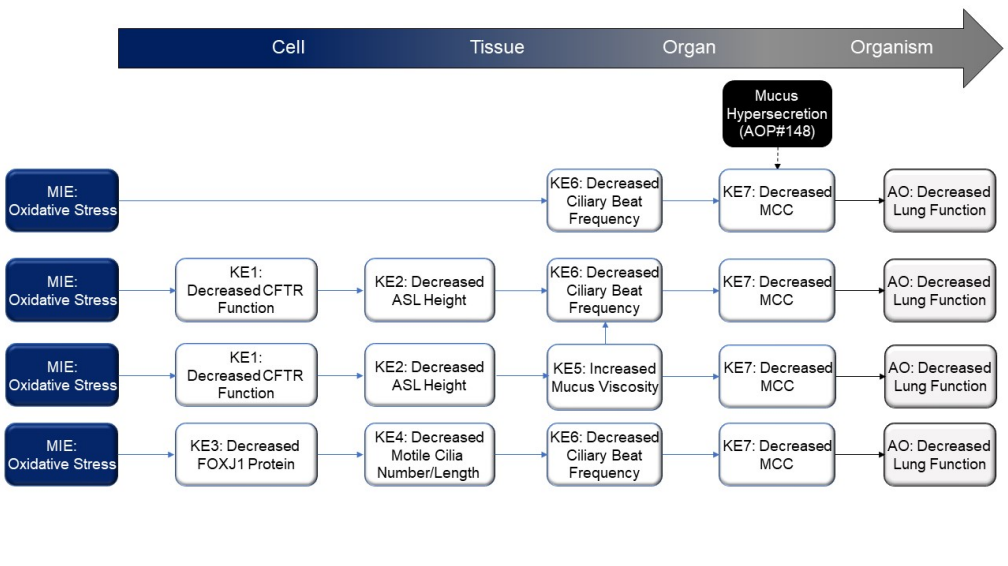
AOP ID and Title:

AOP 411: Oxidative stress [MIE] Leading to Decreased Lung Function [AO]
Short Title: Oxidative stress [MIE] Leading to Decreased Lung Function [AO]

Graphical Representation



Authors

Karsta Luettich, Philip Morris Products S.A., Philip Morris International R&D, Neuchatel, Switzerland

Hasmik Yepiskoposyan, Philip Morris Products S.A., Philip Morris International R&D, Neuchatel, Switzerland

Monita Sharma, PETA Science Consortium International e.V., Stuttgart, Germany

Frazer Lowe, Broughton Nicotine Services, Earby, Lancashire, United Kingdom

Damien Breheny, British American Tobacco (Investments) Ltd., Group Research and Development, Southampton, United Kingdom

Status

Author status	OECD status	OECD project	SAAOP status
Open for comment. Do not cite			

Abstract

This AOP evaluates the major processes known to be involved in regulating efficient mucociliary clearance (MCC). MCC is a key aspect of the innate immune defense against airborne pathogens and inhaled chemicals and is governed by the concerted action of its functional components, the cilia and the airway surface liquid (ASL), which is composed of mucus and periciliary layers (Bustamante-Marin and Ostrowski, 2017). For MCC to be efficient, the depth of the ASL has to be constantly adjusted to allow for efficient cilia beating and mucus transport (Antunes and Cohen, 2007). In healthy subjects, ≥10 mL of airway secretions are continuously produced and transported daily by the mucociliary escalator, and scintigraphy studies showed that MCC is normally accomplished within 24 hours of deposition of radiolabeled aerosols (Rubin, 2002). Disturbances in any of the processes regulating ASL volume, mucus production, mucus viscoelastic properties, or ciliary function can cause MCC dysfunction and are linked to airway diseases such as chronic obstructive pulmonary disease (COPD) or asthma, both of which are characterized by decreased lung function and bear a significant risk of increased morbidity and mortality.

Background

With a surface area of ~100 m^2 and ventilated by 10,000 to 20,000 liters of air per day (National Research Council, 1988; Frohlich et al., 2016), the lungs are a major barrier that protect the body from a host of external factors that enter the respiratory system and may cause lung pathologies. Mucociliary clearance (MCC) is a key aspect of the innate immune defense against airborne pathogens and inhaled particles and is governed by the concerted action of its functional components, the cilia and the airway surface liquid (ASL), which comprises mucus and the periciliary layer (Bustamante-Marin and Ostrowski, 2017). In healthy subjects,

≥10 mL airway secretions are continuously produced and transported daily by the mucociliary escalator. Disturbances in any of the processes regulating ASL volume, mucus production, mucus viscoelastic properties, or ciliary function can cause MCC dysfunction and are linked to airway diseases such as chronic obstructive pulmonary disease (COPD) or asthma, both of which bear a significant risk of increased morbidity and mortality. The mechanism by which exposure to inhaled toxicants might lead to mucus hypersecretion and thereby impact pulmonary function has already been mapped in AOP148 on decreased lung function. However, whether an exposure-related decline in lung function is solely related to excessive production of mucus is debatable, particularly in light of the close relationship between mucus, ciliary function, and efficient MCC. To date, no single event has been attributed to MCC impairment, and it is likely that events described in this AOP network as well as in AOP148 have to culminate to lead to decreased lung function.

Summary of the AOP

Events

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

Sequence	Type	Event ID	Title	Short name
1	MIE	1392	Oxidative Stress	Oxidative Stress
2	KE	1906	Cystic Fibrosis Transmembrane Regulator Function, Decreased	CFTR Function, Decreased
3	KE	1907	Airway Surface Liquid Height, Decreased	ASL Height, Decreased
4	KE	1908	Cilia Beat Frequency, Decreased	CBF, Decreased
5	KE	1909	Mucociliary Clearance, Decreased	MCC, Decreased
6	KE	1910	Mucus Viscosity, Increased	Mucus Viscosity, Increased
7	KE	1911	FOXJ1 Protein, Decreased	FOXJ1 Protein, Decreased
8	KE	1912	Motile Cilia Number/Length, Decreased	Motile Cilia Number/Length, Decreased
9	AO	1250	Decrease, Lung function	Decreased lung function

Key Event Relationships

Upstream Event	Relationship Type	Downstream Event	Evidence	Quantitative Understanding
Cystic Fibrosis Transmembrane Regulator Function, Decreased	adjacent	Airway Surface Liquid Height, Decreased	High	Moderate
Airway Surface Liquid Height, Decreased	adjacent	Cilia Beat Frequency, Decreased	Moderate	Low
Cilia Beat Frequency, Decreased	adjacent	Mucociliary Clearance, Decreased	High	Moderate
Mucociliary Clearance, Decreased	adjacent	Decrease, Lung function	Moderate	Moderate
Airway Surface Liquid Height, Decreased	adjacent	Mucus Viscosity, Increased	Moderate	Low
Mucus Viscosity, Increased	adjacent	Cilia Beat Frequency, Decreased	Moderate	Moderate
Mucus Viscosity, Increased	adjacent	Mucociliary Clearance, Decreased	High	Moderate
FOXJ1 Protein, Decreased	adjacent	Motile Cilia Number/Length, Decreased	High	High
Motile Cilia Number/Length, Decreased	adjacent	Cilia Beat Frequency, Decreased	Moderate	Moderate
Oxidative Stress	adjacent	Cystic Fibrosis Transmembrane Regulator Function, Decreased	High	High
Oxidative Stress	adjacent	FOXJ1 Protein, Decreased	Moderate	Moderate
Oxidative Stress	adjacent	Cilia Beat Frequency, Decreased	High	High

Stressors

Name	Evidence
Acrolein	Moderate
Ozone	Moderate
Cigarette smoke	High
Nitrogen dioxide	Low
Diesel engine exhaust	Low

Acrolein

Acrolein, a ubiquitous environmental pollutant, is a highly reactive unsaturated aldehyde that exerts toxicity through several mechanism, including oxidative stress (Moghe et al., 2015). Acrolein exposure decreased CFTR-mediated Cl– transport in primary murine nasal septal epithelia, in human bronchial epithelial cells grown in monolayers and in human Calu-3 lung cancer cells (Alexander et al., 2012; Raju et al., 2013), transiently reduced CBF at low concentrations (0.5–1 mM) and induced ciliostasis at high concentrations (> 1 mM) in rabbit tracheal epithelial cells (Romet et al., 1990), and significantly increased mucin production in rats (Chen et al., 2013; Liu et al., 2009; Borchers et al., 1998; Wang et al., 2009). In addition, exposure of Fischer rats to acrolein caused a left shift in the quasi-static compliance curves and increased lung volumes, indicative of airway obstruction (Costa et al., 1986).

Ozone

Tracheas of Wistar rats exposed to 1.5 ppm ozone for 1 h/day for 3 days exhibited reduced CFTR protein expression. Similarly, at 4 hours following a 30-min exposure to ozone, CFTR mRNA and protein were down-regulated in 16HBE14o- cells. At 24 hours post-exposure, a reduction in forskolin-stimulated CFTR Cl– conductance was observed (Qu et al., 2009).

Continuous, exposure of human nasal epithelial cells to different concentrations of ozone at 37°C for up to 4 weeks slightly (but not significantly) reduced CBF in healthy mucosa (7.1% at 500 µg/m^3 and 10.3% at 1000 µg/m^3), and significantly in chronically inflamed mucosa (20.5/16.4%) at 2 weeks. During the third and fourth week of exposure at these higher concentrations CBF was significantly reduced in both healthy (after 3 weeks: 18.7/37.5%; after 4 weeks: 11.1/33.3%) and chronically inflamed mucosa (after 3 weeks: 33.8/26.8%; after 4 weeks: 21.4/38.6%). Low ozone concentrations (100 µg/m^3) appeared to not have an effect on CBF (Gosepath et al., 2000).

Acute exposure (2 h) of adult ewes to 1.0 ppm ozone significantly reduced tracheal mucus transport velocity (TMV) at 40 min and 2 h post-exposure. Repeated exposure to 1.0 ppm ozone for 5 hper day, for 4 consecutive days showed a progressively significant decrease in TMV on the first and second days, and stabilized over the third and fourth days, around values ranging from -42% to -55% of the initial baseline. TMV remained depressed even after the end of exposure, persisting up to 5 days post-exposure (Allegra et al., 1991).

Acute exposure of healthy young adult subjects (aged 19 to 35 years, non-smokers) to 0.06 ppm ozone for 6.6 h resulted in a 1.71 + 0.50% (mean + SEM) decrease in FEV1 and a 2.32 + 0.41% decrease in FVC compared with air exposure (Kim et al., 2011).

A US-based study found inverse associations between increasing lifetime exposure to ozone (estimated median: 36 ppm; interquartile range 29–45 ppm; range 19–64 ppm) and FEF75 and FEF25–75 in adolescents (aged 18–20 years) (Tager et al., 2005).

Cigarette smoke

CFTR transcript and protein levels were reduced in human Calu-3 lung cancer cells exposed to the gas phase of cigarette smoke (Cantin et al., 2006b), human immortalized bronchial epithelial 16HBE14o- cells treated with 10% cigarette smoke extract (Hassan et al., 2014; Rasmussen et al., 2014; Xu et al., 2015), differentiated primary human bronchial epithelial cells exposed to whole cigarette smoke (Sloane et al., 2012; Hassan et al., 2014), and in airways of smokers compared to non-smokers (Dransfield et al., 2013). Following exposure to cigarette smoke, Cl^- conductance (i.e., CFTR-mediated Cl^- transport) decreased in primary human bronchial epithelial cells grown in monolayers (Lambert et al., 2014), differentiated primary human bronchial epithelial cells (Schmid et al., 2015; Chinnapaiyan et al., 2018), and nasal respiratory and intestinal epithelia of A/J mice (Raju et al., 2013; Raju et al., 2017).
In the lower airways, healthy smokers and smokers with chronic obstructive pulmonary disease (COPD) showed reduced CFTR-dependent Cl^- transport, whereas COPD former smokers showed an intermediate response to chloride-free isoproterenol solution compared to non-smokers. Similarly, amiloride-sensitive lower airway potential difference was also lower in healthy smokers and COPD smokers than in healthy non-smokers. This was linked to reduced CFTR protein levels in the airways of smokers compared to non-smokers, although there were no significant differences between healthy and COPD subjects (Dransfield et al., 2013). CFTR-dependent Cl^- conductance as measured by nasal potential difference was also significantly reduced in healthy and COPD smokers compared to healthy non-smokers or to former smokers with COPD (Sloane et al., 2012). In addition, healthy never-smokers had higher mean sweat chloride concentrations than COPD smokers and COPD former smokers (Raju et al., 2013;

Courville et al., 2014).

Multiple studies showed that exposure of primary human bronchial epithelial cells, either undifferentiated or differentiated at the air-liquid interface, to cigarette smoke decreased ASL height (Hassan et al., 2014; Lambert et al., 2014; Raju et al., 2016; Rasmussen et al., 2014; Schmid et al., 2015). Treatment of immortalized bronchial epithelial 16HBE14o- cells with 10% cigarette smoke extract for 48 hours also resulted in a significant reduction in ASL height (Xu et al., 2015).

Treatment of human sinonasal epithelial cells with cigarette smoke condensate significantly reduced forskolin-stimulated CBF (Cohen et al., 2009). CBF was also decreased in differentiated normal human bronchial epithelial cells exposed to whole cigarette smoke (Schmid et al., 2015), in cilia-bearing explant adenoid tissues treated with 5 and 10% cigarette smoke extract (Wang et al., 2012), in hamster oviducts treated with various mainstream cigarette smoke fractions (Knoll et al., 1995), and in nasal epithelial cells fom smokers with moderate and severe chronic obstructive pulmonary disease (COPD) (Yaghi et al., 2012).

Whole cigarette smoke exposure or treatment with cigarette smoke extract of normal human bronchial epithelial cells significantly lowered FoxJ1 mRNA and protein levels (Milara et al., 2012; Brekman et al., 2014; Valencia-Gattas et al., 2016; Ishikawa and Ito, 2017). Cigarette smoke extract treatment of normal human bronchial epithelial cells also reduced the expression of cilia-related transcription factor genes, including FOXJ1, RFX2, and RFX3, as well as that of cilia motility and structural integrity genes regulated by FOXJ1, including DNAI1, DNAH5, DNAH9, DNAH10, DNAH11, and SPAG6 (Brekman et al., 2014).

Exposure of human bronchial epithelial cells cultured at the air-liquid interface to cigarette smoke extract during differentiation significantly shortened the average cilia length compared to untreated cultures, and treatment of differentiated cultures prevented elongation of cilia seen in untreated cultures
(Brekman et al., 2014). Whole smoke exposure of mouse tracheal epithelial cells differentiated at the air-liquid interface resulted in cilia shortening and also complete loss of cilia at 24 h post-exposure (Lam et al., 2013). Cilia length was also reduced in mouse nasal septal epithelial cells treated with cigarette smoke condensate (Tamashiro et al., 2009). Cilia length was reduced in endobronchial biopsies and airway brushings of smokers compared to nonsmokers (Leopold et al., 2009) and in COPD smokers compared to healthy smokers and nonsmokers (Hessel et al., 2014). In adults with adults with chronic sputum production, current and former smokers had a higher frequency of axonemal ultrastructural abnormalities than non-smokers and controls (Verra et al., 1994).

Nasomuciliary clearance time (determined by saccharin transit test) was significantly higher in smokers than in non-smokers and correlated positively with cigarettes per day and packs/year index (Proença et al., 2011; Baby et al., 2014; Yadav et al., 2014; Habesoglu et al., 2012; Pagliuca et al., 2015; Xavier et al., 2013; Dülger et al., 2018; Solak et al., 2018; Polosa et al., 2021).

Smoking decreased pulmonary function including forced vital capacity (FVC), forced expiratory volume in one second (FEV1) and FEF25–75 (Kuperman and Riker, 1973; Ashley et al., 1975, Tantisuwat and Thaveeratitham, 2014, Gold et al., 1996; Broekema et al., 2009).

Nitrogen dioxide

Exposure of human bronchial epithelial cells to 100, 400, and 800 ppb NO_2 decreased CBF by 2.3±1.7% , 3.0±2.5%, and 6.8±1.7% respectively, which was not significant relative to incubator controls. Exposure of the cells to 2000 ppb NO_2 significantly decreased CBF by 14.2±2.5% in comparison with controls (Devalia et al., 1993).

Diesel engine exhaust

Incubation of human primary bronchial epithelial cells differentiated at the air-liquid interface with Diesel exhaust particles (DEP; 100 µg/mL = 16.26 ng/mL phenanthrene, 3.65 ng/mL fluoranthene, 2.53 ng/mL pyrene) attenuated CBF in a time- and dose-dependent manner. Exposure to 10 µg/mL DEP decreased CBF by 40% (Q1 = 19, Q3 = 46) from baseline after 24-h incubation. Similarly, exposure to 50 µg/mL DEP, filtered DEP solution, or 100 µg/mL DEP decreased CBF by 51% (Q1 = 49, Q3 = 56), 33% (Q1 = 26, Q3 = 36), and 73% (Q1 = 65, Q3 = 83), respectively, from baseline after 24-h incubation. Changes in CBF started to become significant at 4 h with 50 µg/mL DEP and at 2 h with 100 µg/mL DEP compared to untreated cultures (Bayram et al., 1998).

Overall Assessment of the AOP

The experimental evidence to support the biological plausibility of the KERs from MIE to AO is moderate to strong overall for the AOP presented here, while there is a moderate concordance of dose-response relationships. The weakest evidence is for the KER of decreased CFTR function leading to decreased ASL height (KERC), due to both insufficient experimental evidence of causality and a scarcity of quantitative data on dose-related responses and temporal concordances. In terms of essentiality, we have rated all of the KEs as either moderate or high.

AOPs such as this one can play a central role in risk assessment strategies for a wide variety of regulatory purposes by providing mechanistic support to an integrated approach to testing and assessment (IATA; (Clippinger et al., 2018)). IATAs are flexible frameworks that can be adapted to best address the regulatory question or purpose at hand. More specifically, this AOP can be applied to the risk assessment of inhaled toxicants, by enabling the development of testing strategies through the assembly of existing information and the generation of new data where they are currently lacking. Targeted approaches to fill data gaps can be developed using new approach methodologies (NAMs) informed by this AOP.

Domain of Applicability

Life Stage Applicability

Life Stage	Evidence
All life stages	

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	High	NCBI

Sex Applicability

Sex	Evidence
Mixed	

All KE proposed in this AOP network occur and are measurable in several species, including frogs, mice, rats, guinea pigs, ferrets, cats, dogs, cows, monkeys, and humans. The majority of the supporting empirical evidence derives from studies in rodent and human systems, and—with the exception of CFTR function, which is known to vary from species to species (Higgins, 1992)—experimental findings in animals appear to be highly translatable to humans.

Data regarding the applicability of KE to all life-stages from birth to adulthood are available for the MIE (Oxidative Stress), KE2 (Cystic Fibrosis Transmembrane Regulator Function, Decreased), KE4 (Cilia Beat Frequency, Decreased), KE5 (Mucociliary Clearance, Decreased), KE7 (FOXJ1 Protein, Decreased) and KE8 (Motile Cilia Number/Length, Decreased) and AO (Decreased Lung Function), and indicate that they apply to all life stages. There are no data related to ASL regulation and homeostasis relative to organismal health, but it is reasonable to assume that KE3 (Airway Surface Liquid Height, Decreased), through its impact on MCC, can affect all life-stages. It is also worth noting here that age-dependent decreases in CBF, MCC, and lung function have been demonstrated in several species (e.g., guinea pigs, mice, and humans) and reflect normal physiological aging processes (Bailey et al., 2014; Grubb et al., 2016; Ho et al., 2001; Joki and Saano, 1997; Paul et al., 2013; Sharma and Goodwin, 2006).

Gender-specific data relevant to the AOP network are not as widely available as species-specific data, and to our knowledge, the role of gender has not been systematically evaluated for all KE described here. Informative evidence on gender differences stems from patients with chronic pulmonary diseases, such as cystic fibrosis, asthma, COPD, and bronchiectasis, that are characterized by decreased lung function. For example, epidemiological data indicate more rapid lung function decline and shorter life expectancy in females with cystic fibrosis (genetic CFTR dysfunction; Corey and Farewell, 1996; Harness-Brumley et al., 2014; Olesen et al., 2010; Rosenfeld et al., 1997), and earlier disease onset, more severe disease and more rapid lung function decline in females with COPD (acquired CFTR dysfunction; Prescott et al., 1997; Sørheim et al., 2010) but higher prevalence of COPD in males although this gender gap is closing (Ntritsos et al., 2018). Considering the expression patterns of CFTR and FOXJ1 and their function as well as the importance of efficient MCC—brought about by the interactions of ciliary function, ASL homeostasis and mucus properties—for normal physiological function, we consider this AOP network applicable to both genders.

Essentiality of the Key Events

The definition of essentiality implies that the modulation of upstream KEs impacts the downstream KEs in an expected fashion. If blocked or failed to occur, the KEs in the current AOP will not necessarily stop the progression to subsequent KEs. Due to the complex biology of motile cilia formation and function, ASL homeostasis, mucus properties and MCC, the KEs and AO may be triggered because of alternative pathways or biological redundancies. However, when exacerbated, the KEs promote the occurrence of downstream events eventually leading to the AO. The causal pathway starting from the exposure to oxidants and leading to decreased lung function involves parallel routes with KEs, each of which is sufficient to cause the downstream KE to occur. Different mechanisms, such as oxidant-induced decreases in ASL height via CFTR function decline or oxidant-induced decreases in cilia number and length as a result of decreased FOXJ1 levels, lead to decreased CBF and decreased MCC. Each of these pathways contributes to the AO, but their relative contributions are difficult to evaluate. Based on the evidence we judge the key events MIE (Oxidative Stress), KE2 (Cystic Fibrosis Transmembrane Regulator Function, Decreased), KE4 (Cilia Beat Frequency, Decreased), KE5 (Mucociliary Clearance, Decreased), KE7 (FOXJ1 Protein, Decreased) and KE8 (Motile Cilia Number/Length, Decreased) as highly essential and suggest moderate essentiality for KE3 (Airway Surface Liquid Height, Decreased), and KE6 (Mucus Viscosity, Increased).

Weight of Evidence Summary

We judge the overall biological plausibility of this AOP network as strong. Several KER (i.e., Oxidative stress leading to decreased CFTR function, Decreased CFTR function leading to decreased ASL height, Decreased FOXJ1 protein leading to decreased motile cilia length/number, Oxidative stress leading to decreased CBF) are supported by multiple studies across different species with ample empirical evidence reflecting both dose-response and time concordance. Other KER, such as Oxidative stress leading to decreased FOXJ1 and Decreased ASL height leading to reduced CBF, lack this expanse of empirical evidence, or the evidence does not fully support the causality between the KE (Reduced cilia number/length leading to decreased CBF, Decreased CBF leading to decreased MCC, Increased mucus viscosity leading to decreased MCC) even though the relationship is logical and plausible. Taken together, the AOP network here integrates different plausible pathways from the same MIE (Oxidative stress) leading to a common AO, i.e., decreased lung function. Even though the key elements of the AOP—ASL height, ciliary function and

mucus properties—are closely linked to efficient MCC, the individual contributions of each branch of this AOP network to the AO are, however, currently unclear.

Quantitative Consideration

Overall, our quantitative understanding of the AOP network is moderate.

There is robust evidence that provides an insight into several KER presented here, and the dose response and temporal relationship between the two KE in question are well described and quantified for different stressors across different test systems (Oxidative stress leading to decreased CFTR function, Decreased CFTR function leading to decreased ASL height; Decreased FOXJ1 protein leading to decreased motile cilia length/number; Decreased motile cilia length/number leading to decreased cilia beating frequency; Decreased cilia beat frequency leading to decreased MCC; Increased mucus viscosity leading to decreased MCC; Oxidative stress leading to decreased cilia beat frequency).

In some instances, we are less confident in our quantitative understanding. For example, for the KER Decreased ASL height leading to decreased cilia beat frequency, empirical evidence supporting causality between the two KE is lacking as is quantitative evidence. Dose response data as well as data supportive of the KE causality are limited for the KER Decreased MCC leading to decreased lung function. Similarly, the KER Decreased ASL height leading to increased mucus viscosity is supported by only a small number of studies, most of which evaluated the KEs in parallel, without interrogating dose responses and/or time responses.

Considerations for Potential Applications of the AOP (optional)

Given the individual and public health burden of the consequences of lung function impairment, gaining a greater understanding of the underlying mechanisms is extremely important in the risk assessment of respiratory toxicants. An integrated assessment of substances with the potential to be inhaled, either intentionally or unintentionally, could incorporate inhalation exposure and dosimetry modelling to inform an in vitro approach with appropriate exposure techniques and cell systems to assess KEs in this AOP (EPA's Office of Chemical Safety and Pollution Prevention, 2019). Standardization and robustness testing of assays against explicit performance criteria using suitable reference materials can greatly increase the level of confidence in their use for KE assessment (Petersen et al., In Press). Much of the empirical evidence that supports the KERs in the qualitative AOP described here was obtained from in vitro studies using well-established methodologies for biological endpoint assessment. Being chemical agnostic, this AOP can be applied to a variety of substances that share the AO. For example, impaired MCC and decreased lung function have a long-known relationship with smoking, but little is known about the consequences of long-term use of alternative inhaled nicotine delivery products such as electronic cigarettes and heated tobacco products. This AOP can form the basis of an assessment strategy to evaluate the effects of exposure to aerosol from these products based on the KEs identified here.

References

Allegra, L., Moavero, N., and Rampoldi, C. (1991). Ozone-induced impairment of mucociliary transport and its prevention with N-acetylcysteine. Am. J. Med. 91, S67-S71.

Antunes, M.B., and Cohen, N.A. (2007). Mucociliary clearance–a critical upper airway host defense mechanism and methods of assessment. Curr. Opin. Allergy Clin. Immunol. 7, 5-10.

Bailey, K.L., Bonasera, S.J., Wilderdyke, M., Hanisch, B.W., Pavlik, J.A., DeVasure, J., et al. (2014). Aging causes a slowing in ciliary beat frequency, mediated by PKCε. Am. J. Physiol. Lung Cell. Mol. Physiol. 306, L584-L589.

Bayram, H., Devalia, J.L., Sapsford, R.J., Ohtoshi, T., Miyabara, Y., Sagai, M., et al. (1998). The effect of diesel exhaust particles on cell function and release of inflammatory mediators from human bronchial epithelial cells in vitro. Am. J. Respir. Cell Mol. Biol. 18, 441-448.

Bustamante-Marin, X.M., and Ostrowski, L.E. (2017a). Cilia and Mucociliary Clearance. Cold Spring Harb. Persp. Biol. 9, a028241.

Costa, D.L., Kutzman, R.S., Lehmann, J.R., and Drew, R.T. (1986). Altered Lung Function and Structure in the Rat after Subchronic Exposure to Acrolein. Am. Rev. Respir. Dis. 133, 286-291.

Devalia, J., Sapsford, R., Cundell, D., Rusznak, C., Campbell, A., and Davies, R. (1993). Human bronchial epithelial cell dysfunction following in vitro exposure to nitrogen dioxide. Eur. Respir. J. 6, 1308-1316.

EPA's Office of Chemical Safety and Pollution Prevention (2019). "FIFRA Scientific Advisory Panel Meeting Minutes and Final Report No. 2019-01 Peer Review on Evaluation of a Proposed Approach to Refine the Inhalation Risk Assessment for Point of Contact Toxicity: A Case Study Using a New Approach Methodology (NAM) December 4 and 6, 2018 FIFRA Scientific Advisory Panel Meeting". U.S. Environmental Protection Agency).

Feldman, C., Anderson, R., Kanthakumar, K., Vargas, A., Cole, P.J., and Wilson, R. (1994). Oxidant-mediated ciliary dysfunction in human respiratory epithelium. Free Rad. Biol. Med. 17, 1-10.

Frohlich, E., Mercuri, A., Wu, S., and Salar-Behzadi, S. (2016). Measurements of Deposition, Lung Surface Area and Lung Fluid for Simulation of Inhaled Compounds. Front. Pharmacol. 7, 181.

Gosepath, J., Schaefer, D., Brommer, C., Klimek, L., Amedee, R.G., and Mann, W.J. (2000). Subacute Effects of Ozone Exposure

on Cultivated Human Respiratory Mucosa. Am. J. Rhinol. 14, 411-418.

Gosepath, J., Grebneva, N., Mossikhin, S., and Mann, W.J. (2002). Topical Antibiotic, Antifungal, and Antiseptic Solutions Decrease Ciliary Activity in Nasal Respiratory Cells. Am. J. Rhinol. 16, 25-31.

Grubb, B.R., Livraghi-Butrico, A., Rogers, T.D., Yin, W., Button, B., and Ostrowski, L.E. (2016). Reduced mucociliary clearance in old mice is associated with a decrease in Muc5b mucin. Am. J. Physiol. Lung Cell. Mol. Physiol. 310, L860-L867.

Higgins, C.F. (1992). ABC transporters: from microorganisms to man. Ann. Rev. Cell Biol. 8, 67-113.

Ho, J.C., Chan, K.N., Hu, W.H., Lam, W.K., Zheng, L., Tipoe, G.L., et al. (2001). The effect of aging on nasal mucociliary clearance, beat frequency, and ultrastructure of respiratory cilia. Am. J. Respir. Crit. Care Med. 163, 983-988.

Joki, S., and Saano, V. (1997). Influence of ageing on ciliary beat frequency and on ciliary response to leukotriene D4 in guinea-pig tracheal epithelium. Clin. Exp. Pharmacol. Physiol. 24, 166-169.

Kim, C.S., Alexis, N.E., Rappold, A.G., Kehrl, H., Hazucha, M.J., Lay, J.C., et al. (2011). Lung function and inflammatory responses in healthy young adults exposed to 0.06 ppm ozone for 6.6 hours. Am. J. Respir. Crit. Care Med. 183, 1215-1221.

National Research Council (1988). Air Pollution, the Automobile, and Public Health. Washington, DC: The National Academies Press.

Olesen, H.V., Pressler, T., Hjelte, L., Mared, L., Lindblad, A., Knudsen, P.K., et al. (2010). Gender differences in the Scandinavian cystic fibrosis population. Pediatr. Pulmonol. 45, 959-965.

Paul, P., Johnson, P., Ramaswamy, P., Ramadoss, S., Geetha, B., and Subhashini, A. (2013). The effect of ageing on nasal mucociliary clearance in women: a pilot study. ISRN 2013, 598589.

Petersen, E.J., Sharma, M., Clippinger, A.J., Gordon, J., Katz, A., Laux, P., et al. (2021). Use of Cause-and-Effect Analysis to Optimize the Reliability of In Vitro Inhalation Toxicity Measurements Using an Air–Liquid Interface. Chem. Res. Toxicol. 34, 1370–1385.

Prescott, E., Bjerg, A., Andersen, P., Lange, P., and Vestbo, J. (1997). Gender difference in smoking effects on lung function and risk of hospitalization for COPD: results from a Danish longitudinal population study. Eur. Respir. J. 10, 822-827.

Qu, F., Qin, X.-Q., Cui, Y.-R., Xiang, Y., Tan, Y.-R., Liu, H.-J., et al. (2009). Ozone stress down-regulates the expression of cystic fibrosis transmembrane conductance regulator in human bronchial epithelial cells. Chem. Biol. Interact. 179, 219-226.

Rosenfeld, M., Davis, R., FitzSimmons, S., Pepe, M., and Ramsey, B. (1997). Gender gap in cystic fibrosis mortality. Am. J. Epidemiol. 145, 794-803.

Rubin, B.K. (2002). Physiology of airway mucus clearance. Respir. Care 47, 761.

Sharma, G., and Goodwin, J. (2006). Effect of aging on respiratory system physiology and immunology. Clin. Interv. Aging 1, 253-260.

Sørheim, I.-C., Johannessen, A., Gulsvik, A., Bakke, P.S., Silverman, E.K., and DeMeo, D.L. (2010). Gender differences in COPD: are women more susceptible to smoking effects than men? Thorax 65, 480-485.

Tager, I.B., Balmes, J., Lurmann, F., Ngo, L., Alcorn, S., and Künzli, N. (2005). Chronic exposure to ambient ozone and lung function in young adults. Epidemiology 1, 751-759.

Yoshitsugu, M., Matsunaga, S., Hanamure, Y., Rautiainen, M., Ueno, K., Miyanohara, T., et al. (1995). Effects of oxygen radicals on ciliary motility in cultured human respiratory epithelial cells. Auris Nasus Larynx 22, 178-185.

Appendix 1

List of MIEs in this AOP

Event: 1392: Oxidative Stress

Short Name: Oxidative Stress

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:220 - Cyp2E1 Activation Leading to Liver Cancer	KeyEvent

AOP ID and Name	Event Type
Aop:17 - Binding of electrophilic chemicals to SH(thiol)-group of proteins and /or to seleno-proteins involved in protection against oxidative stress during brain development leads to impairment of learning and memory	KeyEvent
Aop:284 - Binding of electrophilic chemicals to SH(thiol)-group of proteins and /or to seleno-proteins involved in protection against oxidative stress leads to chronic kidney disease	KeyEvent
Aop:377 - Dysregulated prolonged Toll Like Receptor 9 (TLR9) activation leading to Acute Respiratory Distress Syndrome (ARDS) and Multiple Organ Dysfunction (MOD)	KeyEvent
Aop:411 - Oxidative stress [MIE] Leading to Decreased Lung Function [AO]	MolecularInitiatingEvent

Stressors

Name
Acetaminophen
Chloroform
furan

Biological Context

Level of Biological Organization
Molecular

Domain of Applicability

Taxonomic Applicability

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

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Mixed	High

Oxidative stress is produced in, and can occur in, any species from bacteria through to humans.

Key Event Description

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

In addition to the directly damaging actions of ROS, cellular oxidative stress also changes cellular activities on a molecular level. Redox sensitive proteins have altered physiology in the presence and absence of ROS, which is caused by the oxidation of sulfhydryls to disulfides (2SH àSS) on neighboring amino acids (Antelmann and Helmann 2011). Importantly Keap1, the negative regulator of Nrf2, is regulated in this manner (Itoh, et al. 2010).

Protection against oxidative stress is relevant for all tissues and organs, although some tissues may be more susceptible. For example, the brain possesses several key physiological features, such as high O2 utilization, high polyunsaturated fatty acids content, presence of autooxidable neurotransmitters, and low antioxidant defenses as compared to other organs, that make it highly susceptible to oxidative stress (Halliwell, 2006; Emerit and al., 2004; Frauenberger et al., 2016).

How it is Measured or Detected

Oxidative Stress. Direct measurement of ROS is difficult because ROS are unstable. The presence of ROS can be assayed indirectly by measurement of cellular antioxidants, or by ROS-dependent cellular damage:

- Detection of ROS by chemiluminescence (https://www.sciencedirect.com/science/article/abs/pii/S0165993606001683)

- Detection of ROS by chemiluminescence is also described in OECD TG 495 to assess phototoxic potential.

- Glutathione (GSH) depletion. GSH can be measured by assaying the ratio of reduced to oxidized glutathione (GSH:GSSG) using a commercially available kit (e.g., http://www.abcam.com/gshgssg-ratio-detection-assay-kit-fluorometric-green-ab138881.html).

- TBARS. Oxidative damage to lipids can be measured by assaying for lipid peroxidation using TBARS (thiobarbituric acid reactive substances) using a commercially available kit.

- 8-oxo-dG. Oxidative damage to nucleic acids can be assayed by measuring 8-oxo-dG adducts (for which there are a number of ELISA based commercially available kits),or HPLC, described in Chepelev et al. (Chepelev, et al. 2015).

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

- Immunohistochemistry for increases in Nrf2 protein levels and translocation into the nucleus;

- Western blot for increased Nrf2 protein levels;

- Western blot of cytoplasmic and nuclear fractions to observe translocation of Nrf2 protein from the cytoplasm to the nucleus;

- qPCR of Nrf2 target genes (e.g., Nqo1, Hmox-1, Gcl, Gst, Prx, TrxR, Srxn), or by commercially available pathway-based qPCR array (e.g., oxidative stress array from SABiosciences);

- Whole transcriptome profiling by microarray or RNA-seq followed by pathway analysis (in IPA, DAVID, metacore, etc.) for enrichment of the Nrf2 oxidative stress response pathway (e.g., Jackson et al. 2014);

- OECD TG422D describes an ARE-Nrf2 Luciferase test method;

- In general, there are a variety of commercially available colorimetric or fluorescent kits for detecting Nrf2 activation.

References

Antelmann, H., Helmann, J.D., 2011. Thiol-based redox switches and gene regulation. Antioxid. Redox Signal. 14, 1049-1063.

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

Emerit, J., Edeas, M., Bricaire, F., 2004. Neurodegenerative diseases and oxidative stress. Biomed. Pharmacotherapy. 58(1): 39-46.

Frauenberger, E.A., Scola, G., Laliberté, V.L.M., Duong, A., Andreazza, A.C., 2015. Redox modulations, Antioxidants, and Neuropsychitrica Disorders. Ox. Med. Cell. Longevity. Vol. 2016, Article ID 4729192.

Halliwell, B., 2006. Oxidative stress and neurodegeneration: where are we now? J. Neurochem. 97(6):1634-1658.

Itoh, K., Mimura, J., Yamamoto, M., 2010. Discovery of the negative regulator of Nrf2, Keap1: a historical overview. Antioxid. Redox Signal. 13, 1665-1678.

Jackson, A.F., Williams, A., Recio, L., Waters, M.D., Lambert, I.B., Yauk, C.L., 2014. Case study on the utility of hepatic global gene expression profiling in the risk assessment of the carcinogen furan. Toxicol. Applied Pharmacol.274, 63-77.

Nguyen, T., Nioi, P., Pickett, C.B., 2009. The Nrf2-antioxidant response element signaling pathway and its activation by oxidative stress. J. Biol. Chem. 284, 13291-13295.

OECD (2018), Test No. 442D: In Vitro Skin Sensitisation: ARE-Nrf2 Luciferase Test Method, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, https://doi.org/10.1787/9789264229822-en.

OECD (2019), Test No. 495: Ros (Reactive Oxygen Species) Assay for Photoreactivity, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, https://doi.org/10.1787/915e00ac-en.

List of Key Events in the AOP

Event: 1906: Cystic Fibrosis Transmembrane Regulator Function, Decreased

Short Name: CFTR Function, Decreased

Key Event Component

Process	Object	Action
chloride channel activity		decreased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:411 - Oxidative stress [MIE] Leading to Decreased Lung Function [AO]	KeyEvent

Stressors

Name
Acrolein
Ozone
Cigarette smoke
Cadmium

Biological Context

Level of Biological Organization
Molecular

Cell term

Cell term
epithelial cell

Organ term

Organ term
lung

Evidence for Perturbation by Stressor

Acrolein

Acrolein exhibited a complex dose-dependent response with respect to CFTR-mediated Cl^- transport in primary murine nasal septal epithelia: At 100 µM acrolein, Cl^- currents increased, whereas 300 µM acrolein reduced forskolin-induced total apical Cl^- secretion and 300 µM acrolein abolished all Cl^- transport. These effects were independent of cAMP, suggesting that channel activation was not PKA/cAMP phosphorylation-dependent (Alexander et al., 2012).
Acrolein decreased cAMP-mediated CFTR ion transport in human bronchial epithelial cells grown in monolayers and in human Calu-3 lung cancer cells, where the response was dose-dependent. Repeated, low-level exposure of human bronchial epithelial cells to acrolein (2.5 – 10 ng/mL for 7 days) had a similar effect on CFTR function and was shown to be unrelated to modulation of CFTR expression. Pretreatment with the antioxidant N-acetylcysteine could prevent acrolein-induced CFTR inhibition (Raju et al., 2013). Similar effects on CFTR function (as measured by nasal and intestinal transepithelial potential difference) were elicited by subcutaneous administration of 1 mg/kg acrolein for 4 weeks, and these could also be counteracted by co-treatment with NAC (Raju et al., 2013).

Ozone

Tracheas of Wistar rats exposed to 1.5 ppm ozone for 1 h/day for 3 days exhibited reduced CFTR protein expression. Similarly, at 4 hours following a 30-min exposure to ozone, CFTR mRNA and protein were down-regulated in 16HBE14o- cells. At 24 hours post-exposure, a reduction in forskolin-stimulated CFTR Cl– conductance was observed (Qu et al., 2009).

Cigarette smoke

CFTR transcript and protein levels were reduced in human Calu-3 lung cancer cells exposed to the gas phase of cigarette smoke (Cantin et al., 2006b), human immortalized bronchial epithelial 16HBE14o- cells treated with 10% cigarette smoke extract (Hassan et al., 2014; Rasmussen et al., 2014; Xu et al., 2015), differentiated primary human bronchial epithelial cells exposed to whole cigarette smoke (Sloane et al., 2012; Hassan et al., 2014), and in airways of smokers compared to non-smokers (Dransfield et al., 2013).

Following exposure to cigarette smoke, Cl^- conductance (i.e., CFTR-mediated Cl^- transport) decreased in primary human bronchial epithelial cells grown in monolayers (Lambert et al., 2014), differentiated primary human bronchial epithelial cells (Schmid et al., 2015; Chinnapaiyan et al., 2018), and nasal respiratory and intestinal epithelia of A/J mice (Raju et al., 2013; Raju et al., 2017).

In the lower airways, healthy smokers and smokers with chronic obstructive pulmonary disease (COPD) showed reduced CFTR-dependent Cl^- transport, whereas COPD former smokers showed an intermediate response to chloride-free isoproterenol solution compared to non-smokers. Similarly, amiloride-sensitive lower airway potential difference was also lower in healthy smokers and COPD smokers than in healthy non-smokers. This was linked to reduced CFTR protein levels in the airways of smokers compared to non-smokers, although there were no significant differences between healthy and COPD subjects (Dransfield et al., 2013). CFTR-dependent Cl^- conductance as measured by nasal potential difference was also significantly reduced in healthy and COPD smokers compared to healthy non-smokers or to former smokers with COPD (Sloane et al., 2012). In addition, healthy never-smokers had higher mean sweat chloride concentrations than COPD smokers and COPD former smokers (Raju et al., 2013; Courville et al., 2014).

Cadmium

Cadmium (Cd) decreased CFTR protein expression in Calu-3 cells in a dose- and time-dependent manner. CFTR transcript levels, however, appeared to only be transiently affected. Reduced CFTR expression at the plasma membrane was associated with a reduction in CFTR Cl^- conductance. Treatment of cells with NAC did not rescue CFTR expression in Cd-treated cells. In contrast, co-treatment with α-tocopherol prevented CFTR inhibition, and this effect was linked to α-tocopherol suppressing the accumulation of ubiquitinated CFTR (Rennolds et al., 2010).

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	High	NCBI
Mus musculus	Mus musculus	Moderate	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Mixed	High

Phylogenetic analysis of CFTR DNA sequences across multiple species suggests a close evolutionary relationship between human and primate CFTR, followed by rabbit, guinea pig, equine, ovine, and bovine CFTR, whereas rodent CFTR DNA largely diverges from the human DNA (Chen et al., 2001). Of note, CFTR ion permeability differs from species to species (Higgins, 1992). For example, murine CFTR displays reduced channel activity compared with its human counterpart, while ovine CFTR exhibits higher ATP sensitivity, greater single-channel conductance and larger open probability than human CFTR. Moreover, sensitivity to pharmacological agents able to potentiate or block CFTR gating varies greatly from species to species (Bose et al., 2015). Therefore, results from animal studies are not easily and directly transferable to human.

CFTR dysfunction as a consequence of inherited CFTR gene defects is studied in pediatric as well as adult cystic fibrosis patients. Acquired CFTR dysfunction following inhalation exposures (e.g. to cigarette smoke) may also apply to both pediatric and adult populations, depending on the setting and type of exposure.

To our knowledge, the role of gender has not been systematically evaluated in acquired CFTR dysfunction. It is thought that the observed suppression of CFTR expression and impairment of CFTR function in cigarette smokers is a contributing factor to the pathogenesis of chronic obstructive pulmonary disease (COPD) (Dransfield et al., 2013; Raju et al., 2016b). The main risk factor for COPD is cigarette smoking, and COPD is more common in men than in women, which may be directly related to the higher prevalence of smoking in men, although this gender gap is closing (Hitchman and Fong, 2011; Ntritsos et al., 2018; Syamlal et al.,

2014). Nevertheless, the available clinical evidence in support of this AOP suggests that there is no remarkable gender difference.

Key Event Description

The cystic fibrosis transmembrane regulator (CFTR) is a multi-domain membrane protein that belongs to the large family of adenine nucleotide binding cassette transporters consisting of two transmembrane domains, two nucleotide binding domains (NBDs) and a unique regulatory domain (Riordan, 2008). It is an integral membrane glycoprotein that functions as cAMP-activated and phosphorylation-regulated Cl^- channel at the apical membrane of epithelial cells (Farinha et al., 2013).
In respiratory epithelia, CFTR is the major Cl^- channel that mediates fluid and electrolyte transport, and CFTR function is critical to normal ASL homeostasis. Exposure to inhaled oxidants, such as ozone and cigarette smoke, leads to decreased CFTR gene and protein expression as well as CFTR internalization, thereby reducing or abolishing short-circuit currents (Qu et al., 2009; Cantin et al., 2006a; Cantin et al., 2006b; Clunes et al., 2012; Sloane et al., 2012; Rasmussen et al., 2014). Reduced CFTR gene transcription rates were mechanistically linked to mobilization of intracellular Ca^{2+}, resulting in decreased mRNA and protein expression, presumably in a protein kinase-dependent manner (Bargon et al., 1992a; Bargon et al., 1992b). Cigarette smoke exposure of primary human bronchial epithelial cells at the air-liquid interface was shown to rapidly increase intracellular Ca^{2+}, followed by a decrease in cell surface CFTR expression (Rasmussen et al., 2014). Of note, this decrease by CFTR internalization was subsequently linked to decreased active Cl^- transport and a reduction in ASL height/volume (Clunes et al., 2012). Similarly, treatment with pyocyanin, a redox-active virulence factor secreted by Pseudomonas aeruginosa which commonly infects the airways of cystic fibrosis patients, increased hydrogen peroxide levels in CFBE41o- bronchial epithelial cells in a dose- and time-dependent manner, leading to oxidation of the cytosol and inhibited forskolin-stimulated ion transport (Schwarzer et al., 2008). Other possible mechanisms of acquired CFTR dysfunction include direct covalent modification of the protein by cigarette smoke and acrolein (Raju et al., 2013; Raju et al., 2016a) or modulation of channel open probability (Zhang et al., 2013; Woodworth, 2015).

How it is Measured or Detected

In cystic fibrosis patients, who carry a defect in the *CFTR* gene, the determination of the residual levels of normal, full-length CFTR transcripts may have some clinical utility in estimating CFTR function (Amaral et al., 2004). Moreover, decreased *CFTR* mRNA and protein expression were previously shown to result in reduced CFTR-mediated Cl^- transport (Cantin et al., 2006a; Cantin et al., 2006b; Clunes et al., 2012; Sloane et al., 2012; Rasmussen et al., 2014). Therefore, measuring decreased CFTR function could be achieved by a combination of multiple techniques. For example, decreased expression of *CFTR* mRNA and protein in cells and tissues can be directly assessed using RT-PCR, Northern blot and Western blot or immunocyto-/histochemical methods, respectively. Of note, *CFTR* gene expression is generally low as is protein abundance, and protein detection methods in general perform more robustly in cultured cells than in native tissues (Farinha et al., 2004). Other, less frequently used methods include cell surface biotinylation, enabling a distinction between intracellular and cell surface forms of the protein if one wishes to study plasma membrane-expressed CFTR.
In vitro or ex vivo, CFTR channel function can be assessed in real-time using patch-clamping of whole (single) cells or cell patches. In the whole-cell patch-clamp approach, current flow through CFTR can be assessed by voltage-clamp, whereas current-clamping provides insights into the effects of CFTR currents on membrane voltage (Sheppard et al., 2004). Measuring the efflux of radiolabeled tracers is another means of studying CFTR channel function, permitting a higher throughput than patch-clamping (Norez et al., 2004). The most commonly used method to study CFTR ion transport, however, utilizes the Ussing chamber to measure transepithelial voltage or “active transport potential” and short-circuit current (Li et al., 2004).
In vivo, CFTR dysfunction is demonstrated by the chloride sweat test, the gold standard diagnostic tool for cystic fibrosis. The sweat test should be performed according to clinical guidelines using the Gibson and Cooke technique (also known as quantitative pilocarpine iontophoresis sweat test) (Farrell et al., 2017; Smyth et al., 2014). As a complementary diagnostic measure, nasal potential difference (NPD) can be assessed to gauge net transepithelial active ion transport and epithelial ion conductance (Schüler et al., 2004).
An entire issue of the Journal of Cystic Fibrosis dedicated to the Virtual Repository of the CFTR Working Group, including the description of consensus research methods, selected principles, techniques and reagents for the assessment of CFTR expression and function is available here:
https://www.sciencedirect.com/journal/journal-of-cystic-fibrosis/vol/3/suppl/S2

References

- Alexander, N.S., Blount, A., Zhang, S., Skinner, D., Hicks, S.B., Chestnut, M., et al., 2012. Cystic fibrosis transmembrane conductance regulator modulation by the tobacco smoke toxin acrolein. The Laryngoscope. 122(6), 1193-1197.
- Amaral, M.D., Clarke, L.A., Ramalho, A.S., Beck, S., Broackes-Carter, F., Rowntree, R., et al., 2004. Quantitative methods for the analysis of CFTR transcripts/splicing variants. Journal of Cystic Fibrosis. 3, 17-23.
- Bargon, J., Trapnell, B., Yoshimura, K., Dalemans, W., Pavirani, A., Lecocq, J., et al., 1992a. Expression of the cystic fibrosis transmembrane conductance regulator gene can be regulated by protein kinase C. Journal of Biological Chemistry. 267(23), 16056-16060.
- Bargon, J., Trapnell, B.C., Chu, C.-S., Rosenthal, E.R., Yoshimura, K., Guggino, W.B., et al., 1992b. Down-regulation of cystic fibrosis transmembrane conductance regulator gene expression by agents that modulate intracellular divalent cations. Molecular and cellular biology. 12(4), 1872-1878.
- Bose, S.J., Scott-Ward, T.S., Cai, Z. and Sheppard, D.N., 2015. Exploiting species differences to understand the CFTR Cl–

channel. Biochemical Society Transactions. 43(5), 975-982.
- Cantin, A.M., Bilodeau, G., Ouellet, C., Liao, J. and Hanrahan, J.W., 2006a. Oxidant stress suppresses CFTR expression. Am J Physiol Cell Physiol. 290(1), C262-270.
- Cantin, A.M., Hanrahan, J.W., Bilodeau, G., Ellis, L., Dupuis, A., Liao, J., et al., 2006b. Cystic Fibrosis Transmembrane Conductance Regulator Function Is Suppressed in Cigarette Smokers. American journal of respiratory and critical care medicine. 173(10), 1139-1144.
- Chen, J.-M., Cutler, C., Jacques, C., Bœuf, G., Denamur, E., Lecointre, G., et al., 2001. A Combined Analysis of the Cystic Fibrosis Transmembrane Conductance Regulator: Implications for Structure and Disease Models. Molecular Biology and Evolution. 18(9), 1771-1788.
- Chinnapaiyan, S., Dutta, R., Bala, J., Parira, T., Agudelo, M., Nair, M., et al., 2018. Cigarette smoke promotes HIV infection of primary bronchial epithelium and additively suppresses CFTR function. Scientific Reports. 8.
- Clunes, L.A., Davies, C.M., Coakley, R.D., Aleksandrov, A.A., Henderson, A.G., Zeman, K.L., et al., 2012. Cigarette smoke exposure induces CFTR internalization and insolubility, leading to airway surface liquid dehydration. The FASEB Journal. 26(2), 533-545.
- Courville, C.A., Tidwell, S., Liu, B., Accurso, F.J., Dransfield, M.T. and Rowe, S.M., 2014. Acquired defects in CFTR-dependent β-adrenergic sweat secretion in chronic obstructive pulmonary disease. Respiratory research. 15(1), 25.
- Dransfield, M.T., Wilhelm, A.M., Flanagan, B., Courville, C., Tidwell, S.L., Raju, S.V., et al., 2013. Acquired Cystic Fibrosis Transmembrane Conductance Regulator Dysfunction in the Lower Airways in COPD. Chest. 144(2), 498-506.
- Farinha, C.M., Penque, D., Roxo-Rosa, M., Lukacs, G., Dormer, R., Mcpherson, M., et al., 2004. Biochemical methods to assess CFTR expression and membrane localization. Journal of Cystic Fibrosis. 3, 73-77.
- Farinha, C.M., Matos, P. and Amaral, M.D., 2013. Control of cystic fibrosis transmembrane conductance regulator membrane trafficking: not just from the endoplasmic reticulum to the Golgi. The FEBS Journal. 280(18), 4396-4406.
- Farrell, P.M., White, T.B., Ren, C.L., Hempstead, S.E., Accurso, F., Derichs, N., et al., 2017. Diagnosis of Cystic Fibrosis: Consensus Guidelines from the Cystic Fibrosis Foundation. The Journal of Pediatrics. 181, S4-S15.e11.
- Hassan, F., Xu, X., Nuovo, G., Killilea, D.W., Tyrrell, J., Da Tan, C., et al., 2014. Accumulation of metals in GOLD4 COPD lungs is associated with decreased CFTR levels. Respiratory research. 15(1), 69.
- Higgins, C.F., 1992. ABC transporters: from microorganisms to man. Annual review of cell biology. 8(1), 67-113.
- Hitchman, S.C. and Fong, G.T., 2011. Gender empowerment and female-to-male smoking prevalence ratios. Bulletin of the World Health Organization. 89(3), 195-202.
- Lambert, J.A., Raju, S.V., Tang, L.P., Mcnicholas, C.M., Li, Y., Courville, C.A., et al., 2014. Cystic fibrosis transmembrane conductance regulator activation by roflumilast contributes to therapeutic benefit in chronic bronchitis. American journal of respiratory cell and molecular biology. 50(3), 549-558.
- Li, H., Sheppard, D.N. and Hug, M.J., 2004. Transepithelial electrical measurements with the Ussing chamber. Journal of Cystic Fibrosis. 3, 123-126.
- Norez, C., Heda, G.D., Jensen, T., Kogan, I., Hughes, L.K., Auzanneau, C., et al., 2004. Determination of CFTR chloride channel activity and pharmacology using radiotracer flux methods. Journal of Cystic Fibrosis. 3, 119-121.
- Ntritsos, G., Franek, J., Belbasis, L., Christou, M.A., Markozannes, G., Altman, P., et al., 2018. Gender-specific estimates of COPD prevalence: a systematic review and meta-analysis. International journal of chronic obstructive pulmonary disease. 13, 1507.
- Qu, F., Qin, X.-Q., Cui, Y.-R., Xiang, Y., Tan, Y.-R., Liu, H.-J., et al., 2009. Ozone stress down-regulates the expression of cystic fibrosis transmembrane conductance regulator in human bronchial epithelial cells. Chemico-Biological Interactions. 179(2–3), 219-226.
- Raju, S.V., Jackson, P.L., Courville, C.A., Mcnicholas, C.M., Sloane, P.A., Sabbatini, G., et al., 2013. Cigarette smoke induces systemic defects in cystic fibrosis transmembrane conductance regulator function. American journal of respiratory and critical care medicine. 188(11), 1321-1330.
- Raju, S.V., Solomon, G.M., Dransfield, M.T. and Rowe, S.M., 2016a. Acquired CFTR Dysfunction in Chronic Bronchitis and Other Diseases of Mucus Clearance. Clinics in chest medicine. 37(1), 147-158.
- Raju, S.V., Lin, V.Y., Liu, L., Mcnicholas, C.M., Karki, S., Sloane, P.A., et al., 2016b. The Cftr Potentiator Ivacaftor Augments Mucociliary Clearance Abrogating Cftr Inhibition by Cigarette Smoke. American journal of respiratory cell and molecular biology.
- Raju, S.V., Rasmussen, L., Sloane, P.A., Tang, L.P., Libby, E.F. and Rowe, S.M., 2017. Roflumilast reverses CFTR-mediated ion transport dysfunction in cigarette smoke-exposed mice. Respiratory research. 18(1), 173.
- Rasmussen, J.E., Sheridan, J.T., Polk, W., Davies, C.M. and Tarran, R., 2014. Cigarette smoke-induced Ca2+ release leads to cystic fibrosis transmembrane conductance regulator (CFTR) dysfunction. J Biol Chem. 289(11), 7671-7681.
- Rennolds, J., Butler, S., Maloney, K., Boyaka, P.N., Davis, I.C., Knoell, D.L., et al., 2010. Cadmium Regulates the Expression of the CFTR Chloride Channel in Human Airway Epithelial Cells. Toxicological Sciences. 116(1), 349-358.
- Riordan, J.R., 2008. CFTR Function and Prospects for Therapy. Annual Review of Biochemistry. 77(1), 701-726.
- Schmid, A., Baumlin, N., Ivonnet, P., Dennis, J.S., Campos, M., Krick, S., et al., 2015. Roflumilast partially reverses smoke-induced mucociliary dysfunction. Respiratory research. 16(1), 135.
- Schüler, D., Sermet-Gaudelus, I., Wilschanski, M., Ballmann, M., Dechaux, M., Edelman, A., et al., 2004. Basic protocol for transepithelial nasal potential difference measurements. Journal of Cystic Fibrosis. 3, 151-155.
- Schwarzer, C., Fischer, H., Kim, E.-J., Barber, K.J., Mills, A.D., Kurth, M.J., et al., 2008. Oxidative stress caused by pyocyanin impairs CFTR Cl– transport in human bronchial epithelial cells. Free Radical Biology and Medicine. 45(12), 1653-1662.
- Sheppard, D.N., Gray, M.A., Gong, X., Sohma, Y., Kogan, I., Benos, D.J., et al., 2004. The patch-clamp and planar lipid bilayer techniques: powerful and versatile tools to investigate the CFTR Cl– channel. Journal of Cystic Fibrosis. 3, 101-108.
- Sloane, P.A., Shastry, S., Wilhelm, A., Courville, C., Tang, L.P., Backer, K., et al., 2012. A pharmacologic approach to acquired cystic fibrosis transmembrane conductance regulator dysfunction in smoking related lung disease. PloS one. 7(6), e39809.

