Small Airway Susceptibility to Chemical and Particle Injury

Leonie Francina Hendrina Fransen  Martin Oliver Leonard

Toxicology Department, Centre for Radiation, Chemical and Environmental Hazards, Public Health England, Didcot, UK

Keywords
Lung · Airway disease · Toxicity · Cellular differentiation

Abstract
Small airways (SA) in humans are commonly defined as those conducting airways <2 mm in diameter. They are susceptible to particle- and chemical-induced injury and play a major role in the development of airway disease such as COPD and asthma. Susceptibility to injury can be attributed in part to structural features including airflow dynamics and tissue architecture, but recent evidence may indicate a more prominent role for cellular composition in directing toxicological responses. Animal studies support the hypothesis that inherent cellular differences across the tracheobronchial tree, including metabolic CYP450 expression in the distal conducting airways, can influence SA susceptibility to injury. Currently, there is insufficient information in humans to make similar conclusions, prompting further necessary work in this area. An understanding of why the SA are more susceptible to certain chemical and particle exposures than other airway regions is fundamental to our ability to identify hazardous materials, their properties, and accompanying exposure scenarios that compromise lung function. It is also important for the ability to develop appropriate models for toxicity testing. Moreover, it is central to our understanding of SA disease aetiology and how interventional strategies for treatment may be developed. In this review, we will document the structural and cellular airway regional differences that are likely to influence airway susceptibility to injury, including the role of secretory club cells. We will also describe recent advances in single-cell sequencing of human airways, which have provided unprecedented details of cell phenotype, likely to impact airway chemical and particle injury.

Introduction
Human small airways (SA) are those non-cartilaginous airways within the lung with an internal diameter <2 mm and typically range from the 6–8th up to the 19–22nd airway generations [1]. They include proximal, distal, and terminal bronchioles (TBs) [2–4], with some descriptions also including respiratory bronchioles (Fig. 1). They comprise a luminal surface epithelium supported by a collagen-rich basement membrane and in more proximal regions, a smooth muscle layer. They can be innervated and are populated by immune cells predominantly at the mucosal surface [5–7]. The main function of the SA are as pathways of low pulmonary resistance, conducting air for respiratory function. Importantly, they are increasingly recognized as primary targets for pulmonary injury and disease development [8] and as the major site of airflow limitation in most airways diseases [8].

© 2021 The Author(s)
Published by S. Karger AG, Basel

This article is licensed under the Creative Commons Attribution 4.0 International License (CC BY) (http://www.karger.com/Services/OpenAccessLicense). Usage, derivative works and distribution are permitted provided that proper credit is given to the author and the original publisher.

Correspondence to:
Martin Oliver Leonard, martin.leonard@phe.gov.uk
Injury to human SA has been identified for many respiratory and systemic chemical and particulate exposures. The best characterized of these is tobacco smoking, where the SA is the primary site for early manifestations of lung injury and airway obstruction [9–15]. Injury to this region has also been observed for other respiratory exposures including mineral dusts and diesel exhaust [16, 17]. Indeed, fine-sized ambient air pollutant particle (0.1–2.5 μm in diameter [PM2.5]) levels are associated with increased SA resistance in asthmatic children [18] and bronchiolitis in infants [19]. Chemical-induced injury to SAs manifesting as bronchiolitis obliterans is also observed with inhalation of chemical vapours such as sulphur mustard and diacetyl [20, 21]. The systemic route of exposure to chemicals can also target SAs, for example, acute bronchiolitis associated with pharmaceutical exposures such as D-penicillamine and busulfan [22, 23]. Interestingly, ingestion of the Taiwanese plant *Sauropus androgynus* is also associated with bronchiolitis obliterans, with injury attributed to a yet unidentified chemical constituent [24].

Accumulating evidence suggests that there are inherent differences in cells of the same category across different airway generations. Large (bronchial [G1–7]) and SA epithelial cells isolated from the same donors produced fundamentally different morphology and secretory profiles. SA in this study produced surfactant protein B and little mucin (MUC)5B, whereas large airways (LA) produced the opposite [4]. Furthermore, transcriptomic profiling of human SA brushings (G10–12) in a separate study [25] identifies numerous differentially expressed genes including secretoglobin (SCGB)3A2, surfactant protein B, and lactotransferrin, when compared to larger bronchial regions (G4–6). Interestingly, cells of the SA in smokers develop a phenotype characteristic of non-smoker proximal airways, typified by increased basal and secretory cells [25]. In addition, in vitro analysis has also suggested that smoking-induced responses are more severe in the SA than in the LA [26].

An understanding of why the SA are more susceptible to certain chemical and particle exposures is important for our ability to identify hazardous materials, their properties, and to develop appropriate models for toxicity testing. It is also important for our understanding of airway disease development and how therapeutic strategies may be developed. In this review, we will describe current knowledge on SA injury mechanisms and highlight areas of uncertainty for future attention, including how inherent differences in cellular composition and differentiation in airway regions may govern responses to exposures and contribute to injury susceptibility.

**Structural Considerations and Regional Airway Susceptibility**

Susceptibility to toxicity is a consequence of an excessive toxicant dose, beyond the capacity for cellular and tissue processes to eliminate. Accumulation of such doses is a function of several factors, including physical and chemical properties of the toxicant as well as cell-type composition, metabolic, and transport capacities of the tissue. Within the lung, structural features governing airflow dynamics can also influence regional dose exposure upon inhalation [27–30]. Investigations into the relative importance of these factors for SA susceptibility to injury have uncovered several important findings.

Within the human lung, it was observed that PM2.5 ambient air pollutant particles can accumulate in the SA,
particularly in the distal bronchioles at up to 25 times greater doses than bronchial regions [31]. Similar findings have been observed for other fine-sized particles including those from tobacco smoke [32, 33] and inorganic dusts such as silica and asbestos, where concomitant SA disease is also observed [34–40]. Due to airflow and structural differences within the respiratory tract, smaller ultrafine (<0.1 μm) and larger coarse (2.5–10 μm) particles preferentially deposit in nasal regions more than fine-sized particles [41, 42]. In general, while there is substantial deposition of all particles in the nasal region upon inhalation, within the lower respiratory tract (lung), the SA region is consistently the primary site for the largest fraction of deposited particles [42–44]. Smaller particles have an increased surface area relative to their mass compared to larger size. Oxidative potential associated with inhaled particulates has been suggested as a mechanism by which inhaled material may cause toxicity particularly for anthropogenic environmental air pollutant particulates [45]. Toxicity attributable to particle surface area oxidative effects may therefore be exacerbated in regions of smaller particle deposition, including SA.

Whether inhaled particles that reach SA preferentially deposit to a particular sub-airway region is not entirely clear, but some studies have pointed to preferential deposition in more distal bronchiolar regions. An in vitro model of SA regions to study trajectories of inhaled smoke particles (0.1–2 μm in diameter) demonstrated higher particle deposition in the TBs and proximal alveolar regions than that in more distal alveolar regions [46]. This selective deposition in more distal parts of the SA is further supported experimentally using in vivo inhalation studies in rodents, albeit where airway generation and size are different to humans but airflow dynamics and compartmentalisation are comparable [47–50]. This distal pattern of deposition is suggested as a result of the transfer from tidal airflow in the conducting airways to reserve airflow in the alveolar region. According to Einstein’s work on Brownian motion, particulates in a gas moving by diffusion settle more quickly than the gas in which they are suspended, providing a suggestion as to how such particulates deposit in this part of the lung in higher concentrations [51].

