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Cellular Mechanisms of Alveolar Pathology in Childhood Interstitial Lung Diseases: Current Insights from Mouse Genetics

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Abstract

Purpose of Review—Childhood Interstitial Lung Diseases (ILDs) are a diverse class of disorders affecting the alveolar gas exchange region that lack specific treatments and are usually fatal. Here, we integrate recent insights into alveolar cell biology with histopathology from well-characterized mutations of surfactant-associated genes. We take a reductionist approach by parsing discrete histological features and correlating each to perturbation of a particular function of the alveolar epithelial type 2 (AT2) cell, the central driver of disease, in order to generate a working model for the cellular mechanisms of disease pathogenesis.

Recent Findings—The application of genetically modified mice and single cell genomics has yielded new insights into lung biology, including the identification of a bipotent alveolar progenitor in development, mapping of adult AT2 stem cells *in vivo*, and demonstration that latent cooperative interactions with fibroblasts can be pathologically activated by targeted injury of the AT2 cell.

Summary—As we learn more about individual and cooperative roles for alveolar cells in health, we can dissect how perturbations of specific cellular functions contribute to disease in childhood ILDs. We hope our updated model centered around the AT2 cell as the initiator of disease provides a cellular framework that researchers can build upon and revise as they identify the specific molecular signals within and between alveolar cells that mediate the diverse pathologic features, so that targeted pharmacologic and cell-based treatments for patients can ultimately be engineered.

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Conflicts of interest

None

Keywords

Childhood Interstitial Lung Disease; Surfactant; Alveolar epithelial type 2 cell

I. Introduction

The term childhood Interstitial Lung Disease (ILD) is applied to respiratory disorders with defects in gas exchange resulting from diffuse abnormalities of the gas exchange (alveolar) region of the lungs that are not infectious in origin[1][2]. Childhood ILDs are clinically and pathologically heterogeneous, although well-described cohorts of patients and families in which full term newborns presented with severe neonatal respiratory distress led to the discovery of causal mutations in several surfactant-associated genes, including adenosine triphosphate (ATP) binding cassette transporter A3 (ABCA3)[3], surfactant associated protein B (SFTPB)[4], and surfactant associated protein C (SFTPC)[5]. Surgical lung biopsy findings, chest imaging results, and clinical presentations often overlap, but no specific therapies exist and many cases lead to death in the neonatal period[1,6]. There is a growing consensus that diffuse lung diseases presenting in infancy are distinct from those in adults, and therefore should no longer be classified using the adult nomenclature. This awareness has motivated the generation of new classification schemes for childhood ILDs by expert panels, which is an ongoing effort[1,2,7]. A major challenge to meaningful classification is our limited understanding of the underlying cellular and molecular basis for overlapping and unique aspects of the observed alveolar pathologies. Fortunately, modern tools and strategies enabling genome-scale molecular analysis[4,8] and, in mice, *in vivo* cell fate-mapping[5,9] and gene manipulation[10] targeting specific alveolar cell types, have yielded important advances in lung biology. These include recent insights into how alveolar epithelial cells develop[11] and are maintained during aging and following injury[12]. Here, we integrate our updated understanding of alveolar cell biology with detailed histopathological descriptions of childhood ILDs to generate working models for the cellular mechanisms of disease pathogenesis. We focus on ILDs presenting in infancy or early childhood with respiratory distress that are collectively classified as disorders of surfactant dysfunction **[13], for which the causative mutations are known and their molecular consequences well characterized. We hope our cellular framework positioning the alveolar epithelial type 2 (AT2) cell as the central driver of disease provides a scaffold upon which the specific molecular signals that mediate the alveolar pathology can be superimposed. Once a comprehensive understanding of the cellular and molecular basis for disease pathogenesis in childhood ILDs has been achieved, treatments may be devised that target the specific cells and molecular signals driving particular aspects of the alveolar pathology and dysfunction.

II. Recent discoveries in alveolar cell biology with significant implications for childhood ILDs

A recent finding of importance for childhood ILDs is evidence indicating that AT1 and AT2 cells develop independently from a bipotent alveolar progenitor in the embryo, which contradicts the traditional view that AT1 cells derive exclusively by trans-differentiation of existing AT2 cells **[14]. The new model is based on several lines of evidence, including

