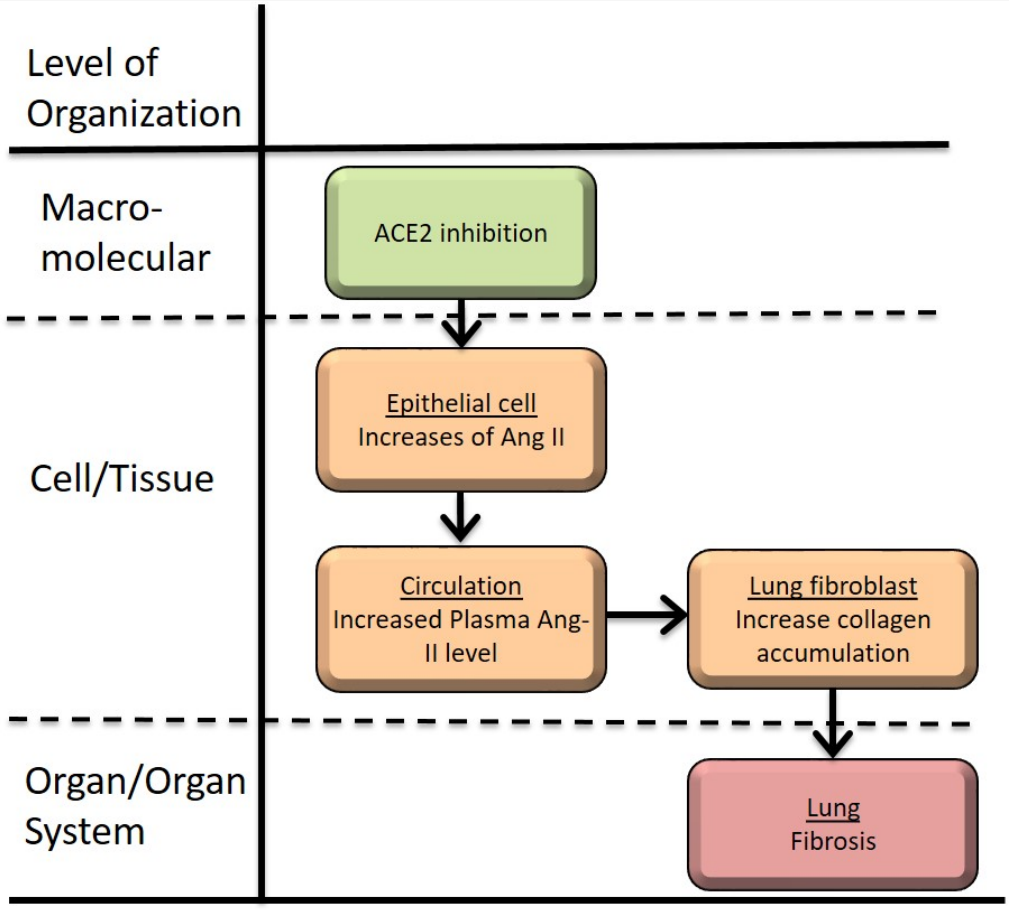
AOP ID and Title:

AOP 319: Binding to ACE2 leading to lung fibrosis
Short Title: Binding to ACE2 leads to lung fibrosis

Graphical Representation



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Status

Author status	OECD status	OECD project	SAAOP status
Open for comment. Do not cite	Under Development	1.96	Included in OECD Work Plan

Abstract

Lung fibrosis has a distinct point in idiopathic pulmonary fibrosis from the renin-angiotensin pathway. this system is reported in many works of literature to share many immune/inflammatory responses to lung damage. When ACE2 activity is inhibited, Ang II is not effectively converted into Ang-(1-7). Because of this, the levels of proinflammatory Ang II are increased and accumulated, while the levels of anti-inflammatory Ang-(1-7) are reduced. Most importantly, the Inhibition of ACE2 was shown to enhance the level of Ang II peptides which is a ligand for type 1 angiotensin receptor (AT1R) and is considered one of the risk factors for lung fibrosis, vasoconstriction, endothelial dysfunction, and cell death. The imbalance of the renin-angiotensin system (RAS) plays a critical role in the fibrogenesis and inflammation damage of many organs. Especially the inhibition of membrane ACE-2 enzymatic activity has shown promising potential in the Molecular initiation event which leads to pulmonary fibrosis. This AOP describes the role of

ACE-2 in fibrotic damage of the lung as an adverse outcome through the fibrogenic components, proinflammatory cytokines, and oxygen deficiency.

Background

ACE2 is an essential enzyme of blood pressure regulation in the renin-angiotensin system. ACE2 is primarily expressed on type II alveolar epithelial cells within the respiratory system. Angiotensin-converting enzyme (ACE) synthesizes the dominant vasoconstriction, inflammatory and profibrotic angiotensin II (Ang II) through its carboxypeptidase function on the decapeptide angiotensin I. In the meantime, Angiotensin-converting enzyme 2 (ACE2) is an exopeptidase that catalyzes the conversion of angiotensin II to the conversion of angiotensin 1-7 function as vasodilation, anti-inflammation, and anti-fibrotic. This AOP describes the dysfunction of membrane ACE2, which results in a high level of angiotensin Ang II synthesized by ACE, which can further lead to pulmonary fibrosis by excessive collagen deposition as the most potent profibrotic factor.

Summary of the AOP

Events

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

Sequence	Type	Event ID	Title	Short name
1	MIE	1740	Induced dysregulation of ACE2	ACE2 enzymatic inhibition
2	KE	1854	Dysregulation, ACE2 expression and activity	ACE2 dysregulation
3	KE	1752	Increased Angiotensin II	Increased AngII
4	KE	1851	Binding of agonist, Angiotensin II receptor type 1 receptor (AT1R)	Binding of agonist, Angiotensin II receptor type 1 receptor (AT1R)
5	KE	1115	Increased, Reactive oxygen species	Increased, Reactive oxygen species
6	KE	1172	Increased activation, Nuclear factor kappa B (NF-kB)	Increased activation, Nuclear factor kappa B (NF-kB)
7	KE	68	Accumulation, Collagen	Accumulation, Collagen
8	KE	1496	Increased, secretion of proinflammatory mediators	Increased proinflammatory mediators
9	AO	1276	Lung fibrosis	Lung fibrosis