Chemical vapour exposure is also subject to regional exposure differences upon inhalation, suggested in part as proportional to the degree of chemical solubility in that airway compartment. Irritant gases with high solubility such as formaldehyde [52] will be preferentially absorbed in the upper airways and will not reach high concentrations in bronchioles, while gases such as phosgene or nitrogen dioxide will and have the potential to exert more distal toxicological effects [53, 54]. Ozone has also been observed to cause injury in the conducting airway and alveolar regions [55, 56], with some recent work in rats suggesting that the bronchial region is particularly susceptible to injury independent of chemical solubility, although the precise mechanism has not been determined [57]. In addition to solubility, regional uptake of chemicals is dependent on airflow rates, blood flow rates, partition chemical concentrations, uptake mechanisms, and tissue thickness [30, 58–61]. Vapour dosimetry modeling of these parameters for the chemicals acrolein and diacetyl highlighted that chemical uptake in bronchial and bronchiolar areas occur in regions, where changes in airflow direction and velocity occur, including airway branch points [58, 59]. Furthermore, chemical uptake in more distal bronchiolar regions was additionally attributed to differences in tissue the compartment metabolic rate, facilitating increased chemical turnover [58, 59]. Much of the contribution of chemical metabolism in these models is based on limited knowledge of this airway region in humans and highlights an underexplored area of significance. Little information exists on systemic exposure to the SA and chemical dosimetry as to underlying causes for toxicity in this context.

Additional structural differences exist between the SA and other airway regions that can influence the exposure dose. The luminal epithelial surface of the conducting airways is covered by a protective mucus layer that functions to capture foreign elements for elimination along the mucociliary escalator. The thickness of this mucus layer decreases from 5 μm in the proximal airways to 0–1 μm in the TBs [62]. Indeed the lack of protective gel-forming mucins in the TBs [4] may contribute to increased levels of toxicant exposure [63] due to decreased physical separation of cells from xenobiotic deposition and reduced mucociliary clearance. This reduced clearance is supported by the observation that in the peripheral human airways after exposure to ultrafine radiolabelled carbon particles, clearance from the upper airways is much faster [64]. Additional differences in airway structural features include cartilage and submucosal glands present within the proximal airways, absent from SA [65], which result in different processes for airway surface liquid circulation and airway regeneration upon injury [66, 67]. Whether differences in fluid movement impact toxicant exposure remains to be explored. Differences in the airway compartment surface area are also likely to contribute to a differential dose and therefore magnitude of toxicological response. For example, combined surface areas of SA
regions (±143,000 cm$^2$), thus providing a dilution of inhaled toxicants when moving to the alveolar region [68, 69]. Ciliated cells are present throughout the airways and account for over 50% of the SA epithelium [70]. Their main function is to move mucus towards the upper pharynx and to regulate secretions [71] and are particularly important in the response to particle deposition. Regional differences in the number and activity of these cells are likely to impact retention time of particles and consequent cellular exposure to toxicants. Indeed, ciliary beat frequency is different in airway regions with a 35% lower frequency in bronchioles than in larger bronchi [72] and may cause increased resident time for inhaled material and resulting increased dose. This aspect therefore represents another source of regional responses to toxicant exposure inherent to cellular composition differences in the airway region.

Further differences exist between airway regions for epithelial progenitor basal cells. Basal and parabasal cells represent 31% and 7% of epithelial cells in human bronchi, respectively, decreasing upon airway branching to 6% and 0% in respiratory bronchioles [73]. Proportional differences in these cells have been argued to confer differential toxicant responses. Inhalation of chlorine in a rabbit exposure model resulted in similar levels of acute injury in the small and large airways but during the recovery period, there was more inflammation and less repair of the epithelium in bronchioles than bronchi [74]. This was suggested due to slower repair processes as fewer progenitor basal cells present in the distal airways. Interestingly, there are differences in ultrastructural and functional responses of basal cells between bronchi and the SA epithelium [75]. More recently, it has been observed using clonal selection techniques that basal cells from human proximal airways differentiate to multiciliated and secretory phenotypes, while human distal SA basal cells do not [76]. Further work by the same group identified different basal cell types in both healthy and diseased lungs, with inherently different differentiation capacity [77]. Characterization of these progenitor cell populations is needed to fully understand their contribution to differential toxicological effects between airway regions.

### Chemical Metabolism and Susceptibility to Injury

Protection from toxicant exposure is a function of constituent and adaptive cellular processes that act to inactivate and eliminate harmful chemical activities. A large part of this protective arsenal is attributable to cellular chemical transformation and transporting capacity. These molecular defence pathways include P450 enzymes and cellular transporters, which can also inadvertently result in toxification of certain chemicals. As structural features of the SA likely contribute to increased toxicological susceptibility to certain inhalation exposures, the relative contribution of cells with different chemical-handling capacity across airway regions is less clear but highly significant. Moreover, the characterization of cell types for inherent defence pathways within the human SA is poor, particularly for the TB, where toxicity is often most prominent. There is however some evidence supporting differential inherent cellular susceptibility independent of exposure dose considerations as a determinant of regional airway toxicity. A study of human lung explants demonstrated that freshly isolated S9 metabolically active fractions from the bronchoalveolar region were more biologically active than the alveolar region in the generation of diesel exhaust chemical-derived mutagens [78]. Furthermore, CYP1A1, a P450 enzyme involved in the bioactivation of these chemicals from diesel exhaust and cigarette smoke [79], was present in the cuboidal epithelium of the TB of smokers but absent in the epithelium of bronchioles larger than 1 mm in diameter [80, 81]. While these observations highlight the TB and more distal SA as a target for toxicity, the influence of an exposure dose in this context may play a part. Indeed, CYP1A1 is an inducible enzyme and has also been observed to be induced in more proximal airway regions by tobacco use [82, 83]. The lack of clarity on inherent chemical responses between airway generations for this type of chemical and others remains.

Phase I cytochrome P450 enzymes (CYPs) are considered the main cellular biotransformation proteins and are involved in metabolic activation of many organic toxicants, with different isoforms having different chemical specificity [28]. Within the airway epithelium of the lung, they are typically localized to secretory cell types [84]. The analysis of CYP isoform regional airway expression and their functional consequences has been examined in some detail in rodents, providing insight on potential cell type-dependent toxicological responses. For example, cytochrome B5 in mice is restricted to the bronchiolar region and not expressed in larger bronchial or smaller alveolar structures [85]. The toxicity of the combustion product furan was found to have specific bronchiolar injury attributable to CYP2E1 expression in secretory club cells [86]. It has also long been recognized that chemicals such as styrene, permethrin, and fluensulfone among
others [87–89] are specifically toxic to the TBs in mice, attributable to the high number of metabolically active secretory club cells in this region enriched for CYP2F2 activity [90]. The relevance of this mechanism to human toxicology has been questioned as CYP2F2 biotransformation is highly species-specific [90]. The concept that regional-specific CYP expression conferring toxicity remains and however may play a significant role in humans. Humanized transgenic mice, where the human orthologues of CYP2F2, CYP2F1, and CYP2A13/2B6 were expressed within the mouse respiratory tract, displayed diminished toxicity to styrene when compared to unmodified murine CYP expressing mice [87, 91]. A similar approach was used to examine naphthalene toxicity, a chemical commonly found in combustion products such as tobacco smoke and diesel exhaust [92, 93] and found some degree of regional-specific toxicity, attributable to more proximal CYP2A13 expression in the olfactory mucosa [94].

