2018. Polystyrene microplastics induce microbiota dysbiosis and hepatic lipid metabolism disorder in mice. Science of the Total Environment 631-632: 449-458.

Luquet, S., Gaudel, C., Holst, D., Lopez-Soriano, J., Jehl-Pietri, C., Fredenrich, A., and Grimaldi, P.A. 2005. Roles of PPAR delta in

lipid absorption and metabolism: A new target for the treatment of type 2 diabetes. Biochimica and Biophysica Acta 1740: 313-317.

Venezia, O., Islam, S., Cho, C., Timme-Laragy, A.R., and Sant, K.E. 2021. Modulation of PPAR signaling disrupts pancreas development in the zebrafish, Danio rerio. Toxicology and Applied Pharmacology 426: 115653.

Relationship: 3094: Alteration, lipid metabolism leads to General Apoptosis

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Reactive Oxygen (ROS) formation leads to cancer via Peroxisome proliferation-activated receptor (PPAR) pathway	adjacent	High	Low

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
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

Life Stage: The life stage applicable to this key event relationship is all life stages.

Sex: This key event relationship applies to both males and females.

Taxonomic: This key event relationship appears to be present broadly, with representative studies on mammals (humans, lab mice, lab rats).

Key Event Relationship Description

Alteration of lipid metabolism leads to changes in cell lipid levels, structural changes in membranes (lipids are key components), and changes in signaling pathways affecting gene and protein expression (Huang and Freter, 2015). Loss of plasma membrane integrity due to disruptions to lipid metabolism results in cellular processes identifying cells as damaged, triggering apoptosis pathways. Oxidation of fatty acids can lead to increases of reactive oxygen species (ROS), creating an additional stress disrupting the cellular environment. As lipids represent a diverse class of molecules, and the basic building blocks for many biologically important compounds, disruption of lipid function will eventually lead to damaged cells and cell death via apoptosis.

Evidence Supporting this KER

Biological Plausibility

The biological plausibility linking alterations in lipid metabolism to apoptosis is moderate. Disruption of lipid metabolism via stressors has been shown to lead to apoptosis, particularly through resulting loss of plasma membrane integrity.

Empirical Evidence

See Huang and Freter (2015) for review of the relationship between lipid metabolism and apoptosis.

Species	Duration	Dose	Alteration lipid metabolism?	General Apoptosis?	Summary	Citation
Lab rats (*Rattus norvegicus*)	4 hours	Injection exposure of methamphetamine.	Yes	Yes	In rats, methamphetamine exposure induced expression genes	Cadet et al. (2010)

					that control lipid metabolism and apoptosis.	
Human (*Homo sapiens*)	48 hours	In vitro exposure of 50-300 uM CPI-613.	Yes	Yes	Human pancreatic cells exposed to PPAR antagonist compounds repressed lipid metabolism and triggered apoptosis.	Gao et al. (2020)
Mussel (*Mytilus galloprovincialis*)	7 days	Aquatic exposure of 0.5, 5, 50 ug/L of <100, 100-1000 um polyethylene and polystyrene microplastics	Yes	Yes	Mussels showed altered gene expression of genes associated with lipid metabolism and apoptosis.	Avio et al. (2015)

References

Avio, C.G., Gorbi, S., Milan, M., Benedetti, M., Fattorini, D., D'Errico, G., Pauletto, M., Bargelloni, L., and Regoli, F. 2015. Pollutants bioavailability and toxicological risk from microplastics to marine mussels. Environmental Pollutants 198: 211-222.

Cadet, J.L., Jayanthi, S., McCoy, M.T., Beauvais, G., and Cai, N.S. 2010. Dopamine D1 receptors, regulation of gene expression in the brain, and neurogeneration. CNS Neurological Disorders - Drug Targets 9: 526-538.

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Huang, C. and Freter, C. 2015. Lipid metabolism, apoptosis and cancer therapy. International Journal of Molecular Sciences 16: 924-949.