- Smyth, A.R., Bell, S.C., Bojcin, S., Bryon, M., Duff, A., Flume, P., et al., 2014. European Cystic Fibrosis Society Standards of Care: Best Practice guidelines. Journal of Cystic Fibrosis. 13, S23-S42.
- Syamlal, G., Mazurek, J.M. and Dube, S.R., 2014. Gender differences in smoking among US working adults. American journal of preventive medicine. 47(4), 467-475.
- Woodworth, B.A., 2015. Resveratrol ameliorates abnormalities of fluid and electrolyte secretion in a hypoxia-Induced model of acquired CFTR deficiency. The Laryngoscope. 125(S7), S1-S13.
- Xu, X., Balsiger, R., Tyrrell, J., Boyaka, P.N., Tarran, R. and Cormet-Boyaka, E., 2015. Cigarette smoke exposure reveals a novel role for the MEK/ERK1/2 MAPK pathway in regulation of CFTR. Biochimica et biophysica acta. 1850(6), 1224-1232.
- Zhang, S., Blount, A.C., Mcnicholas, C.M., Skinner, D.F., Chestnut, M., Kappes, J.C., et al., 2013. Resveratrol enhances airway surface liquid depth in sinonasal epithelium by increasing cystic fibrosis transmembrane conductance regulator open probability. PloS one. 8(11), e81589.

Event: 1907: Airway Surface Liquid Height, Decreased

Short Name: ASL Height, Decreased

Key Event Component

Process	Object	Action
	epithelial lining fluid	decreased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:411 - Oxidative stress [MIE] Leading to Decreased Lung Function [AO]	KeyEvent

Stressors

Name
Cigarette smoke

Biological Context

Level of Biological Organization
Tissue

Organ term

Organ term
lung

Evidence for Perturbation by Stressor

Cigarette smoke

Multiple studies showed that exposure of primary human bronchial epithelial cells, either undifferentiated or differentiated at the air-liquid interface, to cigarette smoke decreased ASL height (Hassan et al., 2014; Lambert et al., 2014; Raju et al., 2016; Rasmussen et al., 2014; Schmid et al., 2015). Treatment of immortalized bronchial epithelial 16HBE14o- cells with 10% cigarette smoke extract for 48 hours also resulted in a significant reduction in ASL height (Xu et al., 2015).

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	High	NCBI

Term	Scientific Term	Evidence	Links
Mus musculus	Mus musculus	Low	NCBI
Sus scrofa	Sus scrofa	Low	NCBI
Ovis aries	Ovis aries	Low	NCBI
Cavia porcellus	Cavia porcellus	Low	NCBI
Bos taurus	Bos taurus	Low	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	Low

Sex Applicability

Sex	Evidence
Mixed	Low

To date, ASL has been investigated in several species including mice, rats, guinea pigs, ferrets, cats, dogs, cows, monkeys, and humans. Although most studies provide data on its composition rather than its height, it is reasonable to assume that regulation of ASL height is equally critical to MCC across these species.

There are no data related to ASL regulation and homeostasis relative to organismal health, but it is reasonable to assume that decreased ASL, through its impact on MCC, can affect all life stages.

There are no gender-specific data on the regulation of ASL height to our knowledge, but it is reasonable to assume that there is no gender difference.

Key Event Description

The airway surface liquid (ASL) is a liquid layer on the apical side of the respiratory epithelium, reportedly between 5 to 100 µm in depth (Widdicombe and Widdicombe, 1995), and consists of an inner aqueous periciliary liquid layer (PCL) that spans the length of cilia and the outer gel-like mucus layer. The PCL has a low viscosity and enables cilia beating, thereby facilitating the forward movement of the outer mucus layer toward the glottis and, ultimately, its removal by cough or ingestion (Antunes and Cohen, 2007). Both ASL composition and height are considered critical for its function (Fischer and Widdicombe, 2006).
Under physiological conditions, ASL composition and height are regulated via vectorial transport of electrolytes, driven by transepithelial transport and apical secretion of Cl^- by (predominantly) CFTR, resulting in passive H_2O secretion and, consequently, increased ASL height. Absorption of Na^+ at the apical side by the epithelial sodium channel ENaC and ENaC's interaction with the basolateral Na^+/K^+-ATPase exchanging Na^+ for K^+ leads to net absorption of Na^+, which in turn drives fluid absorption and therefore decreases ASL height (Althaus, 2013; Hollenhorst et al., 2011). Impairment of CFTR or ENaC function can lead to the dysfunction of the other ion channel (increased CFTR activity leads to decreased ENaC activity and vice versa) (Boucher R., 2003; Boucher, 2004; Schmid et al., 2011), resulting in permanently perturbed ASL height.

How it is Measured or Detected

There is no standardized protocol for the determination of ASL height. In several experimental in vitro studies, confocal fluorescence microscopy scanning in the vertical plane (i.e., in XZ mode) was used to measure ASL height in human and mouse 3D organotypic airway epithelial models, and changes in ASL height could be calibrated using a fluorophore-dextran conjugate to estimate changes in ASL volume (Garcia-Caballero et al., 2009; Lazarowski et al., 2004; Matsui et al., 1998; Roomans et al., 2004; Saint-Criq et al., 2013; Tarran and Boucher, 2002; Tarran et al., 2005; Tarran et al., 2001; Tarran et al., 2006; Zhang et al., 2013). A similar approach was taken for the measurement of ASL height in freshly excised human trachea and bronchi, excised pig tracheas and mouse tracheas in vivo (Jayaraman et al., 2001; Song et al., 2009). A detailed protocol is provided by (Tarran and Boucher, 2002).
In addition, ASL height was measured using micro-optical coherence tomography in differentiated human bronchial epithelial cells (Raju et al., 2016), synchrotron phase contrast x-ray imaging in excised mouse tracheas (Morgan et al., 2013; Siu et al., 2008) and live mice (Donnelley et al., 2014), and low-temperature scanning electron microscopy in excised, rapidly frozen specimens of bovine tracheal epithelium (Wu et al., 1996; Wu et al., 1998) and guinea pig lungs (Yager et al., 1994). Furthermore, a specifically designed chamber allowed for evaluation of ASL height in excised guinea pig and sheep tracheas using videomicroscopy under a cold light source or strobe lights (Seybold et al., 1990; Shephard and Rahmoune, 1994), whereas a microelectrode technique was employed to determine ASL height in live guinea pigs (Rahmoune and Shephard, 1995).

References

Althaus, M. (2013). ENaC inhibitors and airway re-hydration in cystic fibrosis: state of the art. Curr. Mol. Pharmacol. 6, 3-12.

Antunes, M.B. and Cohen, N.A. (2007). Mucociliary clearance–a critical upper airway host defense mechanism and methods of assessment. Curr. Opin. Allergy Clin. Immunol. 7, 5-10.

Boucher, R. (2003). Regulation of airway surface liquid volume by human airway epithelia. Pflügers Arch. 445, 495-498.

Boucher, R.C. (2004). New concepts of the pathogenesis of cystic fibrosis lung disease. Eur. Respir. J. 23, 146-158.

Donnelley, M., Morgan, K.S., Siu, K.K.W., Farrow, N.R., Stahr, C.S., Boucher, R.C., et al. (2014). Non-invasive airway health assessment: Synchrotron imaging reveals effects of rehydrating treatments on mucociliary transit in-vivo. Sci. Rep. 4, 3689.

Fischer, H. and Widdicombe, J.H. (2006). Mechanisms of Acid and Base Secretion by the Airway Epithelium. J. Membr. Biol. 211, 139-150.

Garcia-Caballero, A., Rasmussen, J.E., Gaillard, E., Watson, M.J., Olsen, J.C., Donaldson, S.H., et al. (2009). SPLUNC1 regulates airway surface liquid volume by protecting ENaC from proteolytic cleavage. Proc. Natl. Acad. Sci. U.S.A. 106, 11412-11417.

Hassan, F., Xu, X., Nuovo, G., Killilea, D.W., Tyrrell, J., Da Tan, C., et al. (2014). Accumulation of metals in GOLD4 COPD lungs is associated with decreased CFTR levels. Respir. Res. 15, 69.

Hollenhorst, M.I., Richter, K. and Fronius, M. (2011). Ion transport by pulmonary epithelia. BioMed Res. Int. 174306.

Jayaraman, S., Song, Y., Vetrivel, L., Shankar, L. and Verkman, A.S. (2001). Noninvasive in vivo fluorescence measurement of airway-surface liquid depth, salt concentration, and pH. J. Clin. Invest. 107, 317-324.

Lambert, J.A., Raju, S.V., Tang, L.P., Mcnicholas, C.M., Li, Y., Courville, C.A., et al. (2014). Cystic fibrosis transmembrane conductance regulator activation by roflumilast contributes to therapeutic benefit in chronic bronchitis. Am. J. Respir. Cell Mol. Biol. 50, 549-558.

Lazarowski, E.R., Tarran, R., Grubb, B.R., Van Heusden, C.A., Okada, S. and Boucher, R.C. (2004). Nucleotide release provides a mechanism for airway surface liquid homeostasis. J. Biol. Chem. 279:36855-64.

Matsui, H., Grubb, B.R., Tarran, R., Randell, S.H., Gatzy, J.T., Davis, C.W., et al. (1998). Evidence for periciliary liquid layer depletion, not abnormal ion composition, in the pathogenesis of cystic fibrosis airways disease. Cell 95, 1005-1015.

Morgan, K.S., Donnelley, M., Paganin, D.M., Fouras, A., Yagi, N., Suzuki, Y., et al. (2013). Measuring Airway Surface Liquid Depth in Ex Vivo Mouse Airways by X-Ray Imaging for the Assessment of Cystic Fibrosis Airway Therapies. PloS ONE 8, e55822.

Rahmoune, H. and Shephard, K.L. (1995). State of airway surface liquid on guinea pig trachea. J. Appl. Physiol. 78, 2020-2024.

Raju, S.V., Lin, V.Y., Liu, L., Mcnicholas, C.M., Karki, S., Sloane, P.A., et al. (2016). The Cftr Potentiator Ivacaftor Augments Mucociliary Clearance Abrogating Cftr Inhibition by Cigarette Smoke. Am. J. Respir. Cell Mol. Biol. 56, 99-108.

Rasmussen, J.E., Sheridan, J.T., Polk, W., Davies, C.M. and Tarran, R. (2014). Cigarette smoke-induced Ca2+ release leads to cystic fibrosis transmembrane conductance regulator (CFTR) dysfunction. J. Biol. Chem. 289, 7671-7681.

Roomans, G.M., Kozlova, I., Nilsson, H., Vanthanouvong, V., Button, B. and Tarran, R. (2004). Measurements of airway surface liquid height and mucus transport by fluorescence microscopy, and of ion composition by X-ray microanalysis. J. Cystic Fibr. 3, 135-139.

Schmid, A., Clunes, L.A., Salathe, M., Verdugo, P., Dietl, P., Davis, C.W., et al. (2011). Nucleotide-mediated airway clearance. Purinergic Regulation of Respiratory Diseases. Springer, pp.95-138.

Saint-Criq, V., Kim, S.H., Katzenellenbogen, J.A. and Harvey, B.J. (2013). Non-Genomic Estrogen Regulation of Ion Transport and Airway Surface Liquid Dynamics in Cystic Fibrosis Bronchial Epithelium. PloS ONE 8, e78593.

Schmid, A., Baumlin, N., Ivonnet, P., Dennis, J.S., Campos, M., Krick, S., et al. (2015). Roflumilast partially reverses smoke-induced mucociliary dysfunction. Respir. Res. 16, 135.

Seybold, Z.V., Mariassy, A.T., Stroh, D., Kim, C.S., Gazeroglu, H. and Wanner, A. (1990). Mucociliary interaction in vitro: effects of physiological and inflammatory stimuli. J. Appl. Physiol. 68, 1421-1426.

Shephard, K.L. and Rahmoune, H. (1994). Evaporation-induced changes in airway surface liquid on an isolated guinea pig trachea. J. Appl. Physioly. 76, 1156-1165.

Siu, K.K.W., Morgan, K.S., Paganin, D.M., Boucher, R., Uesugi, K., Yagi, N., et al. (2008). Phase contrast X-ray imaging for the non-invasive detection of airway surfaces and lumen characteristics in mouse models of airway disease. Eur. J. Radiol. 68, S22-S26.

Song, Y., Namkung, W., Nielson, D.W., Lee, J.W., Finkbeiner, W.E. and Verkman, A.S. (2009). Airway surface liquid depth measured in ex vivo fragments of pig and human trachea: dependence on Na+ and Cl- channel function. Am. J. Physiol. Lung Cell. Mol. Physiol. 297, L1131-1140.

Tarran, R., Grubb, B.R., Gatzy, J.T., Davis, C.W. and Boucher, R.C. (2001). The relative roles of passive surface forces and active ion transport in the modulation of airway surface liquid volume and composition. J. Gen. Physiol. 118, 223-236.

Tarran, R. and Boucher, R.C. (2002). Thin-film measurements of airway surface liquid volume/composition and mucus transport rates in vitro. Cystic fibrosis methods and protocols. Springer, pp.479-492.

Tarran, R., Button, B., Picher, M., Paradiso, A.M., Ribeiro, C.M., Lazarowski, E.R., et al. (2005). Normal and cystic fibrosis airway surface liquid homeostasis The effects of phasic shear stress and viral infections. J. Biol. Chem. 280, 35751-35759.

Tarran, R., Trout, L., Donaldson, S.H. and Boucher, R.C. (2006). Soluble mediators, not cilia, determine airway surface liquid volume in normal and cystic fibrosis superficial airway epithelia. J. Gen. Physiol. 127, 591-604.

Widdicombe, J. and Widdicombe, J. (1995). Regulation of human airway surface liquid. Respir. Physiol. 99, 3-12.

Wu, D.X.Y., Lee, C., Widdicombe, J. and Bastacky, J. (1996). Ultrastructure of tracheal surface liquid: low-temperature scanning electron microscopy. Scanning 18, 589-592.

Wu, D.X.-Y., Lee, C.Y.C., Uyekubo, S.N., Choi, H.K., Bastacky, S.J. and Widdicombe, J.H. (1998). Regulation of the depth of surface liquid in bovine trachea. Am. J. Physiol. Lung Cell. Mol. Physiol. 274, L388-L395.

Xu, X., Balsiger, R., Tyrrell, J., Boyaka, P.N., Tarran, R. and Cormet-Boyaka, E., 2015. Cigarette smoke exposure reveals a novel role for the MEK/ERK1/2 MAPK pathway in regulation of CFTR. Biochimica et biophysica acta. 1850(6), 1224-1232.

Yager, D., Cloutier, T., Feldman, H., Bastacky, J., Drazen, J. and Kamm, R., 1994. Airway surface liquid thickness as a function of lung volume in small airways of the guinea pig. Journal of Applied Physiology. 77(5), 2333-2340.

Zhang, S., Blount, A.C., Mcnicholas, C.M., Skinner, D.F., Chestnut, M., Kappes, J.C., et al., 2013. Resveratrol enhances airway surface liquid depth in sinonasal epithelium by increasing cystic fibrosis transmembrane conductance regulator open probability. PloS one. 8(11), e81589.

Event: 1908: Cilia Beat Frequency, Decreased

Short Name: CBF, Decreased

Key Event Component

Process	Object	Action
Abnormal ciliary motility	motile cilium	occurrence

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:411 - Oxidative stress [MIE] Leading to Decreased Lung Function [AO]	KeyEvent

Stressors

Name
Cigarette smoke
Acetaldehyde
Acrolein
Nicotine
Ozone

Biological Context

Level of Biological Organization
Cellular

Cell term

Cell term
multi-ciliated epithelial cell

Organ term

Organ term
lung epithelium

Evidence for Perturbation by Stressor

Cigarette smoke

Treatment of human sinonasal epithelial cells with cigarette smoke condensate for 3 minutes significantly reduced forskolin-stimulated CBF (Cohen et al., 2009). CBF was also decreased in differentiated normal human bronchial epithelial cells exposed to whole cigarette smoke (Schmid et al., 2015), in cilia-bearing explant adenoid tissues treated with 5 and 10% cigarette smoke extract (Wang et al., 2012), in hamster oviducts treated various mainstream cigarette smoke fractions (Knoll et al., 1995), and in nasal epithelial cells fom smokers with moderate and severe chronic obstructive pulmonary disease (COPD) (Yaghi et al., 2012).

Acetaldehyde

A concentration-dependent decrease in CBF has been observed after treatment with aldehydes. For example inhibition of cilia ATPase activity was observed after treatment with acetaldehyde, in ciliated bovine bronchial epithelial cells (Sisson et al., 1991).

Acrolein

Acrolein, an aldehyde in the gas phase of cigarette smoke, induced ciliostasis at high concentrations (> 1 mM), after 5 min of treatment, and cellular necrosis after 3 hr. However, at lower concentrations (from 0.5−1 mM), acrolein transiently reduced the CBF to 4 Hz (Romet et al., 1990).

Nicotine

Normal human bronchial epithelial cells exposed to aerosolized nicotine showed decreased CFTR and BK conductance, CBF, ASL volume, and decreased expression of FOXJ1 and KCNMA1 (Garcia-Arcos et al., 2016).

Ozone

Continuous, exposure of human nasal epithelial cells to different concentrations of ozone at 37°C for up to 4 weeks slightly (but not significantly) reduced CBF in healthy mucosa (7.1% at 500 µg/m3 and 10.3% at 1000 µg/m3), and significantly in chronically inflamed mucosa (20.5/16.4%) at 2 weeks. During the third and fourth week of exposure at these higher concentrations CBF was significantly reduced in both healthy (after 3 weeks: 18.7/37.5%; after 4 weeks: 11.1/33.3%) and chronically inflamed mucosa (after 3 weeks: 33.8/26.8%; after 4 weeks: 21.4/38.6%). Low ozone concentrations (100 µg/m3) appeared to not have an effect on CBF (Gosepath et al., 2000).

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	Moderate	NCBI
Oryctolagus cuniculus	Oryctolagus cuniculus	High	NCBI
Bos taurus	Bos taurus	High	NCBI
Cavia porcellus	Cavia porcellus	Moderate	NCBI
Lithobates catesbeianus	Rana catesbeiana	High	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Mixed	Moderate

Age-dependent decreases in CBF have been demonstrated in several species (e.g. guinea pigs, mice, and human) (Bailey et al., 2014; Grubb et al., 2016; Ho et al., 2001; Joki and Saano, 1997; Paul et al., 2013). In a study with 46 healthy subjects with a wide age distribution (mean 42, range 19–81 years), age was found to be negatively associated with airway clearance of inhaled 6-µm Teflon particles (Svartengren et al., 2005).

Female hormones, i.e. progesterone and estrogen, have been shown to have direct effect on CBF, i.e., progesterone reduces CBF, 17β-estradiol and progesterone receptor antagonists counteract progesterone effects, but estradiol alone has also been shown to have no effect on CBF. However, the mechanism by which these hormones modulate CBF is yet to be elucidated (Jain et al., 2012; Jia et al., 2011).

Key Event Description

Cohesive beating of cilia lining the upper and lower respiratory tract is critical for efficient MCC. CBF is influenced by several factors including changes in the physical and chemical properties of the ASL (especially the periciliary fluid), structural modulation in the cilia, concentration of cyclic nucleotides cAMP and cGMP, and intracellular calcium (Ca^{2+}). Formation of cyclic nucleotides such as cGMP is mediated by nitric oxide (NO), which is released by an enzyme family of nitric oxide synthases (NOSs) when the substrate L-arginine (L-Arg) is transformed to L-citrulline. NO activates its receptor protein, soluble guanylate cyclase (sGC), which catalyzes formation of cGMP from guanosine triphosphate (GTP). cGMP then activates protein kinase G (PKG) which has been implicated in the regulation of CBF (Jiao et al., 2011; Li et al., 2000). NO-dependent stimulation of CBF has also been associated with an increase in cAMP-dependent protein kinase A (PKA) (Di Benedetto et al., 1991; Lansley et al., 1992; Salathe et al., 1993; Sanderson and Dirksen, 1989; Schmid et al., 2007; Sisson et al., 1999; Uzlaner and Priel, 1999). An increase in intracellular endogenous cAMP was observed after treatment with isobutyl-1-methylxanthine that also increased CBF (Tamaoki et al., 1989). cAMP accumulation in the airway cilia has been shown to be dependent on Ca2+–calmodulin-dependent PDE1A and indirectly regulates CBF (Kogiso et al., 2018). Increase in CBF after treatment with NO substrate, L-arginine and inhibition of CBF by a NOS inhibitor, N-omega-nitro-L-arginine methyl ester (L-NAME) further provides evidence for the role of NO in increasing CBF (Jiao J. et al., 2011; Sisson J. H., 1995; Uzlaner and Priel, 1999; Yang et al., 1997).
Modulation of CBF is not always accompanied by changes in cAMP levels. PKC activators, phorbol 12-myristate 13-acetate and L-o~-dioctanoylglycerol have been shown to decrease CBF in a concentration- and time-dependent manner in rabbit tracheal epithelial cells (Kobayashi et al., 1989). CBF has been shown to decrease after exposure to inhaled oxidants such as cigarette smoke across different species. A study with 120 subjects showed a significant decrease in nasal CBF following exposure to tobacco smoke (Agius et al., 1998). Exposure to cigarette smoke extract lead to reduction in forskolin-induced CBF in human sinonasal epithelium (Cohen et al., 2009) and isoproterenol- and methacholine-induced CBF in human adenoid tissues (Wang et al., 2012). This decrease in CBF and unresponsiveness to beta-agonist stimulation occurs in parallel to PKC activation and has been shown to be dependent on the duration of exposure to cigarette smoke in mice (Simet et al., 2010). Normal human bronchial epithelial cells exposed to aerosolized nicotine showed decreased CFTR and BK conductance, impaired CBF, ASL volume, and decreased expression of FOXJ1 and KCNMA1 (Garcia-Arcos et al., 2016).
A concentration-dependent decrease in CBF has been observed after treatment with aldehydes. For example inhibition of cilia ATPase activity was observed after treatment with acetaldehyde, in ciliated bovine bronchial epithelial cells (Sisson et al., 1991). Acrolein, an aldehyde in the gas phase of cigarette smoke, induced ciliostasis at high concentrations (> 1 mM), after 5 min of treatment, and cellular necrosis after 3 hr. However, at lower concentrations (from 0.5−1 mM), acrolein transiently reduced the CBF to 4 Hz (Romet et al., 1990).

How it is Measured or Detected

There is no standardized method for measuring CBF. Digital high-speed video imaging with a manual count of CBF in slow motion video play is the most commonly used method for CBF measurement (Kim et al., 2011; Peabody et al., 2018). Photometry and video-microscopy have been used to measure CBF in vitro and ex vivo, including in ciliated bovine bronchial epithelial cells (Allen-Gipson et al., 2011; Sisson et al., 2003; Uzlaner and Priel, 1999), normal human bronchial epithelial cells (Feriani et al., 2017), human nasal epithelial cells (Dimova et al., 2005; Min et al., 1999b), human nasal ciliated epithelium (nasal brushings) (Agius et al., 1998), and mouse tracheal rings (Simet et al., 2010).
CBF measurement in vitro generally involves mounting the tissue at the air-liquid interface on a stage followed by microscopic analysis and acquisition of images and/or video recordings of beating cilia. For in vivo and ex vivo measurements, Doppler optical coherence tomography (D-OCT) can also be applied, a mesoscopic non-contact imaging modality that provides high-resolution tomographic images and detects micromotion simultaneously (Jing et al., 2017). D-OCT has been used to quantitatively measure CBF in ex vivo rabbit tracheal cultures (Lemieux et al., 2015).

References

Agius, A. M., L. A. Smallman, and A. L. Pahor (1998). Age, smoking and nasal ciliary beat frequency. Clin. Otolaryngol. Allied Sci. 23, 227-230.

Allen-Gipson, D.S., Blackburn, M.R., Schneider, D.J., Zhang, H., Bluitt, D.L., Jarrell, J.C., et al. (2011). Adenosine activation of

A(2B) receptor(s) is essential for stimulated epithelial ciliary motility and clearance. Am. J. Physiol. Lung Cell. Mol. Physiol. 301, L171-L180.

Bailey, K.L., Bonasera, S.J., Wilderdyke, M., Hanisch, B.W., Pavlik, J.A., Devasure, J., et al. (2014). Aging causes a slowing in ciliary beat frequency, mediated by PKCε. Am. J. Physiol. Lung Cell. Mol. Physiol. 306, L584-L589.

Cohen, N.A., Zhang, S., Sharp, D.B., Tamashiro, E., Chen, B., Sorscher, E.J., et al. (2009). Cigarette smoke condensate inhibits transepithelial chloride transport and ciliary beat frequency. Laryngoscope 119, 2269-2274.

Di Benedetto, G., Manara-Shediac, F.S. and Mehta, A. (1991). Effect of cyclic AMP on ciliary activity of human respiratory epithelium. Eur. Respir. J. 4, 789-795.

Dimova, S., Maes, F., Brewster, M.E., Jorissen, M., Noppe, M. and Augustijns, P. (2005). High-speed digital imaging method for ciliary beat frequency measurement. J. Pharmacy Pharmacol 57, 521-526.

Feriani, L., Juenet, M., Fowler, C.J., Bruot, N., Chioccioli, M., Holland, S.M., et al. (2017). Assessing the Collective Dynamics of Motile Cilia in Cultures of Human Airway Cells by Multiscale DDM. Biophys. J. 113, 109-119.

Garcia-Arcos, I., Geraghty, P., Baumlin, N., Campos, M., Dabo, A.J., Jundi, B., et al. (2016). Chronic electronic cigarette exposure in mice induces features of COPD in a nicotine-dependent manner. Thorax 71, 1119-1129.

Gosepath, J., Schaefer, D., Brommer, C., Klimek, L., Amedee, R.G., and Mann, W.J. (2000). Subacute Effects of Ozone Exposure on Cultivated Human Respiratory Mucosa. Am. J. Rhinol. 14, 411-418.

Grubb, B.R., Livraghi-Butrico, A., Rogers, T.D., Yin, W., Button, B. and Ostrowski, L.E. (2016). Reduced mucociliary clearance in old mice is associated with a decrease in Muc5b mucin. Am. J. Physiol. Lung Cell. Mol. Physiol. 310, L860-L867.

Ho, J.C., Chan, K.N., Hu, W.H., Lam, W.K., Zheng, L., Tipoe, G.L., et al. (2001). The Effect of Aging on Nasal Mucociliary Clearance, Beat Frequency, and Ultrastructure of Respiratory Cilia. Am. J. Respir. Crit. Care Med. 163, 983-988.

Jain, R., Ray, J.M., Pan, J.-H. and Brody, S.L. (2012). Sex hormone-dependent regulation of cilia beat frequency in airway epithelium. Am. J. Respir. Crit. Care Med. 46, 446-453.

Jia, S., Zhang, X., He, D.Z., Segal, M., Berro, A., Gerson, T., et al., 2011. Expression and Function of a Novel Variant of Estrogen Receptor–α36 in Murine Airways. Am. J. Respir. Cell Mol. Biol. 45, 1084-1089.

Jiao, J., Wang, H., Lou, W., Jin, S., Fan, E., Li, Y., et al. (2011). Regulation of ciliary beat frequency by the nitric oxide signaling pathway in mouse nasal and tracheal epithelial cells. Exp. Cell Res. 317, 2548-2553.

Jing, J.C., Chen, J.J., Chou, L., Wong, B.J.F. and Chen, Z. (2017). Visualization and Detection of Ciliary Beating Pattern and Frequency in the Upper Airway using Phase Resolved Doppler Optical Coherence Tomography. Sci. Rep. 7, 8522-8522.

Joki, S. and Saano, V. (1997). Influence of ageing on ciliary beat frequency and on ciliary response to leukotriene D4 in guinea-pig tracheal epithelium. Clin. Exp. Pharmacol. Physiol. 24, 166-169.

Kim, W., Han, T.H., Kim, H.J., Park, M.Y., Kim, K.S. and Park, R.W. (2011). An Automated Measurement of Ciliary Beating Frequency using a Combined Optical Flow and Peak Detection. J. Healthc. Inform. Res. 17, 111-119.

Knoll, M., Shaoulian, R., Magers, T. and Talbot, P. (1995). Ciliary beat frequency of hamster oviducts is decreased in vitro by exposure to solutions of mainstream and sidestream cigarette smoke. Biol. Reprod. 53, 29-37.

Kobayashi, K., Tamaoki, J., Sakai, N., Chiyotani, A. and Takizawa, T. (1989). Inhibition of ciliary activity by phorbol esters in rabbit tracheal epithelial cells. Lung 167, 277-284.

Kogiso, H., Hosogi, S., Ikeuchi, Y., Tanaka, S., Inui, T., Marunaka, Y., et al. (2018). [Ca(2+)]i modulation of cAMP-stimulated ciliary beat frequency via PDE1 in airway ciliary cells of mice. Exp. Physiol. 103, 381-390.

Lansley, A.B., Sanderson, M.J. and Dirksen, E.R. (1992). Control of the beat cycle of respiratory tract cilia by Ca2+ and cAMP. Am. J. Physiol. 263, L232-242.

Lemieux, B.T., Chen, J.J., Jing, J., Chen, Z. and Wong, B.J.F. (2015). Measurement of ciliary beat frequency using Doppler optical coherence tomography. Int. Forum Allergy Rhinol. 5, 1048-1054.

Li, D., Shirakami, G., Zhan, X. and Johns, R.A. (2000). Regulation of ciliary beat frequency by the nitric oxide-cyclic guanosine monophosphate signaling pathway in rat airway epithelial cells. Am. J. Respir. Cell Mol. Biol. 23, 175-181.

Min, Y.-G., Ohyama, M., Lee, K.S., Rhee, C.-S., Oh, S.H., Sung, M.-W., et al. (1999). Effects of free radicals on ciliary movement in the human nasal epithelial cells. Auris Nasus Larynx 26, 159-163.

Paul, P., Johnson, P., Ramaswamy, P., Ramadoss, S., Geetha, B. and Subhashini, A.S. (2013). The Effect of Ageing on Nasal Mucociliary Clearance in Women: A Pilot Study. ISRN Pulmonology 2013, 5.

Peabody, J.E., Shei, R.-J., Bermingham, B.M., Phillips, S.E., Turner, B., Rowe, S.M., et al. (2018). Seeing cilia: imaging modalities for ciliary motion and clinical connections. Am. J. Physiol. Lung Cell. Mol. Physiol. 314, L909-L921.

Romet, S., Dubreuil, A., Baeza, A., Moreau, A., Schoevaert, D. and Marano, F. (1990). Respiratory tract epithelium in primary culture: Effects of. Toxicol. In Vitro 4, 399-402.

Salathe, M., Pratt, M.M. and Wanner, A. (1993). Cyclic AMP-dependent phosphorylation of a 26 kD axonemal protein in ovine cilia isolated from small tissue pieces. Am. J. Respir. Cell Mol. Biol. 9, 306-314.

Sanderson, M.J. and Dirksen, E.R. (1989). Mechanosensitive and beta-adrenergic control of the ciliary beat frequency of mammalian respiratory tract cells in culture. Am. Rev. Respir. Dis. 139, 432-440.

Schmid, A., Sutto, Z., Nlend, M.-C., Horvath, G., Schmid, N., Buck, J., et al. (2007). Soluble Adenylyl Cyclase Is Localized to Cilia and Contributes to Ciliary Beat Frequency Regulation via Production of cAMP. J. Gen. Physiol. 130, 99-109.

Schmid, A., Baumlin, N., Ivonnet, P., Dennis, J.S., Campos, M., Krick, S., et al. (2015). Roflumilast partially reverses smoke-induced mucociliary dysfunction. Respir. Res. 16, 135.

Simet, S.M., Sisson, J.H., Pavlik, J.A., Devasure, J.M., Boyer, C., Liu, X., et al. (2010). Long-term cigarette smoke exposure in a mouse model of ciliated epithelial cell function. Am. J. Respir. Cell Mol. Biol. 43, 635-640.

Sisson, J.H. (1995). Ethanol stimulates apparent nitric oxide-dependent ciliary beat frequency in bovine airway epithelial cells. Am. J. Physiol. 268, L596-600.

Sisson, J.H., May, K. and Wyatt, T.A. (1999). Nitric oxide-dependent ethanol stimulation of ciliary motility is linked to cAMP-dependent protein kinase (PKA) activation in bovine bronchial epithelium. Alcohol Clin. Exp. Res. 23, 1528-1533.

Sisson, J.H., Stoner, J., Ammons, B. and Wyatt, T. (2003). All-digital image capture and whole-field analysis of ciliary beat frequency. J. Microsc. 211, 103-111.

Sisson, J.H., Tuma, D.J. and Rennard, S.I. (1991). Acetaldehyde-mediated cilia dysfunction in bovine bronchial epithelial cells. Am. J. Physiol. 260, L29-36.

Svartengren, M., Falk, R. and Philipson, K. (2005). Long-term clearance from small airways decreases with age. Eur. Respir. J. 26, 609-615.

Tamaoki, J., Kondo, M. and Takizawa, T. (1989). Effect of cAMP on ciliary function in rabbit tracheal epithelial cells. J. Appl. Physiol. 66, 1035-1039.

Uzlaner, N. and Priel, Z. (1999). Interplay between the NO pathway and elevated [Ca(2+)](i) enhances ciliary activity in rabbit trachea. J. Physiol. 516, 179-190.

Wang, L.F., White, D.R., Andreoli, S.M., Mulligan, R.M., Discolo, C.M. and Schlosser, R.J. (2012). Cigarette smoke inhibits dynamic ciliary beat frequency in pediatric adenoid explants. Otolaryngol. Head Neck Surg. 146, 659-663.

Yaghi, A., Zaman, A., Cox, G. and Dolovich, M.B. (2012). Ciliary beating is depressed in nasal cilia from chronic obstructive pulmonary disease subjects. Respir. Med. 106, 1139-1147.

Yang, B., Schlosser, R.J. and Mccaffrey, T.V. (1997). Signal transduction pathways in modulation of ciliary beat frequency by methacholine. Ann. Otol. Rhinol. Laryngol. 106, 230-236.

Event: 1909: Mucociliary Clearance, Decreased

Short Name: MCC, Decreased

Key Event Component

Process	Object	Action
mucociliary clearance trait		decreased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:411 - Oxidative stress [MIE] Leading to Decreased Lung Function [AO]	KeyEvent

Stressors

Name

Name
Sulfur dioxide
Formaldehyde
PM10
Nitric oxide
Ozone
Cigarette smoke

Biological Context

Level of Biological Organization
Individual

Evidence for Perturbation by Stressor

Sulfur dioxide

SO_2 exposure of dogs dose-dependently decreased CBF and also caused a marked decrease in mean bronchial mucociliary clearance (from 53.7 ± 5.7% to 32.8 ± 7.7%) after 90 min (Yeates et al., 1997). In guinea pig tracheas, SO_2 exposure affected CBF, albeit non-significantly, and mucociliary activity (Knorst et al., 1994).

Formaldehyde

Treatment of frog palate epithelium with different concentrations of formaldehyde induced significant decreases in CBF and MCC (Fló-Neyret et al., 2001; Morgan et al., 1984). Exposure of F344 rats to formaldehyde caused epithelial adaptation of the nasal epithelium, effectively reducing the number of ciliated cells (and hence cilia beating activity) through squamous metaplasia. At the same time, formaldehyde exposure resulted in “ciliastasis” or loss of ciliary activity in a concentration- and exposure duration-dependent manner as well as in a slowing of mucus flow rates (Morgan et al., 1986).

PM10

Incubation of frog palates with PM10 from Sao Paolo, Brazil, for up to 120 min decreased mucociliary transport at concentrations ≥1000 pg/m^3 (Macchione et al., 1999).

Nitric oxide

In New Zealand white rabbits exposed to 3 ppm NO_2 for 24 h, the average CBF decreased from 764 beats/min to 692 beats/min and the transport velocity decreased from 5.23 mm/min to 3.03 mm/min (Kakinoki, 1998).

Ozone

Acute exposure (2 h) of adult ewes to 1.0 ppm ozone significantly reduced tracheal mucus transport velocity (TMV) at 40 min and 2 h post-exposure. Repeated exposure to 1.0 ppm ozone for 5 hper day, for 4 consecutive days showed a progressively significant decrease in TMV on the first and second days, and stabilized over the third and fourth days, around values ranging from -42% to -55% of the initial baseline. TMV remained depressed even after the end of exposure, persisting up to 5 days post-exposure (Allegra et al., 1991).

Cigarette smoke

Nasomuciliary clearance time (determined by saccharin transit test) was significantly higher in smokers than in non-smokers 8 h after smoking (16 ± 6 min vs 10 ± 4 min) and insignicantly higher immediately after smoking (11 ± 6 min vs 10 ± 4 min). Nasomuciliary clearance time correlated positively with cigarettes per day and packs/year index (Proença et al., 2011).

In a small Indian cross-sectional study, the mean nasomuciliary clearance (determined by saccharin transit test) in smokers was significantly higher than that of nonsmokers (481.2 ± 29.83 s vs 300.32 ± 17.4 s). In addition, mean nasomuciliary clearance increased as the duration of smoking increased (NMC in smoking <1 year = 492.25 ± 79.93 s, NMC in smoking for 1-5 years = 516.7 ± 34.01 s, and NMC in smoking >5 years = 637.5 ± 28.49 s) (Baby et al., 2014).

Nasomuciliary clearance (determined by saccharin transit test) in active and passive smokers was significantly higher than in non-

smokers (23.08 ± 4.60 min; 20.31 ± 2.51 min vs 8.57 ± 2.12 min) (Yadav et al., 2014).

Nasomuciliary clearance (determined by saccharin transit test) was significantly higher in active smokers than in passive smokers and non-smokers (23.59 ± 12.41 min vs 12.6 ± 4.67 min; 6.4 ± 1.55 min) (Habesoglu et al., 2012).

Nasomuciliary clearance time (determined by saccharin transit test) in smokers was significantly higher than in former smokers and non-smokers (15.6 min vs 11.77 min and 11.71 min, respectively) (Pagliuca et al., 2015).

Moderate and heavy smokers had higher saccharin transit test times than light smokers and non-smokers, and there was a positive correlation between STT and cigarettes/day (Xavier et al., 2013).

The median nasal mucociliary clearance time (determined by saccharin transit test) was significantly higher in smokers (who smoked a mean of 20.6 cigarettes (median: 20) per day) than in nonsmokers (12 (interquartile range: 5–33) min vs 9 (interquartile range: 4–12) min) (Dülger et al., 2018).

Nasal mucociliary clearance time (determined by saccharin transit test) in smokers was significantly higher than in non-smokers (536.19 ± 254.81 s vs 320.43 ± 184.98 s) and correlated with the numbers of cigarettes per day, pack-years and smoking duration (Solak et al., 2018).

Current smokers had a median (IQR) mucociliary clearance transit time (determined by saccharin transit test) of 13.15 (9.89–16.08) min, which was significantly longer compared with that of never smokers at 7.24 (5.73–8.73) min, former smokers at 7.26 (6.18–9.17) min, exclusive e-cigarette users at 7.00 (6.38–9.00) min, and exclusive heated tobacco product users at 8.00 (6.00–8.00) min (Polosa et al., 2021).

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	High	NCBI
Sus scrofa domesticus	Sus scrofa domesticus	Moderate	NCBI
Ovis aries	Ovis aries	Moderate	NCBI
Cavia porcellus	Cavia porcellus	Moderate	NCBI
Canis lupus	Canis lupus	Moderate	NCBI
Rana catesbeiana	Rana catesbeiana	Moderate	NCBI
Oryctolagus cuniculus	Oryctolagus cuniculus	Moderate	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Mixed	High

Key Event Description

In healthy adults, tracheal mucus movement varies from 4 to >20 mm/min (Stannard and O'Callaghan, 2006), whereas mucociliary clearance (MCC) in the small airways is slower due to the lower number of ciliated cells (fewer cilia) and their shorter length (Foster et al., 1980; Iravani, 1969; Wanner et al., 1996).
Since optimal MCC is dependent in multiple factors, including cilia number and structure as well as ASL and mucus properties, any disturbances of these can lead to impaired MCC. While high humidity or infection can enhance MCC, long-term exposure to noxious substances (e.g. cigarette smoke) lead to decreased mucus clearance from the airways. In most instances this is reflected by decreased mucus transport rates or velocities.

How it is Measured or Detected

In humans, MCC has been assessed traditionally following inhalation of radio-labeled particles such as 99Tcm-labeled polystyrene particles, resin particles or serum albumin and following their clearance at regular intervals by radioimaging using gamma cameras (Agnew et al., 1986; Kärjä et al., 1982). Taking into account inhalation volumes and flow rates, lung airflow, particle deposition and retention, clearance rates can be calculated and effects of e.g. drugs on MCC can be examined. Alternatively, since MCC occurs at a similar rate in the nose to that in trachea and bronchi (Andersen and Proctor, 1983; Rutland and Cole, 1981) and for ease of use,

measurements of MCC can be restricted to that of nasal MCC only. Probably one of the simplest methods is the saccharin transit test (STT). For this test, a small particle of saccharin is placed behind the anterior end of the inferior turbinate. The saccharin will be transported by mucociliary action toward the nasopharynx, where its sweet taste is perceived. When MCC is impaired, saccharin transit times will increase, with a 10- to 20-minute delay being considered a clinical sign of decreased MCC. Using the same principle, the test can also be performed or complemented with dyes such as indigo carmine or methylene blue (Deborah and Prathibha, 2014).

In experimental animals, MCC has been evaluated by gamma-scintigraphy (Greiff et al., 1990; Hua et al., 2010; Read et al., 1992), fluorescence videography/fluoroscopy (in explanted tracheas etc.) (Grubb et al., 2016; Rogers et al., 2018), or by 3D-SPECT (Ortiz Belda et al., 2016). Direct observation of particle movement across airway epithelia to determine mucus velocity or transport rates by using a fiberoptic bronchoscope may be helpful when working in larger animals such as dogs (King, 1998).
In vitro, freshly excised frog palate preparations have been used to assess cilia function and mucociliary transport by videomicroscopy (Macchione et al., 1995; Macchione et al., 1999; Trindade et al., 2007). Murine and human nasal, bronchial and small airway epithelial models grown at the air-liquid interface are also suitable in vitro test systems for determining mucus transport by tracing inert particle movement with a set-up similar to that used for assessing CBF (Benam et al., 2018; Fliegauf et al., 2013; Knowles and Boucher, 2002; Sears et al., 2015).

References

Agnew, J., Sutton, P., Pavia, D. and Clarke, S. (1986). Radioaerosol assessment of mucociliary clearance: towards definition of a normal range. Brit. J. Radiol. 59, 147-151.

Allegra, L., Moavero, N., and Rampoldi, C. (1991). Ozone-induced impairment of mucociliary transport and its prevention with N-acetylcysteine. Am. J. Med. 91, S67-S71.

Andersen, I. and Proctor, D. (1983). Measurement of nasal mucociliary clearance. Eur. J. Respir. Dis. Suppl. 127, 37-40.

Baby, M.K., Muthu, P.K., Johnson, P., and Kannan, S. (2014). Effect of cigarette smoking on nasal mucociliary clearance: A comparative analysis using saccharin test. Lung India 31, 39-42.

Benam, K.H., Vladar, E.K., Janssen, W.J. and Evans, C.M. (2018). Mucociliary defense: emerging cellular, molecular, and animal models. Ann. Am. Thorac. Soc. 15, S210-S215.

Deborah, S. and Prathibha, K., 2014. Measurement of nasal mucociliary clearance. Clin. Res. Pulmonol. 2, 1019.

Dülger, S., Akdeniz, Ö., Solmaz, F., Şengören Dikiş, Ö., and Yildiz, T. (2018). Evaluation of nasal mucociliary clearance using saccharin test in smokers: A prospective study. Clin. Respir. J. 12, 1706-1710.

Fliegauf, M., Sonnen, A.F.P., Kremer, B. and Henneke, P. (2013). Mucociliary Clearance Defects in a Murine In Vitro Model of Pneumococcal Airway Infection. PloS ONE 8, e59925.

Fló-Neyret, C., Lorenzi-Filho, G., Macchione, M., Garcia, M.L.B. and Saldiva, P.H.N. (2001). Effects of formaldehyde on the frog's mucociliary epithelium as a surrogate to evaluate air pollution effects on the respiratory epithelium. Braz. J. Med. Biol. Res. 34, 639-643.

Foster, W., Langenback, E. and Bergofsky, E. (1980). Measurement of tracheal and bronchial mucus velocities in man: relation to lung clearance. J. Appl. Physiol. 48, 965-971.

Greiff, L., Wollmer, P., Erjefält, I., Pipkorn, U. and Persson, C. (1990). Clearance of 99mTc DTPA from guinea pig nasal, tracheobronchial, and bronchoalveolar airways. Thorax 45, 841-845.

Grubb, B.R., Livraghi-Butrico, A., Rogers, T.D., Yin, W., Button, B. and Ostrowski, L.E. (2016). Reduced mucociliary clearance in old mice is associated with a decrease in Muc5b mucin. Am. J. Physiol. Lung Cell. Mol. Physiol. 310, L860-L867.

Habesoglu, M., Demir, K., Yumusakhuylu, A.C., Sahin Yilmaz, A., and Oysu, C. (2012). Does passive smoking have an effect on nasal mucociliary clearance? Otolaryngol Head Neck Surg. 147, 152-156.

Hua, X., Zeman, K.L., Zhou, B., Hua, Q., Senior, B.A., Tilley, S.L., et al. (2010). Noninvasive real-time measurement of nasal mucociliary clearance in mice by pinhole gamma scintigraphy. J. Appl. Physiol. 108, 189-196.

Iravani, J. (1969). Zum Mechanismus der Ortsabhängigkeit der Flimmeraktivität im Bronchialbaum/Location-Dependent Activity of the Ciliary Movement in the Bronchial Tree and its Possible Mechanism. In: Habermann E. et al. (eds) Naunyn Schmiedebergs Archiv für Pharmakologie. Springer, Berlin, Heidelberg.

Kakinoki Y, Ohashi Y, Tanaka A, Washio Y, Yamada K, Nakai Y, Morimoto K. (1998). Nitrogen dioxide compromises defence functions of the airway epithelium. Acta Oto-Laryngol. 118, 221-226.

Kärjä, J., Nuutinen, J. and Karjalainen, P. (1982). Radioisotopic Method for Measurement of Nasal Mucociliary Activity. Arch. Otolaryngol. 108, 99-101.

King, M. (1998). Experimental models for studying mucociliary clearance. Eur. Respir. J. 11, 222-228.

Knorst, M.M., Kienast, K., Riechelmann, H., Müller-Quernheim, J. and Ferlinz, R. (1994). Effect of sulfur dioxide on mucociliary activity and ciliary beat frequency in guinea pig trachea. Int. Arch. Occup. Environm. Health 65, 325-328.

Knowles, M.R. and Boucher, R.C. (2002). Mucus clearance as a primary innate defense mechanism for mammalian airways. J. Clin. Invest. 109, 571-577.

Macchione, M., Guimarães, E., Saldiva, P. and Lorenzi-Filho, G. (1995). Methods for studying respiratory mucus and mucus clearance. Braz. J. Med. Biol Res. 28, 1347.

Macchione, M., Oliveira, A.P., Gallafrio, C.T., Muchão, F.P., Obara, M.T., Guimarães, E.T., et al. (1999). Acute effects of inhalable particles on the frog palate mucociliary epithelium. Environm. Health Persp. 107, 829-833.