Recent advances in cell and tissue characterization techniques, particularly the development and application of single-cell mRNA sequencing to human tissue analysis have allowed for characterization of cell types and their associated functions at a resolution unachievable to date. Application of these approaches to identify inherent regional differences in cellular composition, differentiation, biotransformation, and biokinetic properties within different airway regions of the lung has not been carried out. However, a recent study has allowed for some preliminary exploration to be carried out in our current review [95]. This study characterized single-cell mRNA expression in different airway regions from 10 normal non-smoking donors, ranging from the nasal region to the SA proximal bronchiolar (12th generation). Some direct comparisons between airway regions for the same broad cell type can be made by comparing cluster-specific marker expression (Fig. 2). This was carried out as described in online supplementary File 1; for all online supplementary materials, see www.karger.com/doi/10.1159/000519344. These comparisons indicate not only significant differences between different cell types but also regional differences within cells of the same lineage. If we examine surface secretory cell groups from nasal versus SA epithelial brushings, we can see that the oligopeptide transporter solute carrier (SLC)15A2 (PEPT2), which transports chemicals such as β-lactam antibiotics [96] and has higher expression in the nasal region. There was also higher expression in submucosal gland goblet cells than in other regions, further supporting more proximal localisation. This pattern parallels observations in humans where protein expression of PEPT2 is greater in more proximal regions of the lung than that in the distal airways [97]. A similar expression pattern can be observed for solute carrier organic anion transporter (SLC)O4C1 (Fig. 2). This analysis focuses on those genes, uniquely clustered to the cell and region type, and may not identify contributors to toxicity, where expression is observed more broadly across airway regions or cell types. This may explain the lack of polyamine transporters identified on analysis of the dataset, genes typically expressed on the membranes of club cells and alveolar type I and II cells and thought to underlie regional paraquat toxicity [29].

In addition to transporters, regional expression of CYP enzymes can also be observed within secretory cells in this dataset (Fig. 2). A greater expression in nasal secretory cells was observed for CYP2A13 consistent with previous histochemical analysis for this protein [98] and is also consistent with expression in murine studies as previously described [94]. The related enzyme CYP2A6 was not observed as specific to a cell type in this analysis and reflects the broad expression of this enzyme across the lung including the alveolar compartment [98]. Both enzymes have differential metabolic activity for chemical toxicants such as naphthalene and phenanthrene (2A13 > 2A6) [99], and therefore, differential expression along the airway is likely to direct differential responses of these airway regions to chemical insult. In addition to nasal region prominent secretory cell CYP gene expression, there was also more distal expression of CYP2F1 and CYP2J2. While this regional difference has been observed previously for CYP2F1 [100, 101], it has yet to be confirmed in other studies for CYP2J2. Again, similar to transporter expression, some CYP enzymes are expressed more broadly across airway regions and were not highlighted in this analysis. CYP2S1, identified prominently in multiciliated cells, is involved in the bioactivation of some aromatic hydrocarbons and is expressed in human bronchial and bronchiolar regions but not in alveolar regions [102]. CYP3A5, identified in bronchial brush cells, is involved in the bioactivation of nitrosamines, polycyclic aromatic hydrocarbons, aflatoxin B1, and numerous other chemical species [28] and is expressed throughout the lung particularly in bronchial regions [84, 103].

As the proportion of different cell types within airway regions is unique, toxicokinetic responses attributable to each region will be dependent not only on differences within each cell type but also on differences present between cell types (e.g., between ciliated and secretory cells). As can be observed from Figure 2, unique expression profiles point to large differences in how chemicals may be...
Airway region

- Small airway
- Bronchial
- Tracheal
- Nasal

Cell type annotation

- AT1/AT2
- SMG goblet
- Deuterosomal
- Multiciliated
- Multiciliated N
- Brush
- Secretory
- Secretory N
- Serous
- PNEC
- Fibroblast
- Endothelial
- Myeloid