Key Event Relationships

Upstream Event	Relationship Type	Downstream Event	Evidence	Quantitative Understanding
Induced dysregulation of ACE2	adjacent	Increased Angiotensin II	High	Moderate
Increased Angiotensin II	adjacent	Binding of agonist, Angiotensin II receptor type 1 receptor (AT1R)	High	High
Binding of agonist, Angiotensin II receptor type 1 receptor (AT1R)	adjacent	Increased, Reactive oxygen species	Moderate	Moderate
Increased, Reactive oxygen species	adjacent	Increased activation, Nuclear factor kappa B (NF-kB)	Moderate	Moderate
Increased activation, Nuclear factor kappa B (NF-kB)	adjacent	Increased, secretion of proinflammatory mediators	Moderate	Moderate
Increased, secretion of proinflammatory mediators	adjacent	Accumulation, Collagen	Moderate	Moderate
Accumulation, Collagen	adjacent	Lung fibrosis	Low	Not Specified

Stressors

Name	Evidence
PM 2.5	High
Streptozocin	High
Losartan	High
DX600	High
cationic polyamidoamine dendrimer (nanoparticle)	High

PM 2.5

Instillation of particulate matter 2.5 induced acute lung injury and attenuated the injury recovery in ACE2 knockout mice,Int. J. Biol. Sci. 2018, Vol. 14

Streptozocin

Streptozocin resulted in decreased expression of ACE2 protein and resulted in increased expression of TGF-Beta1

Losartan

Losartan resulted in decreased expression of ACE2 protein

cationic polyamidoamine dendrimer (nanoparticle)

Cationic nanoparticles directly bind angiotensin-converting enzyme 2 and induce acute lung injury in mice*Sun et al. Particle and Fibre Toxicology (2015) 12:4*

Overall Assessment of the AOP

Domain of Applicability

Life Stage Applicability

Life Stage	Evidence
Not Otherwise Specified	Moderate

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
mouse	Mus musculus	High	NCBI

References

Appendix 1

List of MIEs in this AOP

Event: 1740: Induced dysregulation of ACE2

Short Name: ACE2 enzymatic inhibition

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:319 - Binding to ACE2 leading to lung fibrosis	MolecularInitiatingEvent
Aop:383 - Inhibition of Angiotensin-converting enzyme 2 leading to liver fibrosis	MolecularInitiatingEvent

Biological Context

Level of Biological Organization

Molecular

List of Key Events in the AOP

Event: 1854: Dysregulation, ACE2 expression and activity

Short Name: ACE2 dysregulation

Key Event Component

Process	Object	Action
mRNA transcription	angiotensin-converting enzyme 2	increased
carboxypeptidase activity	angiotensin-converting enzyme 2	increased
protein localization to cell surface	angiotensin-converting enzyme 2	decreased
	angiotensin-converting enzyme 2	increased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:385 - Viral spike protein interaction with ACE2 leads to microvascular dysfunction, via ACE2 dysregulation	KeyEvent
Aop:428 - Binding of S-protein to ACE2 in enterocytes induces ACE2 dysregulation leading to gut dysbiosis	KeyEvent
Aop:319 - Binding to ACE2 leading to lung fibrosis	KeyEvent

Stressors

Name
Sars-CoV-2
SARS-CoV
Influenza Virus

Biological Context

Level of Biological Organization

Molecular

Domain of Applicability

ACE2 is expressed in a wide variety of tissues affecting their function.

ACE2 was initially identified in human lymphoma cDNA library (Tipnis, 2000), and from a human cDNA library of ventricular cells with heart failure (Donoghue 2000). Expression of ACE2 has later been also identified in the heart, kidney, and testis (Donoghue et al. (2000b). However, subsequent studies have shown a much broader distribution, including the upper airways, lungs, gut, and liver (reviewed recently by Saponaro, 2020).

Tissue and sub-cellular distribution of ACE2 (protein and mRNA)

Protein expression patterns

Immunostaining methods show that ACE2 is chiefly bound to cell membranes, predominantly in the smooth muscle cells and in the endothelium of the vasculature, while negligible levels can be detected in the circulation. In blood cells, it has been observed in platelets and macrophages, but not in B and T lymphocytes (Hamming et al., 2004; Fraga-Silva et al., 2011).

ACE2 shows differential sub-cellular distribution which can be significant for its basal/constitutive and modulated function. It is mainly detectable at the cell-surface with little intracellular localization, and the protein does not readily internalize (Warner et al., 2005). In polarized cells, ACE2 is exclusively targeted to the apical surface in vivo in kidney (Warner 2005) and in vitro in polarised cells derived from the colon, lung and kidney (Ren 2006). This is in contrast to its sequence homologue and functional “balancer” ACE, which distributes equally between apical and basolateral surfaces (Warner 2005).

In the small intestines, ACE2 is highly expressed on enterocytes and via its local RAS function participates in the regulation of the intestinal glucose transport. Intestinal ACE2 generates locally Ang 1-7 from luminal Ang II. Ang II was shown to inhibit SGLT1-dependent intestinal glucose uptake in a dose-dependent manner *in vitro* and *in vivo* in rats (Wong et al, 2007; 2009) and in human biopsies via AT1R activation (Casselbrant et al., 2015).

mRNA expression patterns

More recently, Qi. et al 2020 analysed 13 human tissues by scRA sequencing and report that ACE2 mRNA was mainly expressed in the ileum enterocytes, kidney proximal tubules and lung AT2 cells. ACE2 mRNA was also detected but to a lesser extent in the colon enterocytes, esophagus and keratinocytes and minimally in the cholangiocytes (biliary cells of the liver).

Key Event Description

The angiotensin-converting enzyme 2 (ACE2) is a membrane-anchored protein with wide tissue distribution https://www.proteinatlas.org/ENSG00000130234-ACE2. ACE2 has multiple functions, and is highly regulated at the transcriptional, post-transcriptional and post-translational levels. Modulation of the expression levels or functional activity of the ACE2 receptor is described in this KE.

ACE2 is bound to cell membranes, and in polarized cells it is exclusively targeted to the apical surface (Werner 2005; Ren 2006) [for more detailed description of the expression patterns of ACE2 see the applicability domain section].