immunohistochemical analysis during alveolar cell differentiation as well as indelible and heritable genetic marking of newly-formed AT2 cells, which were followed (fate-mapped) and found not to contribute to AT1 cells in development. In a follow-up study, transcriptional profiling of individual alveolar progenitor cells at progressive stages of maturation was performed using single cell RNA-sequencing, providing an incredibly rich (genomic) molecular characterization of the bipotent progenitor and the dynamic changes it undergoes as it differentiates into either an AT1 or AT2 cell*[15]. AT2 cells were also fate-mapped in the adult lung, which showed that rare AT2 cells functioned as stem cells, demonstrating long-term self-renewal, multipotency, and activity throughout the lifespan[14]. Execution of the progenitor function appeared to involve two steps, the first being proliferation of a mature (surfactant-producing) AT2 cell, followed by direct trans-differentiation of one of the daughter AT2 cells into an AT1 cell. In some cases, for instance after selective AT2 cell ablation, the AT1 cell trans-differentiation step did not occur and both daughter cells retained an AT2 identity[16]. Interestingly, rare “back-up” (non-surfactant producing, non-AT2) stem cells have recently been identified that are activated in severe influenza pneumonia with widespread alveolar cell death, but do not appear to function during aging[17,18]. Whether these rare cells are present in embryonic or infant lungs is unknown.

Another mouse genetic experiment relevant for childhood ILDs also focused on the AT2 cell, although it was designed to investigate the pathogenesis of adult Idiopathic Pulmonary Fibrosis (IPF)[19]. This study was performed to test the model that failure to maintain an intact alveolar epithelium, for instance due to loss of AT2 stem cells, results in the myofibroblast activation and collagen deposition seen in IPF[20]. The investigators therefore attempted to specifically ablate AT2 cells in adult mice by genetically expressing the human diphtheria toxin receptor under control of the SFTPC promoter. Surprisingly, targeted AT2 cells were not killed when diphtheria toxin was administered, yet significant local fibrosis resulted after two weeks of daily toxin injections*[21]. This experiment shows that, at least from this particular injury in adult lungs, intrinsic perturbation of AT2 cells (that does not result in cell death) alone is sufficient to trigger a local alveolar fibrotic response. The significance of this finding and of the other recent discoveries in alveolar biology for childhood ILDs will be discussed below.

III. Features of and proposed cellular mechanisms for severe childhood ILDs presenting in infancy

ABCA3 and SFTPB mutations resulting in absent protein

The ABCA3 gene is expressed in AT2 cells and encodes a membrane transporter protein that localizes to cytoplasmic organelles called lamellar bodies (LBs)[22] that contain surfactant phospholipid, of which surfactant associated protein B (SP-B) is an important component [23]. Both ABCA3 and SFTPB are indispensable for functional surfactant, and lamellar bodies are not detectable in AT2 cells of patients with mutations in both copies of either gene that result in loss of protein expression[4,24,25]. Classical reports of ABCA3 deficiency described ultrastructural defects in the AT2 cell cytoplasm, specifically the presence of many dense inclusion bodies, along with the absence of normal LBs[3,26].

Accumulations of alveolar macrophages and proteinaceous material in distal air sacs (originally referred to as an infantile Desquamative Interstitial Pneumonitis (DIP) pattern), AT2 cell hyperplasia, and interstitial “thickening” were also reported with ABCA3 deficiency. The pathologic findings significantly overlap with SFTPB mutations, which similarly result in alveolar proteinosis, AT2 cell hyperplasia, and interstitial “widening.” Fibrosis in the absence of prominent inflammation has also been observed in several cases, but may not be a consistent feature since it was not described in every report[27] (Table 1).

A canonical function of the AT2 cell is synthesizing, secreting, and recycling surfactant that coats the epithelial surface and minimizes surface tension to prevent alveolar collapse[23]. Pulmonary surfactant is primarily composed of phospholipids and its production initiates in maturing AT2 cells during late gestation[32] when the distinctive lamellar bodies can first be detected[33]. As expected, term infants with ABCA3 or SP-B deficiency present with respiratory failure, similar to infants born prematurely who have not yet begun producing surfactant. However, these infants cannot be rescued by exogenous surfactant replacement, suggesting additional consequences of these mutations. Since AT2 cells do not function as progenitors in development, AT1 cells presumably undergo normal differentiation directly from bipotent progenitors, implicating the AT2 cell as the sole driver of the observed pathologies. However, the histological findings of AT2 cell hyperplasia and fibrosis are not easily explained by deficiency of surfactant homeostasis (Figure 1A) alone. We propose the pneumocyte hyperplasia results from inappropriate activation of the proliferative step in the AT2 stem cell program (Figure 1B), although it is not clear that the hyperplasia adversely impacts gas exchange. Alternatively, AT2 cells could be appropriately proliferating in an attempt to repair acute alveolar epithelial injury, in which case the hyperplasia might reflect impairment of the second step of the AT2 stem cell program, AT1 cell trans-differentiation. For the fibrosis, we propose that a fibroblast-activating signal is produced by affected AT2 cells, perhaps in the same signal triggered in the AT2 cell-targeted injury mouse genetic experiment described above (Figure 1C). Why might an injury restricted to the AT2 cell specifically activate alveolar fibroblasts and induce them to produce collagen? AT2 cells have been long known to maintain an intimate physical association with fibroblasts[34], and coordinated activity of these two cell types has been proposed during normal alveolar development [35] and post-pneumonectomy re-growth in rodents[36]. It has also recently been demonstrated that AT2 cell proliferation *in vitro* is significantly enhanced by co-culture with alveolar fibroblasts, providing further evidence of their functional interactions[16]. The identity of the putative fibroblast-activating signal is unknown, as is the nature of the AT2 cell-intrinsic dysfunction that triggers its production, but it does not appear to be a generalized response of AT2 cells to injury, since fibrosis is apparently not a feature of infantile ILD from SFTPC mutations.