Airway region

| Cell type   | Gene 1 | Gene 2 | Gene 3 | Gene 4 | Gene 5 | Gene 6 |
|-------------|--------|--------|--------|--------|--------|--------|
| AGER        | TPL3   | TP63   | TP63   | TP63   | TP63   | TP63   |
| TP63        | KRT19  | KRT6A  | KRT6A  | KRT6A  | KRT6A  | KRT6A  |
| KRT6A       | SGC81A1| SGC81A1| SGC81A1| SGC81A1| SGC81A1| SGC81A1|
| SLPI        | BPIFB2 | BPIFB2 | BPIFB2 | BPIFB2 | BPIFB2 | BPIFB2 |
| BPIFB2      | CDC20B | FOXJ1  | FOXJ1  | FOXJ1  | FOXJ1  | FOXJ1  |
| FOXJ1       | GRP    | GRP    | GRP    | GRP    | GRP    | GRP    |
| GRP         | CDON5  | CDON5  | CDON5  | CDON5  | CDON5  | CDON5  |
| CDON5       | FCER1G | FCER1G | FCER1G | FCER1G | FCER1G | FCER1G |
| FCER1G      | MARCO  | MARCO  | MARCO  | MARCO  | MARCO  | MARCO  |
| MARCO       | SLC2TA2| SLC2TA2| SLC2TA2| SLC2TA2| SLC2TA2| SLC2TA2|
| SLC2TA2     | CYP5TA1| CYP5TA1| CYP5TA1| CYP5TA1| CYP5TA1| CYP5TA1|
| CYP5TA1     | CYP2AA3| CYP2AA3| CYP2AA3| CYP2AA3| CYP2AA3| CYP2AA3|
| CYP2AA3     | FMO2   | FMO2   | FMO2   | FMO2   | FMO2   | FMO2   |
| FMO2        | MGST1  | MGST1  | MGST1  | MGST1  | MGST1  | MGST1  |
| MGST1       | ALDHA3A2| ALDHA3A2| ALDHA3A2| ALDHA3A2| ALDHA3A2| ALDHA3A2|
| ALDHA3A2    | EPHX3  | EPHX3  | EPHX3  | EPHX3  | EPHX3  | EPHX3  |
| EPHX3       | SULT2B1| SULT2B1| SULT2B1| SULT2B1| SULT2B1| SULT2B1|
| SULT2B1     | ABCC1  | ABCC1  | ABCC1  | ABCC1  | ABCC1  | ABCC1  |
| ABCC1       | ALDH2  | ALDH2  | ALDH2  | ALDH2  | ALDH2  | ALDH2  |
| ALDH2       | AKR1A1 | AKR1A1 | AKR1A1 | AKR1A1 | AKR1A1 | AKR1A1 |
| AKR1A1      | CYP4X1 | CYP4X1 | CYP4X1 | CYP4X1 | CYP4X1 | CYP4X1 |
| CYP4X1      | SLC2AA4| SLC2AA4| SLC2AA4| SLC2AA4| SLC2AA4| SLC2AA4|
| SLC2AA4     | GSTA2  | GSTA2  | GSTA2  | GSTA2  | GSTA2  | GSTA2  |
| GSTA2       | GSTA1  | GSTA1  | GSTA1  | GSTA1  | GSTA1  | GSTA1  |
| GSTA1       | CES1   | CES1   | CES1   | CES1   | CES1   | CES1   |
| CES1        | NQ01   | NQ01   | NQ01   | NQ01   | NQ01   | NQ01   |
| NQ01        | ABCC5  | ABCC5  | ABCC5  | ABCC5  | ABCC5  | ABCC5  |
| ABCC5       | SLC04A1| SLC04A1| SLC04A1| SLC04A1| SLC04A1| SLC04A1|
| SLC04A1     | EPHX1  | EPHX1  | EPHX1  | EPHX1  | EPHX1  | EPHX1  |
| EPHX1       | CYP4B1 | CYP4B1 | CYP4B1 | CYP4B1 | CYP4B1 | CYP4B1 |
| CYP4B1      | GSTP1  | GSTP1  | GSTP1  | GSTP1  | GSTP1  | GSTP1  |
| GSTP1       | MGST3  | MGST3  | MGST3  | MGST3  | MGST3  | MGST3  |
| MGST3       | ADH1B  | ADH1B  | ADH1B  | ADH1B  | ADH1B  | ADH1B  |
| ADH1B       | CYP1B1 | CYP1B1 | CYP1B1 | CYP1B1 | CYP1B1 | CYP1B1 |
| CYP1B1      | GSTT1  | GSTT1  | GSTT1  | GSTT1  | GSTT1  | GSTT1  |
| GSTT1       | SLC02B1| SLC02B1| SLC02B1| SLC02B1| SLC02B1| SLC02B1|
| SLC02B1     | CYP2J2 | CYP2J2 | CYP2J2 | CYP2J2 | CYP2J2 | CYP2J2 |
| CYP2J2      | AKR1C1 | AKR1C1 | AKR1C1 | AKR1C1 | AKR1C1 | AKR1C1 |
| AKR1C1      | CYP2B6 | CYP2B6 | CYP2B6 | CYP2B6 | CYP2B6 | CYP2B6 |
| CYP2B6      | CYP3A13| CYP3A13| CYP3A13| CYP3A13| CYP3A13| CYP3A13|
| CYP3A13     | CYP2F1 | CYP2F1 | CYP2F1 | CYP2F1 | CYP2F1 | CYP2F1 |
| CYP2F1      | SLC15A2| SLC15A2| SLC15A2| SLC15A2| SLC15A2| SLC15A2|
| SLC15A2     | ABCG2  | ABCG2  | ABCG2  | ABCG2  | ABCG2  | ABCG2  |
| ABCG2       | ABCB1  | ABCB1  | ABCB1  | ABCB1  | ABCB1  | ABCB1  |
| ABCB1       | ABCC10 | ABCC10 | ABCC10 | ABCC10 | ABCC10 | ABCC10 |
| ABCC10      | CYP3A5 | CYP3A5 | CYP3A5 | CYP3A5 | CYP3A5 | CYP3A5 |
| CYP3A5      | CYP2E1 | CYP2E1 | CYP2E1 | CYP2E1 | CYP2E1 | CYP2E1 |
| CYP2E1      | ABCA4  | ABCA4  | ABCA4  | ABCA4  | ABCA4  | ABCA4  |
| ABCA4       | AKR1B1 | AKR1B1 | AKR1B1 | AKR1B1 | AKR1B1 | AKR1B1 |
| AKR1B1      | AKR1B10| AKR1B10| AKR1B10| AKR1B10| AKR1B10| AKR1B10|
| AKR1B10     | AKR1C2 | AKR1C2 | AKR1C2 | AKR1C2 | AKR1C2 | AKR1C2 |
| AKR1C2      | AKR1C3 | AKR1C3 | AKR1C3 | AKR1C3 | AKR1C3 | AKR1C3 |
| AKR1C3      | ALDHA3A1| ALDHA3A1| ALDHA3A1| ALDHA3A1| ALDHA3A1| ALDHA3A1|
| ALDHA3A1    | DUOX2 | DUOX2 | DUOX2 | DUOX2 | DUOX2 | DUOX2 |
| DUOX2       | CYP2A6 | CYP2A6 | CYP2A6 | CYP2A6 | CYP2A6 | CYP2A6 |
| CYP2A6      | CYP2S1 | CYP2S1 | CYP2S1 | CYP2S1 | CYP2S1 | CYP2S1 |

(For legend see next page.)
Small Airway Susceptibility to Injury

handled by different cell types. As described above, phase I metabolising CYP enzyme expression appeared restricted to classically defined secretory cells. Multiciliated cells on the other hand demonstrated specific expression for the phase II metabolism enzymes glutathione S-transferase 1, glutathione S-transferase 2, and NADH quinone oxidoreductase 1, all nuclear factor erythroid 2-related factor 2-dependent genes [104] that are important for the regulation of oxidative and electrophilic stress. In addition, genes involved in xenobiotic transport and metabolism including SLC22A4 [105] and carboxylesterase 1 [106] were also uniquely expressed in multiciliated cells. Interestingly, some of the highest expression of epoxide hydrolase genes was in suprabasal cells. These enzymes are involved in the metabolism of polycyclic aromatic hydrocarbons such as benzo[a]pyrene to produce toxic and highly mutagenic chemical species [107]. Their localized protein expression to more proximal regions of the lung [108] is consistent with the higher proportion of basal and parabasal cells in larger airways [73].

Further, single-cell sequencing (ScSeq) studies of the lung have provided additional weight for distinct and localized pharmacokinetic and dynamic responses in different airway regions. Inhalation exposure to cigarette smoke was associated with cell type-specific expression of xenobiotic metabolism and stress response genes [109], where specific increases in aldehyde dehydrogenase 3A1 and NADH quinone oxidoreductase 1 in ciliated cells and thioredoxin-interacting protein and aldehyde dehydrogenase 3A2 in basal cells occurred after examination of human SA brushings (airway generations 9–12) [109]. Another recent ScSeq study of the murine trachea [110, 111] revealed that proximal tracheal (C1–4) club cells were phenotypically and functionally different from distal tracheal club cells (C9–12), with over 100 differentially expressed genes including Muc5b and interleukin (IL)13RA1. Following exposure ex vivo of these different airway region cells to allergen, where the exposure dose was controlled, mucus cell hyperplasia was only observed in distal club cells replicating regional effects observed after in vivo exposure [112]. Further, ScSeq analysis study of the human lung identified distinct proximal to distal differences in the expression profile of hundreds of genes within cell types including multiciliated and basal cells [113]. Differences in basal cells indicated greater proliferative and differentiation capacity in the proximal airways, indicating a greater capacity for recovery against injury in this region. Again, this further supports the idea that increased susceptibility of more distal regions may occur due to inherent regional differences within cell types.