ACE2 FUNCTIONS

Enzymatic function of ACE2 in interlinked bioactive peptide systems

ACE2 is mainly known and studied as a type I ectoenzyme i.e. a transmembrane protein with an extracellular amino-terminal domain harbouring a carboxypeptidase active site. ACE2 cleaves the carboxyterminal amino acid from a number of biologically active peptides (Figure 1 and 2), thus activating or deactivating them as agonists within the Renin Angiotensin System (RAS) (Santos et al., 2019) and within the Kinin–kallikrein system (KKS) (Kakoki & Oliver, 2009) in mammals.

The extracellular domain of ACE2 can itself be cleaved (shedding) in vitro and in vivo, releasing a soluble and catalytically active sACE2 (Guy et al., 2008 in human cardiac myofibroblasts; Peng Jia et al., 2009 in human lung epithelial cells and BALF; Werner et al., 2005 in canine epithelial polarised kidney MDCKII cells stabilly expressing ACE2). Constitutive shedding of ACE2 from the cellular membrane is mediated by another membrane-bound metalloprotease from the adamalysin family, ADAM17 (Iwata et al., 2009) also known as TACE, TNFalpha Converting Enzyme (Zunke 2017). Other proteins may also be involved in ACE2 protolithic modulation (Lambert et al, 2008).

The exact role of ACE2 shedding in modulating its function is not well understood, however, being catalytically active, the released sACE2 can, as the membrane-anchored full length ACE2, generate biologically active peptides which activate specific

receptors in different cells/tissues/organs. Thus, the ACE2 function in the organism is mediated via its peptide products activating specific receptors on the same, (autocrine), nearby (paracrine) and potentially distant cells.

ACE2 is a homologue of angiotensin-converting enzyme (ACE), with whom it shares significant sequence similarity (Tipnis, 2000; Donoghue 2000), yet exhibits very distinct enzymatic activity. ACE2, as ACE, is a zink-metallopeptidase, however ACE2 is strictly a carboxypeptidase while ACE is an dipeptidase. Furthermore, the main substrate of ACE2 is the octopeptide Angiotensin II (Ang II), the enzymatic product of its homologue ACE. By cleaving a single amino acid from the C-terminus of Ang II, ACE2 generates a functionally different bioactive peptide Angiotensin 1-7 (Ang1-7) (Figure 1). ACE2 is key to the regulation of local and systemic Ang II levels.

Ultimately, the function of ACE2 at tissue level is mediated via the interaction of its main active product Ang1-7 with the Angiotensin II receptor 2 (AT2R), balanced by the activity of its homologue ACE and other peptidases in the RAS.

Figure1: Enzymatic activity of ACE2 compared to its homologue ACE and another protease relevant to the RAS (from Rice et al., 2004)

[to add]

Figure 2: Simplified representation of the biological function of the enzymatic products of ACE2 activity in the KKS (adopted from Kakoki & Oliver, 2009).

[to add]

Although most studies have focused on the role of ACE2 in angiotensin metabolism in the RAS, the enzyme has broad substrate specificity and it also hydrolyses a number of other biologically active peptides including des-Arg9-bradykinin (DABK), apelin-13, neurotensin(1–11), dynorphin A-1–13), β-casomorphin-(1–7), and ghrelin (Vickers 2002, Humming, 2007.

ACE2 cleavage of DABK to bradykinin 1-7 (Figure 2) has been demonstrated in chemico (Donoghue 2000, Vickers 2002), with human polarised primary lung cells in vitro and in mice broncho-alveolar lavage (BALF) (Sodhi 2018). Deactivation of DBAK, a preferencial bradikynin receptor 1 (B1K) agonist (Coulson et al., 2019), is an important regulatory function of ACE2 in the KKS (Figure 2).

1.2 ACE2 chaperone function for transporters of amino acid transfer (B0AT1)

Somewhat less known is the RAS independent function of ACE2 in the gut, where it regulates intestinal amino acid homeostasis, expression of antimicrobial peptides, and the gut microbiome (Camargo et al., 2020). ACE2 was identified as an important regulator of dietary amino acid homeostasis, innate immunity, gut microbial ecology, and transmissible susceptibility to colitis in mice (Hashimoto et al., 2012). The mechanism by which ACE2 regulates amino acid transport in the intestine involves interaction with the broad neutral (0) amino acid transporter 1 (B0AT1) (Slc6a19) which mediates the uptake of neutral dietary amino acids, such as tryptophan and glutamine, into intestinal cells in a sodium-dependent manner (Camargo et al, 2009). A crystal structure study revealed a complex dimer of ACE2/ B0AT1 heterodimers (Yan et al., 2020), previously suggested by immunoprecipitation of intestinal membrane proteins in mice (Fairweather et al., 2012). Immunofluorescence showed co-localization of B0AT1 with ACE2 at the luminal surface of human small intestine (Vuille-dit-Bille et al., 2015). ACE2 seems to be necessary not only for the amino acid transfer by B0AT1, but also for its membrane expression (Camargo et al., 2008; Hashimoto et al., 2012).

REGULATION OF ACE2 LEVELS and ACTIVITY

ACE2 is regulated at the transcriptional, post-transcriptional and post-translational level, the final potentially differing in the different organisational contexts: cell membrane versus tissue (plasma and/or interstitial). In addition, all of these regulatory processes may be differentially modulated in different tissues.

Age, sex and species specific differences in aspects of the regulation, and also tissue specific regulation, have been reported (reviewed in Saponaro 2020).

Loss of function of ACE2 in vivo in ACE2 knockout (KO) mice has been associated with elevated levels of Ang II in heart, kidney and plasma as well as histological and functional perturbations in the lungs and in the cardiovascular (Crackower et al, Nature 2002) and renal (Oudit et al, 2010) systems, mostly in the presence of a particular stress factor, in some cases potentiated by aging (reviewed in Humming 2007).

Furthermore, Ace2 KO mice exhibited reduced serum levels of tryptophan, together with downregulated expression of small intestinal antimicrobial peptides and altered gut microbiota, which was re-established by tryptophan supplementation (Hashimoto et a., 2012).

At transcriptional level

Overall, the transcriptional regulatory elements of the ace2 gene are not well characterised.