Morgan, K., Patterson, D. and Gross, E. (1986). Responses of the nasal mucociliary apparatus of F-344 rats to formaldehyde gas. Toxicol. Appl. Pharmacol. 82, 1-13.

Morgan, K.T., Patterson, D.L. and Gross, E.A. (1984). Frog palate mucociliary apparatus: structure, function, and response to formaldehyde gas. Fund. Appl. Toxicol. 4, 58-68.

Ortiz Belda, J.L., Ortiz, A., Milara Payá, J., Armengot Carceller, M., Sanz García, C., Compañ Quilis, D., et al. (2016). Evaluation of Mucociliary Clearance by Three Dimension Micro-CT-SPECT in Guinea Pig: Role of Bitter Taste Agonists. Plos ONE 11, e0164399.

Pagliuca, G., Rosato, C., Martellucci, S., De Vincentiis, M., Greco, A., Fusconi, M., et al. (2015). Cytologic and functional alterations of nasal mucosa in smokers: temporary or permanent damage? Otolaryngol Head Neck Surg 152, 740-745.

Proença, M., Xavier, R.F., Ramos, D., Cavalheri, V., Pitta, F., and Ramos, E.C. (2011). Immediate and short term effects of smoking on nasal mucociliary clearance in smokers. Revista Portuguesa de Pneumologia (English Edition) 17), 172-176.

Read, R.C., Roberts, P., Munro, N., Rutman, A., Hastie, A., Shryock, T., et al. (1992). Effect of Pseudomonas aeruginosa rhamnolipids on mucociliary transport and ciliary beating. J. Appl. Physiol. 72, 2271-2277.

Rogers, T.D., Ostrowski, L.E., Livraghi-Butrico, A., Button, B. and Grubb, B.R., 2018. Mucociliary clearance in mice measured by tracking trans-tracheal fluorescence of nasally aerosolized beads. Sci. Rep. 8, 1-12.

Rutland, J. and Cole, P.J. (1981). Nasal mucociliary clearance and ciliary beat frequency in cystic fibrosis compared with sinusitis and bronchiectasis. Thorax 36, 654-658.

Sears, P.R., Yin, W.-N. and Ostrowski, L.E. (2015). Continuous mucociliary transport by primary human airway epithelial cells in vitro. Am. J. Physiol. Lung Cell. Mol. Physiol. 309, L99-L108.

Solak, I., Marakoglu, K., Pekgor, S., Kargin, N.C., Alataş, N., and Eryilmaz, M.A. (2018). Nasal mucociliary activity changes in smokers. Konuralp Med. J. 10, 269-275.

Stannard, W. and O'callaghan, C. (2006). Ciliary function and the role of cilia in clearance. J. Aerosol Med. 19, 110-115.

Trindade, S.H.K., De Mello Júnior, J.F., De Godoy Mion, O., Lorenzi-Filho, G., Macchione, M., Guimarães, E.T., et al. (2007). Methods for Studying Mucociliary Transport. Braz. J. Otorhinolaryngol. 73, 704-712.

Wanner, A., Salathe, M. and O'riordan, T.G. (1996). Mucociliary clearance in the airways. Am. J. Respir. Crit. Care Med. 154, 1868-1902.

Xavier, R.F., Ramos, D., Ito, J.T., Rodrigues, F.M., Bertolini, G.N., Macchione, M., et al. (2013). Effects of cigarette smoking intensity on the mucociliary clearance of active smokers. Respiration 86, 479-485.

Yadav, J., and Kaushik, G. (2014). K Ranga R. Passive smoking affects nasal mucociliary clearance. J. Indian Acad. Clin. Med. 15, 96-99.

Yeates, D.B., Katwala, S.P., Daugird, J., Daza, A.V. and Wong, L.B. (1997). Excitatory and inhibitory neural regulation of tracheal ciliary beat frequency (CBF) activated by ammonia vapour and SO2. Ann. Occup. Hyg. 41, 736-744.

Event: 1910: Mucus Viscosity, Increased

Short Name: Mucus Viscosity, Increased

Key Event Component

Process	Object	Action
glycosylation	mucin-5AC	increased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:411 - Oxidative stress [MIE] Leading to Decreased Lung Function [AO]	KeyEvent

Biological Context

Level of Biological Organization

Organ

Organ term

Organ term

lung

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	High	NCBI
Mus musculus	Mus musculus	Moderate	NCBI
Canis lupus familiaris	Canis lupus familiaris	Moderate	NCBI
Equus caballus	Equus caballus	Moderate	NCBI
Lithobates catesbeianus	Rana catesbeiana	Moderate	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Mixed	High

Key Event Description

Various mucosal surfaces, such as the luminal sides of the urogenital, gastrointestinal, reproductive and respiratory tracts, are lined by mucus, a complex biopolymer that forms a barrier to environmental insults and maintains lubrication (Lai Samuel K. et al., 2009). Chemically, mucus consists of large glycoproteins called mucins (MUCs). Both secreted, gel-forming mucins (e.g. MUC2, MUC5AC, MUC5B and MUC19) and membrane-bound mucins (e.g. MUC1, MUC4, MUC13, MUC16, MUC20, MUC21 and MUC22) are found in the lungs (Atanasova and Reznikov, 2019). Mucins are very heterogeneous, with protein backbones and carbohydrates making up approx. 20% and 80% of their molecular weight, respectively. Cysteine residues in the carboxy and amino terminals of mucin backbones facilitate end-to-end disulfide bonding, resulting in dimerization and multimerization (Ma et al., 2018; Rose and Voynow, 2006). This results in a complex hydrated porous molecular network or gel aggregates that, together with secreted host defense proteins, DNA, lipids, cellular debris and immune cells, make up airway mucus (Atanasova and Reznikov, 2019; Thornton and Sheehan, 2004).

According to Girod et al., “Mucus is a highly non-Newtonian viscoelastic material. Under a discontinuous stress, induced by ciliary motion during active stroke or by cough, the mucus starts to instantaneously deform and, once the stress is removed (as during the recovery period of beating or after cessation of coughing), the mucus relaxes…” (Girod et al., 1992). Mucin content in mucus typically accounts for 2–5% of mucus, with MUC5AC and MUC5AB being the most abundant mucins in airway mucus, whereas water accounts for between 90–98% of mucus mass. Increased mucin production, differential mucin glycosylation or a change in the proportions of the various mucins as is seen in many pulmonary diseases (e.g. cystic fibrosis, asthma, COPD) can therefore increase mucus viscosity. Water availability in and ionic composition of the immediate environment also influence the physical properties of mucus (Hill et al., 2018; Thornton et al., 2008). For example, there is a 5- to 10-fold greater mucin-to-water ratio in patients with cystic fibrosis than in healthy subjects. This results from the CFTR defect-induced inadequate airway hydration and imbalances in ASL ion concentrations that lead to increased mucus viscosity, causes mucus impaction to the consistency of rubber and hence hampers effective mucociliary transport (Fahy and Dickey, 2010; Gheber et al., 1998; Lai et al., 2009).

How it is Measured or Detected

There is no standardized method to determine increased mucus viscosity. In their recent review “Strategies for measuring airway mucus and mucins”, Atanasova and Reznikov as well as Chen and colleagues describe the most widely applied methods for collection and analysis of mucus as well as the associated challenges (Atanasova and Reznikov, 2019; Chen et al., 2019).
Because both physical and chemical properties of mucus are dependent on its composition, many studies examine mucin content of or the contributions of the various mucin proteins to a given mucus sample, by using for example chromatography (historically) and, more recently, mass spectrometry (Atanasova and Reznikov, 2019). The latter not only permits the distinction between the different mucin proteins and their abundances but also facilitates the analysis of glycosylation patterns (Jensen et al., 2010; Mulagapati et al., 2017). This may provide useful insights into mucus viscoelastic properties, because mucin backbone O-glycosylation was linked to higher molecular rigidity, extended conformation and increased hydration (Gum, 1992; Verdugo, 2012).
Simpler methods that do not require mucus or sputum collection include imaging after staining of histological specimens with special stains, such as Alcian Blue and Periodic Acid–Schiff, lectins and mucin antibodies and subsequent quantitative image analysis (Atanasova and Reznikov, 2019). This approach can be applied to both in vitro systems (e.g. 3D organotypic airway cultures) and ex vivo tissues.
In addition to focusing on mucins, direct rheometry–still considered the gold standard to determine viscosity and elasticity–is performed to characterize the physical properties of mucus (Atanasova and Reznikov, 2019). There are dynamic and non-dynamic techniques that can be used and, independent of the method chosen, two parameters are normally examined: (i) viscosity or loss modulus (G″), which is the extent to which the gel resists the tendency to flow, and (ii) elasticity or storage modulus (G′), which measures the tendency for the gel to recover its original shape following stress-induced deformation (Girod et al., 1992; Lai et al., 2009). The most common rheometers are the rotational rheometers, which measure the macrorheological properties of a sample. In a rotational rheometer, a continuous shear motion is applied to the material of interest by the relative rotative motion of two surfaces (https://lsinstruments.ch/en/theory/rheology/rheometers; accessed 4 June 2021). However, other types of rheometers, such as a cone-and-plate rheometer or a capillary viscometer can also be used (Chen et al., 2019; Lai et al., 2014). All these techniques draw on the behavior of mucus when subjected to different shear rates, torques, strains or tractions (Atanasova and Reznikov, 2019). The choice will depend on both the availability of the equipment and the sample size. Because rheometry requires rather large sample volumes, more novel techniques utilizing fluorescence imaging have been developed to interrogate mucus viscoelasticity in, for example, in vitro studies. One of these techniques involves the tracking of particle movement, another fluorescence recovery after photobleaching (FRAP) (Lai et al., 2009).
Current knowledge indicates that airway mucus has intermediate viscoelasticity, with a viscosity in the range of 12–15 Pa ·s, a relaxation time of ca. 40 s and an elastic modulus of 1 Pa (Chen et al., 2019; Lai et al., 2009). In comparison, mucus from cystic fibrosis patients, which exhibits altered mucin glycosylation patterns (Boat et al., 1976) as well as lower water and salt content (Baconnais et al., 1999; Kopito et al., 1973), has a much higher viscosity that can reach up to 110 Pa ·s at shear rates of 0.1 s^{-1} (Carlson et al., 2018; Rose and Voynow, 2006; Rubin, 2007).

References

Atanasova, K.R. and Reznikov, L.R. (2019). Strategies for measuring airway mucus and mucins. Respir. Res. 20, 261.

Baconnais, S., Tirouvanziam, R., Zahm, J.M., De Bentzmann, S., Péault, B., Balossier, G., et al. (1999). Ion composition and rheology of airway liquid from cystic fibrosis fetal tracheal xenografts. Am. J. Respir. Cell Mol. Biol. 20, 605-611.

Carlson, T.L., Lock, J.Y. and Carrier, R.L. (2018). Engineering the Mucus Barrier. Annu. Rev. Biomed. Eng. 20, 197-220.

Chen, Z., Zhong, M., Luo, Y., Deng, L., Hu, Z. and Song, Y. (2019). Determination of rheology and surface tension of airway surface liquid: a review of clinical relevance and measurement techniques. Respir. Res. 20, 1-14.

Fahy, J.V. and Dickey, B.F. (2010). Airway mucus function and dysfunction. New Engl. J. Med. 363, 2233-2247.

Gheber, L., Korngreen, A. and Priel, Z. (1998). Effect of viscosity on metachrony in mucus propelling cilia. Cell Motil. Cytoskeleton 39, 9-20.

Girod, S., Zahm, J., Plotkowski, C., Beck, G. and Puchelle, E. (1992). Role of the physiochemical properties of mucus in the protection of the respiratory epithelium. Eur. Respir. J. 5, 477-487.

Gum, J. (1992). Mucin genes and the proteins they encode: structure, diversity, and regulation. Am. J. Respir. Cell Mol. Biol. 7, 557-557.

Hill, D.B., Long, R.F., Kissner, W.J., Atieh, E., Garbarine, I.C., Markovetz, M.R., et al. (2018). Pathological mucus and impaired mucus clearance in cystic fibrosis patients result from increased concentration, not altered pH. Eur. Respir. J. 52, 1801297.

Jensen, P.H., Kolarich, D. and Packer, N.H. (2010). Mucin-type O-glycosylation–putting the pieces together. FEBS J. 277, 81-94.

Kopito, L.E., Kosasky, H.J. and Shwachman, H. (1973). Water and electrolytes in cervical mucus from patients with cystic fibrosis. Fertil. Steril. 24, 512-516.

Lai, S.K., Wang, Y.-Y., Wirtz, D. and Hanes, J. (2009). Micro- and macrorheology of mucus. Adv. Drug Deliv. Rev. 61, 86-100.

Lai, Y., Dilidaer, D., Chen, B., Xu, G., Shi, J., Lee, R.J., et al. (2014). In vitro studies of a distillate of rectified essential oils on sinonasal components of mucociliary clearance. Am. J. Rhinol. Allergy 28, 244-248.

Ma, J., Rubin, B.K. and Voynow, J.A. (2018). Mucins, mucus, and goblet cells. Chest 154, 169-176.

Mulagapati, S., Koppolu, V. and Raju, T.S. (2017). Decoding of O-linked glycosylation by mass spectrometry. Biochemistry 56, 1218-1226.

Rose, M.C. and Voynow, J.A. (2006). Respiratory Tract Mucin Genes and Mucin Glycoproteins in Health and Disease. Physiol. Rev. 86, 245-278.

Rubin, B.K. (2007). Mucus structure and properties in cystic fibrosis. Paediatr. Respir. Rev. 8, 4-7.

Thornton, D.J. and Sheehan, J.K. (2004). From mucins to mucus: toward a more coherent understanding of this essential barrier. Proc. Am. Thorac. Soc. 1, 54-61.

Thornton, D.J., Rousseau, K. and Mcguckin, M.A. (2008). Structure and function of the polymeric mucins in airways mucus. Ann. Rev. Physiol. 70, 459-486.

Verdugo, P. (2012). Supramolecular dynamics of mucus. Cold Spring Harb. Perspect. Med. 2, a009597.

Event: 1911: FOXJ1 Protein, Decreased

Short Name: FOXJ1 Protein, Decreased

Key Event Component

Process	Object	Action
	forkhead box protein J1	decreased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:411 - Oxidative stress [MIE] Leading to Decreased Lung Function [AO]	KeyEvent

Stressors

Name
Cigarette smoke
Irradiation

Biological Context

Level of Biological Organization
Cellular

Cell term

Cell term
multi-ciliated epithelial cell

Organ term

Organ term
lung epithelium

Evidence for Perturbation by Stressor

Cigarette smoke

Whole cigarette smoke exposure or treatment with cigarette smoke extract of normal human bronchial epithelial cells significantly lowered FoxJ1 mRNA and protein levels (Milara et al., 2012; Brekman et al., 2014; Valencia-Gattas et al., 2016; Ishikawa and Ito, 2017). Cigarette smoke extract treatment of normal human bronchial epithelial cells also reduced the expression of cilia-related transcription factor genes, including FOXJ1, RFX2, and RFX3, as well as that of cilia motility and structural integrity genes regulated by FOXJ1, including DNAI1, DNAH5, DNAH9, DNAH10, DNAH11, and SPAG6 (Brekman et al., 2014).

Irradiation

Irradiation causes excessive levels of free radicals and associated lipid peroxidation, damage to DNA, proteins, leading to wide-spread cellular damage (Azzam et al., 2012; Koc et al., 2003; Rodrigues-Moreira et al., 2017; Shirazi et al., 2013). Thoracic irradiation reduces FOXJ1 mRNA levels in mouse lungs (Bernard et al., 2012).

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Danio rerio	Danio rerio	High	NCBI
Xenopus laevis	Xenopus laevis	High	NCBI
Mus musculus	Mus musculus	High	NCBI
Homo sapiens	Homo sapiens	High	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Mixed	High

FOXJ1 is functionally conserved throughout diverse groups of metazoans including flatworm Schmidtea mediterranea, zebrafish Danio rerio, African clawed frog Xenopus laevis (Stubbs et al., 2008; Vij et al., 2012; Yu et al., 2008). Ectopic expression of FOXJ1 triggers ciliogenesis in zebrafish and frog (Stubbs et al., 2008; Yu et al., 2008). Overexpression of FOXJ1 transcription factor in the neural tube of a chick induces cilia formation (Cruz C. et al., 2010). There are multiple studies of FOXJ1 in mice and in human cells (Boon et al., 2014; Brekman et al., 2014; Brody et al., 2000; Chen et al., 1998; Choksi et al., 2014). Furthermore, the target genes of FOXJ1, for example RFX3, are regulated by FOXJ1 across different species (Alten et al., 2012; Didon et al., 2013a).

FOXJ1 function is important for all life stages from embryo through adulthood (Choksi et al., 2014; Stauber et al., 2017).

FOXJ1 is expressed in the airways of both males and females. In addition to respiratory tract and brain, FOXJ1 is functionally important also in male and female reproductive tissues (Hackett et al., 1995).

Key Event Description

The epithelium of the respiratory tract has a powerful defense mechanism against air-borne pollutants due to the combined performance of mucus-producing goblet cells and ciliated cells that are covered with microtubule-based projections, the cilia. In response to various irritants and pathogens mucus is secreted by goblet cells, and cilia sweep mucus upward by coordinated beating motions thus clearing the airways from these substances. The ciliated airway epithelial cells are typically covered by hundreds of motile cilia. Cilia formation is initiated and coordinated by a distinct gene expression program, led by the transcription factor forkhead box J1 (FOXJ1) (Brody et al., 2000; Zhou and Roy, 2015). In addition to the respiratory tract, FOXJ1 is expressed also in the ciliated cells of the reproductive and central nervous systems (Blatt et al., 1999; Hackett et al., 1995; Lim et al., 1997).

The multiple motile cilia assembly factors MCIDAS and GMNC converge in positive regulation of FOXJ1 (Arbi et al., 2016; Berta et al., 2016; Stubbs et al., 2012), whereas NOTCH signaling, IL-13-or EGF (epidermal growth factor)-triggered signaling antagonize FOXJ1-driven multiciliogenesis (Gerovac and Fregien, 2016; Gerovac et al., 2014; Gomperts et al., 2007; Shaykhiev et al., 2013). Various other factors are involved in multiple motile cilia assembly, including MYB (acts early in multiciliogenesis downstream of MCIDAS), RFX3 (can act as a co-factor for FOXJ1), ULK4 (modulates the expression of FOXJ1), Wnt signaling, etc. (Choksi et al., 2014; Liu et al., 2016; Schmid et al., 2017; Tan et al., 2013). Most of these factors act upstream or parallel to FOXJ1. FOXJ1 appears to be the major factor in multiciliogenesis, whereby its activity is necessary and also sufficient for programming cells to assemble functional motile cilia (Vij et al., 2012).

FOXJ1 is a master regulator of motile ciliogenesis and is essential to program cells to grow motile cilia (Zhou and Roy, 2015). This key event represents the decrease in the levels or absence of FOXJ1 protein in cells of the respiratory tract. The decrease in FOXJ1 levels inhibits ciliogenesis in multiciliated cells of zebrafish and Xenopus (Stubbs et al., 2008). The knockdown of FOXJ1 results in almost complete absence of cilia in mouse epithelial cells (Brody et al., 2000; Chen J. et al., 1998). On the other hand, the

overexpression of FOXJ1 rescues cigarette smoke-mediated suppression of cilia growth in human airway epithelium (Brekman et al., 2014).

How it is Measured or Detected

FOXJ1 protein levels can be measured by Western blot analysis (Brekman et al., 2014; Didon et al., 2013a; Gomperts et al., 2007; Jacquet et al., 2009; Milara et al., 2012), immunofluorescence (Arbi et al., 2016; Gomperts et al., 2007; Valencia-Gattas et al., 2016) or immunohistochemistry (Abedalthagafi et al., 2016; Danielian et al., 2007; Gao et al., 2015). FOXJ1 protein amounts can be inferred from FOXJ1 mRNA levels that can be measured by real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) (Arbi et al., 2016; Brekman et al., 2014; Didon et al., 2013a; Jacquet et al., 2009; Milara et al., 2012; Stubbs et al., 2012), in situ hybridization (Hackett et al., 1995; Stubbs et al., 2012), and Northern blot analysis (Hackett et al., 1995). In addition, FOXJ1 protein activity can be inferred from FOXJ1 target gene expression levels or from reporter gene expression levels (e.g. luciferase assay) of genes harboring FOXJ1 transcription factor binding sites (Brekman et al., 2014; Lim et al., 1997).

References

Alten, L., Schuster-Gossler, K., Beckers, A., Groos, S., Ulmer, B., Hegermann, J., et al. (2012). Differential regulation of node formation, nodal ciliogenesis and cilia positioning by Noto and Foxj1. Development 139, 1276-1284.

Arbi, M., Pefani, D.E., Kyrousi, C., Lalioti, M.E., Kalogeropoulou, A., Papanastasiou, A.D., et al. (2016). GemC1 controls multiciliogenesis in the airway epithelium. EMBO Rep. 17, 400-413.

Azzam, E.I., Jay-Gerin, J.P. and Pain, D. (2012). Ionizing radiation-induced metabolic oxidative stress and prolonged cell injury. Cancer Lett. 327, 48-60.

Bernard, M.E., Kim, H., Rajagopalan, M.S., Stone, B., Salimi, U., Rwigema, J.C., et al. (2012). Repopulation of the irradiation damaged lung with bone marrow-derived cells. In Vivo. 26, 9-18.

Berta, T., Gabriele, P., Sandra, S.-B., Gabriel, G.-G., A, Y.S., Stephan-Otto, A.C., et al. (2016). GEMC1 is a critical regulator of multiciliated cell differentiation. EMBO J. 35, 942-960.

Blatt, E.N., Yan, X.H., Wuerffel, M.K., Hamilos, D.L. and Brody, S.L. (1999). Forkhead transcription factor HFH-4 expression is temporally related to ciliogenesis. Am. J. Respir. Cell Mol. Biol. 21, 168-176.

Boon, M., Wallmeier, J., Ma, L., Loges, N.T., Jaspers, M., Olbrich, H., et al. (2014). MCIDAS mutations result in a mucociliary clearance disorder with reduced generation of multiple motile cilia. Nat. Commun. 5, 4418.

Brekman, A., Walters, M.S., Tilley, A.E. and Crystal, R.G. (2014). FOXJ1 prevents cilia growth inhibition by cigarette smoke in human airway epithelium in vitro. Am. J. Respir. Cell Mol. Biol. 51, 688-700.

Brody, S.L., Yan, X.H., Wuerffel, M.K., Song, S.K. and Shapiro, S.D. (2000). Ciliogenesis and left-right axis defects in forkhead factor HFH-4-null mice. Am. J. Respir. Cell Mol. Biol. 23, 45-51.

Chen, J., Knowles, H.J., Hebert, J.L. and Hackett, B.P. (1998). Mutation of the mouse hepatocyte nuclear factor/forkhead homologue 4 gene results in an absence of cilia and random left-right asymmetry. J. Clin. Invest. 102, 1077-1082.

Choksi, S.P., Lauter, G., Swoboda, P. and Roy, S. (2014). Switching on cilia: transcriptional networks regulating ciliogenesis. Development 141, 1427-1441.

Cruz, C., Ribes, V., Kutejova, E., Cayuso, J., Lawson, V., Norris, D., et al. (2010). Foxj1 regulates floor plate cilia architecture and modifies the response of cells to sonic hedgehog signalling. Development 137, 4271-4282.

Didon, L., Zwick, R.K., Chao, I.W., Walters, M.S., Wang, R., Hackett, N.R., et al. (2013). RFX3 Modulation of FOXJ1 regulation of cilia genes in the human airway epithelium. Respir. Res. 14, 70-70.

Gerovac, B.J. and Fregien, N.L. (2016). IL-13 inhibits multicilin expression and ciliogenesis via janus kinase/signal transducer and activator of transcription independently of Notch cleavage. Am. J. Respir. Cell Mol. Biol. 54, 554-561.

Gerovac, B.J., Valencia, M., Baumlin, N., Salathe, M., Conner, G.E. and Fregien, N.L. (2014). Submersion and hypoxia inhibit ciliated cell differentiation in a notch-dependent manner. Am. J. Respir. Cell Mol. Biol. 51(4), 516-525.

Gomperts, B.N., Gong-Cooper, X. and Hackett, B.P. (2004). Foxj1 regulates basal body anchoring to the cytoskeleton of ciliated pulmonary epithelial cells. J. Cell Sci. 117, 1329-1337.

Gomperts, B.N., Kim, L.J., Flaherty, S.A. and Hackett, B.P. (2007). IL-13 Regulates Cilia Loss and foxj1 Expression in Human Airway Epithelium. Am. J. Respir. Cell Mol. Biol. 37, 339-346.

Hackett, B.P., Brody, S.L., Liang, M., Zeitz, I.D., Bruns, L.A. and Gitlin, J.D. (1995). Primary structure of hepatocyte nuclear factor/forkhead homologue 4 and characterization of gene expression in the developing respiratory and reproductive epithelium. Proc. Natl. Acad. Sci. U. S. A. 92, 4249-4253.

Ishikawa, S. and Ito, S. (2017). Repeated whole cigarette smoke exposure alters cell differentiation and augments secretion of inflammatory mediators in air-liquid interface three-dimensional co-culture model of human bronchial tissue. Toxicol. in Vitro 38, 170-178.

Koc, M., Taysi, S., Buyukokuroglu, M.E. and Bakan, N. (2003). Melatonin protects rat liver against irradiation-induced oxidative injury. J. Radiat. Res. 44, 211-215.

Lim, L., Zhou, H. and Costa, R.H. (1997). The winged helix transcription factor HFH-4 is expressed during choroid plexus epithelial development in the mouse embryo. Proc. Natl. Acad. Sci. U. S. A. 94, 3094-3099.

Liu, M., Guan, Z., Shen, Q., Lalor, P., Fitzgerald, U., O'brien, T., et al., 2016. Ulk4 Is essential for ciliogenesis and CSF flow. J. Neurosci. 36, 7589-7600.

Milara, J., Armengot, M., Bañuls, P., Tenor, H., Beume, R., Artigues, E., et al. (2012). Roflumilast N-oxide, a PDE4 inhibitor, improves cilia motility and ciliated human bronchial epithelial cells compromised by cigarette smoke in vitro. Brit. J. Pharmacol. 166, 2243-2262.

Polosa, R., Emma, R., Cibella, F., Caruso, M., Conte, G., Benfatto, F., et al. (2021). Impact of exclusive e-cigarettes and heated tobacco products use on muco-ciliary clearance. Ther. Adv. Chronic Dis. 12, 20406223211035267-20406223211035267.

Rodrigues-Moreira, S., Moreno, S.G., Ghinatti, G., Lewandowski, D., Hoffschir, F., Ferri, F., et al. (2017). Low-Dose Irradiation Promotes Persistent Oxidative Stress and Decreases Self-Renewal in Hematopoietic Stem Cells. Cell Rep. 20, 3199-3211.

Schmid, A., Sailland, J., Novak, L., Baumlin, N., Fregien, N. and Salathe, M. (2017). Modulation of Wnt signaling is essential for the differentiation of ciliated epithelial cells in human airways. FEBS Lett. 591, 3493-3506.

Shaykhiev, R., Zuo, W.L., Chao, I., Fukui, T., Witover, B., Brekman, A., et al. (2013). EGF shifts human airway basal cell fate toward a smoking-associated airway epithelial phenotype. Proc. Natl. Acad. Sci. U. S. A. 110, 12102-12107.

Shirazi, A., Mihandoost, E., Ghobadi, G., Mohseni, M. and Ghazi-Khansari, M. (2013). Evaluation of radio-protective effect of melatonin on whole body irradiation induced liver tissue damage. Cell J. 14, 292-297.

Stauber, M., Weidemann, M., Dittrich-Breiholz, O., Lobschat, K., Alten, L., Mai, M., et al. (2017). Identification of FOXJ1 effectors during ciliogenesis in the foetal respiratory epithelium and embryonic left-right organiser of the mouse. Dev. Biol. 423, 170-188.

Stubbs, J.L., Vladar, E.K., Axelrod, J.D. and Kintner, C. (2012). Multicilin promotes centriole assembly and ciliogenesis during multiciliate cell differentiation. Nat. Cell Biol. 14, 140-147.

Tan, F.E., Vladar, E.K., Ma, L., Fuentealba, L.C., Hoh, R., Espinoza, F.H., et al. (2013). Myb promotes centriole amplification and later steps of the multiciliogenesis program. Development 140, 4277-4286.

Valencia-Gattas, M., Conner, G.E. and Fregien, N.L. (2016). Gefitinib, an EGFR Tyrosine Kinase inhibitor, Prevents Smoke-Mediated Ciliated Airway Epithelial Cell Loss and Promotes Their Recovery. PloS ONE 11, e0160216.

Vij, S., Rink, J.C., Ho, H.K., Babu, D., Eitel, M., Narasimhan, V., et al. (2012). Evolutionarily ancient association of the FoxJ1 transcription factor with the motile ciliogenic program. PLoS Genet. 8, e1003019.

Yu, X., Ng, C.P., Habacher, H. and Roy, S. (2008). Foxj1 transcription factors are master regulators of the motile ciliogenic program. Nat. Genet. 40, 1445-1453.

Zhou, F. and Roy, S. (2015). SnapShot: Motile Cilia. Cell 162, 224-224 e221.

Event: 1912: Motile Cilia Number/Length, Decreased

Short Name: Motile Cilia Number/Length, Decreased

Key Event Component

Process	Object	Action
	motile cilium	decreased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:411 - Oxidative stress [MIE] Leading to Decreased Lung Function [AO]	KeyEvent

Stressors

Name
Cigarette smoke

Biological Context

Level of Biological Organization
Cellular

Cell term

Cell term
multi-ciliated epithelial cell

Organ term

Organ term
lung epithelium

Evidence for Perturbation by Stressor

Cigarette smoke

Cilia length was reduced in endobronchial biopsies and airway brushings of smokers (average 30 pack-years) compared to nonsmokers (Leopold et al., 2009).

Exposure of human bronchial epithelial cells cultured at the air-liquid interface to 1, 3, and 6% cigarette smoke extract (from the basolateral side) between days 5 and 28 of differentiation significantly shortened the average cilia length of day 28 ALI cultures to 5.7, 5.5, and 4.9 µm, respectively, compared an average cilia
length of 6.7 µm in untreated cultures. Continuous treatment of differentiated cultures with 3 and 6% cigarette smoke extract between days 28 and 42 showed
that ciliated cells in the untreated day 42 cultures had longer cilia than day 28 cultures (ca. +1.5 µm), whereas in the presence of 3 and 6% of CSE, this elongation of cilia was suppressed (+0.5 µm and -0.5 µm, respectively) (Brekman et al., 2014).

Apical exposure of mouse tracheal epithelial cells differentiated at the air-liquid interface to cigarette smoke from 3R4F research cigarettes at a total particular matter concentration of 50 and 100 mg/m^3 for 10 min resulted in cilia shortening (approx. -20% and -50%, respectively) and complete loss of cilia (approx. -25% and -60% of ciliated cells, respectively) at 24 h post-exposure (Lam et al., 2013).

Mean cilia length in the large airway epithelium was 7% shorter in healthy smokers (32.5±10 pack-years) compared to nonsmokers (7.09 vs 7.63 µm), 12% shorter in COPD smokers (39±21 pack-years) compared to healthy smokers (6.16 vs 7.09 µm), and 19% shorter in COPD smokers as compared to nonsmokers. In the small airway epithelium, mean cilia length was 9% shorter in healthy smokers relative to nonsmokers (6.49 vs 7.15 µm), 6% shorter in COPD smokers relative to healthy smokers (6.05 vs 6.49 µm), and 15% shorter in COPD smokers compared to nonsmokers (Hessel et al., 2014).

Exposure of mouse nasal septal epithelial cells to cigarette smoke condensate at concentrations >30 µg/mL for the first 15 days growing at the air-liquid interface inhibited ciliogenesis (ciliated area: 89.9+8.0% in untreated vs 48.8+10.0% [30 µg/mL] and 37.5+12.0% [100 µg/mL]) and resulted in cilia shortening (not quantified) (Tamashiro et al., 2009).

Whole-body exposure of female C57BL/6 mice to mainstream and sidestream cigarette smoke from 1R1 reference cigarettes at 150 mg/m^3 total particular matter for 2 h per day, 5 days per week, for up to 1 year resulted in some areas of sparse or detached ciliated cells by month 6 and an almost complete loss
of ciliated cells by 12 months (Simet et al., 2010).

In a small cohort study in adults with adults with chronic sputum production, current and former smokers had a higher frequency of axonemal ultrastructural abnormalities (16.53 ± 2.66% and 17.66 ± 6.99%, respectively) than non-smokers and controls (5.18 ± 0.9% and 0.7% ± 0.2%, respectively) (Verra et al., 1994).

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Danio rerio	Danio rerio		NCBI
Homo sapiens	Homo sapiens	High	NCBI
Xenopus laevis	Xenopus laevis		NCBI
Mus musculus	Mus musculus		NCBI

Life Stage Applicability

Life Stage	Evidence
During development and at adulthood	High

Sex Applicability

Sex	Evidence
Mixed	High

The ultrastructural features of human and other mammalian respiratory epithelial cilia and those from lower animals (e.g. flatworms and mollusks) are remarkably similar (Meunier and Azimzadeh, 2016; Wanner et al., 1996). The master regulators of multiciliated cell differentiation, such as NOTCH, GEMC1, MCIDAS, FOXJ1, RFX2/3 are conserved throughout vertebrates (e.g. mammals, Xenopus, zebrafish) and multiple motile cilia across these organisms are functionally similar in generating fluid flow through coordinated beating (Choksi et al., 2014; Meunier and Azimzadeh, 2016; Wessely and Obara, 2008).

The motile cilia numbers reach adult levels in the mouse airway epithelium at day 21 after birth (Rawlins et al., 2007; Toskala et al., 2005). At birth, there is no discernable cilia-generated airway fluid flow in mice (Francis et al., 2009). Between postnatal days 3 and 7 the flow is established in trachea correlating with the increase in the density of ciliated cells in the tracheal epithelia (Francis et al., 2009). After airway fluid flow establishment, the KE is applicable to all life stages.

Key Event Description

Motile cilia are microtubule-based organelles that protrude from the cell surface and generate directional flow of fluid with coordinated beating. 50% to 80% of human respiratory epithelium is comprised of ciliated cells covered with multiple motile cilia that move mucus (together with mucus-trapped substances) upward for clearing the airways (Yaghi and Dolovich, 2016). The ciliated airway epithelial cells are typically covered by more than hundred motile cilia (Bustamante-Marin and Ostrowski, 2017). On average, 150 motile cilia were counted per ciliated human epithelial cell in the study by Mao et al. (Mao et al., 2018). In an earlier report, 200 motile cilia per ciliated cell in human trachea is mentioned (Wanner et al., 1996), and, in a more recent study, a range of 100 to 600 ciliary precursors were counted in fully differentiated mouse tracheal epithelial cells correlated with increasing surface area (Nanjundappa et al., 2019). Cilia are 6–7 µm long and 0.2–0.3 µm in diameter (Brooks and Wallingford, 2014; Yaghi and Dolovich, 2016). Ciliated cell density and the motile cilia length and number per cell correlate with ciliary beating frequency which is routinely used as a predictor of the mucociliary clearance efficiency (King, 2006). Morphological changes of airway cilia are expected to impact multiple motile cilia functional integrity. This key event represents the decrease in the numbers or absence of motile cilia or reduction in length of motile cilia.

How it is Measured or Detected

Acetylated tubulin is a common ciliary marker (Kim et al., 2013; Piperno and Fuller, 1985), and apical acetylated tubulin staining with subsequent microscope image scoring is a frequently used method of cilia detection and enumeration (Johnson et al., 2018; Mao et al., 2018; Stubbs et al., 2008). Staining of beta-tubulin IV, a protein enriched in motile cilia, is another common method of cilia detection (Brekman et al., 2014; Milara et al., 2012).
Ciliated cells can also be identified by the presence of axonemal structures on the cell surface using scanning electron microscopy (Gomperts et al., 2007).
Mature cilia numbers could be deduced from ciliary precursors in immunofluorescence assays: ciliary precursors can be calculated from three-dimensional superresolution structured illumination microscopy (3D-SIM) images using e.g. a spot detection tool (Nikon Elements AR 4 Software) (Nanjundappa et al., 2019).

For cilia length measurement, the ciliated cells/tissue needs to be stained (Diff-Quik: Dade Behring stain, hematoxylin and eosin staining, labelling with antibodies for ciliary markers such as alpha-tubulin), visualized by microscopy and cilia length quantified (using e.g. ImageJ software or MetaMorph Microscopy Automation & Image Analysis Software) (Brekman et al., 2014; Leopold et al., 2009b; Li et al., 2014). Generally, multiple measurements of one sample and multiple sample preparations of cells/tissues are imaged for reliable quantitation.

References

Brekman, A., Walters, M.S., Tilley, A.E. and Crystal, R.G. (2014). FOXJ1 prevents cilia growth inhibition by cigarette smoke in human airway epithelium in vitro. Am. J. Respir. Cell Mol. Biol. 51, 688-700.

Brooks, E.R. and Wallingford, J.B. (2014). Multiciliated cells. Curr. Biol. 24, R973-982.

Bustamante-Marin, X.M. and Ostrowski, L.E. (2017). Cilia and Mucociliary Clearance. Cold Spring Harb. Persp. Biol. 9, a028241.

Choksi, S.P., Lauter, G., Swoboda, P. and Roy, S. (2014). Switching on cilia: transcriptional networks regulating ciliogenesis. Development 141, 1427-1441.

Francis, R.J., Chatterjee, B., Loges, N.T., Zentgraf, H., Omran, H. and Lo, C.W. (2009). Initiation and maturation of cilia-generated flow in newborn and postnatal mouse airway. Am. J. Physiol. Lung Cell. Mol. Physiol. 296, L1067-1075.

Gomperts, B.N., Kim, L.J., Flaherty, S.A. and Hackett, B.P. (2007). IL-13 Regulates Cilia Loss and foxj1 Expression in Human Airway Epithelium. Am. J. Respir. Cell Mol. Biol. 37, 339-346.

Hessel, J., Heldrich, J., Fuller, J., Staudt, M.R., Radisch, S., Hollmann, C., et al. (2014). Intraflagellar Transport Gene Expression Associated with Short Cilia in Smoking and COPD. PLoS ONE 9, e85453.

Johnson, J.A., Watson, J.K., Nikolic, M.Z. and Rawlins, E.L. (2018). Fank1 and Jazf1 promote multiciliated cell differentiation in the mouse airway epithelium. Biol. Open 7, bio033944.

Kim, G.W., Li, L., Ghorbani, M., You, L. and Yang, X.J. (2013). Mice lacking alpha-tubulin acetyltransferase 1 are viable but display alpha-tubulin acetylation deficiency and dentate gyrus distortion. J. Biol. Chem. 288, 20334-20350.

King, M. (2006). Physiology of mucus clearance. Paediatr. Respir Rev. 7, S212-214.

Lam, H.C., Cloonan, S.M., Bhashyam, A.R., Haspel, J.A., Singh, A., Sathirapongsasuti, J.F., et al. (2013). Histone deacetylase 6–mediated selective autophagy regulates COPD-associated cilia dysfunction. J. Clin. Invest. 123(12), 5212-5230.

Leopold, P.L., O'mahony, M.J., Lian, X.J., Tilley, A.E., Harvey, B.-G. and Crystal, R.G. (2009). Smoking is associated with shortened airway cilia. PloS ONE 4, e8157.

Li, Y.Y., Li, C.W., Chao, S.S., Yu, F.G., Yu, X.M., Liu, J., et al. (2014). Impairment of cilia architecture and ciliogenesis in hyperplastic nasal epithelium from nasal polyps. J. Allergy Clin. Immunol. 134, 1282-1292.

Mao, S., Shah, A.S., Moninger, T.O., Ostedgaard, L.S., Lu, L., Tang, X.X., et al. (2018). Motile cilia of human airway epithelia contain hedgehog signaling components that mediate noncanonical hedgehog signaling. Proc. Natl. Acad. Sci. U. S. A. 115, 1370-1375.

Meunier, A. and Azimzadeh, J. (2016). Multiciliated Cells in Animals. Cold Spring Harb. Perspect. Biol. 8, a028233.

Milara, J., Armengot, M., Bañuls, P., Tenor, H., Beume, R., Artigues, E., et al. (2012). Roflumilast N-oxide, a PDE4 inhibitor, improves cilia motility and ciliated human bronchial epithelial cells compromised by cigarette smoke in vitro. Brit. J. Pharmacol. 166, 2243-2262.

Nanjundappa, R., Kong, D., Shim, K., Stearns, T., Brody, S.L., Loncarek, J., et al. (2019). Regulation of cilia abundance in multiciliated cells. Elife 8, e44039.

Piperno, G. and Fuller, M.T. (1985). Monoclonal antibodies specific for an acetylated form of alpha-tubulin recognize the antigen in cilia and flagella from a variety of organisms. J. Cell Biol. 101, 2085-2094.

Rawlins, E.L., Ostrowski, L.E., Randell, S.H. and Hogan, B.L. (2007). Lung development and repair: contribution of the ciliated lineage. Proc. Natl. Acad. Sci. U. S. A. 104, 410-417.

Simet, S.M., Sisson, J.H., Pavlik, J.A., Devasure, J.M., Boyer, C., Liu, X., et al. (2010). Long-term cigarette smoke exposure in a mouse model of ciliated epithelial cell function. Am. J. Respir. Cell Mol. Biol. 43, 635-640.

Stubbs, J.L., Oishi, I., Izpisua Belmonte, J.C. and Kintner, C. (2008). The forkhead protein Foxj1 specifies node-like cilia in Xenopus and zebrafish embryos. Nat. Genet. 40, 1454-1460.

Tamashiro, E., Xiong, G., Anselmo-Lima, W.T., Kreindler, J.L., Palmer, J.N., and Cohen, N.A. (2009). Cigarette smoke exposure impairs respiratory epithelial ciliogenesis. Am. J. Rhinol. Allergy 23, 117-122.

Toskala, E., Smiley-Jewell, S.M., Wong, V.J., King, D. and Plopper, C.G. (2005). Temporal and spatial distribution of ciliogenesis in the tracheobronchial airways of mice. Am. J. Physiol. Lung Cell. Mol. Physiol. 289, L454-459.

Verra, F., Escudier, E., Lebargy, F., Bernaudin, J.F., De Cremoux, H., and Bignon, J. (1995). Ciliary abnormalities in bronchial epithelium of smokers, ex-smokers, and nonsmokers. Am. J. Respir. Crit. Care Med. 151, 630-634.

Wanner, A., Salathe, M. and O'riordan, T.G. (1996). Mucociliary clearance in the airways. Am. J. Respir. Crit. Care Med. 154, 1868-1902.

Wessely, O. and Obara, T. (2008). Fish and frogs: models for vertebrate cilia signaling. Front. Biosci. 13, 1866-1880.

Yaghi, A. and Dolovich, M.B. (2016). Airway Epithelial Cell Cilia and Obstructive Lung Disease. Cells. 5, 40.

List of Adverse Outcomes in this AOP

Event: 1250: Decrease, Lung function

Short Name: Decreased lung function

Key Event Component

Process	Object	Action
respiratory function trait		decreased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:148 - EGFR Activation Leading to Decreased Lung Function	AdverseOutcome
Aop:302 - Lung surfactant function inhibition leading to decreased lung function	AdverseOutcome
Aop:411 - Oxidative stress [MIE] Leading to Decreased Lung Function [AO]	AdverseOutcome
Aop:418 - Aryl hydrocarbon receptor activation leading to impaired lung function through AHR-ARNT toxicity pathway	KeyEvent
Aop:419 - Aryl hydrocarbon receptor activation leading to impaired lung function through P53 toxicity pathway	AdverseOutcome

Stressors

Name
Ozone
Nitric oxide
Cigarette smoke
Diesel engine exhaust
PM10

Biological Context

Level of Biological Organization
Individual

Evidence for Perturbation by Stressor

Ozone

Acute exposure of healthy young adult subjects (aged 19 to 35 years, non-smokers) to 0.06 ppm ozone for 6.6 h resulted in a 1.71 ± 0.50% (mean ± SEM) decrease in FEV1 and a 2.32 ± 0.41% decrease in FVC compared with air exposure (Kim et al., 2011).

A US-based study found inverse associations between increasing lifetime exposure to ozone (estimated median: 36; interquartile range 29–45; range 19–64) and FEF75 and FEF25–75 in adolescents (aged 18–20 years) (Tager et al., 2005).

Nitric oxide

In a Dutch cross-sectional study in school children (aged 7–13 years), NOx exposure from industrial emissions per interquartile range of 7.43 µg/m^3 had a significantly lower percent predicted peak expiratory flow (PEF) (-3.67%, 95%CI -6.93% to -0.42%). Children exposed to NOx (per interquartile range of 7.43 µg/m^3) also had a significantly lower percent forced vital capacity (FVC) and percent predicted 1-s forced expiratory volume (FEV1) (– 2.73 95%CI -5.21 to -0.25) (Bergstra et al., 2018).

The European Study of Cohorts for Air Pollution Effects (ESCAPE), a meta-analysis of 5 cohort studies on the association of air pollution with lung function, found that a 10 µg/m^3 increase in NO_2 exposure was associated with lower levels of FEV1 (–14.0 mL, 95% CI –25.8 to –2.1) and FVC (–14.9 mL, 95% CI –28.7 to –1.1), and an increase of 20 µg/m^3 in NOx exposure was associated with a lower level of FEV1, by –12.9 mL (95% CI –23.87 to –2.0) and of FVC, by –13.3 mL (95% CI –25.9 to –0.7) (Adam et al., 2015).

Cigarette smoke

A smoking history of > 20 pack-years decreased pulmonary function including forced vital capacity (FVC), forced expiratory volume in one second (FEV1), FEV1/FVC, and forced expiratory flow at 25–75% (FEF25–75%) (Kuperman and Riker, 1973).

In the Framingham Heart Study, cigarette smoking showed an inverse association with FVC and FEV1% (Ashley et al., 1975).

In the international Seven Countries Study, there was a dose-effect relationship between pack-years and forced expiratory volume in 0.75 s (FEV0.75) in continuous smokers without chronic bronchitis (Pelkonen et al., 2006).

In 34 male subjects aged between 15–18 years who smoked FVC was lower than in an age-matched male group that did not smoke. The most common duration of cigarette smoking was 1–3 years (47%) and the maximal number of cigarettes smoked per day was less than or equal to 10 cigarette(s) per day (88%) (Tantisuwat and Thaveeratitham, 2014).

A dose–response relation was found between smoking and lower levels of FEV1/FVC and FEF25–75 in children between 10-18 years of age (Gold et al., 1996).

In a study of 147 asthmatics, FEV1%predicted was significantly lower in ex-smokers and current smokers compared with never-smokers (Broekema et al., 2009).

In a 6-year longitudinal study in Japanese-American men, FEV1 was lowest in current smokers (2702 mL) and in former smokers (2817 mL) at baseline. These 2 groups experienced a steeper annual decline in FEV1 (-34.4 and -22.8 mL/year, respectively, adjusted by height and age at baseline) compared with never-smokers (-20.3 mL/year) (Burchfiel et al., 1995).

Diesel engine exhaust

In a study of 733 adult females who had lived in the Tokyo metropolitan area for more than 3 years, the higher the level of air pollution, the more significantly the FEV1 was reduced (Sekine et al., 2004).

In a study in 29 healthy subjects, exposure to DE inside diesel-powered trains for 3 days was associated with reduced lung function (Andersen et al., 2019).

In workers who tested diesel engines in an assembly unit of a manufacturing plant, FEV1, FEV1/FVC, FEV25-75 and MEF were significantly reduced compared to non-exposed workers (Zhang et al., 2017).

PM10

A Taiwanese study in 1016 children between 6 and 15 years of age reported that lifetime exposure to to 25–85 µg/m^3 PM10 were associated with lower FEV1, FVC, and FEF25-75 (Tsui et al., 2018).

The Swiss Study on Air Pollution and Lung Diseases in Adults (SAPALDIA) found that an increase of 10 µg/m^3 in annual mean concentration of PM10 was associated with 3.4% lower FVC and 1.6% lower FEV1 (Ackermann-Liebrich et al., 1997).

In the Health Survey for England, a 10 mg/m^3 difference in PM10 across postcode sectors was associated with a lower FEV1 by 111 mL, independent of active and passive smoking, social class, region and month of testing (Forbes et al., 2009).

A 7 µg/m^3 increase in five year means of PM10 (interquartile range) was associated with a 5.1% (95% CI: 2.5%–7.7%) decrease in FEV1, a 3.7% (95% CI: 1.8%–5.5%) decrease in FVC in the German SALIA study (Schikowski et al., 2005).

The ESCAPE project, a meta-analysis of 5 European cohorts/studies from 8 countries, reported that an increase of 10 µg/m^3 in PM10 was associated with a lower level of FEV1 (–44.6 mL, 95% CI:–85.4– –3.8) and FVC (–59.0 mL, 95% CI: –112.3– –5.7) (Adam et al., 2015).

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	High	NCBI

Life Stage Applicability

Life Stage	Evidence
Adult	High

Sex Applicability

Sex	Evidence
Mixed	High

Pulmonary function tests reflect the physiological working of the lungs. Therefore, the AO is applicable to a variety of species, including (but not limited to) rodents, rabbits, pigs, cats, dogs, horses and humans, independent of life stage and gender.

Key Event Description

Lung function is a clinical term referring to the physiological functioning of the lungs, most often in association with the tests used to assess it. Lung function loss can be caused by acute or chronic exposure to airborne toxicants or by an intrinsic disease of the respiratory system.

Although signs of cellular injury are typically exhibited first in the nose and larynx, alveolar-capillary barrier breakdown may ultimately arise and result in local edema (Miller and Chang, 2003). Clinically, bronchoconstriction and hypoxia are seen in the acute phase, with affected subjects exhibiting shortness of breath (dyspnea) and low blood oxygen saturation, and with reduced lung function indices of airflow, lung volume and gas exchange (Hert and Albert, 1994; and How it is Measured or Detected;). When alveolar damage is extensive, the reduced lung function can develop into acute respiratory distress syndrome (ARDS). This severe compromise of lung function is reflected by decreased gas exchange indices (PaO_2/FIO_2 ≤200 mmHg, due to hypoxemia and impaired excretion of carbon dioxide), increased pulmonary dead space and decreased respiratory compliance (Matthay et al., 2019). Acute inhalation exposures to chemical irritants such as ammonia, hydrogen chloride, nitrogen oxides and ozone typically cause local edema that manifests as dyspnea and hypoxia. In cases where a breakdown of the alveolar capillary function ensues, ARDS develops. ARDS has a particularly high risk of mortality, estimated to be 30-40% (Gorguner and Akgun, 2010; Matthay et al., 2018; Reilly et al., 2019).

Lung function decrease due to reduction in lung volume is seen in pulmonary fibrosis, which can be linked to chronic exposures to e.g. silica, asbestos, metals, agricultural and animal dusts (Meltzer and Noble, 2008; Cheresh et al., 2013; Cosgrove, 2015; Trethewey and Walters, 2018). Additionally. decreased lung function occurs in pleural disease, chest wall and neuromuscular disorders, because of obesity and following pneumectomy (Moore, 2012). Decreased lung function can also be a result of narrowing of the airways by inflammation and mucus plugging resulting in airflow limitation. Decreased lung function is a feature of obstructive pulmonary diseases (e.g. asthma, COPD) and linked to a multitude of causes, including chronic exposure to cigarette smoke, dust, metals, organic solvents, asbestos, pathogens or genetic factors.

How it is Measured or Detected

Pulmonary function tests are a group of tests that evaluate several parameters indicative of lung size, air flow and gas exchange. Decreased lung function can manifest in different ways, and individual circumstances, including potential exposure scenarios, determine which test is used. The section outlines the tests used to evaluate lung function in humans (https://www.nhlbi.nih.gov/health-topics/pulmonary-function-tests, accessed 22 March 2021) and in experimental animals.

Lung function tests used to evaluate human lung function

The most common (“gold standard”) lung function test in human subjects is spirometry. Spirometry results are primarily used for diagnostic purposes, e.g. to discriminate between obstructive and restrictive lung diseases, and for determining the degree of lung function impairment. Specific criteria for spirometry tests have been outlined in the American Thoracic Society (ATS) and the European Respiratory Society (ERS) Task Force guidelines (Graham et al., 2019). These guidelines consist of detailed recommendations for the preparation and conduct of the test, instruction of the person tested, as well as indications and contraindications, and are complemented by additional guidance documents on how to interpret and report the test results (Pellegrino et al., 2005; Culver et al., 2017).