While these high-resolution studies have provided unprecedented detail and insight into regional differences in airway phenotype, there is still a lack of fine-tuned spatial resolution to these datasets. For example, while the “SA” or “distal airways” have been profiled for cell-specific gene expression, this categorization encompasses many different SA generations with likely different cellular phenotypes. This is important, particularly for SA regions such as the TB, consistently identified from rodent studies as particularly sensitive to chemical- and particle-induced injury with metabolic gene expression unique to this region. Some evidence also exists in humans that the TB may have functional differences compared to other small airway regions, for example, due to greater numbers of club cells, postulated as the main metabolising cells in the lung. TB-predominant expression of phase I enzymes such as N-acetyltransferase 2 [114, 115] also indicates a potential unique response profile to toxicant exposure. The use of newly developed ScSeq techniques that allow for spatial transcriptomic profiles has the capability to provide the resolution needed to define airway generation level cellular profiling and inherent chemical-handling capacity [116, 117].

Club Cells as a Determinant of SA Susceptibly to Toxicants

Despite the SA being a prominent target for toxicant-induced injury within the lung, few studies in humans have directly examined whether regional cellular compositional differences play a significant role in toxic susceptibility. Animal studies have suggested this may be the case through mechanisms involving higher levels of metabolically active club cells located within the SA region [118–120]. Indeed, club cells have also been identified in humans as absent in the proximal airways and up to 10%
and ~20% of all epithelial cells in the terminal and respiratory bronchioles, respectively [119, 120]. It is therefore possible that increased club cell chemical biotransformation in the SA may lead to a higher proportion of cellular injury in humans. However, difficulties in separating dose effects within the lung and recent work on club cell identification present significant challenges in testing this hypothesis.

Club cells were initially defined as non-ciliated cuboidal epithelial cells possessing a dome-shaped apex projecting into the airway lumen [121]. Using morphological and ultrastructural features, abundant expression of club cells are observed in the human terminal and respiratory bronchiolar epithelium [119, 120, 122, 123], while being absent from nasal, tracheal, and alveolar regions [124, 125]. Using ultrastructural definitions alone, there was proportionally greater numbers of club cells within the TB than within more proximal airways [126]. The secreted protein SCGB1A1 has been used as a marker to identify club cells and interestingly also labelled cells in vitro, which preferentially responded to aryl hydrocarbon receptor chemical toxicant ligands [127]. Using this marker in combination with ultrastructural features within the human airways, club cells were identified as absent from the larger conducting airways and bronchi, while highest expression was within the terminal and respiratory bronchioles [119]. Other studies identified higher staining of SCGB1A1 in the bronchiolar region but also identified positive staining within proximal bronchial regions [128, 129], calling into question the specificity of this marker for classically defined club cells. More recently, a detailed quantitative analysis in the human lung revealed a similar proportion of SCGB1A1 cells within segmental bronchi through to the TBs, with lower levels observed in primary bronchi and trachea [4]. This bronchiolar staining does not correlate with club cells identified based on ultrastructural features and may indicate subcategories of secretory SCGB1A1 cells of differing function including metabolic activity within the conducting airways. It may also indicate that a higher intensity of SCGB1A1 staining correlates with classically defined club cell identity. This subcategorization is further supported by the observation that the SCGB1A1-positive cells within the TBs were negative for MUC5B unlike all other SCGB1A1-stained cells in the lung [4]. The use of SCGB1A1 as a marker to exclusively define club cells is further challenged by studies demonstrating a lack of staining in the human TB and respiratory bronchiole [128, 130]. This incongruity is also observed in closely related primate species, where morphologically and ultrastructurally identified club cells are present within the terminal and respiratory bronchioles of rhesus macaques [131], but only sporadically stain for SCGB1A1, with the greatest staining observed in the proximal and mid-level airways [132]. In addition, single-cell sequencing of the proximal human airway could not clearly distinguish between club cells and mucin-secreting goblet cells based on gene expression [70, 95]. Another ScSeq study revealed that differences among human secretory cells were mostly associated with differences in cellular maturity levels, with less mature secretory cells expressing basal cell markers and more mature cells expressing MUC5B [111]. Observations that goblet cells show higher levels of MUC5AC, MUC5B, and SCGB1A1 than club cells and that goblet cells are rarely present in healthy human bronchi have led to the suggestion that goblet cells could be, in fact, overactive club cells [133]. This further supports the subcategorization of secretory cells that may fall under the umbrella of “club cells.”

Improved markers for human secretory club cells and SA cell types are needed to help define regional differences in cellular metabolism, chemical transport, and toxicity. This is not only important for intact tissue analysis but also for in vitro cell characterization, where airway region- and cell type-specific mechanistic toxicological analysis can be carried out. Some studies have started to explore human airway regional differences in more detail and may help to identify such markers. For example, one study compared human bronchiolar and bronchial airway brushings using RNA-seq analysis [25]. No significant difference in SCGB1A1 expression was observed between SA bronchioles (Generations 10–12) and bronchial airways (Generations 4–6) [25], whereas SCGB3A2 was exclusively expressed in SA samples [25]. This marker and others such as claudin 22, identified within SA SCGB1A1-expressing club cells using ScSeq analysis [134], further support the idea that phenotypically different cells are present within SA. Importantly, no studies to date have focussed on the more distal SAs, including the terminal and respiratory bronchioles. Given the prominence of the distal SA in toxicological injury, analysis of the club cell phenotype in these regions would provide important information on how the SA may possess inherent susceptibility to injury at a cellular level.

Conclusions

Structural features of the lung along the tracheobronchial tree can influence dose levels for particle inhalation and chemical exposure and may explain part of the sus-
ceptibility of SA to toxicant injury. Cell compositional differences between airway regions, such as a greater number of metabolically active club cells within the SA may also contribute to a differential response to toxicant exposure. Animal studies support this inherent susceptibility in distal conducting airway regions, but detailed study in humans has yet to be carried out. Functional toxicological studies targeting human regional variation is hampered by a lack of clarification of regional cell composition along the airway generations. Definitions and localization of metabolically active club cells are increasingly unclear. New technologies including ScSeq and spa-

**Fig. 3.** Overview of SA susceptibility to toxicant-induced injury and airway disease. Structural and cellular compositional differences are summarized as key observations and mechanisms of differential susceptibility to injury. Connections are either facilitatory (→) or inhibitory (⊥). Dashed lines indicate more speculative interactions. SA, small airway; LA, large airway.
tial transcriptomics have the potential to define cell types along airway generations in previously unprecedented detail. This will demonstrate whether human distal airways including the TB possess greater club cell biotransformation activity than other SA and proximal regions. Such information can then be used to facilitate understanding of disease aetiology, therapeutic targets and to refine modelling approaches for toxicological assessments including in vitro systems. A summary of the mechanisms involved in SA susceptibility to injury is provided in Figure 3.

Finally, recent comparisons between the mouse and human lung using scRNAseq revealed <10% conserved expression patterns within cell types across species [113]. This cellular phenotype discordance, along with species-specific CYP chemical reactivity, likely contributes to the difficulties in extrapolation of animal studies to human risk. It also argues strongly for the refinement of human in vitro models to more accurately characterize molecular toxicological profiling.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Funding Sources

The work was funded by the Marie Skłodowska-Curie Action-Innovative Training Network project in3, under Grant No. 721975. This study is also part funded by the UK National Institute for Health Research (NIHR) Health Protection Research Unit in Environmental Exposures and Health, a partnership between Public Health England and Imperial College London. The views expressed are those of the authors and not necessarily those of the NIHR, Public Health England or the Department of Health and Social Care.