Ace2, human but not mouse, was identified as an Interferon Stimulated Gene (ISG) in airway epithelial cells (Ziegler, 2020), indicating species specific regulation and its importance for human viral infections mediated via ACE2 (e.g. SARS-COV2). Influenza virus infection also induced ACE2 mRNA synthesis in human lung tissue (Ziegler, 2020).

In vitro in normal kidney tubular epithelial cell line (HK-2) ACE2 mRNA is down-regulated following Ang II treatment (Koka 2008). The exact transcriptional regulatory mechanism is not clear, but the observed ACE2 mRNA up-regulation in this system appears to be mediated by the activation of the ERK1/2 and p38 MAP kinase pathway and dependent on the activation of AT1R receptor by AngII, as demonstrated by specific AT1R, MAP kinase and ERK1/2 MAP kinase inhibitors (Koka et al., 2008). Regulation of ACE2 expression mediated by AT1R activation is an important endogenous regulatory mechanism for ACE2 activity within the RAS system (ref.....).

Vitamin D Receptor (VDR) may also emerge as an ACE2 transcriptional regulator/repressor (Saponaro 2020 and Glinsky 2020, unreviewed pre-print). VDR has already been implicated in the transcriptional repression of Renin, at least in vitro (Yuan 2007).

17b-estradiol (E2) has also been indicated in the transcriptional regulation of ace2 in a tissue specific manner (recently reviewed by Saponaro 2020). E2 down-regulated ace2 transcription particularly in kidney and differentiated airway epithelial cells. However, in human atrial tissue, E2 appeared to up-regulate ACE2 mRNA and protein. This change was associated with decreased levels of ACE homologue protein. The exact mechanism for this regulation remains to be elucidated as it may represent a significant modulating factor in the differential sex susceptibility to ACE2 dysregulation under varied stress conditions (e.g SARS-COV infection).

Epigenetic transcriptional regulation of ace2 has also been indicated (recently reviewed by Saponaro 2020. Transcription of ace2 is repressed by histone methylation and stimulated by NAD+-dependent deacetylase SIRT1 during cellular energy stress. Interestingly, in children ACE2 is normally hypermethylated and poorly expressed in the lung and in other organs (Saponaro 2020, ref therein).

Gut microbiota have also been implicated in the transcriptional regulation of ACE2 expression in the gut (Yang et al., 2020) but also in the lung (Koester et al., 2021). Whether this is directly or indirectly occurring via microbial metabolites remains to be elucidated, but the study by Koester ar e al, 2020 observed variability in intestinal Ace2 expression in gnotobiotic mice colonized with different microbiota, partially attributable to differences in microbiome-encoded proteases and peptidases.

At post-transcriptional level

Generally, modulation of ACE2 mRNA and protein levels appear to follow consistent pattern. However, it has been demonstrated that under certain conditions and in some tissues, mRNA and protein levels appear to follow a different pattern, suggesting important role of post-transcriptional or post-translational (see next section) regulation of ACE2 expression and function.

For example, hypertension in humans has been associated with different modulation of mRNA and protein levels in the heart tissue (Koka 2008). Specifically, heart tissue from patients with hypertension showed decreased levels of ACE2 mRNA while protein levels were comparable to normal tissue. In contrast, ACE mRNA and protein levels appeared consistently up-regulated in hart tissue of hypertensive patients (Koka 2008). In the same study, in the kidney tissue from patients with hypertensive nephropathy, both, ACE2 mRNA and protein levels, appeared consistently down-regulated compared to normal kidney tissue (Koka 2008). Significant suppression of ACE2 mRNA and protein expression was also observed in vitro in normal kidney tubular epithelial cell line (HK-2) treated with AngII (linked to hypertension in vivo) in a dose and time dependent manner Koka 2008). AngII treatment in vitro with myocardia- derived cells was not examined in this study and the discrepancy of mRNA and protein level modulation in the hypertensive human heart tissue biopsies was attributed by the authors to limitations of the detecting methods (Koka 2008).

Clear discrepancy in the modulation of mRNA versus protein level has been observed in vivo in mice in myocardial tissue (Patel 2014). Namely, up-regulation of mRNA synthesis was associated with down-regulation of ACE2 protein levels following 1 or 2 week treatment by exogenous circulating AngII (Patel 2014). In this study, down-regulation of ACE2 protein levels was alleviated by AT1R blockage/inhibitors, while mRNA up-regulation was not dependent on AT1R signalling. This strongly suggests involvement of post-transcriptional mechanism step(s) mediated by AngII/AT1R fot the regulation of ACE2 protein/function, at least in myocardial tissue under certain stress conditions.

Modulation of ACE2 protein and activity levels by AngII is clinically relevant phenomenon and AngII activity blockers (ACE inhibitors and AT1R blockers) are used to move the balance of the RAS from the ACE/AngII/AT1R axis towards the protective ACE2/Ang1-7/MasR axis. This is particularly relevant in the lung where ACE/ACE2 activity ratio is high (Roca-Ho, 2017-mice, human and other ref.....?..).

The up-regulation of ACE2 mRNA observed in mouse myocardial tissue by Patel et al., 2014 appears contradictory to the finding of ACE2 mRNA down-regulation observed in the heart of hypertensive patients observed in the study of Koka et al., 2008 (if the latter result is accepted despite potential method limitations). However, it should be noted that the base level of ACE2 and also the relative ACE2/ACE ratio in the case of chronic hypertensive patients, many of whom have been on AT1R inhibitor treatment (Koka 2008), and in healthy mice treated with AngII for relatively short time (Patel 2014), may be different leading to response to the stressor (hypertension and AngII) over time. Consistent with this, distinct ACE/ACE2 activity ratios have been demonstrated in different organs of normal, non-obese diabetic (NOD) and insulin treated NOD mice, which varied additionally over the time course after the onset of diabetes (Roca-Ho et al., 2014).

Finally, species specific regulatory differences may be involved that would warrant further examination. But, overall, the

studies discussed above illustrate the complex regulatory mechanisms of ACE2 mRNA, protein and activity levels in different tissues and under different stress conditions for the RAS system.