Spirometry measures several different parameters during forceful exhalation, including:

- Forced expiratory volume in 1 s (FEV1), the maximum volume of air that can forcibly be exhaled during the first second following maximal inhalation
- Forced vital capacity (FVC), the maximum volume of air that can forcibly be exhaled following maximal inhalation
- Vital capacity (VC), the maximum volume of air that can be exhaled when exhaling as fast as possible
- FEV1/FVC ratio
- Peak expiratory flow (PEF), the maximal flow that can be exhaled when exhaling at a steady rate
- Forced expiratory flow, also known as mid-expiratory flow; the rates at 25%, 50% and 75% FVC are given
- Inspiratory vital capacity (IVC), the maximum volume of air that can be inhaled after a full expiration

A reduced FEV1, with normal or reduced VC, normal or reduced FVC, and a reduced FEV1/FVC ratio are indices of airflow

limitation, i.e., airway obstruction as seen in COPD (Moore, 2012). In contrast, airway restriction is demonstrated by a reduction in FVC, normal or increased FEV1/FVC ratio, a normal spirometry trace and potentially a high PEF (Moore, 2012).

Lung capacity or lung volumes can be measured using one of three basic techniques: 1) plethysmography, 2) nitrogen washout, or 3) helium dilution. Plethysmography consists of a series of sequential measurements in a body plethysmograph, starting with the measurement of functional residual capacity (FRC), the volume of gas present in the lung at end-expiration during tidal breathing. Once the FRC is known, expiratory reserve volume (ERV; the volume of gas that can be maximally exhaled from the end-expiratory level during tidal breathing, i.e., the FRC), vital capacity (VC; the volume change at the mouth between the positions of full inspiration and complete expiration), and inspiratory capacity (IC; the maximum volume of air that can be inhaled from FRC) are determined, and total lung capacity (TLC; the volume of gas in the lungs after maximal inspiration, or the sum of all volume compartments) and residual volume (RV; the volume of gas remaining in the lung after maximal exhalation) are calculated (Weinstock and McCannon, 2017).

The other two techniques used to measure lung volumes—helium dilution and nitrogen washout—are based on the principle of conservation of mass: [initial gas concentration] x [initial volume of the system] = [final gas concentration] x [final volume of the system]. The nitrogen washout method is based on the fact that nitrogen is present in the air, at a relatively constant amount. The subject is given 100% oxygen to breathe, and the expired gas, which contains nitrogen in the lung at the beginning of the test, is collected. When no more nitrogen is noted in the expirate, the volume of air expired and the entire amount of nitrogen in that volume are measured, and the initial volume of the system (FRC) can be calculated. In the helium dilution method, a known volume and concentration of helium is inhaled by the subject. Helium, an inert gas that is not absorbed significantly from the lungs, is diluted in proportion to the lung volume to which it is added. The final concentration of helium is then measured and FRC calculated (Weinstock and McCannon, 2017).

Measurements of lung volumes in humans are technically more challenging than spirometry. However, they complement spirometry (which cannot determine lung volumes) and may be a preferred means of lung function assessment when subject compliance cannot be reasonably expected (e.g. in pediatric subjects) or where forced expiratory maneuvers are not possible (e.g. in patients with advanced pulmonary fibrosis). There are recommended standards for lung volume measurements and their interpretation in clinical practice, issued by the ATS/ERS Task Force (Wanger et al., 2005; Criée et al., 2011).

Finally, indices of gas exchange across the alveolar-capillary barrier are tested by diffusion capacity of carbon monoxide (DLCO) studies (also referred to as transfer capacity of carbon monoxide, TLCO). The principle of the test is the increased affinity of hemoglobin to preferentially bind carbon monoxide over oxygen (Weinstock and McCannon, 2017). Complementary to spirometry and lung volume measurements, DLCO provides information about the lung surface area available for gas diffusion. Therefore, it is sensitive to any structural changes affecting the alveoli, such as those accompanying emphysema, pulmonary fibrosis, pulmonary edema, and ARDS. Recommendations for the standardization of the test and its evaluation have been outlined by the ATS/ERS Task Force (Graham et al., 2017). An isolated reduction in DLCO with normal spirometry and in absence of anemia suggests an injury to the alveolar-capillary barrier, as for example seen in the presence of pulmonary emboli or in patients with pulmonary hypertension (Weinstock and McCannon, 2017; Lettieri et al., 2006; Seeger et al., 2013). Reduced DLCO together with airflow obstruction (i.e., reduced FEV1) indicates lung parenchymal damage and is commonly observed in smokers and in COPD patients (Matheson et al., 2007; Harvey et al., 2016), whereas reduced DLCO with airflow restriction is seen in patients with interstitial lung diseases (Dias et al., 2014; Kandhare et al., 2016).

Lung function tests used to evaluate experimental animal lung function

Because spirometry requires active participation and compliance of the subject, it is not commonly used in animal studies. However, specialized equipment such as the flexiVent system (SCIREQ®) are available for measuring FEV, FVC and PEF in anesthetized and tracheotomized small laboratory animals. Other techniques such as plethysmography or forced oscillation are increasingly preferred for lung function assessment in small laboratory animals (McGovern et al., 2013; Bates, 2017).

In small laboratory animals, plethysmography can be used to determine respiratory physiology parameters (minute volume, respiratory rate, time of pause and time of break), lung volume and airway resistance of conscious animals. Both whole body and head-out plethysmography can be applied, although there is a preference for the latter in the context of inhalation toxicity studies, because of its higher accuracy and reliability (OECD, 2018a; Hoymann, 2012).

Gas diffusion tests are not frequently performed in animals, because reproducible samplings of alveolar gas are difficult and technically challenging (Reinhard et al., 2002; Fallica et al., 2011). Modifications to the procedure employed in humans have, however, open possibilities to obtain a human-equivalent DLCO measure or the diffusion factor for carbon monoxide (DFCO)—a variable closely related to DLCO, which can inform on potential structural changes in the lungs that have an effect on gas exchange indices (Takezawa et al., 1980; Dalbey et al., 1987; Fallica et al., 2011; Limjunyawong et al., 2015).

Regulatory Significance of the AO

Established regulatory guideline studies for inhalation toxicity focus on evident clinical signs of systemic toxicity, including death, or organ-specific toxicity following acute and (sub)chronic exposure respectively. In toxicological and safety pharmacological studies with airborne test items targeting the airways or the lungs as a whole, lung function is a relevant endpoint for the characterization of potential adverse events (OECD, 2018a; Hoymann, 2012). Hence, the AO “decreased lung function” is relevant for regulatory decision-making in the context of (sub)chronic exposure (OECD, 2018b; OECD, 2018c).

Regulatory relevance of the AO “decreased lung function” is evident when looking at the increased risk of diseases in humans following inhalation exposure, and because of its links to other comorbidities and mortality.

To aid diagnosis and monitoring of fibrosis, current recommendations include both the recording of potential environmental and occupational exposures as well as an assessment of lung function (Baumgartner et al., 2000). The latter typically confirms decreased lung function as demonstrated by a loss of lung volume. As the disease progresses, dyspnea and lung function worsen, and the prognosis is directly linked to the decline in FVC (Meltzer and Noble, 2008).

Chronic exposure to cigarette smoke and other combustion-derived particles results in the development of COPD. COPD is diagnosed on the basis of spirometry results as laid out in the ATS/ERS Task Force documents on the standardization of lung function tests and their interpretation (Pellegrino et al., 2005; Culver et al., 2017, Graham et al., 2019). Rapid rates of decline in the lung function parameter FEV1 are linked to higher risk of exacerbations, increased hospitalization and early death (Wise et al., 2006; Celli, 2010). Reduced FEV1 also poses a risk for serious cardiovascular events and mortality associated with cardiovascular disease (Sin et al., 2005; Lee et al., 2015).

References

Ackermann-Liebrich, U., Leuenberger, P., Schwartz, J., Schindler, C., Monn, C., Bolognini, G., et al. (1997). Lung function and long term exposure to air pollutants in Switzerland. Study on Air Pollution and Lung Diseases in Adults (SAPALDIA) Team. Am. J. Resp. Crit. Care Med. 155, 122-129.

Adam, M., Schikowski, T., Carsin, A.E., Cai, Y., Jacquemin, B., Sanchez, M., et al. (2015). Adult lung function and long-term air pollution exposure. ESCAPE: a multicentre cohort study and meta-analysis. Eur. Resp. J. 45, 38-50.

Andersen, M.H.G., Frederiksen, M., Saber, A.T., Wils, R.S., Fonseca, A.S., Koponen, I.K., et al. (2019). Health effects of exposure to diesel exhaust in diesel-powered trains. Part. Fibre Toxicol. 16, 21.

Ashley, F., Kannel, W.B., Sorlie, P.D., and Masson, R. (1975). Pulmonary function: relation to aging, cigarette habit, and mortality: the Framingham Study. Ann. Int. Med. 82, 739-745.

Bates, J.H.T. (2017). CO

Bergstra, A.D., Brunekreef, B., and Burdorf, A. (2018). The effect of industry-related air pollution on lung function and respiratory symptoms in school children. Environm. Health 17, 30.

Baumgartner, K.B., Samet, J.M., Coultas, D.B., Stidley, C.A., Hunt, W.C., Colby, T.V., and J.A. Waldron (2000). Occupational and environmental risk factors for idiopathic pulmonary fibrosis: a multicenter case-control study. Collaborating Centers. Am. J. Epidemiol. 152, 307-315.

Broekema, M., ten Hacken, N.H., Volbeda, F., Lodewijk, M.E., Hylkema, M.N., Postma, D.S., et al. (2009). Airway epithelial changes in smokers but not in ex-smokers with asthma. Am. J. Resp. Crit. Care Med. 180, 1170-1178.

Celli, B. R. (2010). Predictors of mortality in COPD. Respir. Med. 104, 773-779.

Cheresh, P., Kim, S.J., Tulasiram, S., and D.W. Kamp (2013). Oxidative stress and pulmonary fibrosis. Biochim. Biophys. Acta, 1832, 1028–1040.

Cosgrove, M.P. (2015). Pulmonary fibrosis and exposure to steel welding fume. Occup. Med. 65, 706-712.

Criée, C.P., Sorichter, S., Smith, H.J., Kardos, P., Merget, R., Heise, D., Berdel, D., Köhler, D., Magnussen, H., Marek, W. and H. Mitfessel (2011). Body plethysmography–its principles and clinical use. Respir. Med. 105, 959-971.

Dalbey, W., Henry, M., Holmberg, R., Moneyhun, J., Schmoyer, R. and S. Lock (1987). Role of exposure parameters in toxicity of aerosolized diesel fuel in the rat. J. Appl. Toxicol. 7, 265-275.

Dias, O.M., Baldi, B.G., Costa, A.N., C.R. Carvalho (2014). Combined pulmonary fibrosis and emphysema: an increasingly recognized condition. J. Bras. Pneumol. 40, 304-312.

Fallica, J., Das, S., Horton, M., and W. Mitzner (2011). Application of carbon monoxide diffusing capacity in the mouse lung. J. Appl. Physiol. 110, 1455–1459.

Forbes, L.J., Kapetanakis, V., Rudnicka, A.R., Cook, D.G., Bush, T., Stedman, J.R., et al. (2009). Chronic exposure to outdoor air pollution and lung function in adults. Thorax 64, 657-663.

Gold, D.R., Wang, X., Wypij, D., Speizer, F.E., Ware, J.H., and Dockery, D.W. (1996). Effects of cigarette smoking on lung function in adolescent boys and girls. N. Engl. J. Med. 335, 931-937.

Gorguner, M., and M. Akgun (2010). Acute inhalation injury. Euras. J. Med. 42, 28–35.

Graham, B.L., Brusasco, V., Burgos, F., Cooper, B.G., Jensen, R., Kendrick, A., MacIntyre, N.R., Thompson, B.R. and J. Wanger

(2017). 2017 ERS/ATS standards for single-breath carbon monoxide uptake in the lung. Eur. Respir. J. 49, 1600016.

Graham, B.L., Steenbruggen, I., Miller, M.R., Barjaktarevic, I.Z., Cooper, B.G., Hall, G.L., Hallstrand, T.S., Kaminsky, D.A., McCarthy, K., McCormack, M.C. and C.E. Oropez (2019). Standardization of spirometry 2019 update. An official American Thoracic Society and European Respiratory Society technical statement. Am. J. Respir. Crit. Care Med. 200, e70-e88.

Harvey, B.G., Strulovici-Barel, Y., Kaner, R.J., Sanders, A., Vincent, T.L., Mezey, J.G. and R.G. Crystal (2016). Progression to COPD in smokers with normal spirometry/low DLCO using different methods to determine normal levels. Eur. Respir. J. 47, 1888-1889.

Hert, R. and R.K. Albert (1994). Sequelae of the adult respiratory distress syndrome. Thorax 49, 8-13.

Hoymann, H.G. (2012). Lung function measurements in rodents in safety pharmacology studies. Front. Pharmacol. 3, 156.

Johnson, J. D., and W. M. Theurer (2014). A stepwise approach to the interpretation of pulmonary function tests. Am. Fam. Phys. 89, 359-366.

Kandhare, A.D., Mukherjee, A., Ghosh, P. and S.L. Bodhankar (2016). Efficacy of antioxidant in idiopathic pulmonary fibrosis: A systematic review and meta-analysis. EXCLI J. 15, 636.

Kim, C.S., Alexis, N.E., Rappold, A.G., Kehrl, H., Hazucha, M.J., Lay, J.C., et al. (2011). Lung function and inflammatory responses in healthy young adults exposed to 0.06 ppm ozone for 6.6 hours. Am. J. Respir. Crit. Care Med. 183, 1215-1221.

Kuperman, A.S., and Riker, J.B. (1973). The variable effect of smoking on pulmonary function. Chest 63, 655-660.

Lee, H. M., Liu, M. A., Barrett-Connor, E., and N. D. Wong (2014). Association of Lung Function with Coronary Heart Disease and Cardiovascular Disease Outcomes in Elderly: The Rancho Bernardo Study. Respir. Med. 108, 1779–1785.

Lettieri, C.J., Nathan, S.D., Barnett, S.D., Ahmad, S. and A.F. Shorr (2006). Prevalence and outcomes of pulmonary arterial hypertension in advanced idiopathic pulmonary fibrosis. Chest 129, 746-752.

Limjunyawong, N., Fallica, J., Ramakrishnan, A., Datta, K., Gabrielson, M., Horton, M., and W. Mitzner (2015). Phenotyping mouse pulmonary function in vivo with the lung diffusing capacity. JoVE 95, e52216.

Matheson, M.C., Raven, J., Johns, D.P., Abramson, M.J. and E.H. Walters (2007). Associations between reduced diffusing capacity and airflow obstruction in community-based subjects. Respir. Med. 101, 1730-1737.

Matthay, M.A., Zemans, R.L., Zimmerman, G.A., Arabi, Y.M., Beitler, J.R., Mercat, A., Herridge, M., Randolph, A.G. and C.S. Calfee (2019). Acute respiratory distress syndrome. Nature Reviews Disease Primers 5, 1-22.

McGovern, T.K., Robichaud, A., Fereydoonzad, L., Schuessler, T.F., and J.G. Martin (2013) Evaluation of respiratory system mechanics in mice using the forced oscillation technique. JoVE 75, e50172.

Meltzer, E.B., and P.W. Noble (2008). Idiopathic pulmonary fibrosis. Orphanet J. Rare Dis. 3, 8.

Miller, K. and A. Chang (2003). Acute inhalation injury. Emerg. Med. Clin. N. Am. 21, 533-557.

Miller, M.R., Crapo, R., Hankinson, J., Brusasco, V., Burgos, F., Casaburi, R., Coates, A., Enright, P., van der Grinten, C.M., and P. Gustafsson (2005a). General considerations for lung function testing. Eur. Respir. J. 26, 153-161.

Miller, M.R., Hankinson, J., Brusasco, V., Burgos, F., Casaburi, R., Coates, A., Crapo, R., Enright, P., van der Grinten, C., and P. Gustafsson (2005b). Standardisation of spirometry. Eur. Respir. J. 26, 319-338.

Moore, V.C. (2012). Spirometry: step by step. Breathe 8, 232-240.

OECD (2018a). OECD Guidance Document on Inhalation Toxicity Studies, GD 39.

OECD (2018b), Test No. 412: Subacute Inhalation Toxicity: 28-Day Study, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, https://doi.org/10.1787/9789264070783-en.

OECD (2018), Test No. 413: Subchronic Inhalation Toxicity: 90-day Study, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, https://doi.org/10.1787/9789264070806-en.

Park, Y., Ahn, C., and T.H. Kim (2021) Occupational and environmental risk factors of idiopathic pulmonary fibrosis: a systematic review and meta-analyses. Sci. Rep. 11, 4318.

Prada-Dacasa, P., Urpi, A., Sánchez-Benito, L., Bianchi, P., A. Quintana (2020). Measuring Breathing Patterns in Mice Using Whole-body Plethysmography. Bio. Protoc. 10, e3741.

Pelkonen, M., Notkola, I.-L., Nissinen, A., Tukiainen, H., and Koskela, H. (2006). Thirty-year cumulative incidence of chronic bronchitis and COPD in relation to 30-year pulmonary function and 40-year mortality: a follow-up in middle-aged rural men. Chest 130, 1129-1137.

Pellegrino, R., Viegi, G., Brusasco, V., Crapo, R., Burgos, F., Casaburi, R., Coates, A., van der Grinten, C., Gustafsson, P., and J.

Hankinson (2005). Interpretative strategies for lung function tests. Eur. Respir. J. 26, 948-968.

Raghu, G., Remy-Jardin, M., Myers, J.L., Richeldi, L., Ryerson, C.J., Lederer, D.J., Behr, J., Cottin, V., Danoff, S.K., Morell, F., and K.R. Flaherty (2018). Diagnosis of idiopathic pulmonary fibrosis. An official ATS/ERS/JRS/ALAT clinical practice guideline. Am. J. Respir. Crit. Care Med. 198, e44-e68.

Reilly, J.P., Zhao, Z., Shashaty, M.G., Koyama, T., Christie, J.D., Lanken, P.N., Wang, C., Balmes, J.R., Matthay, M.A., Calfee, C.S. and L.B. Ware (2019). Low to moderate air pollutant exposure and acute respiratory distress syndrome after severe trauma. Am. J. Respir. Crit. Care Med. 199, 62-70.

Reinhard. C., Eder, G., Fuchs, H., Ziesenis, A., Heyder, J. and H. Schulz H (2002). Inbred strain variation in lung function. Mamm. Genome 13, 429-437.

RP: Measurement of lung function in small animals. J. Appl. Physiol. 123, 1039-1046.

Schikowski, T., Sugiri, D., Ranft, U., Gehring, U., Heinrich, J., Wichmann, H.E., et al. (2005). Long-term air pollution exposure and living close to busy roads are associated with COPD in women. Respir. Res. 6, 152.

Seeger, W., Adir, Y., Barberà, J.A., Champion, H., Coghlan, J.G., Cottin, V., De Marco, T., Galiè, N., Ghio, S., Gibbs, S. and F.J. Martinez (2013). Pulmonary hypertension in chronic lung diseases. J. Am. Coll. Cardiol. 62 Suppl. 25, D109-D116.

Sin, D. D., Wu, L., and S. P. Man (2005). The relationship between reduced lung function and cardiovascular mortality: a population-based study and a systematic review of the literature. Chest 127, 1952-1959.

Takezawa, J., Miller, F.J. and J.J. O'Neil (1980). Single-breath diffusing capacity and lung volumes in small laboratory mammals. J. Appl. Physiol. 48, 1052-1059.

Tantisuwat, A., and Thaveeratitham, P. (2014). Effects of smoking on chest expansion, lung function, and respiratory muscle strength of youths. J. Phys. Ther. Sci. 26, 167-170.

Trethewey, S. P., and G. I. Walters (2018). The Role of Occupational and Environmental Exposures in the Pathogenesis of Idiopathic Pulmonary Fibrosis: A Narrative Literature Review. Medicina (Kaunas, Lithuania) 54, 108.

Tsui, H.-C., Chen, C.-H., Wu, Y.-H., Chiang, H.-C., Chen, B.-Y., and Guo, Y.L. (2018). Lifetime exposure to particulate air pollutants is negatively associated with lung function in non-asthmatic children. Environ. Poll. 236, 953-961.

Vestbo, J., Anderson, W., Coxson, H.O., Crim, C., Dawber, F., Edwards, L., Hagan, G., Knobil, K., Lomas, D.A., MacNee, W. and E.K. Silverman (2008). Evaluation of COPD longitudinally to identify predictive surrogate end-points (ECLIPSE). Eur. Respir. J. 31, 869-73.

Wanger, J., Clausen, J.L., Coates, A., Pedersen, O.F., Brusasco, V., Burgos, F., Casaburi, R., Crapo, R., Enright, P., Van Der Grinten, C.P.M. and P. Gustafsson (2005). Standardisation of the measurement of lung volumes. Eur. Respir. J. 26, 511-522.

Weinstock, T, and J. McCannon (2017). Pulmonary Medicine. Pulmonary Function Testing. https://www.pulmonologyadvisor.com/home/decision-support-in-medicine/pulmonary-medicine/pulmonary-function-testing/ (accessed 22 March 2021). Decision Support in Medicine, LLC.

Wise, R. A. (2006). The value of forced expiratory volume in 1 second decline in the assessment of chronic obstructive pulmonary disease progression. Am. J. Med. 119, 4-11.

Zhang, L.P., Zhang, X., Duan, H.W., Meng, T., Niu, Y., Huang, C.F., et al. (2017). Long-term exposure to diesel engine exhaust induced lung function decline in a cross sectional study. Ind.l Health 55, 13-26.

Appendix 2

List of Key Event Relationships in the AOP

List of Adjacent Key Event Relationships

Relationship: 2440: CFTR Function, Decreased leads to ASL Height, Decreased

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Oxidative stress [MIE] Leading to Decreased Lung Function [AO]	adjacent	High	Moderate

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	High	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Mixed	High

Phylogenetic analysis of CFTR DNA sequences across multiple species suggests a close evolutionary relationship between human and primate CFTR, followed by rabbit, guinea pig, equine, ovine, and bovine CFTR, whereas rodent CFTR DNA largely diverges from the human DNA (Chen et al., 2001). Of note, CFTR ion permeability differs from species to species (Higgins, 1992). For example, murine CFTR displays reduced channel activity compared with its human counterpart, while ovine CFTR exhibits higher ATP sensitivity, greater single-channel conductance and larger open probability than human CFTR. Moreover, sensitivity to pharmacological agents able to potentiate or block CFTR gating varies greatly from species to species (Bose et al., 2015). Therefore, results from animal studies are not directly transferable to human.

To date, ASL has been investigated in several species including mice, rats, guinea pigs, ferrets, cats, dogs, cows, monkeys, and humans. Although most studies provide data on its composition rather than its height, it is reasonable to assume that regulation of ASL height is equally critical to MCC across these species.

CFTR dysfunction as a consequence of inherited CFTR gene defects is studied in pediatric as well as adult cystic fibrosis patients. Acquired CFTR dysfunction following inhalation exposures (e.g. to cigarette smoke) may also apply to both pediatric and adult populations, depending on the setting and type of exposure, and this also applies to decreased ASL height.

To our knowledge, the role of gender has not been systematically evaluated in acquired CFTR dysfunction and its impact on ASL height. It is thought that the observed suppression of CFTR expression and impairment of CFTR function in cigarette smokers is a contributing factor to the pathogenesis of chronic obstructive pulmonary disease (COPD)(Dransfield et al., 2013; Raju et al., 2016). The main risk factor for COPD is cigarette smoking, and COPD is more common in men than in women, which may be directly related to the higher prevalence of smoking in men, although this gender gap is closing (Hitchman and Fong, 2011; Ntritsos et al., 2018; Syamlal et al., 2014). Nevertheless, the available clinical evidence in support of this AOP suggests that there is no remarkable gender difference.

Key Event Relationship Description

Serous and glandular secretions of the airway epithelium contribute to the ASL, and epithelial ion channel (e.g. CFTR, ENaC, CaCC, BK) function is critical to normal ASL homeostasis. Should the PCL decrease in depth, liquid will be absorbed from the mucus layer until the necessary depth is restored. Conversely, the mucus layer will absorb surplus PCL to reduce any increase in its depth. The regulation of these reabsorption processes is complex and not fully elucidated (Boucher, 2004). Experimental evidence suggests that the balance between Na^+ absorption and Cl^- secretion mediated by ENaC and CFTR plays a major role, with the ion channels affecting each other's activity (increased CFTR activity leads to decreased ENaC activity and vice versa) (Boucher, 2003; Boucher, 2004; Schmid et al., 2011). Mechanistic studies with selective CFTR and ENaC inhibitors suggest that the sensors for regulating ASL height lie within the ASL itself (Boucher, 2003; Hobbs et al., 2013). Additionally, ATP, adenosine and other purinergic receptor agonists, adenylate cyclase and cyclic adenosine monophosphate (cAMP)-dependent protein kinases acting on CFTR and/or ENaC ensure that the ASL height is adjusted to the appropriate height, resulting in maintenance of PCL depth at approximately the length of cilia (Antunes and Cohen, 2007). If the CFTR-ENaC interaction is perturbed, the airways become "dehydrated" (i.e., the ASL height decreases), resulting in slowing or inhibition of cilia movement and impaired MCC (Munkholm and Mortensen, 2014).

Evidence Supporting this KER

As a major Cl^- channel in the respiratory epithelium, CFTR levels and function are vital for maintenance of ASL homeostasis. In vitro studies on the effects of cigarette smoke exposure on human lung primary cells and cell lines showed a reduction in ASL height, associated with decreased CFTR levels (Hassan et al., 2014; Rasmussen et al., 2014; Xu et al., 2015; Ghosh et al., 2017) and decreased Cl^- current (Lambert et al., 2014; Raju et al., 2016). Moreover, pharmaceutical stimulation and inhibition of CFTR function and expression directly increased and decreased ASL height, respectively (Song et al., 2009; Van Goor et al., 2009; Van Goor et al., 2011; Tuggle et al., 2014).

Biological Plausibility

Impaired function of the CFTR and ENaC ion channels results in enhanced Na^+ absorption and reduced Cl^- secretion, and as a consequence, reduced ASL height. This phenomenon is well-known from studies in models of cystic fibrosis and acquired CFTR deficiency, even though the exact mechanism of the interaction between these two channels remains to be elucidated (Boucher, 2003; Hassan et al., 2014; Raju et al., 2016a; Rasmussen et al., 2014; Tarran et al., 2001a; Woodworth, 2015; Zhang et al., 2013). Additionally, evidence from studies with pharmacological agents that enhance CFTR expression and/or function or perturb the interaction between CFTR and ENaC provide further support for strong biological plausibility of this KER (Lambert et al., 2014; Van Goor et al., 2009; Van Goor et al., 2011).

Empirical Evidence

Addition of 2% cigarette smoke extract (CSE) to differentiated primary human bronchial epithelial cells (HBECs) stimulated with forskolin acutely decreased Cl^- transport, which was associated with reduced ASL height and cilia beat frequency (Raju et al., 2016a).

Exposure of differentiated primary HBECs to cigarette smoke for up to 120 h significantly reduced plasma membrane CFTR expression and ASL height (Hassan et al., 2014) in one study, and reduced intracellular cAMP levels, Cl^- conductance, ASL volume and cilia beat frequency in another study (Schmid et al., 2015).

Exposure of primary HBECs grown in monolayers to cigarette smoke caused a dose- and time-dependent decrease in forskolin-stimulated Cl^- transport, which could be partially restored by treatment with roflumilast via a cAMP-dependent mechanism. Smoke exposure also decreased ASL height, and this effect could also be ameliorated by roflumilast (Lambert et al., 2014).

Treatment of immortalized 16HBE14o- airway epithelial cells with 10% cigarette smoke extract for 48 h resulted in significant reduction in CFTR total and cell surface protein expression as well as in ASL height (Xu et al., 2015).

Repeated exposure of differentiated primary HBECs to smoke from little cigars significantly decreased CFTR protein expression and ASL height (Ghosh et al., 2017).

CFTR protein expression was also reduced following exposure of baby hamster kidney cells expressing human CFTR (BHK^{CFTR}) or Calu-3 lung cancer cells to cigarette smoke, resulting in cigarette smoke-induced increases in intracellular Ca^{2+}, CFTR internalization and subsequent reduction in ASL height (Rasmussen et al., 2014).

In excised pig and human trachea preparations, addition of amiloride and CFTR activators increased ASL height. In pig trachea, CFTR inhibition prevented the increase in ASL height produced by forskolin/3-isobutyl-1-methylxanthine (IBMX). This study did not investigate dose or time responses (Song et al., 2009).

Experiments with pharmacological compounds shown to enhance CFTR cell surface expression or channel open probability in primary human bronchial epithelial cells demonstrated enhanced Cl– currents and increased ASL height (Van Goor et al., 2009; Van Goor et al., 2011).

ASL height in the excised tracheas of rats without functional CFTR expression was approx. half that of wild-type animals (Tuggle et al., 2014).

Exposure of differentiated primary HBECs to supernatant from mucopurulent material (SMM) of cystic fibrosis airways decreased amiloride-sensitive Cl– current in CFTR wild-type but not CFTR mutant HBECs. Forskolin-induced Cl^- conductance was absent in treated CFTR mutant HBECs and enhanced in wild-type HBECs, where this effect was both dose- and time-dependent. Enhanced UTP-induced currents were seen in HBECs, independent of CFTR genotype. SMM treatment furthermore induced an increase in ASL height in CFTR wild-type but not mutant HBECs (Abdullah et al., 2018).

Knockdown of SPLUNC1, an allosteric regulator of ENaC, in human bronchial epithelial cells significantly reduced ENaC currents and rapidly and significantly reduced ASL height (Garcia-Caballero et al., 2009). SPLUNC1 is degraded in airway epithelia of cystic fibrosis patients, resulting in failure of ENaC internalization and, consequently, increased ENaC activity. Addition of recombinant SPLUNC1 or sputum supernatants from healthy subjects significantly elevated ASL height in HBECs (Hobbs et al., 2013; Webster et al., 2018).
Knock-down of β-ENaC mRNA in HBECs resulted in significantly reduced amiloride-sensitive short-circuit (Na+) currents and significantly increased ASL height (Gianotti et al., 2013). Knockdown of ENaC mRNA in BMI-1 transduced cystic fibrosis BECs resulted in nearly 50% reduction in amiloride short-circuit currents, a ca. 1.5 fold increase in ASL height and increased cilia beat frequency (Tagalakis et al., 2018).
Inhibition of channel activating proteases, including prostasin, matriptase, and furin, in ΔF508 cystic fibrosis airway epithelia by QUB-TL1 increased intracellular ENaC, decreased ENaC-mediated Na^+ transport and increased ASL height (Reihill et al., 2016).
In excised tracheas and bronchi from mice overexpressing the β-ENaC subunit in lower airway epithelia, amiloride-sensitive Na^+ transport was increased ca. 2- to 3-fold, and ASL was significantly depleted (Mall et al., 2004).

Uncertainties and Inconsistencies

The process of reabsorption of excess liquid to regulate ASL height is also known as “isosmotic volume hypothesis” or “isotonic volume transport/mucus clearance hypothesis” and implies that CFTR assumes a critical role in regulating ASL height by inhibiting ENaC activity (Ganesan et al., 2013; Matsui et al., 1998). However, an alternative, opposing hypothesis exists, the “hypotonic hypothesis” which states “*that normal airway epithelia are covered by an ASL with a [NaCl] sufficiently low (≤ 50 mM NaCl) to*

activate defensins and create an antimicrobial "shield" on airway surfaces", and there is evidence to both support and refute it (Cowley et al., 1997; Goldman et al., 1997; Jayaraman et al., 2001; Knowles et al., 1997; Landry and Eidelman, 2001; Matsui et al., 1998; Tarran et al., 2001a; Tarran et al., 2001b; Verkman et al., 2003). Other studies suggest the involvement of additional ion channels such as alternative chloride channels (Grasemann et al., 2007) and cyclic nucleotide-gated cation channels, particularly in the alveolar epithelium (Schwiebert et al., 1997; Wilkinson et al., 2011) in the regulation of ASL height.
In addition, one study showing that instillation of Pseudomonas aeruginosa-laden agarose beads into excised swine tracheas significantly increased ASL height, and that this increase could be blocked by pre-incubation with the CFTR inhibitor CFTRinh172 (100 µM, 30 minutes) (Luan et al., 2014) presents an inconsistency with the available evidence presented here.

Quantitative Understanding of the Linkage

While there are convincing quantitative data in support of this KER, it becomes clear from the review of the evidence that the downstream KE can only be modulated to a certain extent (e.g., maximal decrease in ASL height did not exceed 50% in the majority of studies), independent of the extent of change in the upstream KE. In addition, the available temporal data indicates that acute exposures predominantly cause a transient, rather than a lasting change in the downstream KE. Since chronic treatment data are not available, we judge our quantitative understanding as being moderate.

Response-response relationship

Treatment of fully differentiated primary human bronchial epithelial cells (HBECs) with 2% cigarette smoke extract (CSE; bubbling 10 puffs of smoke from one 3R4F reference into 1 mL DMSO, at 2 s/10 mL puff, 10 puffs over 3 min; defined as 100%) for 20 minutes reduced CFTR channel activity by 50% and ASL by approx. 2-fold (Raju et al., 2016).

Apical treatment of primary HBECs grown in monolayers with 2% CSE (not further described) for 24 h decreased forskolin-induced Cl^- currents by ca. 20% and ASL height by approx. 25%, and this could be counteracted by co-treatment with 10 µM ivacaftor, a CFTR potentiator known to significantly augment cAMP-mediated ion transport activity (Sloane et al., 2012).

Exposure of primary HBECs to cigarette smoke (5 min, ca. 12 puffs at 1 puff every 30 s; generated according to ISO standards) resulted in efficient removal of CFTR from the plasma membrane and a ca. 2-fold reduction in ASL height (Xu et al., 2015).

Exposure of primary HBECs, differentiated at the air-liquid interface, to cigarette smoke from 1 cigarette (ten 35-mL puffs, 2R4F reference cigarette) nearly abolished responses of the transepithelial electric potential difference Vt to ADO (i.e., blocking the ADO-A2b-cAMP-CFTR- active ion transport) and significantly decreased ASL volume/height by approx. 2-fold after 30 min (Clunes et al., 2012).

Exposure of fully differentiated primary HBECs to 30 puffs of whole smoke from 2 cigarettes (generated according to ISO standards) every day for 5 days (120 h) resulted in a ca. 40% reduction in CFTR expression and approx. 50% reduction in ASL height (Hassan et al., 2014).

Exposure of primary human airway epithelial cells grown in monolayers to whole smoke (3R4F reference cigarette; inExpose exposure system; 3L/min) resulted in significant reduction of CFTR Cl^- currents (ca. 40% for a 30-min exposure) and significantly decreased ASL depth from 11.4± 4.1 to 5.6± 2.0 µm (Lambert et al., 2014).

Exposure of fully differentiated primary HBECs to smoke from 1 cigarette or little cigar every day for 5 days (1 × 35 ml puff per 30 second, up to a butt length of 36 mm) significantly reduced CFTR protein expression by ca. 2- to 4-fold and ASL height by 10 to 20% (Ghosh et al., 2017).

Cell surface CFTR protein expression was reduced by ca. 70% following exposure of baby hamster kidney cells expressing human CFTR (BHK^{CFTR}) to cigarette smoke for 10 min (3R4F reference cigarette, 1 puff per min according to ISO standards). This was accompanied by a significant reduction in ASL height by approx. 50% (Rasmussen et al., 2014).

Treatment of primary HBECs from a cystic fibrosis patient with the ΔF508 mutation, grown as monolayer at the air-liquid interface, with the CFTR corrector VX-809 for 48 h increased CFTR maturation by ca. 8-fold and enhanced Cl^- transport by approx. 4-fold, from 1.9±0.4 to 7.8±1.3 µA/cm^2. VX-809 treatment for 5 days increased ASL height from 4.5±0.2 to 6.7±0.5 µm. Addition of 3 µM VX-770 further increased the ASL height to 9.2±0.2 µm (Van Goor et al., 2011). Treatment of primary HBECs from a G551D/ΔF508 cystic fibrosis patient, grown as monolayer at the air-liquid interface, with the CFTR potentiator VX-770 (10 µM) for 72 h dose-dependently increased forskolin-mediated Cl^- currents by ca. 10-fold to 27±2 µA/cm^2 and ASL volume to 125% that of controls (Van Goor et al., 2009).

ASL depth in the excised tracheas of rats without functional CFTR expression was approx. half that of wild-type animals (Tuggle et al., 2014).

Knockdown of mRNAs for the α- and β-ENaC subunits resulted in a ca. 70% decrease in amiloride-sensitive currents and a significant increase in ASL height from 6.8±0.5 and 7.4±0.5 µm to 9.8±0.6 and 9.6±0.8 µm in non-CF and CF epithelia, respectively (Gianotti et al., 2013).

Knockdown of α-ENaC mRNA in BMI1-transduced cystic fibrosis bronchial epithelial cells resulted in ca. 50% reduction in protein expression, reduction in amiloride-sensitive short-circuit current from 11.5 (siRNA control) to 6.4 µA/cm^2 and increase in ASL height

from 7.9 (siRNA control) to 12.1 µm (Tagalakis et al., 2018).

Overexpression of the β-ENaC subunit in mouse airways increased basal and amiloride-sensitive short-circuit currents approx. 2-fold (excised tracheas; compared to wild-type) and significantly reduced ASL height in bronchi and tracheas (by approx. 2 µm) (Mall et al., 2004).

Time-scale

Treatment of fully differentiated primary HBECs with 2% CSE (bubbling 10 puffs of smoke from one 3R4F reference into 1 mL DMSO, at 2 s/10 mL puff, 10 puffs over 3 minutes; defined as 100%) for 24 h decreased total CFTR expression and cell surface CFTR expression by approx. 20 and 25%, respectively, but treatment for 20 min did not. A 50% reduction in CFTR channel activity occurred immediately after addition of CSE and lasted for at least 20 minutes. A 2-fold reduction in ASL height was seen after 20 minutes, and ASL height was only partially restored at 1 h after CSE treatment (Raju et al., 2016a).

Following exposure of primary HBECs, differentiated at the air-liquid interface, to cigarette smoke from 1 cigarette (ten 35-mL puffs, 2R4F reference cigarette), ASL volume/height was significantly decreased by approx. 2-fold after 30 min. This decrease lasted for >2.5 h, and ASL height was restored at 4 h post-exposure (Clunes et al., 2012).
Exposure of BHK^{CFTR} cells to cigarette smoke for 10 min (3R4F reference cigarette, 1 puff per min according to ISO standards) resulted in a reduction in ASL height by approx. 50% within 30 min; the decrease lasted for up to 1 h post-exposure (Rasmussen et al., 2014).

Exposure of fully differentiated primary human HBECs to 30 puffs of whole smoke from 2 cigarettes (generated according to ISO standards) was sufficient to decrease ASL height by approx. 50% within 1 h of exposure, and following daily exposure for another 4 days, ASL height remained at around this level (Hassan et al., 2014).

Exposure of fully differentiated primary HBECs to whole smoke from four 3R4F reference cigarettes (generated according to ISO standard 3308; Vitrocell VC10 exposure system) resulted in a small, non-significant increase in ASL volume 4 h post-exposure. ASL volume decreased to baseline levels 7 h post-exposure and continued to drop below baseline levels until 24 h post-exposure (Schmid et al., 2015).

ASL height of primary HBECs dropped within 30 min of exposure to cigarette smoke (5 min, ca. 12 puffs at 1 puff every 30 seconds). ASL height stayed at that reduced level up to until 70 min post-exposure (Xu et al., 2015).

The maximum effect of VX-809 treatment on Cl^- currents of primary human bronchial epithelial cells, grown as monolayer at the air-liquid interface, occurred following 24 h, and Cl– transport returned to uncorrected levels within 48 h of compound washout (concurrent ASL data not available) (Van Goor et al., 2011).

Known modulating factors

Unknown

Known Feedforward/Feedback loops influencing this KER

Unknown

References

Antunes, M.B., and Cohen, N.A. (2007). Mucociliary clearance–a critical upper airway host defense mechanism and methods of assessment. Curr. Opin. Allergy Clin. Immunol. 7, 5-10.

Bose, S.J., Scott-Ward, T.S., Cai, Z., and Sheppard, D.N. (2015). Exploiting species differences to understand the CFTR Cl– channel. Biochem. Soc. Transact. 43, 975-982.

Boucher, R. (2003). Regulation of airway surface liquid volume by human airway epithelia. Pflügers Arch. 445, 495-498.

Boucher, R.C. (2004). New concepts of the pathogenesis of cystic fibrosis lung disease. Eur. Respir. J. 23, 146-158.

Chen, J.-M., Cutler, C., Jacques, C., Bœuf, G., Denamur, E., Lecointre, G., et al. (2001). A combined analysis of the cystic fibrosis transmembrane conductance regulator: implications for structure and disease models. Mol. Biol. Evol. 18., 1771-1788.

Clunes, L.A., Davies, C.M., Coakley, R.D., Aleksandrov, A.A., Henderson, A.G., Zeman, K.L., et al. (2012). Cigarette smoke exposure induces CFTR internalization and insolubility, leading to airway surface liquid dehydration. FASEB J. 26, 533-545.

Cowley, E., Wang, C., Gosselin, D., Radzioch, D., and Eidelman, D. (1997). Mucociliary clearance in cystic fibrosis knockout mice infected with Pseudomonas aeruginosa. European Respiratory Journal 10, 2312-2318.

Downs, C.A., Kreiner, L.H., Trac, D.Q., and Helms, M.N. (2013). Acute Effects of Cigarette Smoke Extract on Alveolar Epithelial Sodium Channel Activity and Lung Fluid Clearance. Am. J. Respir. Cell Mol. Biol. 49, 251-259.

Dransfield, M.T., Wilhelm, A.M., Flanagan, B., Courville, C., Tidwell, S.L., Raju, S.V., et al. (2013). Acquired cystic fibrosis

transmembrane conductance regulator dysfunction in the lower airways in COPD. Chest 144, 498-506.

Ganesan, S., Comstock, A.T., and Sajjan, U.S. (2013). Barrier function of airway tract epithelium. Tissue Barriers 1, e24997.

Garcia-Caballero, A., Rasmussen, J.E., Gaillard, E., Watson, M.J., Olsen, J.C., Donaldson, S.H., et al. (2009). SPLUNC1 regulates airway surface liquid volume by protecting ENaC from proteolytic cleavage. Proc. Natl. Acad. Sci. U. S. A. 106, 11412-11417.

Ghosh, A., Abdelwahab, S.H., Reeber, S.L., Reidel, B., Marklew, A.J., Garrison, A.J., et al. (2017). Little Cigars are More Toxic than Cigarettes and Uniquely Change the Airway Gene and Protein Expression. Sci. Rep. 7, 46239.

Gianotti, A., Melani, R., Caci, E., Sondo, E., Ravazzolo, R., Galietta, L.J., et al. (2013). Epithelial sodium channel silencing as a strategy to correct the airway surface fluid deficit in cystic fibrosis. Am. J. Respir. Cell Mol. Biol. 49, 445-452.

Goldman, M.J., Anderson, G.M., Stolzenberg, E.D., Kari, U.P., Zasloff, M., and Wilson, J.M. (1997). Human β-defensin-1 is a salt-sensitive antibiotic in lung that is inactivated in cystic fibrosis. Cell 88, 553-560.

Grasemann, H., Stehling, F., Brunar, H., Widmann, R., Laliberte, T.W., Molina, L., et al. (2007). Inhalation of Moli1901 in patients with cystic fibrosis. Chest 131, 1461-1466.

Hassan, F., Xu, X., Nuovo, G., Killilea, D.W., Tyrrell, J., Da Tan, C., et al. (2014). Accumulation of metals in GOLD4 COPD lungs is associated with decreased CFTR levels. Respir. Res. 15, 69.

Higgins, C.F. (1992). ABC transporters: from microorganisms to man. Ann. Rev. Cell Biol. 8, 67-113.

Hitchman, S.C., and Fong, G.T. (2011). Gender empowerment and female-to-male smoking prevalence ratios. Bull. World Health Organ. 89, 195-202.

Hobbs, C.A., Blanchard, M.G., Alijevic, O., Tan, C.D., Kellenberger, S., Bencharit, S., et al. (2013). Identification of the SPLUNC1 ENaC-inhibitory domain yields novel strategies to treat sodium hyperabsorption in cystic fibrosis airway epithelial cultures. Am. J. Physiol. Lung Cell. Mol. Physiol. 305(12), L990-L1001.

Jayaraman, S., Song, Y., Vetrivel, L., Shankar, L., and Verkman, A.S. (2001). Noninvasive in vivo fluorescence measurement of airway-surface liquid depth, salt concentration, and pH. J. Clin. Invest. 107, 317-324.

Knowles, M.R., Robinson, J.M., Wood, R.E., Pue, C.A., Mentz, W.M., Wager, G.C., et al. (1997). Ion composition of airway surface liquid of patients with cystic fibrosis as compared with normal and disease-control subjects. J. Clin. Invest. 100, 2588-2595.

Lambert, J.A., Raju, S.V., Tang, L.P., McNicholas, C.M., Li, Y., Courville, C.A., et al. (2014). Cystic fibrosis transmembrane conductance regulator activation by roflumilast contributes to therapeutic benefit in chronic bronchitis. Am. J. Respir. Cell Mol. Biol. 50, 549-558.

Landry, J.S., and Eidelman, D.H. (2001). Airway surface liquid: end of the controversy? J. Gen. Physiol. 117, 419-422.

Luan, X., Campanucci, V.A., Nair, M., Yilmaz, O., Belev, G., Machen, T.E., et al. (2014). Pseudomonas aeruginosa triggers CFTR-mediated airway surface liquid secretion in swine trachea. Proc. Natl. Acad. Sci. U.S.A. 111, 12930-12935.

Mall, M., Grubb, B.R., Harkema, J.R., O'Neal, W.K., and Boucher, R.C. (2004). Increased airway epithelial Na+ absorption produces cystic fibrosis-like lung disease in mice. Nat. Med. 10, 487.

Matsui, H., Grubb, B.R., Tarran, R., Randell, S.H., Gatzy, J.T., Davis, C.W., et al. (1998). Evidence for periciliary liquid layer depletion, not abnormal ion composition, in the pathogenesis of cystic fibrosis airways disease. Cell 95, 1005-1015.

Munkholm, M., and Mortensen, J. (2014). Mucociliary clearance: pathophysiological aspects. Clin. Physiol. Funct. Imaging 34, 171-177.

Ntritsos, G., Franek, J., Belbasis, L., Christou, M.A., Markozannes, G., Altman, P., et al. (2018). Gender-specific estimates of COPD prevalence: a systematic review and meta-analysis. Int. J. Chron. Obstruct. Pulmon. Dis. 13, 1507.

Raju, S.V., Lin, V.Y., Liu, L., Mcnicholas, C.M., Karki, S., Sloane, P.A., et al. (2016). The Cftr Potentiator Ivacaftor Augments Mucociliary Clearance Abrogating Cftr Inhibition by Cigarette Smoke. Am. J. Respir. Cell Mol. Biol. 56, 99-108.

Raju, S.V., Solomon, G.M., Dransfield, M.T., and Rowe, S.M. (2016). Acquired cystic fibrosis transmembrane conductance regulator dysfunction in chronic bronchitis and other diseases of mucus clearance. Clin. Chest Med. 37, 147-158.

Rasmussen, J.E., Sheridan, J.T., Polk, W., Davies, C.M., and Tarran, R. (2014). Cigarette smoke-induced Ca2+ release leads to cystic fibrosis transmembrane conductance regulator (CFTR) dysfunction. J. Biol. Chem. 289, 7671-7681.

Reihill, J.A., Walker, B., Hamilton, R.A., Ferguson, T.E., Elborn, J.S., Stutts, M.J., et al. (2016). Inhibition of protease–epithelial sodium channel signaling improves mucociliary function in cystic fibrosis airways. Am. J. Respir. Crit. Care Med. 194, 701-710.

Schmid, A., Clunes, L.A., Salathe, M., Verdugo, P., Dietl, P., Davis, C.W., et al. (2011). "Nucleotide-mediated airway clearance," in Purinergic Regulation of Respiratory Diseases. Springer), 95-138.

Schwiebert, E.M., Potter, E.D., Hwang, T.H., Woo, J.S., Ding, C., Qiu, W., et al. (1997). cGMP stimulates sodium and chloride

currents in rat tracheal airway epithelia. Am. J. Physiol. Cell Physiol. 272, C911-C922.

Shlyonsky, V., Boom, A., and Mies, F. (2016). Hydrogen Peroxide and Sodium Transport in the Lung and Kidney. Biomed Res. Int. 2016, 9512807.

Song, Y., Namkung, W., Nielson, D.W., Lee, J.-W., Finkbeiner, W.E., and Verkman, A.S. (2009). Airway surface liquid depth measured in ex vivo fragments of pig and human trachea: dependence on Na+ and Cl– channel function. Am. J. Physiol. Lung Cell. Mol. Physiol. 297, L1131-L1140.

Syamlal, G., Mazurek, J.M., and Dube, S.R. (2014). Gender differences in smoking among US working adults. Am. J. Prev. Med. 47, 467-475.

Tagalakis, A.D., Munye, M.M., Ivanova, R., Chen, H., Smith, C.M., Aldossary, A.M., et al. (2018). Effective silencing of ENaC by siRNA delivered with epithelial-targeted nanocomplexes in human cystic fibrosis cells and in mouse lung. Thorax 73, 847-856.

Tarran, R., Grubb, B., Parsons, D., Picher, M., Hirsh, A., Davis, C., et al. (2001a). The CF salt controversy: in vivo observations and therapeutic approaches. Mol. Cell 8, 149-158.

Tarran, R., Grubb, B.R., Gatzy, J.T., Davis, C.W., and Boucher, R.C. (2001b). The relative roles of passive surface forces and active ion transport in the modulation of airway surface liquid volume and composition. J. Gen. Physiol. 118, 223-236.

Tuggle, K.L., Birket, S.E., Cui, X., Hong, J., Warren, J., Reid, L., et al. (2014). Characterization of Defects in Ion Transport and Tissue Development in Cystic Fibrosis Transmembrane Conductance Regulator (CFTR)-Knockout Rats. PloS ONE 9, e91253.

Van Goor, F., Hadida, S., Grootenhuis, P.D.J., Burton, B., Cao, D., Neuberger, T., et al. (2009). Rescue of CF airway epithelial cell function in vitro by a CFTR potentiator, VX-770. Proc. Natl. Acad. Sci. U. S. A. 106, 18825-18830.

Van Goor, F., Hadida, S., Grootenhuis, P.D.J., Burton, B., Stack, J.H., Straley, K.S., et al. (2011). Correction of the F508del-CFTR protein processing defect in vitro by the investigational drug VX-809. Proc. Natl. Acad. Sci. U. S. A. 108, 18843-18848.

Verkman, A., Song, Y., and Thiagarajah, J.R. (2003). Role of airway surface liquid and submucosal glands in cystic fibrosis lung disease. Am. J. Physiol. Cell Physiol. 28, C2-C15.

Webster, M.J., Reidel, B., Tan, C.D., Ghosh, A., Alexis, N.E., Donaldson, S.H., et al. (2018). SPLUNC1 Degradation by the Cystic Fibrosis Mucosal Environment Drives Airway Surface Liquid Dehydration. Eur. Respir. J. 52, 1800668.

Wilkinson, W.J., Benjamin, A.R., De Proost, I., Orogo-Wenn, M.C., Yamazaki, Y., Staub, O., et al. (2011). Alveolar epithelial CNGA1 channels mediate cGMP-stimulated, amiloride-insensitive, lung liquid absorption. Pflügers Arch. 462, 267-279.

Woodworth, B.A. (2015). Resveratrol ameliorates abnormalities of fluid and electrolyte secretion in a hypoxia-Induced model of acquired CFTR deficiency. Laryngoscope 125, S1-S13.

Xu, X., Balsiger, R., Tyrrell, J., Boyaka, P.N., Tarran, R., and Cormet-Boyaka, E. (2015). Cigarette smoke exposure reveals a novel role for the MEK/ERK1/2 MAPK pathway in regulation of CFTR. Biochim. Biophys. Acta 1850, 1224-1232.

Zhang, S., Blount, A.C., McNicholas, C.M., Skinner, D.F., Chestnut, M., Kappes, J.C., et al. (2013). Resveratrol enhances airway surface liquid depth in sinonasal epithelium by increasing cystic fibrosis transmembrane conductance regulator open probability. PloS ONE 8, e81589.