Author Contributions

M.O.L. and L.F.H.F. had equal roles in the conception, collation of information, and writing of this review.

References

1 Peake JL, Pinkerton KE. Gross and subgross anatomy of lungs, pleura, connective tissue septa, distal airways, and structural units. In: Comparative biology of the normal lung: second edition. Elsevier Inc.; 2015. p. 21–31.

2 Weibel ER. Geometric and dimensional airway models of conductive, transitory and respiratory zones of the human lung. In: Morphometry of the human lung. Berlin, Heidelberg: Springer; 1963. p. 136–42.

3 Wiggs BR, Moreno R, Hogg JC, Hilliam C, Paré PD. A model of the mechanics of airway narrowing. J Appl Physiol. 1990;69(3):849–60.

4 Okada K, Chen G, Subramani DB, Wolf M, Gilmore RC, Kato T, et al. Localization of secretory mucins MUC5AC and MUC5B in normal/healthy human airways. Am J Respir Crit Care Med. 2019;199(6):715–27.

5 Young CD, Moore GW, Hutchins GM. Connective tissue arrangement in respiratory airways. Anat Rec. 1980;198(2):245–54.

6 Nomellini V, Chen H. Murray and Nadel's textbook of respiratory medicine. JAMA. 2012;307(1):92–3.

7 Hunninghake GW, Gadek JE, Kawaiami O, Ferrans VJ, Crystal RG. Inflammatory and immune processes in the human lung in health and disease: evaluation by bronchoalveolar lavage. Am J Pathol. 1979;97(1):149.

8 Burgele PR, Bergeron A, de Blic J, Bonnait P, Bourdin A, Chanez P, et al. Small airways diseases, excluding asthma and COPD: an overview. Eur Respir Rev. 2013;22(128):131–47.

9 Beasley MB. Smoking-related small airway disease – a review and update. Adv Anat Pathol. 2010;17(4):270–6.

10 Hogg JC, Macklem PT, Thurlbeck WM. Site and nature of airway obstruction in chronic obstructive lung disease. N Engl J Med. 1968; 278(25):1355–60.

11 Niewoehner DE, Kleinerman J, Rice DB. Pathologic changes in the peripheral airways of young cigarette smokers. N Engl J Med. 1974;291(15):755–8.

12 Hogg JC, Paré PD, Hackett TL. The contribution of small airway obstruction to the pathogenesis of chronic obstructive pulmonary disease. Physiol Rev. 2017;97(2):529–52.

13 Hogg JC, McDonough JE, Suzuki M. Small airway obstruction in COPD: new insights based on micro-CT imaging and MRI imaging. Chest. 2013;143(5):1436–43.

14 Park JY, Ryu H, Lee B, Ha DH, Ahn M, Kim S, et al. Development of a functional airway-on-a-chip by 3D cell printing. Biofabrication. 2019;11(1):015002.

15 Hogg JC, Chu F, Utkaparch S, Woods R, Elliott WM, Buzatu L, et al. The nature of small-airway obstruction in chronic obstructive pulmonary disease. N Engl J Med. 2004 Jun; 350(26):2645–53.

16 Wu M, Gordon RE, Herbert R, Padilla M, Moline J, Mendelson D, et al. Case report: lung disease in world trade center responders exposed to dust and smoke: carbon nanotubes found in the lungs of world trade center patients and dust samples. Environ Health Perspect. 2010;118(4):499–504.

17 Zhang LP, Zhang X, Duan HW, Meng T, Niu Y, Huang CF, et al. Long-term exposure to diesel engine exhaust induced lung function decline in a cross sectional study. Ind Health. 2017;55(1):13–26.

18 Cui X, Li Z, Teng Y, Barkjohn KK, Norris CL, Fang L, et al. Association between bedroom particulate matter filtration and changes in airway pathophysiology in children with asthma. JAMA Pediatr. 2020;174(6):533–42.

19 Karr C, Lumley T, Schreuder A, Davis R, Larson T, Ritz B, et al. Effects of subchronic and chronic exposure to ambient air pollutants on infant bronchiolitis. Am J Epidemiol. 2007; 165(5):533–60.

20 Ghanei M, Mokhtari M, Mohammad MM, Aslani J. Bronchiolitis obliterans obliterans following exposure to sulfur mustard: chest high resolution computed tomography. Eur J Radiol. 2004;52(2):164–9.

21 Van Rooy FG, Rooyackers JM, Prokop M, Houb Ra, Smit LA, Heederik DJ. Bronchiolitis obliterans syndrome in chemical workers producing diacetyl for food flavorings. Am J Respir Crit Care Med. 2007;176(5):498–504.

22 Devouassoux G, Coutin V, Lioret H, Marchand E, Frachon I, Schuller A, et al. Characterisation of severe obliterative bronchiolitis in rheumatoid arthritis. Eur Respir J. 2009; 33(5):1053–61.

23 Massin F, Fur A, Reybet-Degat O, Camus P, Jeannin L. [Busulfan-induced pneumopathy]. Rev Mal Respir. 1987;4(1):3–10.
Small Airway Susceptibility to Injury

Respiration 2022;101:321–333
DOI: 10.1159/000519344

24 Bunawan H, Bunawan SN, Baharum SN, Noor NM, Saurous androgenus (L.) Merr. induced bronchiolitis obliterans: from botanical studies to toxicology. Evid Based Complement Alternat Med. 2015;2015:14158.

25 Yang J, Zuo WL, Fukui T, Chao I, Gomi K, Lee B, et al. Smoking-dependent distal-to-proxi- mal repatterning of the adult human small airway epithelium. Am J Respir Crit Care Med. 2017;196(3):340–52.

26 Baskoro H, Sato T, Karasutani K, Suzuki Y, Mitsui A, Arano N, et al. Regional heteroge- neity in response of airway epithelial cells to cigarette smoke. BMC Pulm Med. 2018;18(1):148–11.

27 Boucher RC, Van Scott MR, Willumsen N, Stutts MJ. 3. Epithelial injury. Mechanisms and cell biology of airway epithelial injury. Am Rev Respir Dis. 1988;138(6 Pt 2):S41–4.

28 Castell JV, Donato MT, Gómez-Lechón MJ. Metabolism and bioactivation of toxicants in the lung. 1990:472-cellular approach. Exp Toxicol Pathol. 2005;57 Suppl 1(1):189–204.

Dinis-Oliveira RJ, Duarte JA, Sánchez-Navarro A, Remião F, Bastos ML, Carvalho F. Parau-quat poisonings: mechanisms of lung toxicity, clinical features, and treatment. Crit Rev Toxicol. 2008;38(1):13–71.

30 Gloede E, Cichocki JA, Baldino JB, Morris JB. A validated hybrid computational fluid dy- namics-physiologically based pharmacokinet- ics model for respiratory tract vapor absorp- tion in the human and rat and its applica- tion to inhalation dosimetry of diacetyl. Toxicol Sci. 2011;123(1):231–46.