Rapid and transient up-regulation of ACE2 mRNA followed by down-regulation of ACE2 protein levels has been reported in the lung as a result of LPS induced acute inflammation in mice (Sodhi 2018). In this case the increase of ACE2 mRNA appears to be a rapid and transient compensatory effect to ACE2 protein/activity down-regulation mediated by NFk- B signalling in response to acute inflammation. Inhibiting NF- B signaling by Bay11-7082 restored ACE2 activity, again demonstrating post transcriptional or translational regulation of ACE2 in the lungs. In addition, this study examined the effect of ACE2 dysregulation on the KKS and demonstrated that attenuation of ACE2 activity under conditions of LPS induced inflammation leads to impaired DABK inactivation and enhanced BKB1R signalling (Sodhi 2018).

The underlying mechanisms of post-transcriptional mRNA regulation remain to be elucidated further. There is evidence that small non-coding micro RNAs (miRNA or miRs) may be involved (Widiasta 2020; Lu 2020; Fang 2017, Lambert 2014).

At post-translational level – enzymatic activity including shedding

Complexity of analysing the regulation of ACE2 function is emphasized even further when enzymatic activity is considered, including its spatial distribution between cell/tissue versus interstitium/plasma, mediated by shedding.

The exact role of ACE2 shedding is not well understood, but proteolytic ectodomain shedding of membrane proteins is a fundamental post-translational regulatory mechanism of the activity/function of a wide variety of proteins, including growth factors, cytokines, receptors and cell adhesion proteins (Lichtenthaler et al., 2018).

sACE2 activity is increased in patients with heart failure (HF) and correlates with disease severity(Epelman 2008).

In mice in vivo, shedding of ACE2 by TACE was induced by sub-chronic (2 weeks) exogenous AngII treatment (mimicking HF), leading to decreased ACE2 protein level and increased ACE2 mRNA in myocardial tissue with concurrent elevated sACE2 activity (Patel et al., 2014).

How it is Measured or Detected

ACE2 activity

- using fluorescently labeled peptide substrates (Rice, 2004; Sodhi, 2008; Roca-Ho, 2017 1; Lu and Sun, 2020; Xiao 2017)
- measuring catalytic products (direct) or markers of activation of receptors for the products (indirect) of ACE2 activity (e.g Ferrario 2005)

ACE2 levels

- mRNA by RT-PCR (Sodhi 2018; Roca-Ho, 2017) or scRNA seq (e.g. Qi. et al 2020)
- protein in tissue extracts/preparations by immunoprecipitation or Western blotting (Koka 2008)
- protein in live tissues or cultured cells by immunostaining (Humming 2007; Fraga-Silva et al., 2011; Ren 2006; Warner 2005)

high throughput and quantitative measurement of protein by quantitative proteomic analysis (Park 2020; Stegbauer 2020)

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Event: 1752: Increased Angiotensin II

Short Name: Increased AngII

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:319 - Binding to ACE2 leading to lung fibrosis	KeyEvent
Aop:384 - Hyperactivation of ACE/Ang-II/AT1R axis leading to chronic kidney disease	KeyEvent
Aop:385 - Viral spike protein interaction with ACE2 leads to microvascular dysfunction, via ACE2 dysregulation	KeyEvent

AOP ID and Name	Event Type
Aop:381 - Binding of viral S-glycoprotein to ACE2 receptor leading to dysgeusia	KeyEvent

Biological Context

Level of Biological Organization

Cellular

Event: 1851: Binding of agonist, Angiotensin II receptor type 1 receptor (AT1R)

Short Name: Binding of agonist, Angiotensin II receptor type 1 receptor (AT1R)

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:382 - Angiotensin II type 1 receptor (AT1R) agonism leading to lung fibrosis	MolecularInitiatingEvent
Aop:319 - Binding to ACE2 leading to lung fibrosis	KeyEvent

Biological Context

Level of Biological Organization

Molecular

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 ID and Name	Event Type
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: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
Aop:540 - Oxidative Stress in the Fish Ovary Leads to Reproductive Impairment via Reduced Vitellogenin Production	MolecularInitiatingEvent

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, *lifestages, and sexes.*

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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Event: 1172: Increased activation, Nuclear factor kappa B (NF-kB)

Short Name: Increased activation, Nuclear factor kappa B (NF-kB)

Key Event Component

Process	Object	Action
regulation of I-kappaB kinase/NF-kappaB signaling		increased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:382 - Angiotensin II type 1 receptor (AT1R) agonism leading to lung fibrosis	KeyEvent
Aop:377 - Dysregulated prolonged Toll Like Receptor 9 (TLR9) activation leading to Multi Organ Failure involving Acute Respiratory Distress Syndrome (ARDS)	KeyEvent
Aop:319 - Binding to ACE2 leading to lung fibrosis	KeyEvent
Aop:443 - DNA damage and mutations leading to Metastatic Breast Cancer	KeyEvent

Stressors

Name
Reactive oxygen species

Biological Context

Level of Biological Organization
Cellular

Cell term

Cell term
epithelial cell

Organ term

Organ term
tissue

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	High	NCBI

Life Stage Applicability

Life Stage	Evidence
Not Otherwise Specified	Moderate

Sex Applicability

Sex	Evidence

Sex	Evidence
Mixed	Not Specified

The ROS directly influences NF-κB signalling, resulting in differential production of cytokines and chemokines (McKay and Cidlowski, 1999). NIn accordance with the OECD AOP Handbook, the pathway begins with increased levels of reactive oxygen species (ROS), serving as the Molecular Initiating Event (MIE), which subsequently triggers the Activation of the NF-κB Signaling Pathway . This activation, in turn, directly influences the expression of genes involved in the Differential Production of Cytokines and Chemokines , culminating in the regulation of Pro-Inflammatory Responses Transcriptionally Mediated by NF-κB (. The resultant exaggerated and dysregulated pro-inflammatory response contributes to chronic inflammation and tissue damage, representing the Adverse Outcome (AO). This sequence of events is underpinned by the works of McKay and Cidlowski (1999) and aligns with the guidelines set forth in the OECD AOP Handbook.F-κB regulates pro-inflammatory responses that are transcriptionally mediated by NF-κB.

Key Event Description

The NF-kB pathway consists of a series of events where the transcription factors of the NF-kB family play a key role. The proinflammatory cytokine (IL-1beta) can be activated by NF-kB , including Reactive Oxygen Species produced by NADPH oxidase (NOX). Upon pathway activation, the IKK complex will be phosphorylated, which in turn phosphorylates IkBa. There, this transcription factor can express pro-inflammatory and pro-fibrotic genes. This can be achieved by ROS, IKK enhancer or nuclear translocation enhancer.