Relationship: 2441: ASL Height, Decreased leads to CBF, Decreased

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Oxidative stress [MIE] Leading to Decreased Lung Function [AO]	adjacent	Moderate	Low

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	High	NCBI
Mus musculus	Mus musculus	Moderate	NCBI
Sus scrofa	Sus scrofa	Moderate	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Mixed	Moderate

Key Event Relationship Description

The airway surface liquid (ASL) is a liquid layer on the apical side of the respiratory epithelium, reportedly between 5 to 100 µm in depth (Widdicombe and Widdicombe, 1995), and consists of an inner aqueous periciliary liquid layer (PCL) that spans the length of cilia and the outer gel-like mucus layer. Under physiological conditions, ASL composition and height are regulated via vectorial transport of electrolytes, driven by transepithelial transport and apical secretion of Cl^- by (predominantly) CFTR, resulting in passive H_2O secretion and, consequently, increased ASL height. Absorption of Na^+ at the apical side by ENaC and ENaC's interaction with the basolateral Na^+/K^+-ATPase exchanging Na^+ for K^+ leads to net absorption of Na^+, which in turn drives fluid absorption and therefore decreases ASL height (Althaus, 2013; Hollenhorst et al., 2011). Decreased ASL height or ASL dehydration, if not rebalanced, results in cilia collapse and thereby effectively hinders coordinated ciliary beating (Knowles and Boucher, 2002; Matsui et al., 1998; Tarran et al., 2001)

Evidence Supporting this KER

Concurrent ASL height and CBF decreases were noted in human 3D airway epithelial cultures following exposure to cigarette smoke (Åstrand et al., 2014; Xu et al., 2015) and following the addition of large dextran molecules, low-melting point agarose or endogenous mucus (Button et al., 2012). Treatment of human airway epithelial with an ENaC inhibitor prevented the cigarette smoke effect on ASL height and CBF (Åstrand et al., 2014). In addition, treatment of cystic fibrosis airway cultures with a CFTR-modifying drug increased both ASL height and CBF (Van Goor et al., 2009).

Biological Plausibility

Boucher states that "*Morphological studies of normal cultures under these steady-state conditions reveal that the 7 µm height is optimal for the extension and beating of cilia and, therefore, is physiologically suited to efficient mucociliary clearance.*" (Boucher R., 2003). The link between decreased ASL height and reduced cilia beating has been established in multiple in vitro and in vivo studies (Van Goor et al., 2009; Xu et al., 2015; Zhang et al., 2014), and even though the evidence does not describe causality between these two events, this KER is biologically plausible (Button et al., 2012; Mall, 2008).

Empirical Evidence

When increasing the osmotic modulus in human bronchial epithelial cells (differentiated at the air-liquid interface) by treatment with large dextran molecules, low-melting point agarose or endogenous mucus, ASL height decreased and cilia collapsed. Although the cilia were still beating, they did not do so at their full height (Button et al., 2012).

Uncertainties and Inconsistencies

Although the empirical evidence suggests a link between decreased ASL height and reduced cilia beating, causality between the two KEs has not been proven nor has this KER been systematically examined or quantified yet.

Quantitative Understanding of the Linkage

The evidence provided here stems from studies reporting on the effects of stressors such as cigarette smoke on both ASL height and CBF. Although the empirical evidence suggests a link between decreased ASL height and reduced ciliary beating, causality between the two KEs has not been proven nor has this KER been systematically examined or quantified yet. Our quantitative understanding of this KER is therefore poor (weak).

Response-response relationship

Osmotic compression of the ASL between 300 and 800 Pa (using large dextran molecules, endogenous mucin or low-melting point agarose) had minimal effects on cilia height or cilia beating. At osmotic pressures exceeding 800 Pa, the ASL became compressed from 7 to less than 2 µm, and cilia height decreased to approx. the same extent (consequently, cilia were not beating at full height) (Button et al., 2012).

Exposure of primary human airway epithelial cells to cigarette smoke (5 min, ca. 12 puffs at 1 puff every 30 seconds) resulted in a ca. 2-fold reduction in ASL height. CBF decreased from 4.19 ± 0.24 Hz (in air control) to 1.28 ± 0.06 Hz. Replenishment of the ASL by addition of 50 µL PBS restored CBF in air- and smoke-exposed cultures (6.04 ± 0.3 Hz vs 6.82 ± 0.37 Hz) (Xu et al., 2015).

Treatment of murine nasal septal epithelia with Sinupret, a phytomedicine, significantly increased ASL depth from 5.25±0.38 to 9.14±0.42 µm and increased the mean CBF from 1.52±0.10 to 2.05±0.15 when applied apically, from 0.99±0.04 to 1.37±0.09 when

applied basally, and from 1.53±0.09 to 2.17±0.12 when applied to both compartments (Zhang et al., 2014).

An experimental compound targeting ENaC termed “compound A” dose-dependently increased ASL height in ASL-depleted cultures (absorptive mode analysis). Following exposure to cigarette smoke (1 2R4F cigarette, ISO smoking regimen), ASL decreased by approx. 4 µm within 30 min compared to air controls, and this could be prevented by a 2.5-h pre-treatment with 1 µM compound A. In the same cultures, CBF was significantly decreased by more than 1 Hz following cigarette smoke exposure, whereas pre-treatment with compound A completely prevented this (Åstrand et al., 2014).

Time-scale

ASL height of primary human airway epithelial cells dropped within 30 min of exposure to cigarette smoke (5 min, ca. 12 puffs at 1 puff every 30 s). ASL height stayed at that reduced level up until 70 min post-exposure. Significant decreases in CBF in cigarette smoke-exposed cultures were seen 3 h post-exposure (Xu et al., 2015).

Treatment of primary human bronchial epithelial cells from a cystic fibrosis patient with the G551D/ΔF508 genotype, grown as monolayer at the air-liquid interface, with the CFTR potentiator VX-770 (10 µM) for 72 h increased ASL height by approx. 25%, and treatment for 5 days more than doubled CBF (Van Goor et al., 2009).

Exposure of human bronchial epithelial cells to cigarette smoke decreased ASL height by approx. 4 µm within 30 min, whereas pre-treatment with 1 µM compound A prevented this decrease. When compound A was added 30 min after exposure to cigarette smoke, ASL height returned to normal levels significantly more quickly. In the same exposed cultures CBF was decreased by more than 1 Hz within 1 h (Åstrand et al., 2014).

Known modulating factors

Unknown

Known Feedforward/Feedback loops influencing this KER

Unknown

References

- Althaus, M. (2013). ENaC inhibitors and airway re-hydration in cystic fibrosis: state of the art. Curr. Mol. Pharmacol. 6(1), 3-12.
- Åstrand, A.B., Hemmerling, M., Root, J., Wingren, C., Pesic, J., Johansson, E., et al. (2014). Linking increased airway hydration, ciliary beating, and mucociliary clearance through ENaC inhibition. Am. J. Physiol. Lung Cell. Mol. Physiol. 308(1), L22-L32.
- Boucher, R. (2003). Regulation of airway surface liquid volume by human airway epithelia. Pflügers Arch. 445(4), 495-498.
- Button, B., Cai, L.-H., Ehre, C., Kesimer, M., Hill, D.B., Sheehan, J.K., et al. (2012). A periciliary brush promotes the lung health by separating the mucus layer from airway epithelia. Science 337(6097), 937-941.
- Hollenhorst, M.I., Richter, K., and Fronius, M. (2011). Ion transport by pulmonary epithelia. Biomed. Res. Int. 2011(2011), 174306.
- Knowles, M.R., and Boucher, R.C. (2002). Mucus clearance as a primary innate defense mechanism for mammalian airways. J. Clin. Investig. 109(5), 571-577.
- Mall, M.A. (2008). Role of cilia, mucus, and airway surface liquid in mucociliary dysfunction: lessons from mouse models. J. Aerosol Med. Pulm. Drug Deliv. 21(1), 13-24.
- Matsui, H., Grubb, B.R., Tarran, R., Randell, S.H., Gatzy, J.T., Davis, C.W., et al. (1998). Evidence for periciliary liquid layer depletion, not abnormal ion composition, in the pathogenesis of cystic fibrosis airways disease. Cell 95(7), 1005-1015.
- Tarran, R., Grubb, B., Parsons, D., Picher, M., Hirsh, A., Davis, C., et al. (2001). The CF salt controversy: in vivo observations and therapeutic approaches. Mol. Cell 8(1), 149-158.
- Van Goor, F., Hadida, S., Grootenhuis, P.D.J., Burton, B., Cao, D., Neuberger, T., et al. (2009). Rescue of CF airway epithelial cell function in vitro by a CFTR potentiator, VX-770. Proc. Natl. Acad. Sci. U. S. A. 106(44), 18825-18830.
- Widdicombe, J., and Widdicombe, J. (1995). Regulation of human airway surface liquid. Respir. Physiol. 99(1), 3-12.
- Xu, X., Balsiger, R., Tyrrell, J., Boyaka, P.N., Tarran, R., and Cormet-Boyaka, E. (2015). Cigarette smoke exposure reveals a novel role for the MEK/ERK1/2 MAPK pathway in regulation of CFTR. Biochim. Biophys. Acta 1850(6), 1224-1232.
- Zhang, S., Skinner, D., Hicks, S.B., Bevensee, M.O., Sorscher, E.J., Lazrak, A., et al. (2014). Sinupret Activates CFTR and TMEM16A-Dependent Transepithelial Chloride Transport and Improves Indicators of Mucociliary Clearance. PloS one 9(8), e104090. doi: 10.1371/journal.pone.0104090.

Relationship: 2442: CBF, Decreased leads to MCC, Decreased

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Oxidative stress [MIE] Leading to Decreased Lung Function [AO]	adjacent	High	Moderate

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Evidence Supporting Applicability of this Relationship			

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	High	NCBI
Mus musculus	Mus musculus		NCBI
Canis lupus	Canis lupus		NCBI
Cavia porcellus	Cavia porcellus		NCBI
Ovis aries	Ovis aries		NCBI
Lithobates catesbeianus	Rana catesbeiana		NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Mixed	High

Key Event Relationship Description

Synchronized ciliary action transports mucus from the distal lung to the mouth, where it is swallowed or expectorated (Munkholm and Mortensen, 2014). In addition to ASL and mucus properties, the speed of ciliary movement, and hence the effectiveness of mucociliary clearance (MCC), is dependent on ciliary amplitude and beat frequency (Rubin, 2002). CBF itself is influenced by several factors, including changes in the physical and chemical properties of the ASL (especially the periciliary fluid), structural modulation in the cilia, concentration of cyclic nucleotides cAMP and cGMP, and intracellular calcium (Ca^{2+}). Aside from genetic defects leading to ciliopathies, there is ample evidence for prolonged exposure to noxious agents, such as cigarette smoke, nitrogen oxide and sulfur dioxide, playing a major role in decreasing CBF and hampering efficient MCC.

Evidence Supporting this KER

A decrease in CBF resulting from sulfur dioxide exposure reduced mucociliary clearance in dogs (Yeates et al., 1997) and mucociliary activity in guinea pig tracheas (Knorst et al., 1994). In rats, formaldehyde inhalation exposure resulted in lower numbers of ciliated cells, while ciliary activity and mucus flow rates were decreased in a dose and time-dependent manner (Morgan et al., 1986). In humans, CBF positively correlates with nasal mucociliary clearance time (Ho et al., 2001), and bronchiectasis patients have lower nasal CBF and slower mucociliary transport (MCT) (Rutland and Cole, 1981). Administration of nebulized CBF inhibitors and enhancers quantifiably decreased or increased mucociliary clearance, respectively (Boek et al., 1999; Boek et al., 2002). Increased CBF and MCT was also noted in human sinonasal epithelial cell cultures treated with Myrtol®, an essential oil distillate (Lai et al., 2014) and in sheep tracheas and human airway epithelial cultures subjected to temperature changes (Kilgour et al., 2004; Sears et al., 2015). Exposures of frog palate epithelia to formaldehyde and PM10 reduced MCC and mucociliary transport, but only formaldehyde-treated epithelia showed decreases in CBF (Morgan et al., 1984; Macchione et al., 1999; Fló-Neyret et al., 2001).
Ex vivo treatment of sheep trachea with acetylcholine and epinephrine increased CBF, but only acetylcholine increased surface liquid velocity, while both parameters were decreased upon incubation with platelet-activating factor (Seybold et al., 1990).

Biological Plausibility

Ciliary function and mucus transport are invariably linked to effective mucus transport along the mucociliary escalator. Studies in animal models of ciliopathies and in individuals with genetic disorders causing cilia defects demonstrate that absent or asynchronous cilia beating results in defective mucus clearance from the lungs, consequently leading to respiratory infections that may be chronic recurrent in nature and ultimately lead to declining lung function (Knowles et al., 2013; Munkholm and Mortensen, 2014; Tilley et al., 2015). Similarly, indirect effects of airway inflammation, caused for example by respiratory infections or allergies, are known to be responsible for changes in cilia beating and hence mucus clearance (Almeida-Reis et al., 2010; Hisamatsu and Nakajima, 2000; Maurer et al., 1982). Finally, airway epithelial injury following exposure to inhalation toxicants can also damage cilia and inhibit cilia function and thereby impair MCC (Iravani and Van As, 1972; Wanner et al., 1996). Therefore, this KER is biologically plausible.

Empirical Evidence

A decrease in CBF resulting from sulfur dioxide exposure reduced mucociliary clearance in dogs (Yeates et al., 1997) and mucociliary activity in guinea pig tracheas (Knorst et al., 1994). In rats, formaldehyde inhalation exposure resulted in lower numbers

of ciliated cells, while ciliary activity and mucus flow rates were decreased in a dose and time-dependent manner (Morgan et al., 1986). In humans, CBF positively correlates with nasal mucociliary clearance time (Ho et al., 2001), and bronchiectasis patients have lower nasal CBF and slower mucociliary transport (MCT) (Rutland and Cole, 1981). Administration of nebulized CBF inhibitors and enhancers quantifiably decreased or increased mucociliary clearance, respectively (Boek et al., 1999; Boek et al., 2002). Increased CBF and MCT was also noted in human sinonasal epithelial cell cultures treated with Myrtol®, an essential oil distillate (Lai et al., 2014) and in sheep tracheas and human airway epithelial cultures subjected to temperature changes (Kilgour et al., 2004; Sears et al., 2015). Exposures of frog palate epithelia to formaldehyde and PM10 reduced MCC and mucociliary transport, but only formaldehyde-treated epithelia showed decreases in CBF (Morgan et al., 1984; Macchione et al., 1999; Fló-Neyret et al., 2001).

The available evidence does not interrogate the direct relationship between CBF and MCC, but rather evaluates both outcomes in parallel. However, because of the intrinsic linkage of cilia function and MCC, we find the empirical evidence in support of this KER to be moderate.
Ex vivo treatment of sheep trachea with acetylcholine and epinephrine increased CBF, but only acetylcholine increased surface liquid velocity, while both parameters were decreased upon incubation with platelet-activating factor (Seybold et al., 1990).

Uncertainties and Inconsistencies

Although ciliary function is considered a primary determinant for effective MCC (Duchateau et al., 1985; Gizurarson, 2015), there is evidence that suggests that MCC can be impeded by other factors that do not affect CBF. For example, nasal CBF in cigarette smokers regularly exhaling through the nose was not significantly different from that of nonsmokers, although they exhibited significantly longer nasomuciliary clearance times compared to nonsmokers. Possible explanations offered for this discrepancy were a potential loss of cilia in the nasal epithelium or increased mucus viscoelasticity (Stanley et al., 1986). Similarly, formaldehyde exposure of rats resulted in decreased cilia numbers and slower mucus flow rates (Morgan KT et al., 1986). On the other hand, there are a number of pharmacological compounds that improve mucociliary clearance through reductions in mucus viscosity, but have no effect on CBF (Jiao and Zhang, 2019), or through increases in CBF, but have no effect on mucociliary clearance (Phillips et al., 1990).

Quantitative Understanding of the Linkage

There are several studies providing insights into the negative effect of inhalation exposures on CBF and MCC, that are in line with the current thinking on how these two KEs connect. Additionally, pharmacological studies demonstrated that stimulation of CBF typically results in stimulation of MCC. However, since most studies usually evaluated the KEs in parallel, and even though some results support both dose response and temporal sequence of the KEs, none of the available data affirms causal linkage between CBF and MCC. Our understanding of the evidence is therefore moderate.

Response-response relationship

CBF decreased sequentially with increasing SO_2 doses in dogs. CBF decreased from 6.3 ± 0.2 (SE) Hz at baseline to 5.7 ± 0.2 Hz at 5.5 ppm SO_2. Five ppm SO_2 delivered to both the trachea and tracheobronchial airways for 20 min also caused a marked decrease in mean bronchial mucociliary clearance from 53.7 ± 5.7% to 32.8 ± 7.7% after 90 min (Yeates et al., 1997).

The effects of 30-min exposure to SO_2 on mucociliary activity (MCA) and ciliary beat frequency (CBF) were studied in 31 guinea pig tracheas. A 63% reduction in mean MCA and statistically insignificant changes in CBF were recorded at concentrations of 2.5 ppm SO_2. Higher SO_2 concentrations caused further impairment of MCA as well as a dose-dependent decrease in CBF: At 5 ppm SO2, CBF decreased by 45%, at 12.5 ppm by 72%. The maximum decrease in MCA (81%) was observed with 7.5 ppm SO_2; the highest SO_2 concentration did not decrease MCA further. The decrease in MCA was associated with an impairment of CBF only at SO_2 concentrations ≥5.0 ppm (Knorst et al., 1994b).

Administration of a nebulized CBF inhibitor (0.9% NaCl) to 15 healthy volunteers significantly decreased mucociliary transport (MCT) from 7.9±1.5 mm/min (SEM) to 4.5±1.6 mm/min. Salbutamol, a CBF enhancer, significantly increased MCT from 8.0±1.4 to 12.5±1.1 mm/min (Boek et al., 2002; Boek et al., 1999).

Cooling human airway epithelial cultures grown at the air-liquid interface from 37°C to 25°C over the course of approx. 20 min decreased CBF from 12 to 6 Hz and mucociliary transport (MCT) from 140 to 90 µm/s. Extending the range of temperature tested, CBF was found to increase by 0.49±0.06 Hz for every temperature increase by 1°C, and this was mirrored by an increase in MCT. MCT increased on average between 5 and 11 µm/s for every Hz increase in CBF. This study also showed that CBF decreased with increasing mucin concentration, dropping from 12.4 Hz at 2% bovine submaxillary mucin (BSM) to 10.1 Hz at 8% BSM, concurrent with a ca. 70% reduction in MCT. In addition, treatment with 10 µM basolateral forskolin reproducibly increased CBF by 19.3±2.1% and MCT by 24.4±3.1% over baseline (Sears et al., 2015). In sheep trachea CBF and mucus transport velocity (MTV) were 9.8±2.7 beats/s and 5.7±2.6 mm/min, respectively, at baseline. Temperature reductions from 37°C to 34°C caused a progressive decline in CBF (ca. –20% at 2 h and –90% at 4 h) and MTV (ca. –50% at 2 h and –90% at 4 h), which was further exacerbated by additional temperature decreases (30°C; CBF: ca. –75% at 2 h; MTV: –80% at 2 h) (Kilgour et al., 2004).

Frog palate preparations were incubated with 1.25, 2.5 and 5.0 ppm formaldehyde. At formaldehyde doses of 2.5 and 5 ppm, CBF decreased by ca. 25% compared to baseline within 30 min and by 35-50% within 60 min (Fló-Neyret et al., 2001).
Incubation of frog palates with PM10 from Sao Paolo, Brazil, for up to 120 min did not affect CBF but decreased MCT at concentrations ≥1000 pg/m3 (Macchione et al., 1999)

In freshly excised sheep tracheas, a 60-min incubation with 10 µM platelet-activating factor caused a 6% decrease in CBF and a dose-dependent decrease in surface liquid velocity, reaching a maximum of 63% (Seybold et al., 1990).

In patients with bronchiectasis, nasal CBF was 12.8±1.3 Hz and nasal clearance time was 31.8± 18.4 min. In comparison, in healthy controls, nasal CBF was 14.0±1.3 Hz and nasal clearance time was 17.6± 8.3 min (Rutland and Cole, 1981).

Following basolateral treatment of human sinonasal epithelial cell cultures grown at the air-liquid interface with Myrtol®, a phytopharmaceutical mixture of distillates of rectified essential oils of eucalyptus, sweet orange, myrtle, and lemon as the active ingredients, increased CBF in a dose-dependent manner, with a maximum stimulation with 0.1% of 48±7% after 30 min. The same concentration caused a 46±16% increase in MCT at 40 min (Lai et al., 2014).

In New Zealand white rabbits exposed to 3 ppm NO_2 for 24 h, the average CBF decreased from 764 beats/min to 692 beats/min, and the transport velocity decreased from 5.23 mm/min to 3.03 mm/min (Kakinoki, 1998).

Time-scale

A 20-minute exposure of dogs to SO_2 caused a decrease in mean bronchial MCC after 90 min (Yeates et al., 1997).

Frog palate epithelia were incubated with 1.25, 2.5 and 5.0 ppm formaldehyde. At formaldehyde doses of 2.5 and 5 ppm, CBF decreased by ca. 25% compared to baseline within 30 min and by 35-50% within 60 min (Fló-Neyret et al., 2001).

Incubation of freshly excised sheep tracheas with 10 µM platelet-activating factor caused a maximal decrease in CBF of 6% after 60 min and decrease in surface liquid velocity of ca. 30% at 20 min, ca. 50% at 40 min and 63% after 60 min (Seybold et al., 1990).

Following basolateral treatment of human sinonasal epithelial cell cultures grown at the air-liquid interface with different concentrations of Myrtol®, CBF increased rapidly within the first 30 min and then declined thereafter. The maximum response for MCT was seen after 40 min (Lai et al., 2014).

Known modulating factors

Physiological factors such as age, sex, posture, sleep, and exercise were shown to affect MCC, although study findings are not always concordant (Houtmeyers et al., 1999). MCC and CBF, for example, were observed to decrease with age in several species in numerous studies (e.g. guinea pigs, mice, and human) (Bailey et al., 2014; Grubb et al., 2016; Ho et al., 2001; Joki and Saano, 1997; Paul et al., 2013; Yager et al., 1978), but evidence by (Agius et al., 1998) suggests that age does not have a major effect on CBF.

Known Feedforward/Feedback loops influencing this KER

Unknown

References

- Agius, A.M., Smallman, L.A., and Pahor, A.L. (1998). Age, smoking and nasal ciliary beat frequency. Clin. Otolaryngol. Allied Sci. 23(3), 227-230.
- Almeida-Reis, R., Toledo, A.C., Reis, F.G., Marques, R.H., Prado, C.M., Dolhnikoff, M., et al. (2010). Repeated stress reduces mucociliary clearance in animals with chronic allergic airway inflammation. Respiratory physiology & neurobiology 173(1), 79-85.
- Bailey, K.L., Bonasera, S.J., Wilderdyke, M., Hanisch, B.W., Pavlik, J.A., DeVasure, J., et al. (2014). Aging causes a slowing in ciliary beat frequency, mediated by PKCε. American journal of physiology. Lung cellular and molecular physiology 306(6), L584-L589. doi: 10.1152/ajplung.00175.2013.
- Boek, W.M., Graamans, K., Natzijl, H., van Rijk, P.P., and Huizing, E.H. (2002). Nasal Mucociliary Transport: New Evidence for a Key Role of Ciliary Beat Frequency. Laryngoscope 112(3), 570-573.
- Boek, W.M., Keleş, N., Graamans, K., and Huizing, E.H. (1999). Physiologic and hypertonic saline solutions impair ciliary activity in vitro. Laryngoscope 109(3), 396-399.
- Duchateau, G.S., Merkus, F.W., Zuidema, J., and Graamans, K. (1985). Correlation between nasal ciliary beat frequency and mucus transport rate in volunteers. The Laryngoscope 95(7), 854-859.
- Fló-Neyret, C., Lorenzi-Filho, G., Macchione, M., Garcia, M.L.B., and Saldiva, P.H.N. (2001). Effects of formaldehyde on the frog's mucociliary epithelium as a surrogate to evaluate air pollution effects on the respiratory epithelium. Braz. J. Med. Biol. Res. 34, 639-643.
- Gizurarson, S. (2015). The effect of cilia and the mucociliary clearance on successful drug delivery. Biological and Pharmaceutical Bulletin, b14-00398.
- Grubb, B.R., Livraghi-Butrico, A., Rogers, T.D., Yin, W., Button, B., and Ostrowski, L.E. (2016). Reduced mucociliary clearance in old mice is associated with a decrease in Muc5b mucin. American journal of physiology. Lung cellular and molecular physiology 310(9), L860-L867.
- Hisamatsu, K.-i., and Nakajima, M. (2000). Pranlukast protects leukotriene C4- and D4-induced epithelial cell impairment of the nasal mucosa in vitro. Life Sciences 67(22), 2767-2773.
- Ho, J.C., Chan, K.N., Hu, W.H., Lam, W.K., Zheng, L., Tipoe, G.L., et al. (2001). The Effect of Aging on Nasal Mucociliary Clearance, Beat Frequency, and Ultrastructure of Respiratory Cilia. Am. J. Respir. Crit. Care Med. 163(4), 983-988.
- Houtmeyers, E., Gosselink, R., Gayan-Ramirez, G., and Decramer, M. (1999). Regulation of mucociliary clearance in health and disease. European Respiratory Journal 13(5), 1177-1188.

- Iravani, J., and Van As, A. (1972). Mucus transport in the tracheobronchial tree of normal and bronchitic rats. The Journal of pathology 106(2), 81-93.
- Jiao, J., and Zhang, L. (2019). Influence of Intranasal Drugs on Human Nasal Mucociliary Clearance and Ciliary Beat Frequency. Allergy Asthma Immunol Res 11(3), 306-319.
- Joki, S., and Saano, V. (1997). Influence of ageing on ciliary beat frequency and on ciliary response to leukotriene D4 in guinea-pig tracheal epithelium. Clinical and experimental pharmacology and physiology 24(2), 166-169.
- Kakinoki, Y.O., Ayaki Tanaka, Yushi Washio, Koji Yamada, Yoshiaki Nakai, Kazuhiro Morimoto, Yasushi (1998). Nitrogen dioxide compromises defence functions of the airway epithelium. Acta Otolaryngol. 118(538), 221-226.
- Kilgour, E., Rankin, N., Ryan, S., and Pack, R. (2004). Mucociliary function deteriorates in the clinical range of inspired air temperature and humidity. Intensive Care Med. 30(7), 1491-1494.
- Knorst, M.M., Kienast, K., Riechelmann, H., Müller-Quernheim, J., and Ferlinz, R. (1994). Effect of sulfur dioxide on mucociliary activity and ciliary beat frequency in guinea pig trachea. Int. Arch. Occup. Environ. Health 65(5), 325-328.
- Knowles, M.R., Daniels, L.A., Davis, S.D., Zariwala, M.A., and Leigh, M.W. (2013). Primary ciliary dyskinesia. Recent advances in diagnostics, genetics, and characterization of clinical disease. American journal of respiratory and critical care medicine 188(8), 913-922.
- Lai, Y., Dilidaer, D., Chen, B., Xu, G., Shi, J., Lee, R.J., et al. (2014). In vitro studies of a distillate of rectified essential oils on sinonasal components of mucociliary clearance. Am. J. Rhinol. Allergy 28(3), 244-248.
- Macchione, M., Oliveira, A.P., Gallafrio, C.T., Muchão, F.P., Obara, M.T., Guimarães, E.T., et al. (1999). Acute effects of inhalable particles on the frog palate mucociliary epithelium. Environ. Health Perspect. 107(10), 829-833.
- Maurer, D., Sielczak, M., Oliver Jr, W., Abraham, W., and Wanner, A. (1982). Role of ciliary motility in acute allergic mucociliary dysfunction. Journal of Applied Physiology 52(4), 1018-1023.
- Morgan, K., Patterson, D., and Gross, E. (1986). Responses of the nasal mucociliary apparatus of F-344 rats to formaldehyde gas. Toxicol. Appl. Pharmacol. 82(1), 1-13.
- Munkholm, M., and Mortensen, J. (2014). Mucociliary clearance: pathophysiological aspects. Clin. Physiol. Funct. Imaging 34(3), 171-177.
- Paul, P., Johnson, P., Ramaswamy, P., Ramadoss, S., Geetha, B., and Subhashini, A.S. (2013). The Effect of Ageing on Nasal Mucociliary Clearance in Women: A Pilot Study. ISRN Pulmonology 2013, 5.
- Phillips, P.P., McCaffrey, T.V., and Kern, E.B. (1990). The in vivo and in vitro effect of phenylephrine (Neo Synephrine) on nasal ciliary beat frequency and mucociliary transport. Otolaryngology—Head and Neck Surgery 103(4), 558-565.
- Rubin, B.K. (2007). Mucus structure and properties in cystic fibrosis. Paediatr Respir Rev 8(1), 4-7.
- Rutland, J., and Cole, P.J. (1981). Nasal mucociliary clearance and ciliary beat frequency in cystic fibrosis compared with sinusitis and bronchiectasis. Thorax 36(9), 654-658.
- Sears, P.R., Yin, W.-N., and Ostrowski, L.E. (2015). Continuous mucociliary transport by primary human airway epithelial cells in vitro. Am. J. Physiol. Lung Cell. Mol. Physiol. 309(2), L99-L108.
- Seybold, Z.V., Mariassy, A.T., Stroh, D., Kim, C.S., Gazeroglu, H., and Wanner, A. (1990). Mucociliary interaction in vitro: effects of physiological and inflammatory stimuli. J. Appl. Physiol. 68(4), 1421-1426. doi: 10.1152/jappl.1990.68.4.1421.
- Stanley, P., Wilson, R., Greenstone, M., MacWilliam, L., and Cole, P. (1986). Effect of cigarette smoking on nasal mucociliary clearance and ciliary beat frequency. Thorax 41(7), 519-523.
- Tilley, A.E., Walters, M.S., Shaykhiev, R., and Crystal, R.G. (2015). Cilia dysfunction in lung disease. Annu Rev Physiol 77, 379-406.
- Wanner, A., Salathe, M., and O'Riordan, T.G. (1996). Mucociliary clearance in the airways. Am. J. Respir. Crit. Care Med. 154(6 Pt 1), 1868-1902.
- Yager, J., Chen, T.-M., and Dulfano, M.J. (1978). Measurement of frequency of ciliary beats of human respiratory epithelium. Chest 73(5), 627-633.
- Yeates, D.B., Katwala, S.P., Daugird, J., Daza, A.V., and Wong, L.B. (1997). Excitatory and inhibitory neural regulation of tracheal ciliary beat frequency (CBF) activated by ammonia vapour and SO2. Ann. Occup. Hyg. 41, 736-744.

Relationship: 2443: MCC, Decreased leads to Decreased lung function

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Oxidative stress [MIE] Leading to Decreased Lung Function [AO]	adjacent	Moderate	Moderate

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	High	NCBI

Life Stage Applicability

Life Stage	Evidence

All life stages High

Life Stage	Evidence

Sex Applicability

Sex	Evidence
Mixed	High

Key Event Relationship Description

It is very well known that patients suffering from motile ciliopathies, such as primary ciliary dyskinesia, have impaired or absent MCC and lower lung function (reduced FEV1 and FVC) compared to their healthy counterparts (Halbeisen et al., 2018; Marthin et al., 2010; Wallmeier et al., 2020). In cystic fibrosis patients, MCC is impaired by two main mechanisms: 1) decreased airway hydration and 2) changes in mucus chemical and viscoelastic properties. This causes mucus build-up which can lead to mucus plugging in the airways and consequently leads to decreased lung function over time (Kerem et al., 2014; Mossberg et al., 1978; Regnis et al., 1994; Robinson and Bye, 2002; Szczesniak et al., 2017; Wanner et al., 1996). Mucus plugging due to decreased MCC is also considered a major cause of airway obstruction and airflow limitation in COPD patients (Dunican et al., 2021; Okajima et al., 2020) and asthmatics (Kuyper et al., 2003; Maxwell, 1985).

Evidence Supporting this KER

Changes in MCC rate are typically paralleled by effects on lung function in several studies where both endpoints have been assessed. In patients with primary ciliary dyskinesia, absence of cilia motion prevents normal MCC and consequently, lung function is reduced (Denizoglu Kulli et al., 2020). In cystic fibrosis patients, the ASL is depleted resulting in impaired MCC (Boucher, 2004). Although the known CFTR genotypes can result in a variety of phenotypes (Derichs, 2013), clinical data indicate that some specific gene defects, such as the p.Phe508del variant, are more frequently associated with decreased lung function indices (e.g. FEV1 % predicted, FVC % predicted, FEF25-75) (Kerem et al., 1990; Johansen et al., 1991; Schaedel et al., 2002). Both cigarette smoking and occupational exposure to biomass fumes led to slower MCC and reduced FEV1 % predicted and FEV1/FVC (Ferreira et al., 2018). Nasomucociliary clearance was slower in COPD smokers compared to former smokers with COPD or to nonsmokers (Ito et al., 2015). Allergen challenge in asthma patients resulted in both reduced MCC and FEV1, which could be reversed by inhalation of hypertonic saline solution (Alexis et al., 2017). In cystic fibrosis patients, treatment with mucolytic agents (Laube et al., 1996; McCoy et al., 1996; Quan et al., 2001; Elkins et al., 2006; Amin et al., 2011; Donaldson et al., 2018) or a CFTR potentiator (Rowe et al., 2014) improved both MCC and lung function (FEV1, FVC and FEF25-75).

Biological Plausibility

Lung function is known to decrease with age, and several studies showed that mucus transport rates also decrease in older compared to younger individuals (Goodman et al., 1978; Uzeloto et al., 2021). Impaired MCC is also seen in chronic smokers, even prior to a clinically significant drop in lung function and the detection of small airway disease (Clunes et al., 2012a; Goodman et al., 1978; Lourenço et al., 1971; Uzeloto et al., 2021; Vastag et al., 1986), and in patients with obstructive lung disease and hence, poor lung function (Cruz et al., 1974; Vastag et al., 1986). Adult asthmatics also displayed decreased mucus transport rates/velocities in addition to decreased lung function (Ahmed et al., 1981; Bateman et al., 1983; Foster et al., 1982; Mezey et al., 1978).
In patients with primary ciliary dyskinesia, absence of cilia motion prevents normal MCC and consequently, lung function is reduced (Denizoglu Kulli et al., 2020). In cystic fibrosis patients, the ASL is depleted resulting in impaired MCC (Boucher, 2004a). Although the known CFTR genotypes can result in a variety of phenotypes (Derichs, 2013), clinical data indicate that some specific gene defects, such as the p.Phe508del variant, are more frequently associated with decreased lung function indices (e.g. FEV1 % predicted, FVC % predicted, FEF25-75) (Johansen et al., 1991; Kerem et al., 1990; Schaedel et al., 2002). Unsurprisingly, results from studies with pharmacological agents aimed at restoring CFTR function do not only indicate enhanced MCC but also support improvements in lung function (Bennett et al., 2018; Donaldson et al., 2018; Rowe S. M. et al., 2014a). While the available data link these two KEs, causal evidence is not always available, and some inference is present. Therefore, we judge the biological plausibility of this KER as moderate.

Empirical Evidence

Occupational exposure to biomass combustion products resulted in slower MCC and reduced FEV1 % predicted and FEV1/FVC (Ferreira et al., 2018).

Compared to healthy controls, current smokers without airway obstruction and current smokers with COPD exhibited longer saccharin transit times, indicative of impaired MCC, and lower FEV1 % predicted and FEV1/FVC (Uzeloto et al., 2021). Similarly, nasomucociliary clearance was slower in COPD smokers compared to former smokers with COPD or to nonsmokers (Ito et al., 2015). Additionally, mucus plug density—assessed by CT imaging—and mucoid (rather than watery) consistency were inversely related to FEF25–75% and associated with increased RV/TLV (Kesimer et al., 2018).

Asthma patients responded to allergen challenge with a reduction in both MCC and FEV1 (Bennett et al., 2011; Mezey et al., 1978), which could be rescued by inhalation with hypertonic saline solution (Alexis et al., 2017).

Multiple studies interrogating the effect of mucolytic agents such as hypertonic saline solution or recombinant DNase on mucus transport rates or mucus clearance in patients with cystic fibrosis report improvements in both, mucus transport velocities or rates and lung function indices, including FEV1, FVC and FEF25-75 (Amin et al., 2011; Donaldson et al., 2018; Elkins et al., 2006; Laube

et al., 1996; McCoy et al., 1996; Quan et al., 2001).

Both MCC and lung function (FEV1, FVC and FEF25-75) improved in cystic fibrosis patients treated with ivacaftor, a CFTR potentiator that increases the channel open probability (Rowe et al., 2014b).

Some studies with mucolytics such as N-acetylcysteine, bromhexine, theophylline/ambroxol or sorebrol demonstrated improved MCC was connected with small improvements in lung function (FEV1, FVC and FEV1/FVC) in patients with chronic bronchitis (Aylward et al., 1980; Castigiioni and Gramolini, 1986; Thomson et al., 1974; Würtemberger et al., 1988).

Uncertainties and Inconsistencies

Genetic defects leading to motile ciliopathies or defects in CFTR function are linked to impaired MCC. However, because of the genetic variety, not every defect, for example in the CFTR gene, also expresses an overt pulmonary phenotype. Other factors, such as low-level chronic inflammation may drive lung pathology by pathways independent of MCC. This might also explain the absence of differences in MCC between healthy smokers and smokers with COPD (Fleming et al., 2019).
Not all studies looking to elucidate the effect of mucolytics on MCC report an improvement of lung function, even though mucus transport rates or tracheobronchial clearance significantly improve. These studies include, for example, some on the effects of hypertonic saline solution, NAC, ambroxol and 2-mercapto-ethane sulphonate (Clarke et al., 1979; Ericsson et al., 1987; Millar et al., 1985; Robinson et al., 1997; Würtemberger et al., 1988). This could be, at least in part, related to the fact that a sudden drop in lung function served as an indicator of patient distress in these studies, and interventions were halted when they occurred to ensure patient safety (Robinson et al., 1996). Another reason could be related to the mechanisms underlying mucus solubilization that may be completely independent of lung function.
MCC is only one means by which mucus can be cleared from the lungs. Another one is cough clearance, and it is highly dependent on the properties of the ASL, in particular the ASL height (Knowles and Boucher, 2002).

Quantitative Understanding of the Linkage

The available data, though not causally linking decreases in MCC with decreased lung function, provide a good insight into the importance of the physiological role of MCC in maintaining normal lung function. In at least some studies, impairment of MCC correlated with the drop in FEV1 or FEF25-75. Although clinically valuable benefits can be seen in studies with pharmacological agents such as mucolytics and CFTR modifying drugs, they do not cover a wide range of dose responses nor are they supportive of the KER causality. Therefore, we judge our quantitative understanding as moderate.

Response-response relationship

Sixteen Brazilian sugarcane workers aged 25±4 years, with a BMI of 24±3 kg/m2, with exhaled CO of 2.1±1.5 ppm, were examined during the non-harvest season and during the sugarcane burning harvest season. There was a non-significant decrease in saccharin transit time (from 8±1 min to 3±1 min) and a significant decrease FEV1/FVC ratio (from 88.62±5.68 to 84.90±6.47) and %FEV1 (from 92.19±13.24 to 90.44±12.76) during harvest compared with the non-harvest season (Ferreira et al., 2018).

12 (6M/6F) mild allergic, non-smoking asthmatics ages 20–39 with skin sensitivity to house dust mites (HDM) and normal baseline lung function (FEV1 %pred > 80, FEV1/FVC ratio >0.70) inhaled sequential doses of inhaled HDM extract (D. farinae, Greer®, Lenoir, NC) delivered as 5 inhalations from a Devilbiss 646 nebulizer (mass median aerodynamic diameter of 5 um, GSD = 2.0). Five of the 12 patients responded to the allergen challenge with >10% reductions in FEV1 % predicted and reduction in whole lung MCC as evidenced by increased retention rates (mean Central TB Ave120Ret increased from 0.69 to 0.79 for baseline vs. allergen challenge respectively). This reduction in MCC significantly correlated with the post challenge 24 hour FEV1 (Bennett et al., 2011).

Treatment of patients with chronic bronchitis with bromhexine (3 x 16 mg/day) for 14 days resulted in mean changes in FEV1, FVC and FEV1/FVC of + 0.047 L + 0.033 L and +0.6%, respectively, with MCC at 6 h being 6.8% greater after treatment compared to baseline (Thomson et al., 1974).

Treatment of patients with chronic bronchitis with ambroxol alone (2 x 30 mg/day) or with theophyllin (2 x 400 mg/day) and ambroxol (2 x 30 mg/day) for 7 days MCC/h improved from 18.3 ± 11.1% to 23.3 ± 13% and 29.6 ± 15.7%, respectively whereas lung function remained nearly unchanged with FEV1 predicted of 86.0 ± 9.78 at baseline vs 83.7 ± 9.27 (ambroxol only) and 83.1 ± 11.07 (combination) (Würtemberger et al., 1988).

Treatment of chronic bronchitics with N-acetylcysteine (4 mg/day by metered dose inhaler) for 16 weeks significantly improved sputum viscosity (-0.53 vs -0.67; differences between medians to placebo: 0-14 (-0.77 0.64)) and minimally improved FVC (3.0±0.21 vs 2.9± 0.18 L/s) and PEF (356.7 ±29.64 L/min vs 354.6±25.07) but not FEV1 (1.9±0.18 vs 2.0± 0.13 L/s) (Dueholm et al., 1992).

Treatment of asthmatics with salmeterol improved tracheobronchial clearance rates (AUC: 333±24%h vs 347±30%h in placebo) as well as FEV1 (76 ± 8), FVC (100 ± 5) and PEF % predicted (100 ± 7) compared to placebo (73 ± 8; 95 ± 5; 94 ± 7) (Hasani et al., 2003).

Treatment of mild-to-moderate bronchitics with 42 µg salmeterol slightly enhanced whole lung clearance in 2 hr (not significant; C10–2= 25±11% vs 22±10% in placebo), significantly increased mean peripheral lung clearance (C10–2= 22±9% vs 17±10% in placebo) and significantly increased FEV1 %pred and FEF25–75 at 2 h compared to baseline (93±18%predicted, 2.45 ± 1.08 L/s vs 88±19%predicted, 2.27 ± 0.98 L/s in placebo) (Bennett et al., 2006).

Sputum induction by inhalation of hypertonic saline solution (5%) in asthmatics at 6 hr following challenge with LPS significantly improved FEV % predicted by approx. 20% and was accompanied by a ca. 6-fold increase in whole lung clearance (from 0.1 %/min to 0.6%/min) (Alexis et al., 2017).

133 cystic fibrosis patients (age (mean [SD]) was 21.1 (11.4) years and 46.4% were female. All participants had one copy of the G551D mutation, and 72.2% were compound heterozygous with F508del on the other allele.) completed a 6-month course of ivacaftor. Lung function improved from baseline FEV1% predicted of 82.6 (25.6) to 90.1 (25.0) (mean change, 6.7; 95% CI, 4.9–8.5). In a subgroup of 22 patients, particle clearance from the whole right lung was markedly increased. Average clearance through 60 minutes at 1 month post-treatment was more than twice the baseline value, reflecting substantially improved MCC (Rowe et al., 2014b).
Inhalation of hypertonic saline solution (7%, 4 mL twice daily for 48 weeks) by cystic fibrosis patients improved FVC (by 82 mL; 95 percent confidence interval, 12 to 153) and FEV1 (by 68 mL; 95 percent confidence interval, 3 to 132) values, but not FEF25–75 (Elkins et al., 2006).

In cystic fibrosis patients that inhaled hypertonic saline solution without amiloride twice a day over a period of 14 days one-hour mucus clearance rates improved from baseline (9.3±1.6%) to 14.0±2.0% and increased FEV1 by 6.2%. FVC and FEF25-75 also improved by 1.8% and 13.1%, respectively (Donaldson et al., 2006).

Dornase alfa (recombinant human DNase) is currently used as a mucolytic to treat pulmonary disease in cystic fibrosis. It reduces mucus viscosity in the lungs, promoting improved clearance of secretions (Yang and Montgomery, 2021). In children with cystic fibrosis (mean: 8.4 yrs of age with FEV1 ≥95% predicted) treated with dornase alfa for 96 weeks, FEV1 % predicted improved by 3.2 ± 1.2, FVC % predicted improved by 0.7 ± 1.0, and FEF25-75 % predicted improved by 7.9 ± 2.3 compared to placebo (Quan et al., 2001). In young patients with cystic fibrosis (6-18 yrs of age with FEV1 ≥80% predicted) treated with dornase alfa for 96 weeks, FEF25-75 % predicted improved by 6.1±10.34 compared to placebo (Amin et al., 2011). In 10 adult cystic fibrosis patients receiving 2.5 mg rhDNase twice a day for 6 days, FEV1 and FVC increased by an average of 9.4 ± 3.5% and 12.7 ± 2.6%, respectively, as compared with a decrease of 1.8 ± 1.7% and an increase of 0.4±1.1% in the placebo group, respectively, although there were no significant changes in MCC (Laube et al., 1996). In 320 cystic fibrosis patients (7 to 57 yrs of age), dornase alfa treatment at 2.5 mg/day for 12 weeks (McCoy et al., 1996).

Saccharin transit times (a marker of nasal MCC) were higher in healthy current smokers and COPD smokers than in healthy controls (10.87 [7.29–17] min and 16.47 [8.25–20.15] min, respectively, vs 8.52 [5.54–13.91] min). These groups also differed in their lung function indices: FEV1 % predicted was 101.4 ± 12.37 in healthy controls, 96.41 ± 12.3 in healthy current smokers, and 67.96 ± 24.02 in COPD smokers. FVC % predicted was 103.1 ± 13.45 in healthy controls, 97.51 ± 12.88 in healthy current smokers, and 90.33 ± 29.27 in COPD smokers. FEV1/FVC % predicted was 82.15 [78.5–85] in healthy controls, 82.20 [79.2–84.1] in healthy current smokers, and 61.1 [55.3–67.2] in COPD smokers (Uzeloto et al., 2021).
Saccharin transit time of smokers with COPD (16.5 [11–28] min, median [interquartile range 25–75%]) was slightly longer than that of current smokers (15.9 [10 –27] min), and both were longer compared with exsmokers with COPD (10.2 [6 –12] min) and nonsmokers (8 [6 –16] min). Lung function parameters for the groups were as follows: nonsmokers, FEV1/FVC 0.84 ± 0.09, FEV1 % predicted 103.2 ± 11.5, FVC % predicted 102.2 ± 13.3; current smokers, FEV1/FVC 0.76 ± 0.05, FEV1 % predicted 90.7 ± 7.4, FVC % predicted 96.3 ± 13.9; former smokers with COPD, FEV1/FVC 0.49 ± 0.08, FEV1 % predicted 46.8 ± 12.6, FVC % predicted 76.8 ± 18.5; current smokers with COPD, FEV1/FVC 0.66 ± 0.16, FEV1 % predicted 48.7 ± 16.8, FVC % predicted 71.7 ± 13.0 (Ito et al., 2015).

Time-scale

Six asymptomatic patients with bronchial asthma and a history of allergic pollenosis and episodic bronchospasm consistent with ragweed hypersensitivity wer challenged by inhalation of an aqueous, short ragweed antigen extract (Greer Laboratories, Lenoir, N.C.), diluted with a phosphate-buffered saline solution. Mean tracheal mucus velocity (TMV) decreased to 72% of baseline immediately after challenge when specific airway conductance (SGaw), and FEV1 showed a maximal decrease, with a further decrease to 47% of baseline after 1 h, when SGaw and FEV1 had returned to baseline values (Mezey et al., 1978).

Treatment of chronic bronchitics with N-acetylcysteine (3 x 200 mg/day) for 4 weeks significantly decreased sputum thickness, increased sputum pourability from 650% glycerol time (at baseline) to 320% glycerol time on day 21 and PEFR on days 28 (+5%), 35 (+6%) and 42 (+7%) and FEV1 on days 21 (+2%), 28(+3%), 35 (+4%) and 42 (+5%) compared to baseline (ca. 33% predicted and 28% predicted, respectively) (Aylward et al., 1980).

Treatment of mild-to-moderate bronchitics with 42 µg salmeterol slightly enhanced whole lung clearance in 2 hr (not significant; C10–2= 25±11% vs 22±10% in placebo), significantly increased mean peripheral lung clearance (C10–2= 22±9% vs 17±10% in placebo) and significantly increased FEV1 %pred and FEF25–75 at 2 h compared to baseline (93±18%predicted, 2.45 ± 1.08 L/s vs 88±19%predicted, 2.27 ± 0.98 L/s in placebo) , and significantly increased FEV1 %pred and FEF25–75 at both 1 (92±19%predicted, 2.44 ± 1.14 L/s) and 2 h (93±18%predicted, 2.45 ± 1.08 L/s) compared to baseline (pre-dose; 90 ± 20%predicted, 2.16 ± 0.92 L/s) (Bennett et al., 2006).

In cystic fibrosis patients on a 6-month ivacaftor regimen, FEV1% improvement was detectable as soon as the 1-month follow-up visit (mean change, 6.7; 95% CI, 5.2–8.3) (Rowe et al., 2014b). MCC remained at elevated level at the month 3 visit (Donaldson et al., 2018).

One-hour mucus-clearance rates in cystic fibrosis patients receiving hypertonic saline with placebo were significantly faster than in the group receiving hypertonic saline with amiloride (14.0±2.0 vs. 7.0±1.5 %), and the durability of response following the inhalation

of hypertonic saline with placebo was ≥8 hours (Donaldson et al., 2006).

Known modulating factors

Invariably, if mucus viscosity increases (independent of whether that results from increased mucus production (hypersecretion), depletion of the ASL or another cause) and MCC decreases, another mechanism comes into action to clear excess mucus: cough clearance. Cough constitutes a “backup” host defense by which acutely or chronically accumulated mucus is expelled through forceful, high-velocity airflow (Button et al., 2018; King, 2006). Our current understanding of the mechanical principles and biology of cough suggest that failure of cough clearance may also be a contributor to decreased lung function.

Known Feedforward/Feedback loops influencing this KER

Unknown

References

- Ahmed, T., Greenblatt, D.W., Birch, S., Marchette, B., and Wanner, A. (1981). Abnormal mucociliary transport in allergic patients with antigen-induced bronchospasm: role of slow reacting substance of anaphylaxis. American Review of Respiratory Disease 124(2), 110-114.
- Alexis, N.E., Bennett, W., and Peden, D.B. (2017). Safety and benefits of inhaled hypertonic saline following airway challenges with endotoxin and allergen in asthmatics. J. Asthma 54(9), 957-960.
- Amin, R., Subbarao, P., Lou, W., Jabar, A., Balkovec, S., Jensen, R., et al. (2011). The effect of dornase alfa on ventilation inhomogeneity in patients with cystic fibrosis. Eur. Respir. J. 37(4), 806-812.
- Aylward, M., Maddock, J., and Dewland, P. (1980). Clinical evaluation of acetylcysteine in the treatment of patients with chronic obstructive bronchitis: a balanced double-blind trial with placebo control. European Journal of Respiratory Diseases Supplement 111, 81-89.
- Bateman, J., Pavia, D., Sheahan, N., Agnew, J., and Clarke, S. (1983). Impaired tracheobronchial clearance in patients with mild stable asthma. Thorax 38(6), 463-467.
- Bennett, W.D., Zeman, K.L., Laube, B.L., Wu, J., Sharpless, G., Mogayzel, P.J., Jr., et al. (2018). Homogeneity of Aerosol Deposition and Mucociliary Clearance are Improved Following Ivacaftor Treatment in Cystic Fibrosis. Journal of aerosol medicine and pulmonary drug delivery 31(4), 204-211.
- Bennett, W.D., Almond, M.A., Zeman, K.L., Johnson, J.G., and Donohue, J.F. (2006). Effect of salmeterol on mucociliary and cough clearance in chronic bronchitis. Pulmonary pharmacology & therapeutics 19(2), 96-100.
- Bennett, W.D., Herbst, M., Alexis, N.E., Zeman, K.L., Wu, J., Hernandez, M.L., et al. (2011). Effect of inhaled dust mite allergen on regional particle deposition and mucociliary clearance in allergic asthmatics. Clin Exp Allergy 41(12), 1719-1728.
- Boucher, R. (2004). New concepts of the pathogenesis of cystic fibrosis lung disease. European Respiratory Journal 23(1), 146-158.
- Button, B., Goodell, H.P., Atieh, E., Chen, Y.-C., Williams, R., Shenoy, S., et al. (2018). Roles of mucus adhesion and cohesion in cough clearance. Proceedings of the National Academy of Sciences 115(49), 12501-12506.
- Clunes, L.A., Davies, C.M., Coakley, R.D., Aleksandrov, A.A., Henderson, A.G., Zeman, K.L., et al. (2012). Cigarette smoke exposure induces CFTR internalization and insolubility, leading to airway surface liquid dehydration. FASEB J. 26(2), 533-545.
- Cruz, R.S., Landa, J., Hirsch, J., and Sackner, M.A. (1974). Tracheal mucous velocity in normal man and patients with obstructive lung disease; effects of terbutaline. American Review of Respiratory Disease 109(4), 458-463.
- Denizoglu Kulli, H., Gurses, H.N., Zeren, M., Ucgun, H., and Cakir, E. (2020). Do pulmonary and extrapulmonary features differ among cystic fibrosis, primary ciliary dyskinesia, and healthy children? Pediatr. Pulmonol. 55(11), 3067-3073.
- Derichs, N. (2013). Targeting a genetic defect: cystic fibrosis transmembrane conductance regulator modulators in cystic fibrosis. Eur. Respir. J. 22(127), 58-65.
- Donaldson, S.H., Bennett, W.D., Zeman, K.L., Knowles, M.R., Tarran, R., and Boucher, R.C. (2006). Mucus Clearance and Lung Function in Cystic Fibrosis with Hypertonic Saline. New England Journal of Medicine 354(3), 241-250.
- Donaldson, S.H., Laube, B.L., Corcoran, T.E., Bhambhvani, P., Zeman, K., Ceppe, A., et al. (2018). Effect of ivacaftor on mucociliary clearance and clinical outcomes in cystic fibrosis patients with G551D-CFTR. JCI Insight 3(24), e122695.
- Dueholm, M., Nielsen, C., Thorshauge, H., Evald, T., Hansen, N.-C., Madsen, H., et al. (1992). N-acetylcysteine by metered dose inhaler in thetreatment of chronic bronchitis: a multi-centre study. Respiratory medicine 86(2), 89-92.
- Dunican, E.M., Elicker, B.M., Henry, T., Gierada, D.S., Schiebler, M.L., Anderson, W., et al. (2021). Mucus plugs and emphysema in the pathophysiology of airflow obstruction and hypoxemia in smokers. Am. J. Respir. Crit. Care Med. 203(8), 957-968.
- Elkins, M.R., Robinson, M., Rose, B.R., Harbour, C., Moriarty, C.P., Marks, G.B., et al. (2006). A Controlled Trial of Long-Term Inhaled Hypertonic Saline in Patients with Cystic Fibrosis. N. Engl. J. Med. 354(3), 229-240.
- Ferreira, A.D., Ramos, E.M.C., Trevisan, I.B., Leite, M.R., Proença, M., de Carvalho-Junior, L.C.S., et al. (2018). Função pulmonar e depuração mucociliar nasal de cortadores de cana-de-açúcar brasileiros expostos à queima de biomassa. Rev. Bras. Saúde Ocup. 43.
- Foster, W., Langenback, E., and Bergofsky, E. (1982). "Lung mucociliary function in man: interdependence of bronchial and tracheal mucus transport velocities with lung clearance in bronchial asthma and healthy subjects," in Inhaled Particles V. Elsevier), 227-244.
- Goodman, R., Yergin, B., Landa, J., Golinvaux, M., and Sackner, M. (1978). Relationship of smoking history and pulmonary function tests to tracheal mucous velocity in nonsmokers, young smokers, ex-smokers, and patients with chronic bronchitis.