SFTPC gene mutations resulting in misfolded protein

Lungs from infants presenting with respiratory failure from SP-C mutations also share a characteristic set of histologic findings (Table 1), termed chronic pneumonitis of infancy (CPI)[1,28]. This stereotyped pattern was originally described by investigators who used histological features to distinguish and classify childhood diffuse lung diseases[28]. Although this study did not report the associated gene mutations, the constellation of

pneumocyte hyperplasia, interstitial “widening,” and alveolar wall thickening have since been regularly reported in infants with SFTPC mutations[1]. Unlike SP-B and ABCA3, SP-C is not critical for surfactant function and homozygous null mice that lack this protein do not exhibit respiratory failure at birth[37] (in contrast with mice homozygous for deletions of either the SFTPB[38] or the ABCA3 gene[39-41]). Many disease-causing human mutations in the SFTPC gene do not abrogate protein expression, however, but rather map to a C-terminal domain of the pro-SP-C protein required for proper folding and transit through the secretory pathway[42], resulting in cytoplasmic aggregation of misfolded SP-C protein that produces AT2 cell injury and death[42-44]. The presence of only one mutant allele is sufficient to produce this toxicity, consistent with a functionally dominant effect and the observed autosomal dominant pattern of inheritance.

With SFTPC mutations, AT2 cells contain normal lamellar bodies and surfactant function is relatively intact, suggesting that loss of the canonical surfactant function of the AT2 cells is not responsible for the acute respiratory failure. A distinguishing feature of the disease pathophysiology with SFTPC mutations is that because SP-C protein is expressed by epithelial progenitors during lung development, bipotent (and earlier) embryonic progenitors as well as newly-differentiating AT1 cells could also experience toxicity and contribute to the alveolar pathology (Figure 1D). The presence of cholesterol clefts in the air sacs of infants with SFTPC mutations [29] suggests diffuse alveolar cell death, possibly due to widespread epithelial cell toxicity from the misfolded SP-C protein (Figure 1D). Whether the molecular signal producing pneumocyte hyperplasia is the same as in ABCA3 and SFTPB mutations is unknown, but the mechanism of AT2 cell-intrinsic dysfunction is presumably distinct given the differences in ultrastructural features of AT2 cells and absence of alveolar fibrosis in infants with SFTPC mutations. It is also possible that some or even all of the hyperplastic pneumocytes may result from bipotent progenitor toxicity that impairs cellular differentiation into AT1 cells, rather than increased proliferation (Figure 1B). Toxicity in epithelial progenitors at embryonic stages preceding alveolar cell differentiation could additionally dysregulate earlier steps in lung development[44] including epithelial-mesenchymal interactions, which may underlie the observed interstitial “widening” and alveolar wall thickening.

IV. Proposed mechanisms for clinicopathological heterogeneity of ILDs presenting beyond infancy

Interestingly, mutations in the SFTPC gene have also been found in adult ILDs [30,31] and the clinical presentation of familial cases (where affected individuals harbor the identical mutation) can vary widely. The lack of mortality in infancy suggests these mutations are intrinsically less severe or consequential (i.e., “low impact”) than the ones that are uniformly and rapidly fatal. For instance, the same mutations in SP-C have been identified in individuals presenting with pulmonary disease associated with alveolar fibrosis both in adult life and in older children[30]. In such cases, including recently identified patients with ABCA3 mutations that do not abolish protein production[45], we propose clinical disease results from interaction of a “low-impact” mutation with patient-specific genetic modifiers and environmental exposures, as schematized in Figure 2. As they age, such individuals may

experience the cumulative and independent toll of long-term deleterious effects of the mutation and environmental stressors, ultimately presenting with clinically apparent disease. We predict that as subtle lung disease is increasingly detected in adults and next generation sequencing approaches are more widely applied to human cohorts, new mutations in surfactant-associated genes and their modifiers will be identified that contribute to alveolar pathology by dysregulating or depleting an essential AT2 cell function.