How it is Measured or Detected

NF-kB transcriptional activity: Beta lactamase reporter gene assay (Miller et al. 2010). NF-kB transcription: Lentiviral NF-kB GFP reporter with flow cytometry (Moujalled et al. 2012)

NF-κB translocation: RelA-GFP reporter assay (Wink et al 2017)

IκBa phosphorylation: Western blotting (Miller et al. 2010)

NF-κB p65 (Total/Phospho) ELISA

ELISA for IL-6, IL-8, and Cox

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Event: 68: Accumulation, Collagen

Short Name: Accumulation, Collagen

Key Event Component

Process	Object	Action
collagen biosynthetic process	collagen	increased

AOPs Including This Key Event

AOP ID and Name	Event Type

AOP ID and Name	Event Type
Aop:38 - Protein Alkylation leading to Liver Fibrosis	KeyEvent
Aop:241 - Latent Transforming Growth Factor beta1 activation leads to pulmonary fibrosis	KeyEvent
Aop:144 - Endocytic lysosomal uptake leading to liver fibrosis	KeyEvent
Aop:319 - Binding to ACE2 leading to lung fibrosis	KeyEvent
Aop:382 - Angiotensin II type 1 receptor (AT1R) agonism leading to lung fibrosis	KeyEvent
Aop:173 - Substance interaction with the pulmonary resident cell membrane components leading to pulmonary fibrosis	KeyEvent

Biological Context

Level of Biological Organization

Tissue

Organ term

Organ term
connective tissue

Domain of Applicability

Taxonomic Applicability

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

Life Stage Applicability

Life Stage	Evidence
All life stages	

Sex Applicability

Sex	Evidence
Unspecific	

Humans: Bataller and Brenner, 2005; Decaris et al., 2015.

Mice: Dalton et al., 2009; Leung et al., 2008; Nan et al., 2013.

Rats: Hamdy and El-Demerdash, 2012; Li et al., 2012; Luckey and Petersen, 2001; Natajaran et al., 2006.

Key Event Description

Collagen is mostly found in fibrous tissues such as tendons, ligaments and skin. It is also abundant in corneas, cartilage, bones, blood vessels, the gut, intervertebral discs, and the dentin in teeth. In muscle tissue, it serves as a major component of the endomysium. Collagen is the main structural protein in the extracellular space in the various connective tissues, making up from 25% to 35% of the whole-body protein content. In normal tissues, collagen provides strength, integrity, and structure. When tissues are disrupted following injury, collagen is needed to repair the defect. If too much collagen is deposited, normal anatomical structure is lost, function is compromised, and fibrosis results.

The fibroblast is the most common collagen producing cell. Collagen-producing cells may also arise from the process of transition of differentiated epithelial cells into mesenchymal cells. This has been observed e.g. during renal fibrosis (transformation of tubular epithelial cells into fibroblasts) and in liver injury (transdifferentiation of hepatocytes and cholangiocytes into fibroblasts) (Henderson and Iredale, 2007)·

There are close to 20 different types of collagen found with the predominant form being type I collagen. This fibrillar form of collagen represents over 90 percent of our total collagen and is composed of three very long protein chains which are wrapped around each other to form a triple helical structure called a collagen monomer. Collagen is produced initially as a larger precursor molecule called procollagen. As the procollagen is secreted from the cell, procollagen proteinases remove the extension peptides from the ends of the molecule. The processed molecule is referred to as collagen and is involved in fiber formation. In the extracellular spaces the triple helical collagen molecules line up and begin to form fibrils and then fibers. Formation of stable crosslinks within and between the molecules is promoted by the enzyme lysyl oxidase and gives the collagen fibers tremendous strength (Diegelmann,2001)· The overall amount of collagen deposited by fibroblasts is a regulated balance between collagen synthesis and collagen catabolism. Disturbance of this balance leads to changes in the amount and composition of collagen. Changes in the composition of the extracellular matrix initiate positive feedback pathways that increase collagen production.

Normally, collagen in connective tissues has a slow turn over; degradating enzymes are collagenases, belonging to the family of matrix metalloproteinases. Other cells that can synthesize and release collagenase are macrophages, neutrophils, osteoclasts, and tumor cells (Di Lullo et al., 2002; Kivirikko and Risteli, 1976; Miller and Gay, 1987; Prockop and Kivirikko, 1995).

How it is Measured or Detected

Determination of the amount of collagen produced *in vitro* can be done in a variety of ways ranging from simple colorimetric assays to elaborate chromatographic procedures using radioactive and non-radioactive material. What most of these procedures have in common is the need to destroy the cell layer to obtain solubilized collagen from the pericellular matrix. Rishikof et al. describe several methods to assess the *in vitro* production of type I collagen: Western immunoblotting of intact alpha1(I) collagen using antibodies directed to alpha1(I) collagen amino and carboxyl propeptides, the measurement of alpha1(I) collagen mRNA levels using real-time polymerase chain reaction, and methods to determine the transcriptional regulation of alpha1(I) collagen using a nuclear run-on assay (Rishikof et al., 2005).

Histological staining with stains such as Masson Trichrome, Picro-sirius red are used to identify the tissue/cellular distribution of collagen, which can be quantified using morphometric analysis both *in vivo* and *in vitro*. The assays are routinely used and are quantitative.

Sircol Collagen Assay for collagen quantification:

The Serius dye has been used for many decades to detect collagen in histology samples. The Serius Red F3BA selectively binds to collagen and the signal can be read at 540 nm (Chen and Raghunath, 2009; Nikota et al., 2017).

Hydroxyproline assay:

Hydroxyproline is a non-proteinogenic amino acid formed by the prolyl-4-hydroxylase. Hydroxyproline is only found in collagen and thus, it serves as a direct measure of the amount of collagen present in cells or tissues. Colorimetric methods are readily available and have been extensively used to quantify collagen using this assay (Chen and Raghunath, 2009; Nikota et al., 2017).

Ex vivo precision cut tissue slices

Precision cut tissue slices mimic the whole organ response and allow histological assessment, an endpoint of interest in regulatory decision making. While this technique uses animals, the number of animals required to conduct a dose-response study can be reduced to 1/4th of what will be used in whole animal exposure studies (Rahman et al., 2020).