American Review of Respiratory Disease 117(2), 205-214.
- Halbeisen, F.S., Goutaki, M., Spycher, B.D., Amirav, I., Behan, L., Boon, M., et al. (2018). Lung function in patients with primary ciliary dyskinesia: an iPCD Cohort study. European Respiratory Journal 52(2), 1801040.
- Hasani, A., Toms, N., O'Connor, J., Dilworth, J., and Agnew, J. (2003). Effect of salmeterol xinafoate on lung mucociliary clearance in patients with asthma. Respiratory medicine 97(6), 667-671.
- Ito, J.T., Ramos, D., Lima, F.F., Rodrigues, F.M., Gomes, P.R., Moreira, G.L., et al. (2015). Nasal Mucociliary Clearance in Subjects With COPD After Smoking Cessation. Respir. Care 60(3), 399-405.
- Johansen, H.K., Nir, M., Koch, C., Schwartz, M., and Høiby, N. (1991). Severity of cystic fibrosis in patients homozygous and heterozygous for ΔF508 mutation. Lancet 337(8742), 631-634.
- Kerem, E., Corey, M., Kerem, B.-s., Rommens, J., Markiewicz, D., Levison, H., et al. (1990). The relation between genotype and phenotype in cystic fibrosis—analysis of the most common mutation (ΔF508). N. Engl. J. Med. 323(22), 1517-1522.
- Kerem, E., Viviani, L., Zolin, A., MacNeill, S., Hatziagorou, E., Ellemunter, H., et al. (2014). Factors associated with FEV1 decline in cystic fibrosis: analysis of the ECFS Patient Registry. Eur. Respir. J. 43(1), 125-133.
- Kesimer, M., Smith, B.M., Ceppe, A., Ford, A.A., Anderson, W.H., Barr, R.G., et al. (2018). Mucin concentrations and peripheral airway obstruction in chronic obstructive pulmonary disease. Am. J. Respir. Crit. Care Med. 198, 1453-1456.
King, M. (2006). Physiology of mucus clearance. Paediatr. Respir. Rev. 7 Suppl 1, S212-214.
- Kuyper, L.M., Paré, P.D., Hogg, J.C., Lambert, R.K., Ionescu, D., Woods, R., et al. (2003). Characterization of airway plugging in fatal asthma. Am. J. Med. 115(1), 6-11.
- Laube, B.L., Auci, R.M., Shields, D.E., Christiansen, D.H., Lucas, M.K., Fuchs, H.J., et al. (1996). Effect of rhDNase on airflow obstruction and mucociliary clearance in cystic fibrosis. Am. J. Respir. Crit. Care Med. 153(2), 752-760.
- Lourenço, R.V., Klimek, M.F., and Borowski, C.J. (1971). Deposition and clearance of 2 μ particles in the tracheobronchial tree of normal subjects—smokers and nonsmokers. The Journal of clinical investigation 50(7), 1411-1420.
- Marthin, J.K., Petersen, N., Skovgaard, L.T., and Nielsen, K.G. (2010). Lung function in patients with primary ciliary dyskinesia: a cross-sectional and 3-decade longitudinal study. American journal of respiratory and critical care medicine 181(11), 1262-1268.
- Maxwell, G. (1985). The problem of mucus plugging in children with asthma. J. Asthma 22(3), 131-137.
- McCoy, K., Hamilton, S., and Johnson, C. (1996). Effects of 12-Week Administration of Dornase Alfa in Patients with Advanced Cystic Fibrosis Lung Disease. Chest 110(4), 889-895.
- Mezey, R.J., Cohn, M.A., Fernandez, R.J., Januszkiewicz, A.J., and Wanner, A. (1978). Mucociliary transport in allergic patients with antigen-induced bronchospasm. American Review of Respiratory Disease 118(4), 677-684.
- Mossberg, B., Afzelius, B., Eliasson, R., and Camner, P. (1978). On the pathogenesis of obstructive lung disease. A study on the immotile-cilia syndrome. Scand. J. Respir. Dis. 59(2), 55-65.
- Okajima, Y., Come, C.E., Nardelli, P., Sonavane, S.K., Yen, A., Nath, H.P., et al. (2020). Luminal Plugging on Chest CT Scan: Association With Lung Function, Quality of Life, and COPD Clinical Phenotypes. Chest 158(1), 121-130.
- Quan, J.M., Tiddens, H.A.W.M., Sy, J.P., McKenzie, S.G., Montgomery, M.D., Robinson, P.J., et al. (2001). A two-year randomized, placebo-controlled trial of dornase alfa in young patients with cystic fibrosis with mild lung function abnormalities. J. Pediatr. 139(6), 813-820.
- Regnis, J., Robinson, M., Bailey, D., Cook, P., Hooper, P., Chan, H., et al. (1994). Mucociliary clearance in patients with cystic fibrosis and in normal subjects. Am. J. Respir. Crit. Care Med. 150(1), 66-71.
- Robinson, M., and Bye, P.T.B. (2002). Mucociliary clearance in cystic fibrosis. Pediatr. Pulmonol. 33(4), 293-306.
- Rowe, S.M., Heltshe, S.L., Gonska, T., Donaldson, S.H., Borowitz, D., Gelfond, D., et al. (2014). Clinical mechanism of the cystic fibrosis transmembrane conductance regulator potentiator ivacaftor in G551D-mediated cystic fibrosis. Am J Respir Crit Care Med 190(2), 175-184.
- Schaedel, C., de Monestrol, I., Hjelte, L., Johannesson, M., Kornfält, R., Lindblad, A., et al. (2002). Predictors of deterioration of lung function in cystic fibrosis. Pediatr. Pulmonol. 33(6), 483-491.
- Szczesniak, R., Heltshe, S.L., Stanojevic, S., and Mayer-Hamblett, N. (2017). Use of FEV(1) in cystic fibrosis epidemiologic studies and clinical trials: A statistical perspective for the clinical researcher. J. Cyst. Fibros. 16(3), 318-326.
- Thomson, M., Pavia, D., Gregg, I., and Stark, J. (1974). Bromhexine and mucociliary clearance in chronic bronchitis. British journal of diseases of the chest 68, 21-27.
- Uzeloto, J.S., Ramos, D., Silva, B.S.d.A., Lima, M.B.P.d., Silva, R.N., Camillo, C.A., et al. (2021). Mucociliary Clearance of Different Respiratory Conditions: A Clinical Study. Int Arch Otorhinolaryngol 25(1), e35-e40.
- Vastag, E., Matthys, H., Zsamboki, G., Köhler, D., and Daikeler, G. (1986). Mucociliary clearance in smokers. European journal of respiratory diseases 68(2), 107-113.
- Wallmeier, J., Nielsen, K.G., Kuehni, C.E., Lucas, J.S., Leigh, M.W., Zariwala, M.A., et al. (2020). Motile ciliopathies. Nature Reviews Disease Primers 6(1), 1-29.
- Würtemberger, G., Michaelis, K., and Matthys, H. (1988). [Additive action of theophylline and ambroxol on bronchial clearance?]. Prax Klin Pneumol 42 Suppl 1, 300-303.

Relationship: 2444: ASL Height, Decreased leads to Mucus Viscosity, Increased

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Oxidative stress [MIE] Leading to Decreased Lung Function [AO]	adjacent	Moderate	Low

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	High	NCBI
Rattus norvegicus	Rattus norvegicus		NCBI
Sus scrofa	Sus scrofa		NCBI
Mustela furo	Mustela putorius furo		NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	Low

Sex Applicability

Sex	Evidence
Mixed	Moderate

Key Event Relationship Description

The phenomenon of ASL volume changes determining mucus viscosity is well described in the cystic fibrosis literature. In patients with this genetic defect, impaired CFTR function results in ASL depletion and mucus hyperviscosity. Mechanistically, the imbalance of Cl^- and $HCO3^-$ secretion and increased Na+ absorption by the airway epithelium results in dehydration of airway mucus, making it more viscous and adhesive (Knowles and Boucher, 2002; Mall et al., 2004; Puchelle et al., 2002; Tarran, 2004). Studies with transgenic mice overexpressing βENaC in the airways corroborate the link between ASL hydration and mucociliary impairment as evidenced by the increased incidence of airway mucus plugging (Mall et al., 2004; Mall, 2008).
Increased mucus viscosity may also play a significant role in asthma and chronic bronchitis, although the mechanisms are less well explored. In a ferret model of cigarette smoke-induced COPD, Lin et al. identified ASL depletion as one of the drivers of increased mucus viscosity and decreased MCC (Lin et al., 2020). The authors also showed that mucus from COPD patients, obtained from 3D organotypic airway epithelial cultures from different smoking donors with COPD, was significantly more viscous than that of healthy, non-smoking individuals and smokers without disease (Lin et al., 2020). Considering the known effects of cigarette smoke exposure on the ASL height (Hassan et al., 2014; Lambert et al., 2014; Raju et al., 2016; Schmid et al., 2015; Xu et al., 2015), this links decreased ASL height to increased mucus viscosity in the context of chronic bronchitis.

Evidence Supporting this KER

In patients with cystic fibrosis, impaired CFTR function results in ASL depletion and mucus hyperviscosity (Knowles and Boucher, 2002; Puchelle et al., 2002; Mall et al., 2004; Tarran, 2004). This has been confirmed experimentally in pig and rat models of this disease (Birket et al., 2014; Birket et al., 2016; Birket et al., 2018). Studies with transgenic mice overexpressing βENaC in the airways also corroborate the link between ASL dehydration and increased mucus viscosity, evidenced by the increased incidence of airway mucus plugging [129, 195]. In a ferret model of cigarette smoke-induced COPD, ASL depletion was shown to be one of the drivers of increased mucus viscosity and decreased MCC (Lin et al., 2020). The same study also showed that mucus from COPD patients, obtained from 3D organotypic airway epithelial cultures from different smoking donors with COPD, is significantly more viscous than that from healthy, non-smoking individuals and smokers without disease (Lin et al., 2020).

Biological Plausibility

There are only few studies that report ASL height and mucus viscosity, and although studies on cystic fibrosis in animal models or human cell cultures show the dependencies between these two KEs, the causal evidence is sparse. However, because the underlying mechanism is well-described and translatable across different species and is amenable to positive modulation by e.g. CFTR drugs, we consider the biological plausibility of this KER to be moderate.

Empirical Evidence

ASL height was inversely related to mucociliary transport (MCT) rate, an outcome of particle tracking measurements and hence an indicator of mucus viscosity, in tracheas of CFTR-deficient piglets. Complementary studies in airway cell monolayer cultures also identified elevated effective viscosity of the ASL in cystic fibrosis compared to non-cystic fibrosis cultures (Birket et al., 2014).

ASL height was decreased and mucus viscosity was increased in human airway epithelial cultures from cystic fibrosis donors grown at the air-liquid interface (Birket et al., 2014). Treatments with CFTR function-restoring drugs improve both ASL height and mucus viscosity (Birket et al., 2016).

Deficiency of Cftr in rats results in decreased ASL height and increased mucus viscosity compared to their wild-type littermates (Birket et al., 2018).

Exposure of rats to nebulized N-acetylcysteine, assumed to function as a mucolytic (i.e., to decrease mucus viscosity), for 20 or 90 min increased the viscoelastic properties of mucus and decreased mucus transport speed (Lorenzi et al., 1992).

Computational modeling of the MCT process indicated that mucus velocity is not only dependent on the cilia beat frequency and the numbers of cilia, but also on ASL height (Jayathilake et al., 2015; Lee et al., 2011).

Uncertainties and Inconsistencies

At least one study in primary cultures of human bronchial epithelial cells grown at the air-liquid interface found mucus viscosity to be higher in cultures from cystic fibrosis donors compared to those from healthy donors, but did not observe differences in ASL height (Derichs et al., 2011).
In asthmatics, airway mucus is very viscous, and its viscosity increases even more in acute exacerbations (Innes et al., 2009). However, unlike in individuals with cystic fibrosis or chronic bronchitis, changes in ASL height because of impaired anion transport might not be the primary cause for increased mucus viscosity in patients with asthma. Instead a more acidic ASL may lead to improper unpacking of secreted mucins and tethering of mucins to the epithelial surface, where they form protein tangles that result in mucus airway plugs (Abdullah et al., 2017; Bonser and Erle, 2017; Bonser et al., 2016; Evans et al., 2015; Shimura et al., 1988; Tang et al., 2016). Alternatively, the composition of mucus may be altered by production of more acidic mucins thereby skewing the pH balance of mucus itself (Gearhart and Schlesinger, 1989; Holma, 1989; Kim et al., 2014). In addition, mucus viscosity is also affected by the presence of DNA, lipids and proteins other than mucins, such as lactoferrin, albumin and immunoglobulins, all of which may be present in higher amounts in the presence of airway inflammation (Puchelle et al., 2002; Rogers, 2004).

Quantitative Understanding of the Linkage

There are a small number of studies reporting on ASL and mucus viscosity that provide insights into the quantitative relationship between these two KEs. Some of these studies use pharmacological agents with well described effects on ASL height and mucus viscosity. However, most studies evaluated the KEs in parallel, without interrogating dose responses and/or time responses. Therefore, we only have a rudimentary quantitative understanding of this KER, leading us to judge this as weak.

Response-response relationship

In primary human bronchial epithelial cultures grown at the air-liquid interface, the ASL depth of cystic fibrosis cells was significantly lower than that of non-cystic fibrosis cells (2.4±0.6 µm vs. 6.7±0.2 µm) and mucus viscosity was significantly higher (80.6±26.5 cP vs. 12.0±3.6 mm/min) by particle-tracking microrheology. FRAP indicated an increased half-life of fluorescence recovery (evidence of increased viscosity) of approx. 16 s in cystic fibrosis cultures vs ca. 11 s in non-cystic fibrosis cultures (Birket et al., 2014).

ASL height in the tracheas of $^{-/-}$ CFTR piglets was significantly lower than in wild-type littermates (3.2±0.8 µm vs. 6.5±0.2 µm) and mucus viscosity was significantly higher by FRAP, which indicated an increased half-life of approx. 13 s in CFTR-deficient tracheas vs ca. 9.5 s in wild-type controls (Birket et al., 2014). Treatment of normal adult pig trachea with 100 µM bumetanide to block $HCO3^-$ transport reduced ASL height from 7.8±0.5 µm (untreated) to 6.4±0.4 µm and mucus viscosity from approx. 600 cP (untreated) to 2000 cP at frequencies between 0.5 and 10 Hz (Birket et al., 2014).

In tracheas of Cftr-deficient rats, ASL depths were diminished in both basal and stimulated conditions, from weaning until at least 6 months of age (WT 1 month 22.9 ± 4.6 µm, 6 months 43.6 ± 12.5 µm vs. KO 1 month 6.9 ± 0.7 µm, 6 month 19.5 ± 4.8 µm). Under baseline conditions at 1 month of age, effective viscosity was no different in KO tracheae compared with WT tracheae (1.76 ± 1.0 cP WT vs. 1.90 ± 1.7 cP KO). At 3 months, effective viscosity of KO airway mucus increased slightly but was still no different than that of WT airway mucus (5.12 ± 1.0 cP WT vs. 10.72 ± 2.7 cP KO). By 6 months of age, KO tracheal mucus was 20-fold more viscous compared with WT littermates (2.91 ± 0.9 cP WT vs. 65.09 ± 3.6 cP KO) (Birket et al., 2018).

Treatment of primary bronchial epithelial cell monolayer cultures from G551D/F508del cystic fibrosis patients with ivacaftor, a CFTR potentiator, at concentrations ≥ 100 nM for 24 h increased ASL height from ca. 6 to 17.5 µm, which was similar to the ASL height of non-cystic fibrosis cultures (18.03 ± 1.6 µm). The half-life of time to recovery measured by FRAP shortened from 12.39 ± 1.3 to 7.57 ± 0.8 s, indicative of decreased mucus viscosity. Effective viscosity of cells treated with ivacaftor (600 cP) was significantly lower than control (2,600 cP) at the physiological frequency of 0.9 Hz (Birket et al., 2016).

Treatment of primary bronchial epithelial cell monolayer cultures from homozygous F508del cystic fibrosis patients with the combination of 10 µM ivacaftor and 3 µM C18 (a ivacaftor homolog) with 20 µM forskolin significantly increased ASL height (23.4 ± 2.6 vs. 9.01 ± 1.4 µm C18 + forskolin, 10.99 ± 1.7 µm ivacaftor + forskolin; 13.03 ± 2.8 µm forskolin alone, and 12.53 ± 2.3 µm vehicle). Effective mucus viscosity effective in situ was reduced from ca. 1200 cP (vehicle) to ca. 100 cP by 48-h treatment only with the combination of ivacaftor and C18 (Birket et al., 2016).

Time-scale

No data

Known modulating factors

Unknown

Known Feedforward/Feedback loops influencing this KER

Unknown

References

- Abdullah, L.H., Evans, J.R., Wang, T.T., Ford, A.A., Makhov, A.M., Nguyen, K., et al. (2017). Defective postsecretory maturation of MUC5B mucin in cystic fibrosis airways. JCI Insight 2(6), e89752.
- Birket, S.E., Chu, K.K., Houser, G.H., Liu, L., Fernandez, C.M., Solomon, G.M., et al. (2016). Combination therapy with cystic fibrosis transmembrane conductance regulator modulators augment the airway functional microanatomy. Am. J. Physiol. Lung Cell. Mol. Physiol. 310(10), L928-L939.
- Birket, S.E., Chu, K.K., Liu, L., Houser, G.H., Diephuis, B.J., Wilsterman, E.J., et al. (2014). A functional anatomic defect of the cystic fibrosis airway. Am. J. Respir. Crit. Care Med. 190(4), 421-432.
- Birket, S.E., Davis, J.M., Fernandez, C.M., Tuggle, K.L., Oden, A.M., Chu, K.K., et al. (2018). Development of an airway mucus defect in the cystic fibrosis rat. JCI Insight 3(1). e97199.
- Bonser, L.R., and Erle, D.J. (2017). Airway mucus and asthma: the role of MUC5AC and MUC5B. Journal of clinical medicine 6(12), 112.
- Bonser, L.R., Zlock, L., Finkbeiner, W., and Erle, D.J. (2016). Epithelial tethering of MUC5AC-rich mucus impairs mucociliary transport in asthma. J Clin Invest 126(6), 2367-2371.
- Derichs, N., Jin, B.-J., Song, Y., Finkbeiner, W.E., and Verkman, A.S. (2011). Hyperviscous airway periciliary and mucous liquid layers in cystic fibrosis measured by confocal fluorescence photobleaching. FASEB journal : official publication of the Federation of American Societies for Experimental Biology 25(7), 2325-2332.
- Evans, C.M., Raclawska, D.S., Ttofali, F., Liptzin, D.R., Fletcher, A.A., Harper, D.N., et al. (2015). The polymeric mucin Muc5ac is required for allergic airway hyperreactivity. Nat Commun 6, 6281.
- Gearhart, J.M., and Schlesinger, R.B. (1989). Sulfuric acid-induced changes in the physiology and structure of the tracheobronchial airways. Environmental health perspectives 79, 127-136.
- Hassan, F., Xu, X., Nuovo, G., Killilea, D.W., Tyrrell, J., Da Tan, C., et al. (2014). Accumulation of metals in GOLD4 COPD lungs is associated with decreased CFTR levels. Respir. Res. 15(1), 69.
- Holma, B. (1989). Effects of inhaled acids on airway mucus and its consequences for health. Environmental health perspectives 79, 109-113.
- Innes, A.L., Carrington, S.D., Thornton, D.J., Kirkham, S., Rousseau, K., Dougherty, R.H., et al. (2009). Ex vivo sputum analysis reveals impairment of protease-dependent mucus degradation by plasma proteins in acute asthma. American journal of respiratory and critical care medicine 180(3), 203-210.
- Jayathilake, P.G., Le, D.V., Tan, Z., Lee, H.P., and Khoo, B.C. (2015). A numerical study of muco-ciliary transport under the condition of diseased cilia. Comput. Methods Biomech. Biomed. Engin. 18(9), 944-951.
- Kim, D., Liao, J., and Hanrahan, J.W. (2014). The buffer capacity of airway epithelial secretions. Frontiers in Physiology 5(188). doi: 10.3389/fphys.2014.00188.
- Knowles, M.R., and Boucher, R.C. (2002). Mucus clearance as a primary innate defense mechanism for mammalian airways. J. Clin. Investig. 109(5), 571-577.
- Lambert, J.A., Raju, S.V., Tang, L.P., McNicholas, C.M., Li, Y., Courville, C.A., et al. (2014). Cystic fibrosis transmembrane conductance regulator activation by roflumilast contributes to therapeutic benefit in chronic bronchitis. Am. J. Respir. Cell Mol. Biol. 50(3), 549-558.
- Lee, W., Jayathilake, P., Tan, Z., Le, D., Lee, H., and Khoo, B. (2011). Muco-ciliary transport: effect of mucus viscosity, cilia beat frequency and cilia density. Comput. Fluids 49(1), 214-221.
- Lin, V.Y., Kaza, N., Birket, S.E., Kim, H., Edwards, L.J., LaFontaine, J., et al. (2020). Excess mucus viscosity and airway dehydration impact COPD airway clearance. Eur. Respir. J. 55(1), 1900419.
- Lorenzi, G., Böhm, G., Guimarães, E., Vaz, C., King, M., and Saldiva, P. (1992). Correlation between rheologic properties and in vitro ciliary transport of rat nasal mucus. Biorheology 29(4), 433-440.
- Mall, M.A. (2008). Role of cilia, mucus, and airway surface liquid in mucociliary dysfunction: lessons from mouse models. J. Aerosol Med. Pulm. Drug Deliv. 21(1), 13-24.
- Mall, M., Grubb, B.R., Harkema, J.R., O'Neal, W.K., and Boucher, R.C. (2004). Increased airway epithelial Na+ absorption produces cystic fibrosis-like lung disease in mice. Nat. Med. 10(5), 487.
- Puchelle, E., Bajolet, O., and Abély, M. (2002). Airway mucus in cystic fibrosis. Paediatr. Respir. Rev. 3(2), 115-119.
- Raju, S.V., Lin, V.Y., Liu, L., Mcnicholas, C.M., Karki, S., Sloane, P.A., et al. (2016). The Cftr Potentiator Ivacaftor Augments Mucociliary Clearance Abrogating Cftr Inhibition by Cigarette Smoke. Am. J. Respir. Cell Mol. Biol. 56(1), 99-108.
- Rogers, D.F. (2004). Airway mucus hypersecretion in asthma: an undervalued pathology? Current opinion in pharmacology 4(3), 241-250.
- Schmid, A., Baumlin, N., Ivonnet, P., Dennis, J.S., Campos, M., Krick, S., et al. (2015). Roflumilast partially reverses smoke-induced mucociliary dysfunction. Respir. Res. 16(1), 135.
- Shimura, S., Sasaki, T., Sasaki, H., and Takishima, T. (1988). Chemical properties of bronchorrhea sputum in bronchial asthma. Chest 94(6), 1211-1215.
- Tang, X.X., Ostedgaard, L.S., Hoegger, M.J., Moninger, T.O., Karp, P.H., McMenimen, J.D., et al. (2016). Acidic pH increases airway surface liquid viscosity in cystic fibrosis. J Clin Invest 126(3), 879-891.
- Tarran, R. (2004). Regulation of airway surface liquid volume and mucus transport by active ion transport. Proc. Am. Thorac. Soc. 1(1), 42-46.
- Xu, X., Balsiger, R., Tyrrell, J., Boyaka, P.N., Tarran, R., and Cormet-Boyaka, E. (2015). Cigarette smoke exposure reveals a novel role for the MEK/ERK1/2 MAPK pathway in regulation of CFTR. Biochim. Biophys. Acta 1850(6), 1224-1232.

Relationship: 2445: Mucus Viscosity, Increased leads to CBF, Decreased

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Oxidative stress [MIE] Leading to Decreased Lung Function [AO]	adjacent	Moderate	Moderate

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens		NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	

Sex Applicability

Sex	Evidence
Mixed	

Key Event Relationship Description

Under physiological conditions, the viscosity of mucus has been shown to range from 1 to 100 Pa.s under low shear rate conditions and from 0.01 to 1 Pa.s under high shear rate conditions. Mucus viscoelastic properties, whether altered by airway dehydration or mucus hypersecretion, directly influence MCC. Toxicant exposures, such as to nicotine, as well as inflammation can also affect the physicochemical properties of mucus (Chen et al., 2014). Increased mucus viscosity in turn decreases CBF and slows transport of mucus on the mucociliary escalator.

Evidence Supporting this KER

Several studies have shown that there is an optimal range of viscoelastic mucus properties that facilitates efficient MCC and that changes in mucus viscosity beyond that optimal range impact CBF and alter MCC. Studies in humans, mice, hamsters, horses and frogs have shown that increased mucus viscosity correlates with a decrease in CBF (King, 1979; Gheber et al., 1998; Matsui et al., 1998; Andrade et al., 2005; González et al., 2016; Kikuchi et al., 2017; Birket et al., 2018).

Biological Plausibility

Mucus viscoelastic properties, whether altered by airway dehydration or mucus hypersecretion, directly influence MCC. In fact, there is an inverse relationship between mucus viscosity and CBF and mucus transport/MCC, as demonstrated in several in vivo and ex vivo studies. A large proportion of these studies have employed (bio)polymers or other large organic molecules to mimic the mucus layer in the airways and the increase in its viscosity. In addition, some of these studies have shown that decreased mucus viscosity may also result in impairment of MCC. Therefore, a causal link is only tentatively supported. Because cilia function, ASL height, and mucus properties are intricately linked to each other as evidenced by cystic fibrosis studies, we consider the plausibility as moderate.

Empirical Evidence

Exposure of primary cultures of hamster oviductal ciliated cells to increased viscous loading reduced the CBF (Andrade et al., 2005).

The tracheal samples from mice were used to measure ciliary beat frequencies and beat amplitudes of ciliary motion in viscous culture media over the range of η= 0.9–303.8 mPa.s. The CBF decreased with increasing viscosity, up to about 32.0 mPa.s, while it was nearly constant above 32.0 mPa.s (Kikuchi et al., 2017). In tracheal samples from mice, CBF decreased with increasing viscosity, up to about 32.0 mPa.s, while it was nearly constant above 32.0 mPa.s. CBF were calculated from the averaged cycles of beat velocity, with 14.8 ± 3.0 Hz and 9.0 ± 2.4 Hz at η = 0.9 (0% methylcellulose solution) and 32.0 mPa.s (0.3% methylcellulose solution), respectively (Kikuchi et al., 2017).

When the viscosity of medium 199 was increased from 7.8 to 58 mP by adding polyvinylpyrrolidone, CBF of bronchial epithelial cell explants was decreased by ca. 10% from the control value. Medium viscosity of 87 millipoises decreased CBF to 25% from the control value (Luk and Dulfano, 1983).

Treatment of primary bronchial epithelial cell monolayer cultures from G551D/F508del cystic fibrosis patients with 10 µM ivacaftor, a CFTR potentiator, at concentrations ≥ 100 nM for 24 hr and 10 µM forskolin decreased mucus viscosity from 2600 cP to 600 cP at

the physiological frequency of 0.9 Hz and increased CBF from ca. 3 Hz to ca. 5 Hz (Birket et al., 2016).

Epithelial cell monolayers from explants of pediatric adenoid tissues were used to assess the impact of viscosity on CBF. A decrease in CBF was observed immediately after the viscosity of the medium was increased, with a greater decrease in CBF in cultures exposed to 20% dextran (González et al., 2016).

Uncertainties and Inconsistencies

Studies interrogating the link between CBF and/or mucus viscosity and MCC found the optimal range of viscoelastic mucus properties to be between 10 and 30 cP and 11 to 25 dyn/cm2 (Chen and Dulfano, 1978; King, 1979; King, 2006; King et al., 1997). These studies also documented that both increases and decreases in mucus viscosity beyond that optimal range impact CBF and decrease and increases, respectively, MCC. A large proportion of these studies utilize (bio)polymers or other large organic molecules to mimic the mucus layer in the airways and increases in its viscosity. Therefore, there may be limitations to the translatability of these findings.

There is at least one study showing that increased mucus viscosity not only slows CBF, but also alters cilia beat metachrony, with medium viscosities in the range of 30–1500 cP increasing metachronal wave velocities by up to 50% and changes in wave direction in cultured frog esophagus (Gheber et al., 1998; Stafanger et al., 1987).
CBF also appears to be, at least in part, autoregulated by ciliated respiratory cells, which adjust cilia beating to differences in viscous load via a mechanosensory mechanism (Johnson et al., 1991).

Quantitative Understanding of the Linkage

The bulk of quantitative data supports the inverse relationship between mucus viscosity and MCC, either via slowing of cilia beating or decreased mucus transport speed. In particular, studies mimicking changes in mucus viscosity by using (bio)polymers or large molecules such as dextran provide insights into the dose-response effects of increasing mucus viscosity on mucociliary transport rates. They do, however, suggest that the effects are transient in nature, at least in ex vivo and in vitro systems. These studies also indicate that there is an optimal range of viscoelastic mucus properties that facilitates efficient MCC and that changes in mucus viscosity beyond that optimal range impact CBF and alter MCC. Because MCC can both decrease and increase dependent on mucus viscosity and because not all studies provide evidence of a causal relationship between these two KEs, we judge our quantitative understanding moderate.

Response-response relationship

Exposure of primary cultures of hamster oviductal ciliated cells to increased viscous loading reduced the CBF (Andrade et al., 2005).

The tracheal samples from mice were used to measure ciliary beat frequencies and beat amplitudes of ciliary motion in viscous culture media over the range of η= 0.9–303.8 mPa.s. The CBF decreased with increasing viscosity, up to about 32.0 mPa.s, while it was nearly constant above 32.0 mPa.s (Kikuchi et al., 2017).

When the viscosity of medium 199 was increased from 7.8 to 58 millipoises (by adding polyvinylpyrrolidone), CBF of bronchial epithelial cell explants was decreased by ca. 10% from the control value. Medium viscosity of 87 millipoises decreased CBF to 25% from the control value (Luk and Dulfano, 1983).

Treatment of primary bronchial epithelial cell monolayer cultures from G551D/F508del cystic fibrosis patients with 10 µM ivacaftor, a CFTR potentiator, at concentrations ≥ 100 nM for 24 hr and 10 µM forskolin decreased mucus viscosity (from 2600 cP to 600 cP) at the physiological frequency of 0.9 Hz and increased CBF (from ca. 3 Hz to ca. 5 Hz) (Birket et al., 2016).

Epithelial cell monolayers from explants of pediatric adenoid tissues were used to assess the impact of viscosity on CBF. A decrease in CBF was observed immediately after the viscosity of the medium was increased, with a greater decrease in CBF in cultures exposed to 20% dextran (González et al., 2016).

Time-scale

Within the first 10 min, the CBF of human oviductal cells dropped ~35% within the range of 2–37 cP (2–15% dextran solutions), but no further decrease was observed at higher viscosities in the range of 37–200 cP (15–30% dextran solutions) (Andrade et al., 2005).

The CBF decreased with increasing viscosity, up to about 32.0 mPa.s, while it was nearly constant above 32.0 mPa.s. The beat frequencies were calculated from the averaged cycles of beat velocity, 14.8 ± 3.0 Hz and 9.0 ± 2.4 Hz (0% methylcellulose solution) and 32.0 mPa.s (0.3% methylcellulose solution), respectively (Kikuchi et al., 2017).

In epithelial cell monolayers from explants of pediatric adenoid tissues , a decrease in CBF was observed immediately after the viscosity of the medium was increased, with a greater decrease in CBF in cultures exposed to 20% dextran. When cultures, prior to viscosity change, were treated with TNFa, CBF decreased furthermore only in culture exposed to 10% dextran. This effect of TNFa occurs in the first 10 min of viscous load, then TNFa-treated cells seem to adjust the CBF to control values (González et al., 2016).

Known modulating factors

Unknown

Known Feedforward/Feedback loops influencing this KER

Unknown

References

- Andrade, Y.N., Fernandes, J., Vázquez, E., Fernández-Fernández, J.M., Arniges, M., Sánchez, T.M., et al. (2005). TRPV4 channel is involved in the coupling of fluid viscosity changes to epithelial ciliary activity. J. Cell Biol. 168(6), 869-874.
- Birket, S.E., Chu, K.K., Houser, G.H., Liu, L., Fernandez, C.M., Solomon, G.M., et al. (2016). Combination therapy with cystic fibrosis transmembrane conductance regulator modulators augment the airway functional microanatomy. Am. J. Physiol. Lung Cell. Mol. Physiol. 310(10), L928-L939.
- Birket, S.E., Davis, J.M., Fernandez, C.M., Tuggle, K.L., Oden, A.M., Chu, K.K., et al. (2018). Development of an airway mucus defect in the cystic fibrosis rat. JCI Insight 3(1), e97199.
- Chen, E.Y., Sun, A., Chen, C.-S., Mintz, A.J., and Chin, W.-C. (2014). Nicotine alters mucin rheological properties. American Journal of Physiology-Lung Cellular and Molecular Physiology 307(2), L149-L157.
- Chen, T., and Dulfano, M. (1978). Mucus viscoelasticity and mucociliary transport rate. The Journal of laboratory and clinical medicine 91(3), 423-431.
- Gheber, L., Korngreen, A., and Priel, Z. (1998). Effect of viscosity on metachrony in mucus propelling cilia. Cell motility and the cytoskeleton 39(1), 9-20.
- González, C., Droguett, K., Rios, M., Cohen, N.A., and Villalón, M. (2016). TNFα Affects Ciliary Beat Response to Increased Viscosity in Human Pediatric Airway Epithelium. Biomed. Res. Int. 2016, 3628501.
- Johnson, N.T., Villalón, M., Royce, F.H., Hard, R., and Verdugo, P. (1991). Autoregulation of beat frequency in respiratory ciliated cells. The American review of respiratory disease 144, 1091-1094.
- Kikuchi, K., Haga, T., Numayama-Tsuruta, K., Ueno, H., and Ishikawa, T. (2017). Effect of fluid viscosity on the cilia-generated flow on a mouse tracheal lumen. Ann. Biomed. Eng. 45(4), 1048-1057.
- King, M. (1979). Interrelation between mechanical properties of mucus and mucociliary transport: effect of pharmacologic interventions. Biorheology 16(1-2), 57-68.
- King, M. (2006). Physiology of mucus clearance. Paediatr. Respir. Rev. 7 Suppl 1, S212-214. doi: 10.1016/j.prrv.2006.04.199.
- King, M., Dasgupta, B., Tomkiewicz, R.P., and Brown, N.E. (1997). Rheology of cystic fibrosis sputum after in vitro treatment with hypertonic saline alone and in combination with recombinant human deoxyribonuclease I. American journal of respiratory and critical care medicine 156(1), 173-177.
- Luk, C.K., and Dulfano, M.J. (1983). Effect of pH, viscosity and ionic-strength changes on ciliary beating frequency of human bronchial explants. Clin Sci (Lond) 64(4), 449-451.
- Matsui, H., Grubb, B.R., Tarran, R., Randell, S.H., Gatzy, J.T., Davis, C.W., et al. (1998). Evidence for periciliary liquid layer depletion, not abnormal ion composition, in the pathogenesis of cystic fibrosis airways disease. Cell 95(7), 1005-1015.
- Stafanger, G., Bisgaard, H., Pedersen, M., Mørkassel, E., and Koch, C. (1987). Effect of N-acetylcysteine on the human nasal ciliary activity in vitro. European journal of respiratory diseases 70(3), 157-162.

Relationship: 2446: Mucus Viscosity, Increased leads to MCC, Decreased

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Oxidative stress [MIE] Leading to Decreased Lung Function [AO]	adjacent	High	Moderate

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	High	NCBI
Rattus norvegicus	Rattus norvegicus		NCBI
Sus scrofa	Sus scrofa		NCBI
Equus ferus	Equus caballus ferus		NCBI

Life Stage Applicability

Life Stage	Evidence

All life stages High

Life Stage Evidence

Sex Applicability

Sex	Evidence
Mixed	High

Key Event Relationship Description

Under physiological conditions, the viscosity of mucus has been shown to range from 1 to 100 Pa.s under low shear rate conditions and from 0.01 to 1 Pa.s under high shear rate conditions. Mucus viscoelastic properties, whether altered by airway dehydration or mucus hypersecretion, directly influence mucociliary clearance (MCC). Toxicant exposures as well as inflammation can also affect the physicochemical properties of mucus (Chen et al., 2014). Increased mucus viscosity in turn decreases CBF and slows transport of mucus on the mucociliary escalator.

Evidence Supporting this KER

Mucus viscoelastic properties, whether altered by airway dehydration or mucus hypersecretion, directly influence MCC. Studies in cystic fibrosis models and those on mimicking changes in mucus viscosity by using (bio)polymers or large molecules such as dextran have indicated a dose-response effect of increasing mucus viscosity on mucociliary transport rates, although these changes are transient in nature in ex vivo and in vitro systems (Birket et al., 2018; Fernandez-Petty et al., 2019). Increased mucus viscosity also has a negative impact on MCC in horses with recurrent airway obstruction (Gerber et al., 2000). Conversely, inhalation of hypertonic saline solution decreases mucus viscosity and enhances MCC in cystic fibrosis patients (Robinson et al., 1997).

Biological Plausibility

Mucus viscoelastic properties, whether altered by airway dehydration or mucus hypersecretion, directly influence MCC. In fact, there is an inverse relationship between mucus viscosity and CBF and mucus transport/MCC, as demonstrated in several in vivo and ex vivo studies. A large proportion of these studies have employed (bio)polymers or other large organic molecules to mimic the mucus layer in the airways and the increase in its viscosity. In addition, some of these studies have shown that decreased mucus viscosity may also result in impairment of MCC. Therefore, a causal link is only tentatively supported. Because cilia function, ASL height, and mucus properties are intricately linked to each other as evidenced by cystic fibrosis studies, we consider the biological plausibility of this KER moderate.

Empirical Evidence

Exposure of primary cultures of hamster oviductal ciliated cells to increased viscous loading reduced the CBF, reaching a new stable value within the first 10 min (Fig. 1 a).The CBF dropped 35% within the range of 2–37 cP (2–15% dextran solutions), but no further decrease was observed at higher viscosities in the range of 37–200 cP (15–30% dextran solutions) (Andrade et al., 2005).

In tracheal and bronchial sections of mice, the CBF decreased with increasing viscosity up to about 32.0 mPa s, while it was nearly constant above 32.0 mPa s. The amplitude of the ciliary beat was kept at ~1.5 lm regardless of the viscosity (Kikuchi et al., 2017).

In control cultures of human nasopharyngeal pediatric airway ciliated cells, a decrease in CBF was observed immediately after the viscosity of the medium was increased, with a greater decrease in CBF in cultures exposed to 20% dextran (González et al., 2016).

CBF in bronchial biopsies from patients undergoing bronchoscopy for diagnostic purposes was inhibited by increased viscosity of the polyvinylpyrrolidone-supplemented medium (Luk and Dulfano, 1983).

In cultured ciliary cells from the frog esophagus photoelectric measurements were performed in the viscosity range of 1–2000 cP. In solutions of viscosity >30 cP. the decrease in CBF was more pronounced but the general trend of a relatively moderate decrease in the frequency at higher viscosity applies for the whole viscosity range (Gheber et al., 1998).

Sputum from chronic bronchitis patients placed on mucus-depleted frog palates moved slower if their Newtonian viscosity exceeded 1000-3000 P (Chen TM and Dulfano, 1978).

In human cystic fibrosis airway epithelial cultures grown at the air-liquid interface, ASL height was lower than that in non-cystic fibrosis cultures thereby increasing mucus viscosity, and mucus velocity was lower than that in non-cystic fibrosis cultures (Matsui et al., 1998).

In tracheas of 6-month old Cftr-deficient rats, mucus viscosity was higher than that of their wild-type littermates and mucus transport was significantly slowed down (Birket S. E. et al., 2018).

Polycarbophil and Carbopol 1342 polymers of increasing viscosity caused ever greater reductions MCT rates [cm/min] across bovine trachea (Shah and Donovan, 2007a). A similar observation was made for the transport of anionic polysaccharide gels of increasing viscosity and linear polyanionic samples across the frog palate (Lin et al., 1993; Shah and Donovan, 2007b).

In a study on children with cystic fibrosis, the mucus dry weights had a borderline significant inverse correlation with the average percent clearance through the first 60 minutes (MCC60) (Laube et al., 2020).

Mucus from patients with chronic bronchial inflammatory diseases treated with 600 mg/day of Sobrerol (decreases viscosity of sputum) traveled faster the pre-established distance on frog palate ('reference' speed of MCT) than from mucus from untreated patients (Allegra et al., 1981).

Acetylcysteine reduced the viscosity of human tracheobronchial secretions in vitro (Sheffner et al., 1964). In non-smokers, treatment with 0.6 g N-acetylcysteine/day po. for 60 days increased mucociliary clearance rates that returned to baseline values after a washout period (Todisco et al., 1985).

When hypertonic saline is added to mucus/sputum, its viscosity is markedly reduced (Elkins and Bye, 2011), and this greatly enhances its transportability in bovine trachea (King et al., 1997; Wills and Cole, 1995; Wills et al., 1998). Inhalation of hypertonic saline solution increased MCC in asthmatic and healthy subjects (Daviskas et al., 1996), adult patients with cystic fibrosis (Robinson et al., 1997; Robinson et al., 1996) and improved MCT times in patients with acute/chronic sinusitis and those having undergone sinus surgery (Talbot et al., 1997).

Inhaled mannitol reduced the viscosity of the sputum in asthmatics with mucus hypersecretion (Daviskas et al., 2007). Inhaled mannitol also improved MCC in asthmatics and patients with cystic fibrosis and bronchiectasis (Daviskas et al., 1997; Daviskas et al., 2008; Daviskas et al., 2010).

Poly(acetyl, arginyl) glucosamine (PAAG) improved the viscoelasticity of sputum. The treatment of cystic fibrosis bronchial epithelial cell culture monolayers with sputum samples that were treated with PAAG had MCT rates significantly faster than those treated with PBS (Fernandez-Petty et al., 2019).

A three times daily dose of 16 mg bromhexine, which modifies the physicochemical characteristics of mucus/is a mucolytic agent (Zanasi et al., 2017), for 14 days resulted in improved MCC in subjects with chronic bronchitis (Thomson et al., 1974).

Uncertainties and Inconsistencies

Studies interrogating the link between CBF and/or mucus viscosity and MCC found the optimal range of viscoelastic mucus properties to be between 10 and 30 cP and 11 to 25 dyn/cm2 (Chen and Dulfano, 1978; King , 1979; King, 2006; King et al., 1997). These studies also documented that both increases and decreases in mucus viscosity beyond that optimal range impact CBF and decrease and increases, respectively, MCC. A large proportion of these studies utilize (bio)polymers or other large organic molecules to mimic the mucus layer in the airways and increases in its viscosity. Therefore, there may be limitations to the translatability of these findings.
There is at least one study showing that increased mucus viscosity not only slows CBF, but also alters cilia beat metachrony, with medium viscosities in the range of 30–1500 cP increasing metachronal wave velocities by up to 50% and changes in wave direction in cultured frog esophagus (Gheber et al., 1998; Stafanger et al., 1987).
CBF also appears to be, at least in part, autoregulated by ciliated respiratory cells, which adjust cilia beating to differences in viscous load via a mechanosensory mechanism (Johnson et al., 1991).

Quantitative Understanding of the Linkage

The bulk of quantitative data supports the inverse relationship between mucus viscosity and MCC, either via slowing of cilia beating or decreased mucus transport speed. In particular, studies mimicking changes in mucus viscosity by using (bio)polymers or large molecules such as dextran provide insights into the dose-response effects of increasing mucus viscosity on mucociliary transport rates. They do, however, suggest that the effects are transient in nature, at least in ex vivo and in vitro systems. These studies also indicate that there is an optimal range of viscoelastic mucus properties that facilitates efficient MCC and that changes in mucus viscosity beyond that optimal range impact CBF and alter MCC. Because MCC can both decrease and increase dependent on mucus viscosity and because not all studies provide evidence of a causal relationship between these two KEs, we judge our quantitative understanding moderate.

Response-response relationship

In tracheas of 6-month old Cftr-deficient rats, mucus was 20-fold more viscous than that of their wild-type littermates (2.91 ± 0.9 cP WT vs. 65.09 ± 3.6 cP KO) and mucus transport rate was significantly slowed down (ca. 0.8 mm/min WT vs ca. 0.3 mm/min KO). Following addition of sodium bicarbonate, which is known to improve airway hydration and hence decrease mucus viscosity, at concentrations of 23 mM, 69 mM, 92 mM, and 115 mM to the apical surface of Cftr-deficient rat tracheas ex vivo (6-month old animals) increased MCT rates in a dose-dependent manner, up to ca. 2.5 mm/min (Birket et al., 2018).

Sputum dry weights more than 151.0 mg/mL were linked with 6.8 (3.3-17.7) median percent MCC60, and sputum dry weights less than 151.0 mg/mL were linked with 13.2 (6.2-28.6) median percent MCC60. Percent MCC60 was borderline significantly higher in children with dry weights less than 151.0 mg/mL than in children with dry weights more than 151.0 mg/mL (Laube et al., 2020).

Adult CF patients receiving hypertonic saline via Omron-NE-U06 ultrasonic nebulizer exhibited increased MCC as evidenced by increased Tc particle clearance rates. The amount cleared at 90 min on the control day was 12.7% (95% confidence interval (CI) 9.8 to 17.2) compared with 19.7% (95% CI 13.6 to 29.5) for 3% hypertonic saline, 23.8% (95% CI 15.9 to 36.7) for 7% hypertonic saline and 26.0% (95% CI 19.8 to 35.9) for 12% hypertonic saline (Robinson et al., 1997).

Treatment with 100-500 µg/mL poly(acetyl, arginyl) glucosamine (PAAG) for 2 h significantly reduced the dynamic viscosity of CF sputum at low shear rates. Effective viscosities measured at a shear rate of 0.8 s^{-1} indicated that the treatment effect was particularly prominent in CF sputum samples that exhibited high dynamic viscosity at baseline (359 ± 561 Pa•s for sputum treated

with PBS compared with 62 ± 97 Pa•s for sputum treated with PAAG). When PAAG-treated (250 µg/mL) sputum was added to trachea sputum, it was more rapidly transported in a homogenous fashion than untreated sputum (3.91 ± 1.89 mm/min PAAG vs 1.62 ± 0.56 mm/min PBS) (Fernandez-Petty et al., 2019).

In bronchial epithelial cultures grown at the air-liquid interface, treatment with between 250 and 500 µg/mL PAAG caused a 2-log reduction in effective viscosity across all frequency ranges (597.0 ± 56.1 cP for PBS control versus 2.84 ± 0.11 cP PAAG at 1.0 Hz) and a 57% increase in MCT rate in cells treated with PAAG (250 µg/mL) compared with the PBS control (Fernandez-Petty et al., 2019).

Using $Cftr^{-/-}$ rats aged 6 months in which delayed MCT occurs due to abnormally viscous mucus (Birket et al., 2018), PAAG (250 µg/mL × 20 mL over 45 minutes) or glycerol vehicle control was nebulized once daily for 14 days. Mucus transport increased approx. 3.5-fold in PAAG-treated rats compared to vehicle-treated rats, achieving approximately 44% of MCT rates in normal rats (Fernandez-Petty et al., 2019).

At 24 h following environmental challenge (i.e., stabling in stalls with straw as bedding and hay as feed) of horses with recurrent airway obstruction, the viscoelasticity of mucus increased ca. 3-fold (to log G* averaging 3.0 ± 0.3 dyn/cm^2 vs 2.38 ± 0.11 dyn/cm^2 in controls) and mucociliary clearance index (MCI) decreased to 0.69 ± 0.05 (vs 0.92 ± 0.06 in controls) (Gerber et al., 2000).

In dogs receiving a high dose of methacholine chloride (16-32 mg/mL) acutely, mucus viscosity increased by 203 ± 23% from control, while mucus transport rate on frog palates decreased to 79 ± 6% of control (King, 1979).

Time-scale

Within the first 10 min following addition of 2–15% dextran, the CBF of human oviductal cells dropped ~35% within the range of 2–37 cP. No further decrease was observed at higher viscosities (15–30% dextran solutions) in the range of 37–200 cP (Andrade et al., 2005).

Following addition of sodium bicarbonate, which is known to improve airway hydration and hence decrease mucus viscosity, at concentrations of 23 mM, 69 mM, 92 mM, and 115 mM to the apical surface of Cftr-deficient rat tracheas ex vivo (6-month old animals) increased MCT rates in a dose-dependent manner, up to ca. 2.5 mm/min. The peak effect of bicarbonate addition occurred at 20 minutes after addition and returned to baseline by 35 minutes after addition, consistent with the short half-life of bicarbonate at the surface of the airway (Birket et al., 2018).

At 24 h following environmental challenge (i.e., stabling in stalls with straw as bedding and hay as feed) of horses with recurrent airway obstruction, the viscoelasticity of mucus increased ca. 3-fold (to log G* averaging 3.0 ± 0.3 dyn/cm2 vs 2.38 ± 0.11 dyn/cm2 in controls) and mucociliary clearance index (MCI) decreased to 0.69 ± 0.05 (vs 0.92 ± 0.06 in controls). Significant changes in mucus viscoelasticity and MCI were only observed at 24 and 48 h, but not at 6 h post-challenge (Gerber et al., 2000).

In dogs receiving a high dose of methacholine chloride (16-32 mg/mL) acutely, mucus viscosity increased, while mucus transport rate on frog palates decreased at approx. 2 to 5 min post-treatment (King, 1979).