V. Conclusion

Genetic disorders of surfactant metabolism provide a valuable opportunity for dissecting the underlying pathogenesis of childhood ILDs, in particular how the AT2 cell participates in mediating various features of the pathology. Because absence or disruption of a single gene is sufficient to produce disease, these mutations are ideal for study using genetically engineered mice[44], which afford a high degree of precision and enable robust experimental modeling not possible in patients. In parallel, increasingly powerful and affordable genomic technologies continue to emerge that can be harnessed to deeply profile distinct cell types and identify specific molecular pathways mediating the alveolar pathology in mouse or human lungs. We hope that our proposed framework for the cellular mechanisms of childhood ILD disease pathogenesis facilitates these efforts.

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Key Points

1. Recent discoveries in lung developmental and alveolar cell biology provide novel insights into the cellular mechanisms of disease pathology in childhood ILDs.
2. Under- or over-activity of distinct alveolar epithelial type 2 (AT2) cell functions can account for specific aspects of childhood ILD pathology.
3. As genomic technologies become more widely applied, new mutations and candidate genes contributing to disease will continue to be discovered in patients with ILDs.
4. Mouse genetic technology provides an opportunity to interrogate the cellular and molecular mechanisms of putative causal mutations in hereditary surfactant associated disorders.

Figure 1. Schematic of canonical AT2 cell functions and proposed perturbations producing pathological features seen in chILD

Distinct AT2 cell functions and proposed dysregulation with surfactant-associated gene mutations (A) The alveolar epithelial type 2 (AT2) cell synthesizes and packages surfactant phospholipid in lamellar bodies prior to secretion into the airspace and assembly into tubular myelin that reduces alveolar surface tension, which is subsequently taken up by alveolar macrophages and AT2 cells and recycled. With ABCA3 or SP-B deficiency, AT2 cells contain abnormal LBs and dense vesicles, and lipo-proteinaceous material accumulates in the alveolar spaces. The combination of surfactant deficiency and alveolar proteinosis impair gas exchange. (B) AT2 stem cells are intermittently active in adult alveoli when they self-duplicate and trans-differentiate into AT1 cells, although they do not appear to execute this function in development. Pneumocyte hyperplasia is observed in surfactant associated gene mutations, which we propose results from inappropriate proliferation or failed differentiation of AT2 cells and/or bipotent progenitors. (C) AT2 cells and alveolar fibroblasts maintain intimate connections and exhibit functional interactions during alveolar formation and repair. With ABCA3 or SP-B deficiency, we propose the AT2 cells are perturbed (indicated by lines outside of cell) but not eliminated, and that this directly results in activation of fibroblasts promoting collagen deposition that impairs gas diffusion. (D) The alveolar epithelial monolayer constitutes the functional gas exchange surface. Cholesterol clefts and cellular debris are found in the air sacs with SFTPC mutations that result in a misfolded and toxic protein. We propose this debris reflects widespread AT2 (and perhaps also bipotent progenitor and nascent AT1) cell death from the toxic protein. Abbreviations: AT, alveolar epithelial cell; BP, bipotent alveolar progenitor.

Figure 2.

Classes of fixed and variable factors and stages of influence on clinical severity in non-lethal hereditary interstitial lung diseases

Abbreviations: BP = bipotent alveolar progenitor; AT1, alveolar epithelial type 1 cell; AT2, alveolar epithelial type 2 cell

Table 1

Characteristic features of childhood interstitial lung diseases associated with surfactant pathway gene mutations

Mutated gene	ABCA3 ^[3,26]	SFTPB ^[4,6,25]	SFTPC ^[1,28,29]	SFTPC ^[30,31]
Clinical onset	infant	infant	infant	child, adult
Inheritance pattern	AR	AR	AD	AD
Mutation consequence on protein	absent	absent	misfolded/toxic	misfolded/toxic
Pathologic feature				
AT 2 ultrastructural defects *	+	+	–	–
Pneumocyte hyperplasia	+	+	+	–
Alveolar proteinosis and lipid-laden (“foamy”) macrophages	+	+	+/-	–
Alveolar fibrosis	+/-	+/-	–	+

AD, autosomal dominant; AR, autosomal recessive

* abnormal or absent lamellar bodies, electron-dense inclusions

(+) consistently reported (–) not reported (+/-) variably reported