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Event: 1496: Increased, secretion of proinflammatory mediators

Short Name: Increased proinflammatory mediators

Key Event Component

Process	Object	Action
cytokine production involved in inflammatory response	Cytokine	increased
chemokine secretion	Chemokine	increased
complement activation		increased
	Interleukin	increased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:173 - Substance interaction with the pulmonary resident cell membrane components leading to pulmonary fibrosis	KeyEvent
Aop:320 - Binding of SARS-CoV-2 to ACE2 receptor leading to acute respiratory distress associated mortality	KeyEvent
Aop:382 - Angiotensin II type 1 receptor (AT1R) agonism leading to lung fibrosis	KeyEvent
Aop:392 - Decreased fibrinolysis and activated bradykinin system leading to hyperinflammation	KeyEvent
Aop:409 - Frustrated phagocytosis leads to malignant mesothelioma	KeyEvent
Aop:377 - Dysregulated prolonged Toll Like Receptor 9 (TLR9) activation leading to Multi Organ Failure involving Acute Respiratory Distress Syndrome (ARDS)	KeyEvent
Aop:39 - Covalent Binding, Protein, leading to Increase, Allergic Respiratory Hypersensitivity Response	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:468 - Binding of SARS-CoV-2 to ACE2 leads to hyperinflammation (via cell death)	KeyEvent
Aop:237 - Substance interaction with lung resident cell membrane components leading to atherosclerosis	KeyEvent

Biological Context

Level of Biological Organization
Cellular

Cell term

Cell term
eukaryotic cell

Domain of Applicability

Taxonomic Applicability

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

Life Stage Applicability

Life Stage	Evidence
Adults	High

Sex Applicability

Sex	Evidence
Male	High
Female	High

Human, mouse, rat

Cytokines are the common pro-inflammatory mediators secreted following inflammogenic stimuli. Cytokines can be defined as a diverse group of signaling protein molecules. They are secreted by different cell types in different tissues and in all mammalian species, irrespective of gender, age or sex. A lot of literature is available to support cross species, gender and developmental stage application for this KE. The challenge is the specificity; most cytokines exhibit redundant functions and many are pleotropic.

Key Event Description

Pro-inflammatory mediators are the chemical and biological molecules that initiate and regulate inflammatory reactions. Pro-inflammatory mediators are secreted following exposure to an inflammogen in a gender/sex or developmental stage independent manner. They are secreted during inflammation in all species. Different types of pro-inflammatory mediators are secreted during innate or adaptive immune responses across various species (Mestas and Hughes, 2004). Cell-derived pro-inflammatory mediators include cytokines, chemokines, and growth factors. Blood derived pro-inflammatory mediators include vasoactive amines, complement activation products and others. These modulators can be grouped based on the cell type that secrete them, their cellular localisation and also based on the type of immune response they trigger. For example, members of the interleukin (IL) family including IL-2, IL-4, IL-7, IL-9, IL-15, IL-21, IL-3, IL-5 and Granulocyte-macrophage colony stimulating factor (GM-CSF) are involved in the adaptive immune responses. The pro-inflammatory cytokines include IL-1 family (IL-1α , IL-1β, IL-1rα, IL-18, IL-36α, IL-36β, IL-36γ, IL-36Rα, IL-37), IL-6 family, Tumor necrosis factor (TNF) family, IL-17, and Interferon gamma (IFN-γ) (Turner et al., 2014). While IL-4 and IL-5 are considered T helper (Th) cell type 2 response,IFN-γ is suggested to be Th1 type response.

Different types of pro-inflammatory mediators are secreted during innate or adaptive immune responses across various species (Mestas and Hughes, 2004). However, IL-1 family cytokines, IL-4, IL-5, IL-6, TNF-α, IFN-γ are the commonly measured mediators in experimental animals and in humans. Similar gene expression patterns involving inflammation and matrix remodelling are observed in human patients of pulmonary fibrosis and mouse lungs exposed to bleomycin (Kaminski, 2002).

Literature evidence for its perturbation:

Several studies show increased proinflammatory mediators in rodent lungs and bronchoalveolar lavage fluid, and in cell culture supernatants following exposure to a variety of carbon nanotube (CNT) types and other materials. Poland et al., 2008 showed that long and thin CNTs (>5 µm) can elicit asbestos-like pathogenicity through the continual release of pro-inflammatory cytokines and reactive oxygen species. Exposure to crystalline silica induces release of inflammatory cytokines (TNF-α, IL-1, IL-6), transcription factors (Nuclear factor kappa B [NFκB], Activator protein-1 [AP-1]) and kinase signalling pathways in mice that contain NF-κB luciferase reporter (Hubbard et al., 2002). Boyles et al., 2015 found that lung responses to long multi-walled carbon nanotubes (MWCNTs) included high expression levels of pro-inflammatory mediators Monocyte chemoattractant protein 1 (MCP-1), Transforming growth factor beta 1 (TGF-β1), and TNF-α (Boyles et al., 2015). Bleomycin administration in rodents induces lung inflammation and increased expression of pro-inflammatory mediators (Park et al., 2019). Inflammation induced by bleomycin, paraquat and CNTs is characterised by the altered expression of pro-inflammatory mediators. A large number of nanomaterials induce expression of cytokines and chemokines in lungs of rodents exposed via inhalation (Halappanavar et al., 2011; Husain et al., 2015a). Similarities are observed in gene programs involving pro-inflammatory event is observed in both humans and experimental mice (Zuo et al., 2002).

How it is Measured or Detected

The selection of pro-inflammatory mediators for investigation varies based on the expertise of the lab, cell types studied and the availability of the specific antibodies.