Relationship: 2977: General Apoptosis leads to Increase, Cancer

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Reactive Oxygen Species (ROS) formation leads to cancer via inflammation pathway	adjacent	High	Low
Reactive Oxygen (ROS) formation leads to cancer via Peroxisome proliferation-activated receptor (PPAR) pathway	adjacent	High	Low

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

Life Stage: The life stage applicable to this key event relationship is all life stages.

Sex: This key event relationship applies to both males and females.

Taxonomic: This key event relationship appears to be present broadly, with representative studies focused in mammals (humans, lab mice, lab rats).

Key Event Relationship Description

Cancer is a general key event for related diseases each exhibiting uncontrolled proliferation of abnormal cells (for review see Hanahan and Weinberg 2011). A cancer often is initially associated with a specific organ, with malignant tumors developing ability to metastasize, or travel to other areas of the body. Most cancers develop from genetic mutations in normal cells; in this key event relationship we are focusing on disruption of apoptosis and necrosis pathways, leading to cancer. Exposure to chemical stressors, radiation, tobacco smoke, or viruses can increase the likelihood that cancer will develop. Pathways leading to apoptosis, or single cell death, have traditionally been studied as both independent and simultaneous from pathways leading to necrosis, or tissue-wide cell death, with both overlap and distinct mechanisms (Elmore 2007). For the purposes of this key event relationship, we are characterizing cancer due to widespread cell-death.

Cancer cells proliferate due to capabilities summarized by Hanahan and Weinberg (2011):

1. Sustained proliferation signaling – by deregulating normal cell signals, cancer cells can sustain chronic proliferation.
2. Evading growth suppressors – by evading activities of tumor suppressor genes, cancer cells continue to proliferate.
3. Activating invasion and metastasis – by altering shape and attachment to cells in the extracellular matrix, cancer cells gain ability to move to other locations.
4. Enabling replicative immortality – by disabling senescence pathways, cancer cells have extended lifespans.
5. Inducing angiogenesis – by enabling neovasculature, cancer cells receive nutrients and oxygen and get rid of waste products.
6. Resisting cell death – by evading apotosis and necrosis defense pathways, cancer cells avoid elimination.

Evidence Supporting this KER

Biological Plausibility

The biological plausibility linking cancer to avoidance of apoptosis is strong. Apoptosis is a series of related pathways that eliminate abnormal cells. Cancer cells proliferate due to evasion of cellular defenses (apoptosis pathways) and tissue-level defenses (necrosis pathways). Specific modifications to cancer cells that enable proliferation rather than elimination are listed under the Key Event Relationship Description. For review see:

1. Heinlein and Chang (2004): Role of androgen receptor in apoptosis, loss of androgen pathway function resulting in increases in mammalian prostate cancer.

2. Hanahan and Weinberg (2011): Biological capabilities gained by cancer cell to enable proliferation of tumor cells and evasion of normal regulating mechanisms of apoptosis and necrosis pathways in mammals.

3. Pavet et al. (2014): Role of tumor necrosis factor-related apoptosis-inducing ligandin to induce apoptosis in mammalian cells and reduce incidence of cancer.

4. Vihervaara and Sistonen (2014): Role of increased rate of transcription of heat shock factor 1 in mammalian cancer cells enhancing survival and metastasis, as well as evasion of cellular defenses.

Empirical Evidence

References cited by Jeong and Choi (2020) are review articles and gene expression studies. Empirical studies linking apoptosis to cancer were not provided.

References

Elmore, S. 2007. Apoptosis: A Review of Programmed Cell Death. Toxicologic pathology 35 (4): 495-516.

Hanahan, D. and Weinberg, R.A. 2011. Hallmarks of cancer: the next generation. Cell 144(5): 646-674.

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Pavet, V., Shlyakhtina, Y., He, T., Ceschin, D.G., Kohonen, P., Perala, M., Kallioniemi, O., and Gronemeyer, H. 2014. Plasminogen activator urokinase expression reveals TRAIL responsiveness and support fractional survival of cancer cells. Cell Death and Disease 5: e1043.

Vihervaara, A. and Sistonen, L. 2014. HSF1 at a glance. Journal of Cell Scientce 127: 261-266.