Known modulating factors

Unknown

Known Feedforward/Feedback loops influencing this KER

Unknown

References

- Allegra, L., Bossi, R., and Braga, P.C. (1981). Action of sobrerol on mucociliary transport. Respiration 42(2), 105-109.
- Andrade, Y.N., Fernandes, J., Vázquez, E., Fernández-Fernández, J.M., Arniges, M., Sánchez, T.M., et al. (2005). TRPV4 channel is involved in the coupling of fluid viscosity changes to epithelial ciliary activity. J. Cell Biol. 168(6), 869-874.
- Birket, S.E., Davis, J.M., Fernandez, C.M., Tuggle, K.L., Oden, A.M., Chu, K.K., et al. (2018). Development of an airway mucus defect in the cystic fibrosis rat. JCI Insight 3(1), e97199.
- Chen, E.Y., Sun, A., Chen, C.-S., Mintz, A.J., and Chin, W.-C. (2014). Nicotine alters mucin rheological properties. American Journal of Physiology-Lung Cellular and Molecular Physiology 307(2), L149-L157.
- Chen, T., and Dulfano, M. (1978). Mucus viscoelasticity and mucociliary transport rate. The Journal of laboratory and clinical medicine 91(3), 423-431.
- Daviskas, E., Anderson, S., Brannan, J., Chan, H., Eberl, S., and Bautovich, G. (1997). Inhalation of dry-powder mannitol increases mucociliary clearance. European Respiratory Journal 10(11), 2449-2454.
- Daviskas, E., Anderson, S., Eberl, S., and Young, I. (2008). Effect of increasing doses of mannitol on mucus clearance in patients with bronchiectasis. European Respiratory Journal 31(4), 765-772.
- Daviskas, E., Anderson, S., Gonda, I., Eberl, S., Meikle, S., Seale, J., et al. (1996). Inhalation of hypertonic saline aerosol enhances mucociliary clearance in asthmatic and healthy subjects. European Respiratory Journal 9(4), 725-732.
- Daviskas, E., Anderson, S.D., Jaques, A., and Charlton, B. (2010). Inhaled mannitol improves the hydration and surface properties of sputum in patients with cystic fibrosis. Chest 137(4), 861-868.
- Daviskas, E., Anderson, S.D., and Young, I.H. (2007). Inhaled mannitol changes the sputum properties in asthmatics with

mucus hypersecretion. Respirology 12(5), 683-691.
- Elkins, M.R., and Bye, P.T. (2011). Mechanisms and applications of hypertonic saline. Journal of the Royal Society of Medicine 104(1_suppl), 2-5.
- Fernandez-Petty, C.M., Hughes, G.W., Bowers, H.L., Watson, J.D., Rosen, B.H., Townsend, S.M., et al. (2019). A glycopolymer improves vascoelasticity and mucociliary transport of abnormal cystic fibrosis mucus. JCI Insight 4(8). e125954.
- Gerber, V., King, M., Schneider, D., and Robinson, N. (2000). Tracheobronchial mucus viscoelasticity during environmental challenge in horses with recurrent airway obstruction. Equine Vet. J. 32(5), 411-417.
- Gheber, L., Korngreen, A., and Priel, Z. (1998). Effect of viscosity on metachrony in mucus propelling cilia. Cell motility and the cytoskeleton 39(1), 9-20.
- González, C., Droguett, K., Rios, M., Cohen, N.A., and Villalón, M. (2016). TNFα Affects Ciliary Beat Response to Increased Viscosity in Human Pediatric Airway Epithelium. Biomed. Res. Int. 2016. 3628501.
- Johnson, N.T., Villalón, M., Royce, F.H., Hard, R., and Verdugo, P. (1991). Autoregulation of beat frequency in respiratory ciliated cells. The American review of respiratory disease 144, 1091-1094.
- Kikuchi, K., Haga, T., Numayama-Tsuruta, K., Ueno, H., and Ishikawa, T. (2017). Effect of fluid viscosity on the cilia-generated flow on a mouse tracheal lumen. Ann. Biomed. Eng. 45(4), 1048-1057.
- King, M. (1979). Interrelation between mechanical properties of mucus and mucociliary transport: effect of pharmacologic interventions. Biorheology 16(1-2), 57-68.
- King, M., Dasgupta, B., Tomkiewicz, R.P., and Brown, N.E. (1997). Rheology of cystic fibrosis sputum after in vitro treatment with hypertonic saline alone and in combination with recombinant human deoxyribonuclease I. American journal of respiratory and critical care medicine 156(1), 173-177.
- King, M. (2006). Physiology of mucus clearance. Paediatr. Respir. Rev. 7 Suppl 1, S212-214. doi: 10.1016/j.prrv.2006.04.199.
- Laube, B.L., Carson, K.A., Evans, C.M., Richardson, V.L., Sharpless, G., Zeitlin, P.L., et al. (2020). Changes in mucociliary clearance over time in children with cystic fibrosis. Pediatr Pulmonol 55(9), 2307-2314.
- Lin, S.Y., Amidon, G.L., Weiner, N.D., and Goldberg, A.H. (1993). Viscoelasticity of anionic polymers and their mucociliary transport on the frog palate. Pharm Res 10(3), 411-417.
- Luk, C.K., and Dulfano, M.J. (1983). Effect of pH, viscosity and ionic-strength changes on ciliary beating frequency of human bronchial explants. Clin Sci (Lond) 64(4), 449-451.
- Robinson, M., Hemming, A.L., Regnis, J.A., Wong, A.G., Bailey, D.L., Bautovich, G.J., et al. (1997). Effect of increasing doses of hypertonic saline on mucociliary clearance in patients with cystic fibrosis. Thorax 52(10), 900.
- Robinson, M., Regnis, J.A., Bailey, D.L., King, M., Bautovich, G.J., and Bye, P. (1996). Effect of hypertonic saline, amiloride, and cough on mucociliary clearance in patients with cystic fibrosis. American journal of respiratory and critical care medicine 153(5), 1503-1509.
- Shah, A.J., and Donovan, M.D. (2007a). Rheological characterization of neutral and anionic polysaccharides with reduced mucociliary transport rates. AAPS PharmSciTech 8(2), Article 32.
- Shah, A.J., and Donovan, M.D. (2007b). Formulating gels for decreased mucociliary transport using rheologic properties: polyacrylic acids. AAPS PharmSciTech 8(2), Article 33.
- Stafanger, G., Bisgaard, H., Pedersen, M., Mørkassel, E., and Koch, C. (1987). Effect of N-acetylcysteine on the human nasal ciliary activity in vitro. European journal of respiratory diseases 70(3), 157-162.
- Talbot, A.R., Herr, T.M., and Parsons, D.S. (1997). Mucociliary clearance and buffered hypertonic saline solution. the Laryngoscope 107(4), 500-503.
- Thomson, M., Pavia, D., Gregg, I., and Stark, J. (1974). Bromhexine and mucociliary clearance in chronic bronchitis. British journal of diseases of the chest 68, 21-27.
- Todisco, T., Polidori, R., Rossi, F., Iannacci, L., Bruni, B., Fedeli, L., et al. (1985). Effect of N-acetylcysteine in subjects with slow pulmonary mucociliary clearance. Eur J Respir Dis Suppl 139, 136-141.
- Wills, P., and Cole, P. (1995). Sodium chloride improves ciliary transportability of sputum. Am J Respir Crit Care Med 151, A720.
- Wills, P., Pritchard, K., and Cole, P. (1998). Mucus transportability: the bovine trachea and frog palate models compared. European Respiratory Journal 12(4), 837-841.
- Zanasi, A., Mazzolini, M., and Kantar, A. (2017). A reappraisal of the mucoactive activity and clinical efficacy of bromhexine. Multidisciplinary respiratory medicine 12(1), 1-14.

Relationship: 2447: FOXJ1 Protein, Decreased leads to Motile Cilia Number/Length, Decreased

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Oxidative stress [MIE] Leading to Decreased Lung Function [AO]	adjacent	High	High

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	Moderate	NCBI
Xenopus laevis	Xenopus laevis		NCBI
Mus musculus	Mus musculus		NCBI
Schmidtea mediterranea	Schmidtea mediterranea		NCBI
Danio rerio	Danio rerio		NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	

Sex Applicability

Sex	Evidence
Mixed	

Key Event Relationship Description

Forkhead box J1 (FOXJ1) is a master regulator of motile ciliogenesis which is necessary and also sufficient to program cells to grow functional motile cilia (Vij et al., 2012; Zhou and Roy, 2015). Studies in different model organisms have shown that the loss of FOXJ1 results in a loss of motile cilia (Brody et al., 2000; Chenet al., 1998; Stubbs et al., 2008; Vij et al., 2012).

Evidence Supporting this KER

Homozygous null mutation of Foxj1 results in complete absence of cilia in mouse respiratory epithelium (Chen et al., 1998; Brody et al., 2000). In a previous study, wild-type mice had approximately 20% heavily ciliated cells in the proximal pulmonary epithelium, while explanted Foxj1$^{-/-}$ mouse trachea had no ciliated cells (Gomperts et al., 2004). Loss of FOXJ1 orthologs FoxJ1–4 in flatworm Schmidtea mediterranea results in loss of ciliation of the ventral epithelium which closely resembles the human airway epithelium (Rompolas et al., 2009; Vij et al., 2012). Loss of Foxj1 activity in *Xenopus* and zebrafish—through antisense morpholino oligonucleotides—reduces cilia formation, while, conversely, ectopic Foxj1 overexpression results in formation of multiple motile cilia (Stubbs et al., 2008; Yu et al., 2008). There is a strong correlation between FOXJ1 and expression of the FOXJ1 ciliogenesis program genes in zebrafish, Xenopus and mouse cells (Abedalthagafi et al., 2016).
Treatment with cigarette smoke extract downregulates FOXJ1 mRNA and protein expression, which is accompanied by a reduction in cilia length and number in human bronchial epithelial cells in vitro (Milara et al., 2012; Brekman et al., 2014). This can be prevented by overexpression of FOXJ1 (Brekman et al., 2014) or treatment with roflumilast N-oxide, which reduces intracellular free radical levels and increases FOXJ1 mRNA and protein expression (Milara et al., 2012).

Biological Plausibility

The requirement of FOXJ1 for cells to grow functional motile cilia was demonstrated in 1998 in a mouse model study where targeted disruption of FOXJ1 resulted in the absence of motile cilia in the respiratory epithelium, oviduct, haploid sperm, and choroid plexus (Chen et al., 1998). Subsequently, many research groups consistently showed FOXJ1 requirement for cilia growth in various model organisms (Brody et al., 2000; Gomperts et al., 2007; Stubbs et al., 2008; Vij et al., 2012; Yu et al., 2008). In addition, overexpression of FOXJ1 in ectopic locations prompted cilia growth (Stubbs et al., 2008; Yu et al., 2008), and FOXJ1 overexpression could rescue cigarette smoke extract-caused cilia growth suppression in human airway epithelium (Brekman et al., 2014). The causal association of FOXJ1 to ciliogenesis gene expression program was computationally reinforced (Abedalthagafi et al., 2016). Taken together, the empirical support for this KER based on the research in the motile ciliogenesis field implies a high (strong) confidence for the biological plausibility of the linkage.

Empirical Evidence

Two research groups independently showed that homozygous null mutation of Foxj1 causes complete absence of cilia in the respiratory airways of mice (Brody et al., 2000; Chen et al., 1998).

Loss of Foxj1a activity in zebrafish (antisense morpholino oligonucleotides designed to block Foxj1a protein translation) compromises formation of motile cilia (Yu et al., 2008). In the same study, Foxj1a ectopic expression in the neural tube (through hyperactivation of the hedgehog pathway using dominant negative PKA) triggered motile cilia development (Yu et al., 2008).

In Xenopus, FoxJ1 morpholino oligo treatments dose-dependently reduced cilia formation, and FoxJ1 overexpression in ectopic locations was sufficient to drive multiciliogenesis (Stubbs et al., 2008). In addition, authors also used a zebrafish model where FOXJ1 morpholino resulted in cilia number and length reduction (Stubbs et al., 2008).

The closest ortholog of mammalian FOXJ1 in the flatworm *Schmidtea mediterranea* is FoxJ1-4 which is expressed in the motile ciliated cells of the ventral epithelium. Flatworm ventral epithelium closely resembles the mammalian airway epithelium (Rompolas et al., 2009; Vij et al., 2012). S. mediterranea deficient for foxJ1-4 (RNAi) lost the ciliation of the ventral epithelium (Vij et al., 2012).

Abedalthagafi et al. defined the genes that comprise the FOXJ1 ciliogenesis program using public mRNA expression profiling data from Xenopus, zebrafish and mouse cells. A strong correlation of FOXJ1 expression and the expression of the FOXJ1 ciliogenesis

program was confirmed (Abedalthagafi et al., 2016).

Cigarette smoke extract (CSE) down-regulated FOXJ1 mRNA and protein levels and cilia length in differentiating human airway basal cells in air–liquid interface (ALI) cultures, while lentivirus-mediated FOXJ1 expression prevented CSE-elicited cilia growth suppression (Brekman et al., 2014).

Wild-type mouse tracheas cultured at air-liquid interface demonstrated nearly 20% of the proximal pulmonary epithelial cells to be ciliated with abundant cilia on each cell. In explanted Foxj1$^{-/-}$ tracheas no ciliated epithelial cells were observed (Gomperts et al., 2004).

Exposure of differentiated human bronchial epithelial cells to CSE decreased FOXJ1 expression alongside with reduction of the average number of cells with cilia motility. Roflumilast N-oxide (which reduced intracellular reactive oxygen species levels) prevented the CSE-elicited decrease in the expression levels of Foxj1 mRNA and protein. Concurrently, roflumilast N-oxide partly prevented the loss in cells with cilia motility (Milara et al., 2012).

FOXJ1 overexpression increased the percentage of ciliated cells in mouse trachea organ culture 1.51-fold (Johnson et al., 2018).

Uncertainties and Inconsistencies

Foxj1 overexpression failed to promote ciliogenesis in mouse polarized epithelial cell lines and primary cultured alveolar epithelial cells (You et al., 2004). Also, the overexpression of Foxj1 in wild-type airway epithelial cells did not enhance the total number of ciliated cells. However, delivery of Foxj1 to null cells resulted in cilia formation (You et al., 2004).

Quantitative Understanding of the Linkage

There is ample empirical evidence of FOXJ1 requirement for motile cilia formation, where complete removal of FOXJ1 results in cilia loss, and different levels of FOXJ1 downregulation result in proportional reduction in cilia number and length. Accordingly, FOXJ1 overexpression leads to higher cilia numbers. Based on these data of strong causality between upstream and downstream KEs, we judge our quantitative understanding to be high.

Response-response relationship

Complete removal of FOXJ1 by means of homologous recombination in mouse embryonic stem cells resulted in absence of cilia in mouse airways (as well as in other typically multiciliated tissues such as oviduct, haploid sperm, choroid plexus and epithelial cells of the brain but not in embryonic node) (Brody et al., 2000; Chen J. et al., 1998).

Newly fertilized zebrafish eggs were injected with antisense morpholino oligonucleotides designed to block Foxj1a protein translation. Motile cilia numbers were severely reduced in Kupffer's vesicle (KV), the floor plate and pronephric ducts 14 and 24 hpf (Yu et al., 2008).

Downregulation of Xenopus FoxJ1 produced a dose-dependent defect in skin cilia formation. When 20 ng or 40 ng morpholino oligonucleotides were injected, cilia formed, but were reduced in number and shortened in length. After injection of 75 ng morpholino oligos, most cilia were lost. Cilia length decreased from ~11 microns to 4 microns (Stubbs et al., 2008). Morpholino oligo knockdown of zebrafish FoxJ1 caused a two-fold decrease in the number of KV cilia and a 3.5-fold decrease in the average length of KV cilia (Stubbs et al., 2008).

RNAi against *Schmidtea mediterranea* foxJ1-4 substantially reduced the expression levels of foxJ1-4 which lead to almost complete loss of motile cilia (Vij et al., 2012).

In the presence of CSE, in FOXJ1 overexpressing human airway epithelial cells the average cilia length was significantly higher (5.2 µm) than in lentivirus-control–infected cells (4.1 µm) (Brekman et al., 2014). CSE was obtained from one Marlboro Red commercial cigarette bubbled in 12.5 ml of differentiation medium that was then 0.2 mm pore filtered. The absorbance was measured at 320 nm on a spectrophotometer and the optical density of 1 was defined as 100%. Homozygous FOXJ1 mutant mice were obtained by mating foxj1$^{+/-}$ male and female animals. The explanted trachea of the foxj1$^{-/-}$ mice harbors no motile cilia in contrast to wild-type trachea (Gomperts et al., 2004).

Exposure of differentiated human bronchial epithelial cells to 10% CSE decreased FOXJ1 expression by about 40% at 24 h and 70% at 72 h exposure. The smoke of one 2R4F research cigarette was bubbled into a flask containing 25 mL of pre-warmed (37°C) differentiation medium using a respiratory pump model (Harvard Apparatus Rodent Respirator 680, Harvard Apparatus, Holliston, MA, USA) that generates three puffs min–1; 35 mL per each puff of 2 s duration with a volume of 0.5 cm above the filter. The CS solution was filtered (0.22 µm pore size) to remove particles and the tar phase. The resulting sterile solution was defined as 100% CSE and used within 30 min of preparation. Exposure to CSE concentration- and time-dependently reduced the average number of cells with cilia motility, which was significant after 3 days of incubation with CSE at 2.5% (about 30% inhibition), and reached a maximum of about 75% inhibition versus control after 7 days of incubation with CSE at 10% (Milara et al., 2012). Roflumilast N-oxide at 2 nM or 1 µM concentration-dependently prevented the decrease in the expression levels of Foxj1 mRNA and protein following 3 days of exposure of differentiated bronchial epithelial cells to CSE at 10%. Concurrently, roflumilast N-oxide partly prevented the loss in cells with cilia motility (Milara et al., 2012).

Electroporation using negative control (GFP-only) plasmid resulted in 45±1.4% (mean±s.e.m.) GFP+ ciliated cells in mouse trachea

organ culture. FOXJ1 significantly increased the percentage of GFP+ ciliated cells to 68±3.6% (1.51-fold) (Johnson et al., 2018).

Time-scale

14- or 24-hr treatment with antisense morpholino oligonucleotides designed to block Foxj1a protein translation results in absence of motile cilia in zebrafish (Yu et al., 2008).

Xenopus embryos were injected with FOXJ1 morpholino oligos at two-cell stage (1.5 h of embryo life) and the embryos were analyzed at stage 26 (1 day, 5 h and 30 min of embryo life) for cilia phenotype (Stubbs et al., 2008).

Schmidtea mediterranea worms received three feedings of foxJ1-4 RNAi (2 days in between feeds) and were analyzed for cilia phenotype 14 days after the last feed. RNAi against Schmidtea mediterranea foxJ1-4 substantially reduced the expression levels of foxJ1-4 which lead to almost complete loss of motile cilia (Vij et al., 2012).

Exposure of differentiated human bronchial epithelial cells to CSE concentration- and time-dependently reduced the average number of cells with cilia motility, which was significant after 3 days of incubation with CSE at 2.5% (about 30% inhibition), and reached a maximum of about 75% inhibition versus control after 7 days of incubation with CSE at 10% (Milara et al., 2012).

Known modulating factors

Regulatory factor X3 (RFX3) is a transcriptional co-activator of FOXJ1 (Didon et al., 2013) and is involved in motile cilia biogenesis (El Zein et al., 2009). Fluctuations in RFX3 levels can modulate the outcome that the upstream KE has on the downstream KE.

Known Feedforward/Feedback loops influencing this KER

Unknown

References

- Abedalthagafi, M.S., Wu, M.P., Merrill, P.H., Du, Z., Woo, T., Sheu, S.H., et al. (2016). Decreased FOXJ1 expression and its ciliogenesis programme in aggressive ependymoma and choroid plexus tumours. J. Pathol. 238(4), 584-597.
- Brekman, A., Walters, M.S., Tilley, A.E., and Crystal, R.G. (2014). FOXJ1 prevents cilia growth inhibition by cigarette smoke in human airway epithelium in vitro. Am. J. Respir. Cell Mol. Biol. 51(5), 688-700.
- Brody, S.L., Yan, X.H., Wuerffel, M.K., Song, S.K., and Shapiro, S.D. (2000). Ciliogenesis and left-right axis defects in forkhead factor HFH-4-null mice. Am. J. Respir. Cell Mol. Biol. 23(1), 45-51.
- Chen, J., Knowles, H.J., Hebert, J.L., and Hackett, B.P. (1998). Mutation of the mouse hepatocyte nuclear factor/forkhead homologue 4 gene results in an absence of cilia and random left-right asymmetry. J. Clin. Investig. 102(6), 1077-1082.
- Didon, L., Zwick, R.K., Chao, I.W., Walters, M.S., Wang, R., Hackett, N.R., et al. (2013). RFX3 Modulation of FOXJ1 regulation of cilia genes in the human airway epithelium. Respir. Res. 14(1), 70-70.
- El Zein, L., Ait-Lounis, A., Morle, L., Thomas, J., Chhin, B., Spassky, N., et al. (2009). RFX3 governs growth and beating efficiency of motile cilia in mouse and controls the expression of genes involved in human ciliopathies. J Cell Sci 122(Pt 17), 3180-3189.
- Gomperts, B.N., Gong-Cooper, X., and Hackett, B.P. (2004). Foxj1 regulates basal body anchoring to the cytoskeleton of ciliated pulmonary epithelial cells. J. Cell Sci. 117(Pt 8), 1329-1337.
- Gomperts, B.N., Kim, L.J., Flaherty, S.A., and Hackett, B.P. (2007). IL-13 Regulates Cilia Loss and foxj1 Expression in Human Airway Epithelium. Am. J. Respir. Cell Mol. Biol. 37(3), 339-346.
- Johnson, J.A., Watson, J.K., Nikolic, M.Z., and Rawlins, E.L. (2018). Fank1 and Jazf1 promote multiciliated cell differentiation in the mouse airway epithelium. Biol Open 7(4). bio033944.
- Milara, J., Armengot, M., Bañuls, P., Tenor, H., Beume, R., Artigues, E., et al. (2012). Roflumilast N-oxide, a PDE4 inhibitor, improves cilia motility and ciliated human bronchial epithelial cells compromised by cigarette smoke in vitro. Br. J. Pharmacol. 166(8), 2243-2262.
- Rompolas, P., Patel-King, R.S., and King, S.M. (2009). Schmidtea mediterranea: a model system for analysis of motile cilia. Methods Cell Biol. 93, 81-98.
- Stubbs, J.L., Oishi, I., Izpisua Belmonte, J.C., and Kintner, C. (2008). The forkhead protein Foxj1 specifies node-like cilia in Xenopus and zebrafish embryos. Nat. Genet. 40(12), 1454-1460.
- Vij, S., Rink, J.C., Ho, H.K., Babu, D., Eitel, M., Narasimhan, V., et al. (2012). Evolutionarily ancient association of the FoxJ1 transcription factor with the motile ciliogenic program. PLoS Genet. 8(11), e1003019. d
- You, Y., Huang, T., Richer, E.J., Schmidt, J.-E.H., Zabner, J., Borok, Z., et al. (2004). Role of f-box factor foxj1 in differentiation of ciliated airway epithelial cells. American Journal of Physiology-Lung Cellular and Molecular Physiology 286(4), L650-L657.
- Yu, X., Ng, C.P., Habacher, H., and Roy, S. (2008). Foxj1 transcription factors are master regulators of the motile ciliogenic program. Nat. Genet. 40(12), 1445-1453.
- Zhou, F., and Roy, S. (2015). SnapShot: Motile Cilia. Cell 162(1), 224-224 e221.

Relationship: 2448: Motile Cilia Number/Length, Decreased leads to CBF, Decreased

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Oxidative stress [MIE] Leading to Decreased Lung Function [AO]	adjacent	Moderate	Moderate

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	High	NCBI
Danio rerio	Danio rerio		NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	

Sex Applicability

Sex	Evidence
Mixed	

Key Event Relationship Description

The cilia in the respiratory epithelium beat in a coordinated fashion at a frequency of approximately 10 to 16 Hz, propelling mucus upwards (Joki et al., 1998; Smith et al., 2012). Many factors, including cilia length, number, structure, orientation as well as mucus viscosity, temperature, pH, chemicals, airway surface liquid height, exposure to bacterial and viral pathogens have been shown to affect ciliary function (Clary-Meinesz et al., 1998; Ho et al., 2001b; Jing et al., 2017; Joki et al., 1998; Kanthakumar et al., 1996; Mall, 2008; Smith et al., 2012; Snyder et al., 2017). Alteration from normal physiological conditions and from healthy cilia number/length/structure typically reduces the cilia beat frequency (CBF) (Clary-Meinesz et al., 1998; Jayathilake et al., 2012).

Evidence Supporting this KER

In Chlamydomonas, ciliary motion is directly related to the length of the cilia (Bottier et al., 2018). Similar observations have been made in zebrafish, where modulation of cilia length and number by FOR20 (centrosomal protein 20) deletion/knockdown directly impairs ciliary motility (Xie et al., 2019). There is also a positive correlation between cilia number and CBF in sinusitis patients (Joki et al., 1998), while cilia number, length and orientation correlate positively with mucociliary transport rate in patients with recurrent or longstanding respiratory infections (Toskala et al., 1995; Joki et al., 1998). Comparisons of strips of normal and disrupted ciliated epithelium have shown that CBF is decreased in the latter (Thomas et al., 2009).
Mathematical models and simulations have shown that periciliary liquid and mucus velocity are directly affected by cilia number and length (Lee et al., 2011; Jayathilake et al., 2012; Jayathilake et al., 2015).

Biological Plausibility

Many research groups have shown positive correlations between cilia number/length and ciliary function in studies dating as early as 1995 . In some cases, the authors measured mucociliary clearance as the endpoint, thus the evidence does not describe causality between cilia number/length and CBF. However, the commonly held assumption is that reduced ciliary function, i.e., reduced CBF, leads to decreased mucociliary clearance. Based on the evidence, we judge the biological plausibility of this KER as moderate.

Empirical Evidence

Cilia beating frequency analysis in sinusitis patient samples shows positive correlation between cilia number and CBF (Joki et al., 1998).

Cilia study from patients with recurrent or longstanding respiratory infections demonstrates correlation between mucociliary transport rate and cilia number, length, and orientation (Toskala et al., 1995).

According to numerical method formulated by Jayathilake et al., the average periciliary liquid layer (PCL) velocity decreases when cilia are shortened (Jayathilake et al., 2015; Jayathilake et al., 2012).

A study of Chlamydomonas cilia motion concludes that cilia shorter than 4 µm show variable or no periodicity of beating. For cilia longer than 4 µm, the ratio of frequency estimated from body motion to frequency of cilia motion is very close to 1.0, as expected. In short (2 µm, 4 µm) cilia, the ratio was significantly different from 1; apparently, even if the short cilium beats periodically, the frequency of beating is not reliably transmitted to the body motion (Bottier et al., 2018).

In a simulation study by Lee et al., it was calculated that an increase in cilia number increases the mucus velocity (Lee et al., 2011).

In zebrafish, deletion or knockdown of FOR20 causes reduced number and length of cilia. Ciliary motility is impaired in the animals with reduced FOR20 (Xie et al., 2019).

Cilia beating frequency measurements on normal ciliated or disrupted epithelial strips of tissue revealed lower CBF for tissues with cilia disruptions (Thomas et al., 2009).

Uncertainties and Inconsistencies

Although the majority of studies discuss the effect of shorter cilia on ciliary function, there are also reports on longer than normal cilia impairing cilia function (Jayathilake et al., 2015; Li et al., 2014b). A range of factors other than cilia length and number can influence cilia beat frequency. Often a combination of two or more factors affect ciliary function making it difficult to discern the impact of an individual factor on CBF (Toskala et al., 1995; Xie et al., 2019).

Quantitative Understanding of the Linkage

Cilia beating frequency correlates with cilia size and numbers such that higher number of motile cilia with a healthy length show efficient CBF and reduction of cilia number and/or length result in proportionate reduction of CBF. In some studies, mucociliary clearance was measured as an indicator of CBF. Based on evidence presented here, we judge the quantitative understanding to be moderate.

Response-response relationship

A study in nasal mucosa samples from sinusitis patients demonstrates that reduced cilia numbers account for decreased CBF. In normally ciliated samples the CBF was 11.2±3.7 Hz, in samples with some ciliated cells CBF was 8.9±6.3 Hz, and in samples with no detectable cilia CBF was 2.1±3.8 Hz (Joki S. et al., 1998).

Patients with recurrent or longstanding respiratory infections were divided into 3 groups based on mucociliary transport rates (MTR). The group with slowest MTR had the biggest number of non-ciliated columnar cells in the nasal mucosa, and the highest number of short and disoriented cilia. Loss of ciliated cells was seen in 50% of specimens with good MTR (7 to 10.8 mm per minute), in 71% with moderate MTR (3 to 6.9 mm per minute) and in 86% with poor MTR (0 to 2.9 mm per minute). Percentage of short cilia was 1% in the first group, 6% in the second, and 10% in the third. In this study, ciliary disorientation also contributed to the low MTR (Toskala et al., 1995).

Jayathilake et al. developed a two-dimensional numerical model for computing how the length of cilia is affecting fluid flow. In the model, the cilia length was reduced by 5%, 15%, 25%, and 35% from 6 µm while other parameters were kept unchanged. The average PCL velocity in stream-wise direction decreases when cilia are shortened . When the cilia are shortened by 10%, the average stream-wise PCL velocity is reduced by about 11%, while the average span-wise PCL velocity is reduced by about 62% (Jayathilake et al., 2012). In a similar study by the same group, the length of cilia was defined as 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130% and 140% of the length of the healthy cilia (i.e., 6 µm). The mucus velocity reaches its maximum value when the ciliary length is around the length of the healthy cilia (or standard length of 6 µm) (Jayathilake et al., 2015).

Cilia shorter than 4 µm have anomalies in the ciliary beating periodicity. Cilia shorter than 2 µm were never found to beat periodically. Cilia between 2 and 4 µm in length exhibited more variable periodicity. Cilia with lengths between 4 and 12 µm beat periodically with conserved frequency (Bottier et al., 2018).

Mucus velocity increased in concordance with cilia number increase. Mean mucus velocity increased from 26.82 µm/s to 49.53 µm/s when cilia number grew from 10 to 24 (Lee et al., 2011).

FOR20 deletion or knockdown in zebrafish reduced the number and length of cilia. Cilia in FOR20 morphants had impaired ciliary motility. When cilia length was reduced from an average 5.5 µm to an average 3 µm due to FOR20 depletion, about 80% cilia displayed consistently paralyzed or arrhythmically beating pattern (Xie et al., 2019).

Nasal brush biopsy samples from pediatric patients with primary ciliary dyskinesia were analyzed. The median CBF was 13.4 Hz for normal ciliated epithelia, 11.4 Hz for the ciliated edge with minor projections and 8.7 Hz for the ciliated edge with major projections (Thomas et al., 2009).

Time-scale

No data

Known modulating factors

Unknown

Known Feedforward/Feedback loops influencing this KER

Unknown

References

- Bottier, M., Thomas, K., Dutcher, S., and Bayly, P. (2018). (Preprint) How does cilium length affect beating? bioRxiv, 474346.
- Clary-Meinesz, C., Mouroux, J., Cosson, J., Huitorel, P., and Blaive, B. (1998). Influence of external pH on ciliary beat frequency in human bronchi and bronchioles. Eur. Respir. J. 11(2), 330-333.
- Ho, J.C., Chan, K.N., Hu, W.H., Lam, W.K., Zheng, L., Tipoe, G.L., et al. (2001). The Effect of Aging on Nasal Mucociliary Clearance, Beat Frequency, and Ultrastructure of Respiratory Cilia. Am. J. Respir. Crit. Care Med. 163(4), 983-988.
- Jayathilake, P.G., Le, D.V., Tan, Z., Lee, H.P., and Khoo, B.C. (2015). A numerical study of muco-ciliary transport under the condition of diseased cilia. Comput. Methods Biomech. Biomed. Engin. 18(9), 944-951.
- Jayathilake, P.G., Tan, Z., Le, D.V., Lee, H.P., and Khoo, B.C. (2012). Three-dimensional numerical simulations of human pulmonary cilia in the periciliary liquid layer by the immersed boundary method. Comput. Fluids 67, 130-137.
- Jing, J.C., Chen, J.J., Chou, L., Wong, B.J.F., and Chen, Z. (2017). Visualization and Detection of Ciliary Beating Pattern and Frequency in the Upper Airway using Phase Resolved Doppler Optical Coherence Tomography. Sci. Rep. 7(1), 8522-8522.
- Joki, S., Toskala, E., Saano, V., and Nuutinen, J. (1998). Correlation between ciliary beat frequency and the structure of ciliated epithelia in pathologic human nasal mucosa. Laryngoscope 108(3), 426-430.
- Kanthakumar, K., Taylor, G., Cundell, D., Dowling, R., Johnson, M., Cole, P., et al. (1996). The effect of bacterial toxins on levels of intracellular adenosine nucleotides and human ciliary beat frequency. Pulm. Pharmacol. 9(4), 223-230.
- Lee, W., Jayathilake, P., Tan, Z., Le, D., Lee, H., and Khoo, B. (2011). Muco-ciliary transport: effect of mucus viscosity, cilia beat frequency and cilia density. Comput. Fluids 49(1), 214-221.
- Li, Y.Y., Li, C.W., Chao, S.S., Yu, F.G., Yu, X.M., Liu, J., et al. (2014). Impairment of cilia architecture and ciliogenesis in hyperplastic nasal epithelium from nasal polyps. J. Allergy Clin. Immunol. 134(6), 1282-1292.
- Mall, M.A. (2008). Role of cilia, mucus, and airway surface liquid in mucociliary dysfunction: lessons from mouse models. J. Aerosol Med. Pulm. Drug Deliv. 21(1), 13-24.
- Smith, C.M., Djakow, J., Free, R.C., Djakow, P., Lonnen, R., Williams, G., et al. (2012). ciliaFA: a research tool for automated, high-throughput measurement of ciliary beat frequency using freely available software. Cilia 1(1), 14.
- Snyder, R.J., Hussain, S., Tucker, C.J., Randell, S.H., and Garantziotis, S. (2017). Impaired Ciliogenesis in differentiating human bronchial epithelia exposed to non-Cytotoxic doses of multi-walled carbon Nanotube. Part. Fibre Toxicol. 14(1), 1-14.
- Thomas, B., Rutman, A., and O'Callaghan, C. (2009). Disrupted ciliated epithelium shows slower ciliary beat frequency and increased dyskinesia. Eur. Respir. J. 34(2), 401-404.
- Toskala, E., Nuutinen, J., Rautiainen, M., and Torkkeli, T. (1995). The correlation of mucociliary transport and scanning electron microscopy of nasal mucosa. Acta Otolaryngol. 115(1), 61-65.
- Xie, S., Jin, J., Xu, Z., Huang, Y., Zhang, W., Zhao, L., et al. (2019). Centrosomal protein FOR20 is essential for cilia-dependent development in zebrafish embryos. FASEB J. 33(3), 3613-3622.

Relationship: 2449: Oxidative Stress leads to CFTR Function, Decreased

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Oxidative stress [MIE] Leading to Decreased Lung Function [AO]	adjacent	High	High

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	High	NCBI
Mus musculus	Mus musculus	Moderate	NCBI
Sus scrofa	Sus scrofa	Low	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Mixed	High

Available evidence indicates that this KER is applicab le to human, mouse and pig, independent of life stage and gender.

Key Event Relationship Description

Exposure to inhaled oxidants (such as cigarette smoke) leads to decreased CFTR gene and protein expression as well as CFTR

internalization, thereby reducing or abolishing open probabilities, short-circuit currents and subsequently ASL height/volume (Cantin et al., 2006a; Cantin et al., 2006b; Clunes et al., 2012; Rasmussen et al., 2014; Sloane et al., 2012). Decreased *CFTR* mRNA expression was previously linked to reduced mRNA stability following treatment with tert-butylhydroquinone (BHQ) (Cantin et al., 2006a) as well as increased intracellular Ca^{2+}, which is thought to activate protein kinases, thereby decreasing transcription rates (Bargon et al., 1992a; Bargon et al., 1992b; Rasmussen et al., 2014). Other evidence suggests that the STAT1 pathway is involved in CFTR down-regulation following ozone exposure or in the presence of interferon-γ (Kulka et al., 2005; Qu et al., 2009). In addition, transcriptional activation of an antioxidant response element in the CFTR promoter by Nrf2 was shown to regulate *CFTR* gene expression in airway epithelial cells under oxidative stress conditions, leading to an upregulation of transcript levels in the short-term, but a decline in the long-term (Zhang et al., 2015). On the post-transcriptional level, CFTR function was shown to be affected in multiple ways due to oxidative stress: For example, cell surface CFTR expression was drastically diminished in airway epithelial cells following cigarette smoke exposure, involving a change in protein solubility and trafficking to a perinuclear aggresome-like structure rather than to lysosomes or the proteasome (Bodas et al., 2017; Clunes et al., 2012). ATP depletion as a surrogate for lung ischemia also resulted in decreased CFTR expression at the plasma membrane. This was shown to be a consequence of irreversible actin filament depolymerization, which resulted in loss of cell polarity and relocalization of CFTR to the cytoplasm (Brézillon et al., 1997) .

Evidence Supporting this KER

Inducers of oxidative stress such as cigarette smoke reduced CFTR expression at both the RNA (Cantin et al., 2006a; Cantin et al., 2006b; Qu et al., 2009; Rennolds et al., 2010) and protein (Cantin et al., 2006b; Qu et al., 2009; Rennolds et al., 2010; Sloane et al., 2012; Hassan et al., 2014; Rasmussen et al., 2014; Xu et al., 2015) level in vitro. CFTR protein expression was lower in the airways of smokers compared to non-smokers (Dransfield et al., 2013). In some of these studies, an accompanying decrease in Cl– conductance was also observed (Qu et al., 2009; Rennolds et al., 2010; Sloane et al., 2012). There are many studies that support a direct link between oxidative stress and decreased CFTR function in vitro, ex vivo, in vivo and in human subjects. Human primary epithelial cells and cell lines of respiratory epithelial origin have consistently decreased conductance of Cl– and other ions following exposure to cigarette smoke and other oxidants (Cantin et al., 2006b; Schwarzer et al., 2008; Raju et al., 2013; Lambert et al., 2014; Schmid et al., 2015; Raju et al., 2016; Chinnapaiyan et al., 2018), which could be reversed upon antioxidant treatment (Raju et al., 2013; Lambert et al., 2014; Schmid et al., 2015). Similar observations were made under hypoxic conditions (Brézillon et al., 1997; Zhang et al., 2013; Woodworth, 2015). Antioxidants could also increase Cl– conductance and anion transport in the absence of oxidant treatment or hypoxia induction in human and murine respiratory cells in vitro and in ex vivo tissues (Azbell et al., 2010; Alexander et al., 2011; Conger et al., 2013). Healthy smokers and smokers with COPD have reduced Cl– conductance (Sloane et al., 2012; Dransfield et al., 2013) and increased sweat chloride concentrations (Raju et al., 2013; Courville et al., 2014).

Biological Plausibility

A link between CFTR and chronic bronchitis, which shares some of the features of cystic fibrosis lung disease, was proposed more than 20 years ago. Gene association studies, however, found no clear link between CFTR genotype and COPD except for hereditary disseminated bronchiectasis (Artlich et al., 1995; Joos et al., 2002), leading to the ion channel being of more interest in cystic fibrosis than chronic bronchitis research for some time. More recent evidence points toward a mechanism of acquired CFTR dysfunction in the context of cigarette smoking, which remains the leading risk factor for COPD. Multiple studies clearly demonstrated that CFTR Cl^- conductance is significantly inhibited following cigarette smoke exposure in vitro and in vivo, resulting in reductions of ASL height/volume which ultimately impair MCC (Cantin. et al., 2006b; Clunes et al., 2012; Courville et al., 2014; Dransfield et al., 2013; Hassan et al., 2014; Raju et al., 2013; Rasmussen et al., 2014; Sailland et al., 2017; Schmid et al., 2015). Although there appear to be different schools of thought as to how cigarette smoke modulates CFTR (directly or indirectly), the available evidence on the inhibitory effects of cigarette smoke on CFTR anion transport is conclusive. Considering the similarities between cigarette smoke exposure-related oxidative stress in the airways and oxidative stress arising from e.g. the exposure to other inhalation toxicants and pathogens, the described mechanisms are likely to apply to other stressors eliciting an imbalance in the lung's redox state. Moreover, studies with antioxidants such as NAC, resveratrol, genistein and hesperidin confirm the role of oxidative stress in modulating CFTR function (Alexander et al., 2011; Conger et al., 2013; Raju et al., 2013; Woodworth, 2015; Zhang et al., 2013). Taken together, the described cause-effect relationship is biologically plausible, and our confidence in biological plausibility is strong.

Empirical Evidence

CFTR transcript and protein levels were reduced in Calu-3 lung cancer cells exposed to the gas phase of cigarette smoke (Cantin et al., 2006b; Rasmussen et al., 2014), immortalized human bronchial epithelial 16HBE14o- cells treated with 10% cigarette smoke extract (Hassan et al., 2014; Xu et al., 2015), and differentiated primary human bronchial epithelial cells exposed to cigarette smoke extract (Sloane et al., 2012) and whole cigarette smoke (Hassan et al., 2014). Consequently, CFTR membrane localization was decreased as was channel function (i.e., cAMP-dependent ^{125}I efflux or CFTR Cl^- conductance).

Ozone exposure decreased CFTR mRNA and protein expression in immortalized human bronchial epithelial 16HBE14o- cells and in tracheas of Wistar rats, leading to a reduction in forskolin-stimulated CFTR Cl^- conductance (Qu et al., 2009).

Cadmium (Cd) decreased CFTR protein expression in Calu-3 lung cancer cells in a dose- and time-dependent manner, but only transiently affected *CFTR* gene expression. However, reduced CFTR expression at the plasma membrane was associated with a reduction in CFTR Cl^- conductance (Rennolds et al., 2010).

Acrolein exhibited a complex dose-dependent response with respect to CFTR-mediated Cl^- transport in primary murine nasal

septal epithelia: At 100 µM acrolein, Cl^- currents increased, whereas 300 µM acrolein reduced forskolin-induced total apical Cl^- secretion and 300 µM acrolein abolished all Cl^- transport. These effects were independent of cAMP, suggesting that channel activation was not PKA/cAMP phosphorylation-dependent (Alexander et al., 2012). Acute acrolein exposure also decreased cAMP-mediated CFTR ion transport in human bronchial epithelial cells grown in monolayers and in Calu-3 lung cancer cells, where the response was dose-dependent. Repeated, low-level exposure to acrolein (2.5 – 10 ng/mL for 7 days) had a similar effect on CFTR function and was shown to be unrelated to modulation of CFTR expression. However, pretreatment with the antioxidant N-acetylcysteine could prevent acrolein-induced CFTR inhibition (Raju et al., 2013).

Uncertainties and Inconsistencies

There is currently only limited knowledge about the mechanism by which oxidative stress may affect CFTR expression and function. At least one study indicates that CFTR channel gating is decreased following exposure to acrolein, possibly by protein carbonylation (Raju et al., 2013). Other studies reports that cell surface CFTR expression was drastically diminished in airway epithelial cells following cigarette smoke exposure, involving a change in protein solubility and trafficking to a perinuclear aggresome-like structure rather than to lysosomes or the proteasome (Bodas et al., 2017; Clunes et al., 2012b).

Quantitative Understanding of the Linkage

There is a ample quantitative evidence that provides an insight into this KER, with respect to both a response-response relationship and the temporal relationship between the two KEs across different test systems. Based on the evidence presented here, we judge our quantitative understanding to be high.

Response-response relationship

CFTR Gene & Protein Expression

In Calu-3 cells exposed to cigarette smoke (drawn into a syringe and injected into the 5-L exposure chamber at a rate of 35 mL/min) for 10 min every 2 h for a total of four exposures CFTR mRNA expression decreased by approx. half, from 0.87 ± 0.03 to 0.47 ± 0.03 units (CFTR/GAPDH ratio; northern blot). CFTR protein expression decreased from 2.07 ± 0.24 to 1.24 ± 0.14 units (CFTR/actin ratio; western blot) (Cantin et al., 2006b).

Exposure of fully differentiated primary human bronchial epithelial cells (HEBCs) to 30 puffs of whole smoke from 2 cigarettes (generated according to ISO standard) every day for 5 days (total exposure 120 hr) resulted in a ca. 40% reduction in CFTR expression. Exposure of 16HBE14o- airway epithelial cells to cigarette smoke extract (CSE; smoke from one non-filtered Camel cigarette was bubbled using a peristaltic pump apparatus into 10 mL of complete culture media, defined as 100%) led to a dose-dependent decrease in CFTR protein expression which, with approx. –70% from baseline, was significant for concentrations ≥ 10% (Hassan et al., 2014).

Apical treatment of fully differentiated primary HBECs with 2% CSE (not further described) for 24 h resulted in approx. 30% reduction in CFTR mRNA expression, 50% reduction in total CFTR protein and 30% reduction in CFTR protein cell surface expression (Sloane et al., 2012).

Treatment of 16HBE14o- immortalized HBECs with 10% CSE (smoke from one non-filtered Camel cigarette was bubbled using a peristaltic pump apparatus into 10 mL of complete culture media, defined as 100%) resulted in more than 50% reduction of CFTR total and cell surface protein expression. Co-treatment with 10 mM NAC prevented this decrease in CFTR expression (0.5 mM NAC was not effective) (Xu et al., 2015).

Treatment of fully differentiated primary HBECs with 2% CSE (bubbling 10 puffs of smoke from one 3R4F reference into 1 mL DMSO, at 2 s/10 mL puff, 10 puffs over 3 min; defined as 100%) for 24 h decreased total CFTR expression and cell surface CFTR expression by approx. 20 and 25%, respectively (Raju et al., 2016a).

CFTR protein expression was significantly lower in endobronchial biopsies of healthy smokers (33 [12-54] pack-years; CFTR/tubulin ratio: 0.70), smokers with COPD (52.5 [35-147] pack-years; CFTR/tubulin ratio: 0.50-0.88), and former smokers (45 [39-80] pack-years; CFTR/tubulin ratio: 1.75) with COPD than in healthy non-smokers (0 pack-years; CFTR/tubulin ratio: 4.09 – 4.49) (Dransfield et al., 2013).

Exposure of Calu-3 lung cancer cells to 50 µM cadmium sulfate for 1 or 3 days significantly reduced CFTR protein expression by approx. 20 and 50%, respectively, whereas exposure to lower doses of cadmium (2 µM) for 3 days did not affect CFTR protein levels (Rennolds et al., 2010).

At 4 hours post-exposure to 1.5 ppm ozone for 30 min, CFTR mRNA and protein expression were more than 2-fold decreased (CFTR/GAPDH ratio: 1.35±0.3 (control) vs 0.46±0.07; CFTR/actin ratio: 0.81±0.02 vs 0.33±0.02) in 16HBE14o- airway epithelial cells (Qu et al., 2009).

CFTR Channel Function

Treatment of T84 cells with 100 µM tert-butylhydroquinone (BHQ) for 6 h caused oxidative stress, evidenced by a significant increase in intracellular glutathione concentrations (44.8 ± 0.6 vs. 32.3 ± 0.3 nmol/10^6 cells at 0 hr) and reduced peak cAMP-dependent ^{125}I effluxes in a dose-dependent manner; however, only at BHQ concentrations ≥ 300 µM was the reduction significant (Cantin et al., 2006a).

In murine nasal septal epithelia grown at the air-liquid interface, resting Cl^- secretion was maximally stimulated by 100 µM acrolein (15.9±2.2 vs. 2.4±0.8 µA/cm^2 (control)), whereas forskolin-sensitive ion current was inhibited by 300 µM (13.3±1.2 vs. 19.9±1.0 µA/cm^2 (control); max. and significant) and completely eliminated by 500 µM acrolein (Alexander et al., 2012).

Following exposure of primary HBECs to 3.2 µg/mL acrolein for 24 h, forskolin-stimulated ion currents were inhibited by approx. 50%. Noticeable inhibition occurred at concentrations above 2 µg/ml, and maximum inhibition was seen with ca. 5 µg/mL acrolein; higher concentrations did not further decrease ion currents. Repeated exposure to 10 ng/mL acrolein for 7 days also resulted in an approx. 50% decrease in forskolin-stimulated ion currents (Raju et al., 2013).

Exposure of fully differentiated primary HBECs to whole smoke from four 3R4F reference cigarettes (generated according to ISO standard 3308; Vitrocell VC10 exposure system) resulted in a significant decrease in Cl^- currents (2.1±0.26 vs 4.8±0.71 µA/cm^2 vs 4.8 ± 0.71 µA/cm^2 (control)) (Schmid et al., 2015).

Resveratrol dose-dependently increased CFTR-mediated anion transport in murine sinonasal epithelial cells, with no effect seen for 50 µM, maximal increase (while not affecting total stimulation) seen for 100 µM, and slight inhibitory effects seen for concentrations ≥200 µM (Alexander et al., 2011; Woodworth, 2015). Forskolin-stimulated Cl^- currents increased in murine (14.2±1.5 vs. 0.8±0.2 mA/cm^2 (control)), human (17.4±.7 vs. 1.0±0.2 mA/cm^2 (control)(Woodworth, 2015), 15.69±2.66 vs. 2.49±0.98 mA/cm^2 (control) (Alexander et al., 2011)) and porcine (6.8±0.3 vs. 1.1±0.3 mA/cm^2 (control)) sinonasal epithelial cells following treatment with 100 µM resveratrol. Resveratrol treatment (100 µM) also restored CFTR Cl^- transport in hypoxic murine sinonasal epithelial cells (11.51±0.23 vs. 0.2±0.05 mA/cm^2 (control)) and human sinonasal epithelial cells (10.8±0.7 vs. 0.3±0.05 mA/cm^2 (control)) (Woodworth, 2015; Zhang et al., 2013).
In C57B/L6 mice perfused with 100 µM resveratrol, mean nasal potential difference polarization increased to –4.0±1.87 mV (vs. –0.93±1.69 mV, control), which was slightly higher than results with forskolin (–1.65±1.78 mV), but not significant (Alexander et al., 2011).

Genistein (50 µM) enhanced basal CFTR-mediated anion transport in human sinonasal epithelial cells (23.1±1.8 vs. 0.7±0.2 µA/cm^2 (control)), but had no effect on forskolin-sensitive current (Conger et al., 2013).

Hesperidine dose-dependently increased CFTR-mediated anion transport in murine sinonasal epithelial cells, with the maximum responses seen for 2 mM (16.67±0.43 µA/cm^2) which was not further investigated due to the precipitation of hesperidine. Instead, 1 mM hesperidine was used as it also significantly increased CFTR Cl^- currents (mouse cells: 13.51±0.77 vs. 4.4±0.66 µA/cm^2 (control); human cells: 12.28±1.08 vs. 0.69±0.32 µA/cm^2 (control)). In C57B/L6 mice perfused with 1 mM hesperidine, mean nasal potential difference polarization was increased (–2.3±1.0 mV vs. –0.8±0.8 mV (control)), similar to results with forskolin (–1.9±1.4 mV) (Azbell et al., 2010).

Basal lower airway potential difference (LAPD) was lower in healthy smokers (-7.71±0.88 mV; 33 [12-54] pack-years) and COPD smokers (-7.33±1.30 mV; 52.5 [32-147] pack-years) than in former smokers with COPD (no data; 45 [39-80] pack-years) or healthy non-smokers (-12.61±1.94 mV)(Dransfield et al., 2013).

Healthy smokers, current and former smokers with COPD had lower mean sweat chloride than healthy non-smokers (51.3±4.4 [31.08±14 pack-years], 41.9±3.4 [38±19 pack-years], and 39.0±5.4 [44±19 pack-years], respectively, vs. 53.6±3.3 mmol/L). The association between sweat chloride and pack-years was significant (Courville et al., 2014).

Healthy smokers and smokers with COPD exhibited significantly lower nasal potential difference than healthy non-smokers in response to isoproterenol (-6.3±1.4 [33.2; 10-78 pack-years] and -8.0±2.0 [55.1; 35-78 pack-years], respectively, vs. -15.2±2.7 mV (control)) (Sloane et al., 2012).

Treatment of wild-type CFTR expressing HEK293 cells with 1% CSE (bubbling 10 puffs of smoke from one 3R4F reference into 1 mL DMSO, at 2 s/10 mL puff, 10 puffs over 3 min; defined as 100%) decreased CFTR channel-open probability by 59% (Raju et al., 2016a).

Single channel recordings in apical membrane patches of murine sinonasal epithelial cells demonstrated that 100 µM resveratrol significantly enhanced channel-open probability (0.329± 0.116 vs. 0.119±0.059 NPo/N (control)) (Woodworth, 2015).

Time-scale

In T84 cells exposed to 100 µM BHQ, intracellular GSH levels were significantly increased from 6 hours onwards (from 32.3±0.3 to 44.8±0.6 nmol/10^6 cells), up to 24 hours (from 45.5±0.9 to 94.9±2.5 nmol/10^6 cells), whereas CFTR mRNA expression was significantly decreased over time (from 8.2±0.8 to 1.8±0.3 CFTR/GAPDH mRNA ratio at 6 hours). As a consequence, CFTR protein expression was significantly decreased following treatment of T84 cells with 100 µM BHQ for 24 hours (from 101.3±4.6 to 84.7±4.1 CFTR/actin protein, density as % control) (Cantin et al., 2006a).