Real-time reverse transcription-polymerase chain reaction (qRT-PCR) – will measure the abundance of cytokine mRNA in a given sample. The method involves three steps: conversion of RNA into cDNA by reverse transcription method, amplification of cDNA using the PCR, and the real-time detection and quantification of amplified products (amplicons) (Nolan et al., 2006). Amplicons are detected using fluorescence, increase in which is directly proportional to the amplified PCR product. The number of cycles required per sample to reach a certain threshold of fluorescence (set by the user – usually set in the linear phase of the amplification, and the observed difference in samples to cross the set threshold reflects the initial amount available for amplification) is used to quantify the relative amount in the samples. The amplified products are detected by the DNA intercalating minor groove-binding fluorophore SYBR green, which produces a signal when incorporated into double-stranded amplicons. Since the cDNA is single stranded, the dye does not bind enhancing the specificity of the results. There are other methods such as nested fluorescent probes for detection, but SYBR green is widely used. RT-PCR primers specific to several pro-inflammatory mediators in several species including mouse, rat and humans, are readily available commercially.

Enzyme-linked immunosorbent assays (ELISA) – permit quantitative measurement of antigens in biological samples. The method is the same as described for the MIE. Both ELISA and qRT-PCR assays are used *in vivo* and are readily applicable to *in vitro* cell culture models, where cell culture supernatants or whole cell homogenates are used for ELISA or mRNA assays. Both assays are straight forward, quantitative and require relatively a small amount of input sample.

Apart from assaying single protein or gene at a time, cytokine bead arrays or cytokine PCR arrays can also be used to detect a whole panel of inflammatory mediators in a multiplex method (Husain et al., 2015b). This method is quantitative and especially advantageous when the sample amount available for testing is scarce. Lastly, immunohistochemistry can also be used to detect specific immune cell types producing the pro-inflammatory mediators and its downstream effectors in any given tissue (Costa et al., 2017). Immunohistochemistry results can be used as weight of evidence; however, the technique is not quantitative and depending on the specific antibodies used, the assay sensitivity may also become an issue (Amsen and De Visser, 2009).

Cell models - of varying complexity have been used to assess the expression of pro-inflammatory mediators. Two dimensional submerged monocultures of the main fibrotic effector cells – lung epithelial cells, macrophages, and fibroblasts – have routinely been used *in vitro* due to the large literature base, and ease of use, but do not adequately mimic the *in vivo* condition (Sharma *et al.,* 2016; Sundarakrishnan *et al.,* 2018). Recently, the EpiAlveolar *in vitro* lung

model (containing epithelial cells, endothelial cells, and fibroblasts) was used to predict the fibrotic potential of MWCNTs, and researchers noted increases in the pro-inflammatory molecules TNF-α, IL-1β, and the pro-fibrotic TGF-β using ELISA (Barasova *et al.,* 2020). A similar, but less complicated co-culture model of immortalized human alveolar epithelial cells and idiopathic pulmonary fibrosis patient derived fibroblasts was used to assess pro-fibrotic signalling, and noted enhanced secretion of Platelet derived growth factor (PDGF) and Basic fibroblast growth factor (bFGF), as well as evidence for epithelial to mesenchymal transition of epithelial cells in this system (Prasad et al., 2014). Models such as these better capitulate the *in vivo* pulmonary alveolar capillary, but have lower reproducibility as compared to traditional submerged mono-culture experiments.

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List of Adverse Outcomes in this AOP

Event: 1276: Lung fibrosis

Short Name: Lung fibrosis

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:206 - Peroxisome proliferator-activated receptors γ inactivation leading to lung fibrosis	AdverseOutcome
Aop:319 - Binding to ACE2 leading to lung fibrosis	AdverseOutcome
Aop:382 - Angiotensin II type 1 receptor (AT1R) agonism leading to lung fibrosis	AdverseOutcome
Aop:414 - Aryl hydrocarbon receptor activation leading to lung fibrosis through TGF-β dependent fibrosis toxicity pathway	KeyEvent
Aop:415 - Aryl hydrocarbon receptor activation leading to lung fibrosis through IL-6 toxicity pathway	AdverseOutcome

Biological Context

Level of Biological Organization
Organ

Key Event Description

This consecutive KE resulting in the acquisition of the accumulation of excess fibrous connective tissue, the adverse outcome on pulmonary fibrosis. Scar formation, the accumulation of excess fibrous connective tissue (the process called fibrosis), leads to thickening of the walls, and causes reduced oxygen supply in the blood. As a consequence patients suffer from perpetual shortness of breath.

Appendix 2

List of Key Event Relationships in the AOP

List of Adjacent Key Event Relationships

Relationship: 2065: ACE2 enzymatic inhibition leads to Increased AngII

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Binding to ACE2 leading to lung fibrosis	adjacent	High	Moderate

Relationship: 2593: Increased AngII leads to Binding of agonist, Angiotensin II receptor type 1 receptor (AT1R)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Binding to ACE2 leading to lung fibrosis	adjacent	High	High

Relationship: 2319: Binding of agonist, Angiotensin II receptor type 1 receptor (AT1R) leads

to Increased, Reactive oxygen species

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Angiotensin II type 1 receptor (AT1R) agonism leading to lung fibrosis	adjacent	Moderate	Moderate
Binding to ACE2 leading to lung fibrosis	adjacent	Moderate	Moderate

Relationship: 2320: Increased, Reactive oxygen species leads to Increased activation, Nuclear factor kappa B (NF-kB)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Angiotensin II type 1 receptor (AT1R) agonism leading to lung fibrosis	adjacent	Moderate	Moderate
Binding to ACE2 leading to lung fibrosis	adjacent	Moderate	Moderate

Relationship: 2309: Increased activation, Nuclear factor kappa B (NF-kB) leads to Increased proinflammatory mediators

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Angiotensin II type 1 receptor (AT1R) agonism leading to lung fibrosis	adjacent	Moderate	Moderate
Dysregulated prolonged Toll Like Receptor 9 (TLR9) activation leading to Multi Organ Failure involving Acute Respiratory Distress Syndrome (ARDS)	adjacent	High	High
Binding to ACE2 leading to lung fibrosis	adjacent	Moderate	Moderate

Relationship: 2307: Increased proinflammatory mediators leads to Accumulation, Collagen

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Angiotensin II type 1 receptor (AT1R) agonism leading to lung fibrosis	adjacent	Moderate	Moderate
Binding to ACE2 leading to lung fibrosis	adjacent	Moderate	Moderate

Relationship: 2139: Accumulation, Collagen leads to Lung fibrosis

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Angiotensin II type 1 receptor (AT1R) agonism leading to lung fibrosis	adjacent	Low	Not Specified
Binding to ACE2 leading to lung fibrosis	adjacent	Low	Not Specified