33 Asgharian B, Hofmann W, Bergmann R. Partic- le deposition in a multiple-path model of the human lung. Aerosol Sci Technol. 2001; 34(4):332–9.

34 Schenker MB, Pinkerton KE, Mitchell D, Vallyathan V, Elvine-Kreis B, Green FH, Scherer PW. A model of cigarette smoke particle deposition. Am Ind Hyg Assoc J. 1990;51(5):245–56.

35 Tsuchiya K, Toyoshima M, Kiamiya Y, Naka- mura Y, Baba S, Suda T. Non-smoking chronic obstructive pulmonary disease attributed to occupational exposure to silica dust. Intern Med. 2017;56(13):1701–4.

36 Bernard AM, Gonzalez-Lorenzo JM, Siles T, Trujillano G, Lauweys R. Early decrease of serum Clara cell protein in silica-exposed workers. Eur Respir J. 1994;7(11):1932–7.

37 Choudat D, Frisch C, Barrat G, el Khohtou A, Conso F. Occupational exposure to amorph- ous silica dust and pulmonary function. Br J Ind Med. 1990;47(11):763–6.

38 Hjortsberg U, Ørbaek P, Arboelius M, Rans- tam J, Welinder H. Railroad workers with pleural plaques: II. Small airway dysfunction among asbestos-exposed workers. Am J Ind Med. 1988;14(6):643–6.

39 Craighead JE, Abraham JL, Churg A, Green FH, Kleinerman J, Pratt PC, et al. The pathol- ogy of asbestos-associated diseases of the lungs and pleural cavities: diagnostic criteria and proposed grading schema. Report of the Pneumoconiosis Committee of the College of American Pathologists and the National In- stitute for Occupational Safety. Arch Pathol Lab Med. 1982;106(11):544–96.

40 Churg A, Brauer M, Vedal S, Stevens B. Ampli- ment mineral particles in the small airways of the normal human lung. J Environ Med. 1999; 1(1):39–45.

41 Riebeling C, Luch A, Götze ME. Comparative modeling of exposure to airborne nanoparti- cles released by consumer spray products. Nanotoxicology. 2016;10(3):343–51.

42 Manojkumar N, Srimuruganandam B, Shiva Nagendra SM. Application of multiple-path particle dosimetry model for quantifying age specified deposition of particulate matter in human airway. Ecotoxicol Environ Saf. 2019; 168:241–8.

43 Asgharian B, Hofmann W, Bergmann R. Particle deposition in a multiple-path model of the human lung. Aerosol Sci Technol. 2001; 34(4):332–9.

44 Kolanjiyl AV, Kleinstreuer C. Nanoparticle mass transfer from lung airways to systemic regions-part I: whole-lung aerosol dynamics. J Biomed Eng. 2013;15(2):121003–11.

45 Daellenbach KR, Uzu G, Jiang J, Cassagines LE, Leni Z, Vlachou A, et al. Sources of particulate-matter air pollution and its oxidative potential in Europe. Nature. 2020;587(7834): 414–9.

46 Fishler R, Hofmeier P, Etzion Y, Dubowsky Y, Snitman J, Particle dynamics and deposi- tion in true-scale pulmonary ancin models. Sci Rep. 2015;5(1):14071–11.

47 Brody AR, Roe MW. Deposition pattern of in- organic particles at the alveolar level in the lungs of rats and mice. Am Rev Respir Dis. 1983;128(1):24–9.

48 Kuehl PJ, Anderson TL, Candelaria G, Gersh- man B, Harlin K, Hesterman JY, et al. Regional- ized particle-matter air pollution and its oxidative potential in Europe. Nature. 2020;587(7834): 414–9.

49 Silva RM, Xu J, Saiki C, Anderson DS, Franz LM, Vulpe CD, et al. Short versus long silver nanowires: a comparison of in vivo pulmonary effects post instillation. Part Fibre Toxi- col. 2014;11(1):52–21.

50 Guo C, Buckley A, Marczylo T, Seiffert J, Meyer G, Meyer P, et al. Deposition, reten- tion, and translocation of ultrafine particles from the central airways and lung periphery. Am J Respir Crit Care Med. 2008;177(4):426–32.

51 Wine JJ, Joo NS. Submucosal glands and air- way defense. Proc Am Thorac Soc. 2004(11): 47–53.

52 Wine JM, Durie RL. Site specificity of newt skin injury. J Biomech. 2016;51:312–8.

53 Schachter EN, Zuckin E, Saric M. Occupa- tional airway diseases. Rev Environ Health. 2001;16(2):87–95.

54 Weiss SM, Lakshminarayan S. Acute inhalation injury. Clin Chest Med. 1994;15(1):103–16.

55 Diller WF. Pathogenesis of phosgene poison- ing. Toxicol Ind Health. 1985;11(2):7–15.

56 Cho HY, Zhang LY, Kleeberger SR. Ozone- induced lung inflammation and hyperreactiv- ity are mediated via tumor necrosis factor-alpha receptors. Am J Physiol Lung Cell Mol Physiol. 2001;280(3):L537–46.

57 Prysor WA, Church DF. Aldehydes, hydrogen peroxide, and organic radicals as mediators of ozone toxicity. Free Radic Biol Med. 1991; 11(1):41–6.

58 Sunil VR, Yayas KN, Massa CB, Gow AJ, Laskin JD, Laskin DL. Ozone-induced injury and oxidative stress in bronchiolar epithelium are associated with altered pulmonary me- chanics. Toxicol Sci. 2013;133(2):309–19.

59 Corley RA, Kabilan S, Kuprat AP, Carson JP, Minard KR, Jacob RE, et al. Comparative computational modeling of airflow and vapor dosimetry in the respiratory tracts of rat, monkey, and human. Toxicol Sci. 2012; 128(2):500–16.

60 Morris JB, Hubbs AF. Inhalation dosimetry of diacetyl and butyric acid, two components of butter flavoring vapors. Toxicol Sci. 2009; 108(1):173–83.

61 Kreis K, Gomaa A, Mulligan G, Fedan K, Si- moes EJ, Enright PL. Clinical bronchiolitis obliterans in workers at a microwave-popcorn plant. N Engl J Med. 2002;347(5):330–8.

62 US Department of Health and Human Ser- vices. How tobacco smoke causes disease. The biology and behavioral basis for smoking-at- tributable disease. A report of the surgeon general. 2010.

63 Mauroy B, Flaud P, Pelca D, Faussier C, Mer- cix J, Mitchell BR. Toward the modeling of mucus draining from human lung: role of air- ways deformation on air-mucus interaction. Front Physiol. 2015 Aug;6:214.

64 Manke A, Wang L, Rojanasakul Y. Mechani- nisms of nanoparticle-induced oxidative stress and toxicity. Biomed Res Int. 2013; 2013:942916.

65 Möller W, Felten K, Sommerer K, Scheuch G, Meyer G, Meyer P, et al. Deposition, reten- tion, and translocation of ultrafine particles from the central airways and lung periphery. Am J Respir Crit Care Med. 2008;177(4):426–32.
82 Imkamp K, Bernal V, Grzegorzcyk M, Horvathová P, Vermeulen CJ, Hejink IH, et al. Gene network approach reveals co-expression patterns in nasal and bronchial epithelium. Sci Rep. 2019;9(1):15835–13.