Exposure of T84 cells to cigarette smoke condensate (CSC; prepared by drawing 35 mL/min cigarette smoke for 5 min into a syringe and injecting this smoke into a tonometer containing 10 mL culture media; defined as 100%) for 10 min resulted in a significant increase in GCLC mRNA expression (from 0.69±0.08 to 2.44± 0.25 GCLC/GAPDH mRNA ratio) and decrease in CFTR mRNA expression (from 0.57±0.04 to 0.21±0.02 CFTR/GAPDH mRNA ratio) at 6 hours post-exposure and a significant increase in GSH at 24 hours post-exposure (from 41.7±1.0 to 89.8±1.5 nmol/mg protein) (Cantin et al., 2006b).

Exposure of immortalized 16HBE14o- airway epithelial cells to 10% cigarette smoke extract (CSE; cigarette smoke from one non-

filtered cigarette was bubbled using a peristaltic pump apparatus into 10 mL of complete culture media; defined as 100%) led to an approx. 50% decrease in CFTR mRNA expression and approx. 70% decrease in CFTR protein expression after 24 hours, with little change at later time points (Hassan et al., 2014).

Treatment of Calu-3 lung cancer cells with 50 µM cadmium sulfate significantly reduced CFTR protein expression after 1 day, with maximum decrease observed after 3 days (54 ± 5% of control). Exposure to lower doses of cadmium (2 µM) required 5-day treatment before CFTR protein levels were affected (Rennolds et al., 2010).

Treatment of fully differentiated primary HBECs with 2% CSE (bubbling 10 puffs of smoke from one 3R4F reference into 1 mL DMSO, at 2 s/10 mL puff, 10 puffs over 3 minutes; defined as 100%) for 24 hours decreased total CFTR expression and cell surface CFTR expression by approx. 20 and 25%, respectively, but treatment for 20 minutes did not. A 50% reduction in CFTR channel activity occurred immediately after addition of CSE and lasted for at least 20 minutes (Raju et al., 2016a).

Calu-3 lung cancer cells exposed to cigarette smoke (drawn into a syringe and injected into the 5-L exposure chamber at a rate of 35 mL/min) for 10 min every 2 h for a total of four exposures were loaded with ^{125}I for 1 hour, prior to stimulation with isobutyl methylxanthine, forskolin, and dibutyryl cAMP. cAMP-dependent anion efflux was significantly decreased compared to controls within 3 min of stimulation and remained significantly decreased for a further 3 min (Cantin et al., 2006b).

In untreated monolayers of CFTR-corrected CFBE41o- airway epithelial cells, cAMP-stimulated Cl^- currents remained at stimulated levels for 2 to 3 h, whereas currents were inhibited by 86.0±5.8 and 40.0±2.7% in cells treated with 100 µM pyocyanin or 100 µM H_2O_2, respectively, in the same time period. The effect of pyocyanin occurred at a faster rate than that of H_2O_2; washout of the compounds partly restored cAMP-stimulated Cl^- currents (Schwarzer et al., 2008).

Exposure of fully differentiated primary HBECs to whole smoke (3R4F reference cigarette; inExpose exposure system) resulted in a time-dependent decrease in CFTR short-circuit currents, with significant differences (approx. 15% reduction) from control after 10 min of exposure. Maximal reduction (ca. 50%) was seen after 30 min of exposure (Lambert et al., 2014).

Culture of fully differentiated primary human and murine sinonasal epithelial cells (HSNECs; MSNECs) in 1% O_2 for 12 or 24 h significantly reduced CFTR-mediated (forskolin-sensitive) Cl^- current: HSNECs, 19.55±0.56 mA/cm^2 (12 h); 17.67±1.13 mA/cm^2 (24 h) vs. 25.49±1.48 mA/cm^2 (control); MSNECs, 13.55±0.46 mA/cm^2 (12 h); 12.75±0.07 mA/cm^2 (24 h) vs. 19.23±0.18 mA/cm^2 (control). Transfer of cultures to physiologic O_2 conditions (21%) restored CFTR ion currents (HSNECs, 25.12±1.24 mA/cm^2) after 24 h (Woodworth, 2015).

Known modulating factors

CFTR has also been implicated in transmembrane glutathione transport (Linsdell and Hanrahan, 1998; Roum et al., 1993). Multiple studies suggest that oxidative injury of the lungs, e.g. following inhalation exposures or infections, can be effectively counteracted, if not prevented, by CFTR-mediated elevations of ASL glutathione levels (Day et al., 2004; Gould et al., 2010; Jungas et al., 2002; Kariya et al., 2007; Velsor et al., 2001). The antioxidant properties of glutathione may temporarily delay the acquisition of CFTR dysfunction by neutralizing reactive oxygen species that would otherwise contribute to downregulation of gene and protein expression.

Known Feedforward/Feedback loops influencing this KER

Not known

References

- Alexander, N.S., Hatch, N., Zhang, S., Skinner, D., Fortenberry, J., Sorscher, E.J., et al. (2011). Resveratrol has Salutary Effects on Mucociliary Transport and Inflammation in Sinonasal Epithelium. Laryngoscope 121(6), 1313-1319. doi: 10.1002/lary.21798.
- Alexander, N.S., Blount, A., Zhang, S., Skinner, D., Hicks, S.B., Chestnut, M., et al. (2012). Cystic fibrosis transmembrane conductance regulator modulation by the tobacco smoke toxin acrolein. Laryngoscope 122(6), 1193-1197.
- Azbell, C., Zhang, S., Skinner, D., Fortenberry, J., Sorscher, E.J., and Woodworth, B.A. (2010). Hesperidin stimulates CFTR-mediated chloride secretion and ciliary beat frequency in sinonasal epithelium. Otolaryngol. Head Neck Surg. 143(3), 397.
- Bargon, J., Trapnell, B., Yoshimura, K., Dalemans, W., Pavirani, A., Lecocq, J., et al. (1992). Expression of the cystic fibrosis transmembrane conductance regulator gene can be regulated by protein kinase C. J. Biol. Chem. 267(23), 16056-16060.
- Bargon, J., Trapnell, B.C., Chu, C.-S., Rosenthal, E.R., Yoshimura, K., Guggino, W.B., et al. (1992). Down-regulation of cystic fibrosis transmembrane conductance regulator gene expression by agents that modulate intracellular divalent cations. Mol. Cell. Biol. 12(4), 1872-1878.
- Bodas, M., Silverberg, D., Walworth, K., Brucia, K., and Vij, N. (2017). Augmentation of S-Nitrosoglutathione Controls Cigarette Smoke-Induced Inflammatory–Oxidative Stress and Chronic Obstructive Pulmonary Disease-Emphysema Pathogenesis by Restoring Cystic Fibrosis Transmembrane Conductance Regulator Function. Antioxid. Redox Signal. 27(7), 433-451.
- Brézillon, S., Zahm, J.-M., Pierrot, D., Gaillard, D., Hinnrasky, J., Millart, H., et al. (1997). ATP Depletion Induces a Loss of Respiratory Epithelium Functional Integrity and Down-regulates CFTR (Cystic Fibrosis Transmembrane Conductance Regulator) Expression. J. Biol. Chem. 272(44), 27830-27838. doi: 10.1074/jbc.272.44.27830.

- Cantin, A.M., Bilodeau, G., Ouellet, C., Liao, J., and Hanrahan, J.W. (2006a). Oxidant stress suppresses CFTR expression. Am. J. Physiol. Cell Physiol. 290(1), C262-270. doi: 10.1152/ajpcell.00070.2005.
- Cantin, A.M., Hanrahan, J.W., Bilodeau, G., Ellis, L., Dupuis, A., Liao, J., et al. (2006b). Cystic Fibrosis Transmembrane Conductance Regulator Function Is Suppressed in Cigarette Smokers. Am. J. Respir. Crit. Care Med. 173(10), 1139-1144. doi: 10.1164/rccm.200508-1330OC.
- Clunes, L.A., Davies, C.M., Coakley, R.D., Aleksandrov, A.A., Henderson, A.G., Zeman, K.L., et al. (2012). Cigarette smoke exposure induces CFTR internalization and insolubility, leading to airway surface liquid dehydration. FASEB J. 26(2), 533-545. doi: 10.1096/fj.11-192377.
- Conger, B.T., Zhang, S., Skinner, D., Hicks, S.B., Sorscher, E.J., Rowe, S.M., et al. (2013). Comparison of cystic fibrosis transmembrane conductance regulator (CFTR) and ciliary beat frequency activation by the CFTR modulators genistein, VRT-532, and UCCF-152 in primary sinonasal epithelial cultures. JAMA Otolaryngol. Head Neck Surg. 139(8), 822-827.
- Courville, C.A., Tidwell, S., Liu, B., Accurso, F.J., Dransfield, M.T., and Rowe, S.M. (2014). Acquired defects in CFTR-dependent β-adrenergic sweat secretion in chronic obstructive pulmonary disease. Respir. Res. 15(1), 25.
- Day, B.J., van Heeckeren, A.M., Min, E., and Velsor, L.W. (2004). Role for Cystic Fibrosis Transmembrane Conductance Regulator Protein in a Glutathione Response to Bronchopulmonary Pseudomonas Infection. Infection and Immunity 72(4), 2045-2051. doi: 10.1128/iai.72.4.2045-2051.2004.
- Dransfield, M.T., Wilhelm, A.M., Flanagan, B., Courville, C., Tidwell, S.L., Raju, S.V., et al. (2013). Acquired Cystic Fibrosis Transmembrane Conductance Regulator Dysfunction in the Lower Airways in COPD. Chest 144(2), 498-506. doi: 10.1378/chest.13-0274.
- Gould, N.S., Gauthier, S., Kariya, C.T., Min, E., Huang, J., and Brian, D.J. (2010). Hypertonic saline increases lung epithelial lining fluid glutathione and thiocyanate: two protective CFTR-dependent thiols against oxidative injury. Respiratory research 11(1), 119-119. doi: 10.1186/1465-9921-11-119.
- Hassan, F., Xu, X., Nuovo, G., Killilea, D.W., Tyrrell, J., Da Tan, C., et al. (2014). Accumulation of metals in GOLD4 COPD lungs is associated with decreased CFTR levels. Respir. Res. 15(1), 69.
- Joos, L., Pare, P.D., and Sandford, A.J. (2002). Genetic risk factors of chronic obstructive pulmonary disease. Swiss medical weekly 132(3-4), 27-37.
- Jungas, T., Motta, I., Duffieux, F., Fanen, P., Stoven, V., and Ojcius, D.M. (2002). Glutathione levels and BAX activation during apoptosis due to oxidative stress in cells expressing wild-type and mutant cystic fibrosis transmembrane conductance regulator. Journal of Biological Chemistry 277(31), 27912-27918.
- Kariya, C., Leitner, H., Min, E., van Heeckeren, C., van Heeckeren, A., and Day, B.J. (2007). A role for CFTR in the elevation of glutathione levels in the lung by oral glutathione administration. American journal of physiology. Lung cellular and molecular physiology 292(6), L1590-L1597. doi: 10.1152/ajplung.00365.2006.
- Kulka, M., Dery, R., Nahirney, D., Duszyk, M., and Befus, A.D. (2005). Differential regulation of cystic fibrosis transmembrane conductance regulator by interferon γ in mast cells and epithelial cells. J. Pharmacol. Exp. Ther. 315(2), 563-570.
- Linsdell, P., and Hanrahan, J.W. (1998). Glutathione permeability of CFTR. The American journal of physiology 275(1 Pt 1), C323-326.
- Qu, F., Qin, X.-Q., Cui, Y.-R., Xiang, Y., Tan, Y.-R., Liu, H.-J., et al. (2009). Ozone stress down-regulates the expression of cystic fibrosis transmembrane conductance regulator in human bronchial epithelial cells. Chem.-Biol. Interact. 179(2–3), 219-226. doi: 10.1016/j.cbi.2008.10.059.
- Raju, S.V., Jackson, P.L., Courville, C.A., McNicholas, C.M., Sloane, P.A., Sabbatini, G., et al. (2013). Cigarette smoke induces systemic defects in cystic fibrosis transmembrane conductance regulator function. Am. J. Respir. Crit. Care Med. 188(11), 1321-1330.
- Rasmussen, J.E., Sheridan, J.T., Polk, W., Davies, C.M., and Tarran, R. (2014). Cigarette smoke-induced Ca2+ release leads to cystic fibrosis transmembrane conductance regulator (CFTR) dysfunction. J. Biol. Chem. 289(11), 7671-7681. doi: 10.1074/jbc.M113.545137.
- Rennolds, J., Butler, S., Maloney, K., Boyaka, P.N., Davis, I.C., Knoell, D.L., et al. (2010). Cadmium Regulates the Expression of the CFTR Chloride Channel in Human Airway Epithelial Cells. Toxicol. Sci. 116(1), 349-358. doi: 10.1093/toxsci/kfq101.
- Sailland, J., Grosche, A., Baumlin, N., Dennis, J.S., Schmid, A., Krick, S., et al. (2017). Role of Smad3 and p38 Signalling in Cigarette Smoke-induced CFTR and BK dysfunction in Primary Human Bronchial Airway Epithelial Cells. Scientific Reports 7, 10506. doi: 10.1038/s41598-017-11038-x.
- Schmid, A., Baumlin, N., Ivonnet, P., Dennis, J.S., Campos, M., Krick, S., et al. (2015). Roflumilast partially reverses smoke-induced mucociliary dysfunction. Respir. Res. 16(1), 135. doi: 10.1186/s12931-015-0294-3.
- Schwarzer, C., Fischer, H., Kim, E.-J., Barber, K.J., Mills, A.D., Kurth, M.J., et al. (2008). Oxidative stress caused by pyocyanin impairs CFTR Cl– transport in human bronchial epithelial cells. Free Radical Biology and Medicine 45(12), 1653-1662. doi: http://dx.doi.org/10.1016/j.freeradbiomed.2008.09.011.
- Sloane, P.A., Shastry, S., Wilhelm, A., Courville, C., Tang, L.P., Backer, K., et al. (2012). A pharmacologic approach to acquired cystic fibrosis transmembrane conductance regulator dysfunction in smoking related lung disease. PloS one 7(6), e39809. doi: 10.1371/journal.pone.0039809.
- Velsor, L.W., van Heeckeren, A., and Day, B.J. (2001). Antioxidant imbalance in the lungs of cystic fibrosis transmembrane conductance regulator protein mutant mice. American Journal of Physiology-Lung Cellular and Molecular Physiology 281(1), L31-L38.
- Woodworth, B.A. (2015). Resveratrol ameliorates abnormalities of fluid and electrolyte secretion in a hypoxia-Induced model of acquired CFTR deficiency. Laryngoscope 125(S7), S1-S13.
- Xu, X., Balsiger, R., Tyrrell, J., Boyaka, P.N., Tarran, R., and Cormet-Boyaka, E. (2015). Cigarette smoke exposure reveals a novel role for the MEK/ERK1/2 MAPK pathway in regulation of CFTR. Biochim. Biophys. Acta 1850(6), 1224-1232. doi: 10.1016/j.bbagen.2015.02.004.
- Zhang, Z., Leir, S.-H., and Harris, A. (2015). Oxidative Stress Regulates CFTR Gene Expression in Human Airway Epithelial Cells through a Distal Antioxidant Response Element. Am. J. Respir. Cell Mol. Biol. 52(3), 387-396. doi: 10.1165/rcmb.2014-

0263OC.

Relationship: 2450: Oxidative Stress leads to FOXJ1 Protein, Decreased

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Oxidative stress [MIE] Leading to Decreased Lung Function [AO]	adjacent	Moderate	Moderate

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	High	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	

Sex Applicability

Sex	Evidence
Mixed	

Key Event Relationship Description

Oxidative stress (such as that caused by cigarette smoke exposure or irradiation) leads to decreased forkhead box J1 (FOXJ1) gene and protein expression, as well as to decreased FOXJ1 target gene expression (Brekman et al., 2014; Garcia-Arcos et al., 2016; Ishikawa and Ito, 2017; Milara et al., 2012; Valencia-Gattas et al., 2016). FOXJ1 is a key factor of multiple motile cilia assembly in the respiratory airways (Zhou and Roy, 2015). Thus oxidative stress blocks the multiple ciliogenesis program in the airway epithelium.

Evidence Supporting this KER

Cigarette smoke-induced oxidative stress downregulates FOXJ1 expression at both the gene and protein levels in human lung cells in vitro (Milara et al., 2012; Brekman et al., 2014; Valencia-Gattas et al., 2016; Ishikawa and Ito, 2017). Oxidative stress induced by human respiratory syncytial virus reduces FOXJ1 mRNA levels, which can be restored by treatment with antioxidants or the phosphodiesterase 4 inhibitor roflumilast N-oxide (Akaike et al., 1990; Geiler et al., 2010; Mata et al., 2012). In mice, thoracic irradiation results in free radical generation and subsequent reduction in FOXJ1 mRNA expression (Bernard et al., 2012). Many genes that are transcriptionally regulated by FOXJ1 are also downregulated following exposure to cigarette smoke, which implies a reduction in FOXJ1 transcriptional activity (Brekman et al., 2014).

Biological Plausibility

The negative association between cigarette smoke exposure and FOXJ1 levels in airways was shown in multiple studies and can be estimated as a strong linkage. Yet, the notion that oxidative stress as a result of cigarette smoke exposure is leading to decreased FOXJ1 levels is not well demonstrated. As a complex mixture of thousands of chemicals, cigarette smoke exposure could lead to reduced FOXJ1 levels via different routes. Indirect evidence, such as antioxidant molecules that restore cigarette smoke exposure-reduced FOXJ1 levels, as well evidences from other oxidative stress generating insults that decrease FOXJ1 levels add confidence to this KER. However, studies showing a link between oxidative stress generating agents and reduced FOXJ1 levels are scarce. Collectively, the empirical evidence and uncertainties of the linkage imply a moderate ranking for the KER.

Empirical Evidence

Whole cigarette smoke exposed normal human bronchial epithelial cells (HBECs) had significantly lower FoxJ1 protein levels than air treated controls (measured by immunofluorescence). In addition, FOXJ1 mRNA levels were reduced in whole smoke-exposed differentiating and differentiated cells (TaqMan quantitative RT-PCR). (Valencia-Gattas et al., 2016).

Treatment with cigarette smoke extract (CSE) significantly down-regulated FOXJ1 mRNA and protein levels in differentiating human airway basal cells in air–liquid interface (ALI) cultures (TaqMan quantitative RT-PCR, Western analysis) (Brekman et al., 2014).

Treatment with cigarette smoke extract (CSE) provokes a reduction of FOXJ1 gene and protein expression in differentiated human

bronchial epithelial cells (HBECs) through IL13-mediated mechanism (RT-qPCR, Western analysis) (Milara et al., 2012). Treatment with roflumilast N-oxide (which reduced intracellular reactive oxygen species levels) prevents FOXJ1 loss in CSE treated cells (Milara et al., 2012).

Exposure of 3D co-cultures with HBECs and fibroblasts to whole cigarette smoke decreased FOXJ1 gene expression in a concentration-dependent manner (TaqMan quantitative RT-PCR) (Ishikawa Shinkichi and Ito, 2017).

Human respiratory syncytial virus (RSV) infections involve reactive oxygen intermediates (ROIs) that cause cellular damage (Akaike et al., 1990; Mata et al., 2012). Treatment with the free radical scavenger N-acetylcysteine (NAC) reduces the RSV inflammatory response (Geiler et al., 2010). RSV infection reduced FOXJ1 gene expression (RT-PCR), which was restored in a dose-dependent manner by NAC treatment (Mata et al., 2012). In another study FOXJ1 mRNA levels were consistently low after RSV infection and were restored with Roflumilast N-oxide (Mata et al., 2013).

Thoracic irradiation reduces FOXJ1 mRNA levels in mouse lungs (Bernard et al., 2012). Irradiation causes excessive levels of free radicals and associated lipid peroxidation, damage to DNA, proteins, leading to wide-spread cellular damage (Azzam et al., 2012; Koc et al., 2003; Rodrigues-Moreira et al., 2017; Shirazi et al., 2013).

The expression of cilia-related transcription factor genes, including FOXJ1, RFX2, and RFX3 was significantly down-regulated by CSE treatment. The expression of cilia motility and structural integrity genes, including DNAI1, DNAH5, DNAH9, DNAH10, DNAH11, and SPAG6 was also significantly down-regulated by CSE treatments (Brekman et al., 2014). Many of these genes (RFX2, RFX3, DNAI1, DNAH9, DNAH11, SPAG6) are transcriptionally regulated by FOXJ1 (Causal biological network database, 2019). The downregulation of FOXJ1-controlled genes infer reduced FOXJ1 transcription factor activity. Indeed, overexpression of FOXJ1 led to partial restoration of CSE treatment-induced downregulation of cilia-related genes (Brekman et al., 2014).

Uncertainties and Inconsistencies

Schamberger et al. did not find any alterations in FOXJ1 mRNA levels or FOXJ1 target gene (DNAI1, DNALI1, SPAG6, TEKT1) transcription upon 2.5% or 5% CSE exposure of HBECs for 28 days. However, in this study, cigarette smoke exposure reduced ciliated cell numbers (Schamberger et al., 2015).

The evidences listed suggest several mechanisms on how oxidative stress could lead to decreased FOXJ1 levels, including EGFR-, MCIDAS- or IL-13-mediated mechanisms. Most of the studies, however, do not corroborate on how oxidative stress mechanistically leads to reduced FOXJ1 levels. Since there are several other factors (GMNC, NOTCH, ULK4 etc.) known to regulate FOXJ1 levels, further pathways might be involved in passing the oxidative stress signal to FOXJ1.

Quantitative Understanding of the Linkage

High oxidative stress causes reduction in FOXJ1 levels measured at 24 h and for up to 15 days after exposure to an oxidative stress-causing agent. The data on cigarette smoke-reduced FOXJ1 levels are convincing. Indirect evidence such as antioxidants restoring CS-reduced FOXJ1 levels suggest that oxidative stress plays a major role in the CS-induced effects (Milara et al., 2012). However, given the complexity of the CS mixtures (Baumung et al., 2016), we cannot exclude that factors other than oxidative stress are involved in FOXJ1 level reduction. Other sources of oxidative stress such as RSV infection or GY radiation reduce FOXJ1 levels to similar degree as CS-caused FOXJ1 reduction. Based on the available evidence, we classify the quantitative understanding of this KER as moderate.

Response-response relationship

Normal HBECs were exposed to whole cigarette smoke from 3R4F research grade cigarettes using the Vitrocell® VC 10® Smoking Robot (35-mL puff volume, 2 s duration and 1 min between puffs or air as a control). For differentiated cells, treatment was done every 2 d for 5 d (3 exposures) and samples were collected 48 h after treatment. Differentiating cells were exposed 3 times per week to smoke from 1 cigarette, and samples were collected after 14, 21 and 27 days. FOXJ1 protein and mRNA level changes were decreased 2.5-fold in differentiated and 2-fold in differentiating NHBE cells. There was a significantly lower percentage of FoxJ1 positive cells in the WCS exposed cells at 27 d of differentiation (4.3 +/- 4.2% vs. 13.0 +/- 7.3%, air) (Valencia-Gattas et al., 2016).

CSE was obtained from one Marlboro Red commercial cigarette bubbled in 12.5 mL of differentiation medium that was then filtered (0.2-µm pore filter). The absorbance was measured at 320 nm on a spectrophotometer, and the optical density of 1 was defined as 100%. HBECs were differentiated at the air-liquid interface while being exposed to 0, 3, and 6% CSE between days 5 and 28. 3% and 6% CSE treatment reduced FOXJ1 mRNA levels to approx. 65% and 55% of control levels, respectively. Treatment of differentiating cultures with 3% CSE reduced FOXJ1 protein levels by 2-fold by day 28 (Brekman et al., 2014).

The smoke of one 2R4F research cigarette was bubbled into a flask containing 25 mL of pre-warmed (37°C) differentiation medium using a respiratory pump model (Harvard Apparatus Rodent Respirator 680, Harvard Apparatus, Holliston, MA, USA) that generates three puffs min^{-1}; 35 mL per each puff of 2 s duration with a volume of 0.5 cm above the filter. The solution was then filtered (0.22 µm pore size) to remove particles and the tar phase. The resulting sterile solution was defined as 100% CSE and used within 30 min of preparation. Treatment of differentiated human bronchial epithelial cells with 10% CSE decreased FOXJ1 expression by about 40% at 24 h and 70% at 72 h exposure (Milara et al., 2012).

3R4F reference cigarettes were smoked in accordance with the ISO smoking protocol (35-mL puffs of 2 s each minute). Whole CS,

generated by a VC10 smoking robot, was released into a mixing device in 2.8-s exhaust and diluted with humidified clean air at 1.0 L/min dilution flow. Diluted smoke was introduced into the CULTEX RFS module and guided into the exposure chamber (5 mL/min) using a vacuum pump. FOXJ1 mRNA levels were reduced to 60% and 40% of the control after exposure to CS from 1 or 4 cigarettes, respectively (Ishikawa and Ito, 2017).

RSV infection elicits ROI-mediated effects manifested by changes in the expression of NRF2 and HMOX-1 genes (approx. 6- and 9-fold increase at day 15 post-RSV infection, respectively), H_2O_2 generation (7-fold increase in intracellular levels at day 15 post-infection) and severe reduction in total antioxidant capacity. These data together indicate the presence of oxidative stress following infection which leads to decreased FOXJ1 mRNA levels (ca. 25% of control at 15 days post-RSV infection (Mata et al., 2012) and ca. 45% of control at 10 days after RSV infection (Mata et al., 2013).

FOXJ1 mRNA levels were reduced by 50% in murine lungs 14 days after thoracic irradiation at 15 Gy (Bernard et al., 2012).

Time-scale

Whole cigarette smoke exposure from one 3R4F research grade cigarette using the Vitrocell® VC 10® Smoking Robot (35-mL puff volume, 2 s duration and 1 min between puffs or air as a control) once a day on alternate days for 5 days decreased FOXJ1 mRNA levels by 2.5-fold in differentiated HBECs (Valencia-Gattas et al., 2016).

Whole cigarette smoke exposure from one 3R4F research grade cigarette using the Vitrocell® VC 10® Smoking Robot (35-mL puff volume, 2 s duration and 1 min between puffs or air as a control) for 3 times per week for 4 weeks (27 days) decreased FOXJ1 mRNA levels by 2-fold in differentiating HBECs (Valencia-Gattas et al., 2016).

Treatment of differentiating HBECs between days 5 and 28 with 3% CSE reduced FOXJ1 protein levels by 2-fold by day 28. Treatment of differentiating HBECs between days 5 and 28 with 6% CSE reduced FOXJ1 protein levels by approx. 55% by day 28 (Brekman et al., 2014).

Treatment of differentiated human bronchial epithelial cells with 10% CSE decreased FOXJ1 expression by about 40% at 24 h and 70% at 72 h (Milara et al., 2012).

Repeated exposure of 3D bronchial epithelial cultures to whole smoke of 4 cigarettes (every other day, treatment started on ALI culture day 7) resulted in 2.5-fold decrease of FOXJ1 mRNA levels by day 21 (Ishikawa and Ito, 2017).

At 15 days post-RSV infection, FOXJ1 mRNA levels were four-fold reduced compared to untreated samples (Mata et al., 2012). In another study from the same research group, FOXJ1 mRNA levels were reduced to 45% of the FOXJ1 levels in uninfected sample 10 days post-RSV infection (Mata et al., 2013).

At 7 days and 14 day after 15 GY thoracic irradiation, FOXJ1 mRNA levels were reduced to approx. 70% and 50% of controls, respectively (Bernard et al., 2012).

Known modulating factors

Unknown

Known Feedforward/Feedback loops influencing this KER

Unknown

References

- Akaike, T., Ando, M., Oda, T., Doi, T., Ijiri, S., Araki, S., et al. (1990). Dependence on O2- generation by xanthine oxidase of pathogenesis of influenza virus infection in mice. J. Clin. Investig. 85(3), 739-745.
- Azzam, E.I., Jay-Gerin, J.P., and Pain, D. (2012). Ionizing radiation-induced metabolic oxidative stress and prolonged cell injury. Cancer Lett 327(1-2), 48-60.
- Baumung, C., Rehm, J., Franke, H., and Lachenmeier, D.W. (2016). Comparative risk assessment of tobacco smoke constituents using the margin of exposure approach: the neglected contribution of nicotine. Sci Rep 6, 35577.
- Bernard, M.E., Kim, H., Rajagopalan, M.S., Stone, B., Salimi, U., Rwigema, J.C., et al. (2012). Repopulation of the irradiation damaged lung with bone marrow-derived cells. In Vivo 26(1), 9-18.
- Brekman, A., Walters, M.S., Tilley, A.E., and Crystal, R.G. (2014). FOXJ1 prevents cilia growth inhibition by cigarette smoke in human airway epithelium in vitro. Am. J. Respir. Cell Mol. Biol. 51(5), 688-700.
- Garcia-Arcos, I., Geraghty, P., Baumlin, N., Campos, M., Dabo, A.J., Jundi, B., et al. (2016). Chronic electronic cigarette exposure in mice induces features of COPD in a nicotine-dependent manner. Thorax 71(12), 1119-1129.
- Ishikawa, S., and Ito, S. (2017). Repeated whole cigarette smoke exposure alters cell differentiation and augments secretion of inflammatory mediators in air-liquid interface three-dimensional co-culture model of human bronchial tissue. Toxicol. In Vitro 38, 170-178.
- Koc, M., Taysi, S., Buyukokuroglu, M.E., and Bakan, N. (2003). Melatonin protects rat liver against irradiation-induced oxidative injury. J Radiat Res 44(3), 211-215.
- Mata, M., Martinez, I., Melero, J.A., Tenor, H., and Cortijo, J. (2013). Roflumilast inhibits respiratory syncytial virus infection in human differentiated bronchial epithelial cells. PLoS One 8(7), e69670.
- Mata, M., Sarrion, I., Armengot, M., Carda, C., Martinez, I., Melero, J.A., et al. (2012). Respiratory syncytial virus inhibits

ciliagenesis in differentiated normal human bronchial epithelial cells: effectiveness of N-acetylcysteine. PloS one 7(10), e48037.
- Milara, J., Armengot, M., Bañuls, P., Tenor, H., Beume, R., Artigues, E., et al. (2012). Roflumilast N-oxide, a PDE4 inhibitor, improves cilia motility and ciliated human bronchial epithelial cells compromised by cigarette smoke in vitro. Br. J. Pharmacol. 166(8), 2243-2262.
- Rodrigues-Moreira, S., Moreno, S.G., Ghinatti, G., Lewandowski, D., Hoffschir, F., Ferri, F., et al. (2017). Low-Dose Irradiation Promotes Persistent Oxidative Stress and Decreases Self-Renewal in Hematopoietic Stem Cells. Cell Rep 20(13), 3199-3211.
- Schamberger, A.C., Staab-Weijnitz, C.A., Mise-Racek, N., and Eickelberg, O. (2015). Cigarette smoke alters primary human bronchial epithelial cell differentiation at the air-liquid interface. Scientific reports 5, 8163.
- Shirazi, A., Mihandoost, E., Ghobadi, G., Mohseni, M., and Ghazi-Khansari, M. (2013). Evaluation of radio-protective effect of melatonin on whole body irradiation induced liver tissue damage. Cell J 14(4), 292-297.
- Valencia-Gattas, M., Conner, G.E., and Fregien, N.L. (2016). Gefitinib, an EGFR Tyrosine Kinase inhibitor, Prevents Smoke-Mediated Ciliated Airway Epithelial Cell Loss and Promotes Their Recovery. PloS one 11(8), e0160216.
- Zhou, F., and Roy, S. (2015). SnapShot: Motile Cilia. Cell 162(1), 224-224 e221.

Relationship: 2451: Oxidative Stress leads to CBF, Decreased

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Oxidative stress [MIE] Leading to Decreased Lung Function [AO]	adjacent	High	High

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	High	NCBI
Cavia porcellus	Cavia porcellus		NCBI
Oryctolagus cuniculus	Oryctolagus cuniculus		NCBI
Bos taurus	Bos taurus		NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	

Sex Applicability

Sex	Evidence
Mixed	

Key Event Relationship Description

Because the lung interfaces with the external environment, it is frequently exposed to airborne oxidant gases and particulates, and thus prone to oxidant-mediated cellular damage (Ciencewicki et al., 2008). Oxidant stress—through the action of exogenous and endogenous free radicals, such as super oxides, hydroxyl radicals, and hydrogen peroxides—is a common factor in lung inflammation and various respiratory diseases. The presence of redox-sensitive proteins in motile cilia suggests that oxidant stresses may impact ciliary function negatively (Price and Sisson, 2019). Indeed, exposure of human or rodent ciliated airway epithelial cells to hydrogen peroxide, acetaldehyde, ozone or cigarette smoke—all of which are known to cause oxidative stress—decreases CBF in a dose- and time-dependent manner (Bayram et al., 1998; Burman and Martin, 1986; Gosepath et al., 2000; Hastie et al., 1990; Helleday et al., 1995; Kienast et al., 1994; Knorst et al., 1994a; Min et al., 1999; Simet et al., 2010).

Evidence Supporting this KER

Experimental studies in vitro have shown that exposure of ciliated respiratory cells directly or indirectly to sources of oxidative stress leads to decreased CBF (Burman and Martin, 1986; Wilson et al., 1987; Feldman et al., 1994; Yoshitsugu et al., 1995; Min et al., 1999), which can be reversed by treatment with antioxidants (Schmid et al., 2015). Cigarette smoke condensate, a known inducer of oxidative stress, also causes a decrease in CBF in vitro (Cohen et al., 2009), while, in human subjects exposed to different oxygen levels, oxygen stress causes a decrease in nasal CBF (Stanek et al., 1998).

Biological Plausibility

One mode of antimicrobial defense in the airway epithelium is generation of free radicals by neutrophils and monocytes/macrophages. Some microbes have also been shown to produce oxidants in significant amounts, e.g. H_2O2 production by pneumococcus. Several studies have shown that oxidants, irrespective of the source (microbial or host-derived) inhibit ciliary function. Additionally, there is a large body of experimental evidence demonstrating that exposures to environmental oxidants, including volatile aldehydes, peroxides, sulfur dioxide, nitric dioxide and Diesel exhaust particles have a detrimental impact on ciliary function. Therefore, this KER is plausible.

Empirical Evidence

Tretament with H_2O_2 causes a dose-dependent decline in ciliary beat frequency (CBF) in tracheal rings from male Sprague-Dawley rats (Burman and Martin, 1986).

Exposure of ciliated epithelial cells to enzymatically generated oxidants (xanthine/xanthine oxidase) was accompanied by ciliary slowing, which was maximal at the end of the experiment (4 h). After 4 h, the reduction in CBF was 37.4%. Catalase alone, or in combination with SOD, completely protected the epithelial strips from oxidant-mediated ciliary dyskinesia. Exposure of respiratory epithelium to glucose/glucose oxidase resulted in similar effects to those obtained with the xanthine/xanthine oxidase system, with 38% and 57% CBF reduction after 4 h in systems containing 25 and 100 mU of glucose oxidase, respectively. Treatments with H_2O_2 and HOCl at concentrations of ≥100 µM caused dose-dependent ciliary dyskinesia after 4 h (Feldman et al., 1994).

Marked ciliary slowing was observed after exposure of human nasal epithelial cells to free radicals within the first 5 min. There was a significant difference in CBF between experimental and control groups. Pretreatment with 300 U/mL SOD or with 5 mM 3-ABA prevented CBF changes. (Min et al., 1999).

Pyocyanin dissolved in PBS produced gradual slowing of CBF in strips of normal human nasal ciliated epithelium without recovery. Ciliary dyskinesia was observed only late in the experiments when ciliostasis and epithelial disruption were also noted. In contrast, 1-hydroxyphenazine (1-hp) produced rapid onset of ciliary slowing, dyskinesia, and immediate ciliostasis but also some recovery of ciliary beating during the experiment (Wilson et al., 1987).

Baseline CBF was analyzed in human sinonasal epithelial cells for 4 min (time 0) followed by administration of either cigarette smoke condensate (CSC) or DMSO for 3 min. Forskolin was then administered to the apical surface and stimulated CBF measured. Following addition of forskolin, stimulated CBF was significantly decreased in the CSC-exposed group compared to DMSO controls (Cohen et al., 2009).

Treatment of fully differentiated normal human bronchial epithelial cells with 100 nM roflumilast raised the CBF at 1 h after exposure. Subsequent air or cigarette smoke exposure increased CBF significantly roflumilast-treated cultures (Schmid et al., 2015).

In superficial mucosae of the inferior nasal turbinates from non-smoking healthy volunteers exposed to three different oxygen concentrations—21%, 60% and 95%—for 2 h, CBF dose-dependently increased. Extending exposure to extreme oxygen concentrations to 4 h, however, decreased CBF, which is indicative of “oxygen stress” (Stanek et al., 1998).

Within 2 min of exposure of human respiratory epithelial cell monolayers to 10 µM H_2O_2, a decrease in CBF was observed. All cilia stopped moving within 10 min without obvious surface structural change in the ciliated cells. Catalase significantly reduced the ciliotoxic effect of H_2O_2 (Yoshitsugu et al., 1995).

Uncertainties and Inconsistencies

Several studies show that oxidants decrease CBF which can be reversed by addition of antioxidants, suggesting a direct effect. However, there is evidence suggesting that oxidant-mediated decreases in CBF cannot be prevented by addition of antioxidants. For example, a polycyanin-induced decrease in CBF in human nasal epithelium could be reversed by treatment with isobutylmethylxanthine and forskolin, both of which increase intracellular cAMP, and also by the cAMP analog dibutyryl cAMP, while antioxidants did not seem to have any effect on CBF (Kanthakumar et al., 1993). Like polycyanin, two other P. aeruginosa toxins, 1-hydroxyphenazine (1-HP) and rhamnolipid reduced CBF which was associated with a decrease in intracellular adenosine nucleotides (Kanthakumar et al., 1996).

Inconsistent with several studies, there are studies that suggest that exposure to cigarette smoke does not inhibit CBF. A study involving 56 human subjects (27 non-smokers and 29 smokers) showed no differences in CBF between the 2 groups. However, a decrease in nasal mucociliary clearance was observed in smokers who exhaled smoke through their noses (Stanley et al., 1986).

While several studies have shown age dependence of CBF, we found evidence that suggests otherwise (Agius et al., 1998).

Quantitative Understanding of the Linkage

Several studies in various species, including humans and rodents, provide evidence in support of this KER. The empirical evidence confirms both a dose- and time-dependence between the upstream KE/MIE and the downstream KE. Our quantitative understanding of this KER is therefore strong.

Response-response relationship

Treatment of human nasal ciliated epithelial cells with 0.4 mM xanthine + 100 mU/mL xanthine oxidase—producing 159 ± 4.0 µM/h H_2O_2—decreased CBF by ca. 1 Hz at 1 h and ca. 2.5 Hz (37.4%) at 4 h. Catalase alone (500 U/mL), or in combination with superoxide dismutase (SOD; 300 U/mL) completely protected the cells from oxidant-mediated ciliary dyskinesia (Feldman et al., 1994).

Treatment of human nasal ciliated epithelial cells with 5 mM glucose + 25 mU/mL glucose oxidase— producing 114 ± 7.7 µM/h H_2O_2—decreased CBF by ca. 2 Hz at 1 h and ca. 4 Hz (38%) at 4 h. The decline in CBF was even larger with 57% (approx. 6 Hz) at 4 h when 100 mU/mL glucose oxidase was used (producing 322 ± 11.5 µM/h H_2O_2). Catalase alone (500 U/mL) completely protected the cells from oxidant-mediated ciliary dyskinesia (Feldman et al., 1994).

Treatment of human nasal ciliated epithelial cells with H_2O_2 at concentrations ≥100 µM dose-dependently decreased CBF in human nasal ciliated epithelial cells, with 100 µM causing a 22.4% reduction and the maximal decrease (51.6%) seen with 500 µM H_2O_2 at 4 h. Adding 100 mU/mL MPO to 150 µM H_2O_2 enhanced the H_2O_2-mediated decrease in CBF (control:11.7 ±0.6 Hz; H_2O_2: 8.2 ± 1.1 Hz, 30% decrease; H_2O_2 + MPO: 5.4±0.2 Hz, 53.8% decrease). (Feldman et al., 1994).

Treatment of human nasal ciliated epithelial cells with HOCl at concentrations ≥100 µM dose-dependently decreased CBF in human nasal ciliated epithelial cells, with 100 µM causing a 26.1% reduction and the maximal decrease (100%) seen with 500 µM HOCl at 4 h (Feldman et al., 1994).

Treatment of human nasal epithelial cells with 0.4 mM xanthine + 100 mU/mL xanthine oxidase decreased CBF by ca. 50% within 2 min. Addition of 300 U/mL SOD abolished this effect (Min et al., 1999).

Treatment of human nasal epithelial cells with 10 mM H_2O_2 decreased CBF to 36.5 ±4.4% of baseline within 5 min, with a maximal decrease in CBF of 100% seen after 10 min, whereas 1 mM H_2O_2 had no effect on CBF. Treatment of human nasal ciliated epithelial cells with 0.8 mM xanthine + 100 mU/mL xanthine oxidase transiently increased CBF by 12.1±1.0% from baseline. When xanthine concentration was increased to 4 and 8 mM, CBF decreased by 26.8±1.7 and 25.6±1.5%, respectively (Yoshitsugu et al., 1995).

Treatment of bovine ciliated bronchial epithelial cells with acetaldehyde, an oxidative stressor, decreased CBF in a dose-dependent manner. Significant slowing of ciliary beating by ca. 50% was observed with concentrations as low as 15-30 µM, and ciliary beating was completely abrogated at concentrations > 250 µM. Ciliary beating also decreased following treatment with 15-30 µM propionaldehyde (40-50% of control), butyraldehyde (35-65% of control), isobutyraldehyde (20-40% of control), and benzaldehyde (80-90% of control) (Sisson et al., 1991).

Exposure of rabbit tracheal explants to formaldehyde dose-dependently decreased CBF. At 66 µg formaldehyde/cm^3, CBF decreased from 12.6 to 11.8 Hz; at 33 µg formaldehyde/cm^3, CBF decreased from 13.0 to 10.9 Hz (Hastie et al., 1990).

Exposure of guinea pig trachea to SO_2 at concentrations of 2.5-12.5 ppm for 30 min dose-dependently decreased CBF. Exposure to 2.5 ppm SO_2 caused a small, non-significant decrease in mean CBF, and exposure to 5 ppm SO_2 caused a 45% decrease. The greatest decrease (72 %) in mean CBF was recorded after exposure to 12.5 ppm SO_2 (Knorst et al., 1994a).

Exposure of human nasal epithelial cells (cultured in Ringer's solution) to SO_2 at concentrations of 2.5-12.5 ppm for 30 min dose-dependently decreased CBF. Exposure to 2.5 ppm yielded a 42.8% decrease, whereas exposure to 12.5 ppm yielded a 96.5% decrease in CBF (Kienast et al., 1994).

A 20-min exposure to NO_2, a known air pollutant, at concentrations of 1.5 or 3.5 ppm did not affect CBF in healthy human subjects at 45 min post-exposure (Helleday et al., 1995).

Exposure of human bronchial epithelial cells from healthy volunteers to 10, 50, and 100 µg/mL Diesel exhaust particles (DEP) significantly decreased CBF by 15.9%, 31.0%, and 55.5%, respectively, from baseline after 24 h (Bayram et al., 1998).

A 4-week exposure of human nasal epithelial cells to 100 µg/m^3 ozone had no effect on CBF, whereas 5- and 10-times that concentration significantly decreased CBF (-11.1% at 500 µg/m^3; -33.3% at 1000 µg/m^3) (Gosepath et al., 2000).

Baseline CBF in tracheal rings from C57Bl/6 mice exposed to cigarette smoke (whole body exposure to mainstream and sidestream cigarette smoke via inhalation from 1R1 reference cigarettes, at 150 mg/m^3 total particulate matter for 2 h/day, 5 days/week, for up to 1 year) for 1.5 to 3 months was slightly, but not significantly, increased (~1 Hz). After 6 months of smoke exposure, however, baseline CBF significantly decreased (~2–3 Hz) (Simet et al., 2010).

Time-scale

Treatment of human nasal ciliated epithelial cells with 0.4 mM xanthine + 100 mU/mL xanthine oxidase decreased CBF over time, with a noticeable decrease by ca. 1 Hz at 1 h and a maximal decrease of 37.4% reached at 4 h (Feldman et al., 1994).

Treatment of human nasal ciliated epithelial cells with 5 mM glucose + 25 mU/mL glucose oxidase decreased CBF by ca. 2 Hz at 1 h and a maximal decrease of ca. 4 Hz (38%) at 2 h, that did not change until the end of the experiment at 4 h. When 100 mU/mL

glucose oxidase was used, CBF decreased by ca. 2 Hz at 1 h, 4 Hz at 2 h, 5.5 Hz at 3 h, reaching a maximum of 57% (approx. 6 Hz) at 4 h (Feldman et al., 1994).

Treatment of human nasal epithelial cells with 0.4 mM xanthine + 100 mU/mL xanthine oxidase decreased CBF maximally by ca. 50% within 2 min, after which it began to increase again, reaching approx. 80% of the baseline value after 30 min (Min et al., 1999).

Treatment of human nasal ciliated epithelial cells with 0.8 mM xanthine + 100 mU/mL xanthine oxidase transiently increased CBF by 12.1±1.0% from baseline within 15 s, after which it rapidly returned to baseline levels (within 30 min). When xanthine concentrations were increased to 4 and 8 mM, CBF decreased by 26.8±1.7 and 25.6±1.5%, respectively (Yoshitsugu et al., 1995).

Treatment of bovine ciliated bronchial epithelial cells with acetaldehyde reduced CBF rapidly, with a significant drop in CBF occurring within 30 s and a maximal decrease by 3 min (Sisson et al., 1991).

Exposure of rabbit tracheal explants to formaldehyde time-dependently decreased CBF: At 66 µg/cm^3, CBF decreased from 12.6 to 11.8 Hz immediately upon addition of HCHO to complete cessation of beating by 10 min. At 33 µg/cm^3, CBF decreased from 13.0 to 10.9 Hz by 30 min (Hastie et al., 1990).

At 24 h following a 4-h exposure of healthy human subjects to 3.5 ppm NO_2, there was a significant elevation in CBF from 12.4±0.9 Hz (at baseline, pre-exposure) to 13.8±0.8 Hz (Helleday et al., 1995).

Exposure of human bronchial epithelial cells to DEP significantly decreased CBF from 2 h onward after incubation with 50 to 100 µg/mL DEP and from 6 hours onward after incubation with 10 µg/mL DEP (Bayram et al., 1998).

A 4-week exposure of human nasal epithelial cells to ozone significantly reduced CBF, with effects becoming noticeable at the higher concentrations (-7.1% at 500 µg/m^3;
-10.3% at 1000 µg/m^3) after 2 weeks of exposure and a maximal decrease after 4 weeks (-11.1% at 500 µg/m^3; -33.3% at 1000 µg/m^3) (Gosepath et al., 2000).

Known modulating factors

Unknown

Known Feedforward/Feedback loops influencing this KER

Unknown

References

- Agius, A.M., Smallman, L.A., and Pahor, A.L. (1998). Age, smoking and nasal ciliary beat frequency. Clin. Otolaryngol. Allied Sci. 23(3), 227-230.
- Bayram, H., Devalia, J.L., Khair, O.A., Abdelaziz, M.M., Sapsford, R.J., Sagai, M., et al. (1998). Comparison of ciliary activity and inflammatory mediator release from bronchial epithelial cells of nonatopic nonasthmatic subjects and atopic asthmatic patients and the effect of diesel exhaust particles in vitro. Journal of Allergy and Clinical Immunology 102(5), 771-782.
- Burman, W.J., and Martin, W.J. (1986). Oxidant-Mediated Ciliary Dysfunction: Possible Role in Airway Disease. Chest 89(3), 410-413.
- Ciencewicki, J., Trivedi, S., and Kleeberger, S.R. (2008). Oxidants and the pathogenesis of lung diseases. J Allergy Clin Immunol 122(3), 456-470.
- Cohen, N.A., Zhang, S., Sharp, D.B., Tamashiro, E., Chen, B., Sorscher, E.J., et al. (2009). Cigarette smoke condensate inhibits transepithelial chloride transport and ciliary beat frequency. Laryngoscope 119(11), 2269-2274.
- Feldman, C., Anderson, R., Kanthakumar, K., Vargas, A., Cole, P.J., and Wilson, R. (1994). Oxidant-mediated ciliary dysfunction in human respiratory epithelium. Free Radic. Biol. Med. 17(1), 1-10.
- Gosepath, J., Schaefer, D., Brommer, C., Klimek, L., Amedee, R.G., and Mann, W.J. (2000). Subacute effects of ozone exposure on cultivated human respiratory mucosa. American journal of rhinology 14(6), 411-418.
- Hastie, A.T., Patrick, H., and Fish, J.E. (1990). Inhibition and recovery of mammalian respiratory ciliary function after formaldehyde exposure. Toxicology and applied pharmacology 102(2), 282-291.
- Helleday, R., Huberman, D., Blomberg, A., Stjernberg, N., and Sandstrom, T. (1995). Nitrogen dioxide exposure impairs the frequency of the mucociliary activity in healthy subjects. European Respiratory Journal 8(10), 1664-1668.
- Kanthakumar, K., Taylor, G., Cundell, D., Dowling, R., Johnson, M., Cole, P., et al. (1996). The effect of bacterial toxins on levels of intracellular adenosine nucleotides and human ciliary beat frequency. Pulm. Pharmacol. 9(4), 223-230.
- Kanthakumar, K., Taylor, G., Tsang, K., Cundell, D., Rutman, A., Smith, S., et al. (1993). Mechanisms of action of Pseudomonas aeruginosa pyocyanin on human ciliary beat in vitro. Infection and Immunity 61(7), 2848-2853.
- Kienast, K., Riechelmann, H., Knorst, M., Schlegel, J., Müller-Quernheim, J., Schellenbergt, J., et al. (1994). An experimental model for the exposure of human ciliated cells to sulfur dioxide at different concentrations. The clinical investigator 72(3), 215-219.
- Knorst, M.M., Kienast, K., Riechelmann, H., Müller-Quernheim, J., and Ferlinz, R. (1994). Effect of sulfur dioxide on mucociliary activity and ciliary beat frequency in guinea pig trachea. Int. Arch. Occup. Environ. Health 65(5), 325-328.
- Min, Y.-G., Ohyama, M., Lee, K.S., Rhee, C.-S., Oh, S.H., Sung, M.-W., et al. (1999). Effects of free radicals on ciliary movement in the human nasal epithelial cells. Auris, nasus, larynx 26(2), 159-163.
- Price, M.E., and Sisson, J.H. (2019). Redox regulation of motile cilia in airway disease. Redox Biol 27, 101146-101146.

- Schmid, A., Baumlin, N., Ivonnet, P., Dennis, J.S., Campos, M., Krick, S., et al. (2015). Roflumilast partially reverses smoke-induced mucociliary dysfunction. Respir. Res. 16(1), 135.
- Simet, S.M., Sisson, J.H., Pavlik, J.A., DeVasure, J.M., Boyer, C., Liu, X., et al. (2010). Long-term cigarette smoke exposure in a mouse model of ciliated epithelial cell function. American journal of respiratory cell and molecular biology 43(6), 635-640.
- Sisson, J.H., Tuma, D.J., and Rennard, S.I. (1991). Acetaldehyde-mediated cilia dysfunction in bovine bronchial epithelial cells. The American journal of physiology 260(2 Pt 1), L29-36.
- Stanek, A., Brambrink, A., Latorre, F., Bender, B., and Kleemann, P. (1998). Effects of normobaric oxygen on ciliary beat frequency of human respiratory epithelium. Br. J. Anaesth. 80(5), 660-664.
- Stanley, P., Wilson, R., Greenstone, M., MacWilliam, L., and Cole, P. (1986). Effect of cigarette smoking on nasal mucociliary clearance and ciliary beat frequency. Thorax 41(7), 519-523.
- Wilson, R., Pitt, T., Taylor, G., Watson, D., MacDermot, J., Sykes, D., et al. (1987). Pyocyanin and 1-hydroxyphenazine produced by Pseudomonas aeruginosa inhibit the beating of human respiratory cilia in vitro. J. Clin. Investig. 79(1), 221-229.
- Yoshitsugu, M., Matsunaga, S., Hanamure, Y., Rautiainen, M., Ueno, K., Miyanohara, T., et al. (1995). Effects of oxygen radicals on ciliary motility in cultured human respiratory epithelial cells. Auris, nasus, larynx 22(3), 178-185.