83 Zhang X, Sebastiani P, Liu G, Schembri F, Zhong X, Dumas YM, et al. Similarities and differences between smoking-related gene expression in nasal and bronchial epithelium. *Physiol Genomics*. 2010;41(1):1–8.

84 Hukkanen J, Pelkonen O, Hakkola J, Raunio H. Expression and regulation of xenobiotic-metabolizing cytochrome P450 (CYP) enzymes in human lung. *Crit Rev Toxicol*. 2002;32(5):391–411.

85 Menoret A, Kumar S, Vella AT. Cytochrome b5 and cytochrome P450 1A1 are biomarkers in bronchoalveolar fluid signifying onset of acute lung injury. *PLoS One*. 2012;7(7):e40184.

86 Tábárán AF, O’Sullivan MG, Seabloom DE, Cosson J, Schoevaert D, Blaive B. Ciliary beat frequency in human bronchi and bronchioles. *Sci Rep*. 2019;9(1):15835–13.

87 Jersey DA, Holgate ST. The airway epithelial cell atlas of the human bronchiole. *Respir Physiol Neurobiol*. 2016;224:51–64.
Small Airway Susceptibility to Injury

107 Fretland AJ, Omiecinski CJ. Epoxide hydrolases: biochemistry and molecular biology. Chem Biol Interact. 2000;129(1–2):41–59.

108 Collier JK, Fritz P, Zanger UM, Siegel I, Eichelbaum M, Kroemer HK, et al. Distribution of microsomal epoxide hydrolase in humans: an immunohistochemical study in normal tissues, and benign and malignant tumours. Histochem J. 2001;33(6):329–36.

109 Zhilionis R, Choo-Wing R, Fretland AJ, Omiecinski CJ. Epoxide hydrolase in human bronchial mucosa and peripheral lung tissues. Eur J Cancer. 1998;34(6):914–20.

110 Pardo-Saganta A, Law BM, Gonzalez-Mace K, Bowman ED, Vautravers P, Shields PG, Harris CC, Pfeifer AM. Characterisation of xenobiotic-metabolising enzyme expression in human bronchial mucosa and peripheral lung tissues. Eur J Cancer. 1998;34(6):914–20.

111 Windmill KF, Gaedigk A, HallPauline de la PM, Samarutunga H, Grant DM, Mcmanus ME. Localization of N-Acetyltransferases NAT1 and NAT2 in human tissues. Toxicol Sci. 2000;54:19–29.

112 Wang X, Allen WE, Wright MA, Sylwestrak CM, Vinarsky V, Rajagopal J. Ciliated cell atlas of the human lung from single-cell RNA sequencing. Nature. 2020;587(7835):eaat5691.

113 Celeiro M, Vinarsky V, Rajagopal J. Ciliated cell atlas of the human lung from single-cell RNA sequencing. Nature. 2020;587(7835):eaat5691.

114 Windmill KF, Gaedigk A, HallPauline de la PM, Samarutunga H, Grant DM, Mcmanus ME. Localization of N-Acetyltransferases NAT1 and NAT2 in human tissues. Toxicol Sci. 2000;54:19–29.

115 Fretland AJ, Omiecinski CJ. Epoxide hydrolases: biochemistry and molecular biology. Chem Biol Interact. 2000;129(1–2):41–59.

116 Wang X, Allen WE, Wright MA, Sylwestrak CM, Vinarsky V, Rajagopal J. Ciliated cell atlas of the human lung from single-cell RNA sequencing. Nature. 2020;587(7835):eaat5691.

117 Eng CL, Lawson M, Zhu Q, Dries R, Koulena N, Takei Y, et al. Transcriptome-scale super-resolved imaging in tissues by RNA seq-FISH. Nature. 2019;568(7751):235–9.

118 Singh G, Katsal PL. Clara cell proteins. Ann N Y Acad Sci. 2000;923:43–58.

119 Boers JE, Ambergen AW, Thunnissen FB. Number and proliferation of clara cells in normal human airway epithelium. Am J Respir Crit Care Med. 1999;159(5 Pt 1):1585–91.

120 Lumsdon AB, Mclean A, Lamb D. Goblet and Clara cells of human distal airways: evidence for smoking induced changes in their numbers. Thorax. 1984;39(11):844–9.

121 Plopper CG. Comparative morphologic features of bronchiolar epithelial cells. The Clara cell. Am Rev Respir Dis. 1983;128(2P2):S37–41.

122 Cutz E, Conen PE. Ultrastructure and cytochemistry of Clara cells. Am J Pathol. 1971;62(1):127.

123 André-Bougaran J, Pariente R, Legrand M, Cayrol E. Normal ultrastructure of the pseudostratified airway epithelium do not become mucous cells after ovalbumin challenge. Am J Respir Cell Mol Biol. 2013;48(3):364–73.

124 McDowell EM, Barrett LA, Glavin F, Harris CC, Trump BF. The respiratory epithelium. I. Human bronchus. J Nati Cancer Inst. 1978;61(2):539–49.

125 Rhodin JA. The ciliated cell. Ultrastructure and function of the human tracheal mucosa. Am Rev Respir Dis. 1969;93(3P2):Suppl1–15.

126 Mercer RR, Russell ML, Roggli VL, Crapo JD. Cell number and distribution in human and rat airways. Am J Respir Cell Mol Biol. 1994;10(6):613–24.

127 Chang H, Chang LW, Cheng YH, Tsai WT, Tsai MX, Lin P. Preferential induction of CYP1A1 and CYP1B1 in CCSP-positive cells. Toxicol Sci. 2006;89(1):205–13.

128 Singh G, Singh J, Katyal SL, Brown WE, Kramps JA, Paradis IL, et al. Identification, cellular localization, isolation, and characterization of human Clara cell-specific 10 KD protein. J Histochem Cytochem. 1988;36(1):73–80.

129 Asabe K, Tsuji K, Handa N, Kajiwara M, Saita S. Expression of Clara cell 10-kDa protein (CC10) in congenital diaphragmatic hernia. Pediatr Surg Int. 1998;14(1–2):36–9.

130 Engelhardt JF, Zepeda M, Cohn JA, Yankaskas JR, Wilson JM. Expression of the cystic fibrosis gene in adult human lung. J Clin Invest. 1994;93(2):737–49.

131 Castleman WL, Dungworth DL, Tyler WS. Intrapulmonary airway morphology in three species of monkeys: a correlated scanning and transmission electron microscopic study. Am J Anat. 1975;142(1):107–21.

132 Coppens JT, Van Winkle LS, Pinkerton K, Plopper CG. Distribution of Clara cell secretory protein expression in the tracheobronchial airways of rhesus monkeys. Am J Physiol Lung Cell Mol Physiol. 2007;292(5):L1155–62.

133 Ruiz García S, Deprez M, Lebrigrand K, Cavard A, Paquet A, Arguel MJ, et al. Novel dynamics of human mucociliary differentiation revealed by single-cell RNA sequencing of nasal epithelial cultures. Development. 2019;146(20):17.

134 Zuo WL, Shenoy SA, Li S, O’Beirne SL, Strulovici-Barel Y, Leopold PL, et al. Ontogeny and biology of human small airway epithelial club cells. Am J Respir Crit Care Med. 2018;198(11):1375–88.