Current Technology in the Diagnosis of Developmentally Related Lung Disorders

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Abstract

Respiratory disorders that present in the newborn period may result from structural, functional, or acquired mechanisms that limit gas exchange between the airspace and vascular bed. Exciting new imaging, gene sequencing, mass spectrometry, and molecular and cell-based techniques are enhancing our understanding of mechanisms of disease; highlighting the complexity of interactions between genes, development, and environment in the manifestation of health and disease; and becoming part of the clinical armamentarium for the care of patients. Some of these technologies and their clinical potential are briefly reviewed in this paper.

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Respiratory disorders that present in the newborn period may result from structural, functional, or acquired mechanisms that limit gas exchange between the airspace and vascular bed. On the one hand, an underdeveloped airway or pulmonary vascular bed limits the surface area available for gas exchange. On the other hand, the lung

may be functionally (biochemically) unprepared to exchange gas, although the structural development of the lung is perfectly normal. Finally, the lung may be structurally and functionally mature, but an acquired superimposed condition, such as an infection or meconium aspiration, may lead to respiratory dysfunction.

Disorders of Lung Development

Disorders of Structural Lung Development

Structural disorders of the lung result from a disruption in the normal sequence of development of the pulmonary vascular system, conducting airways, terminal gas exchanging units, or any combination thereof. An exquisitely coordinated interaction of genes and proteins dictates this sequence of development through successive dichotomous branching from the embryonic foregut endoderm [1]. Although the exact cascade of events that results in lung formation has not been completely delineated, it is clear that mutations in some of these genes result in incomplete development. For example, the thyroid transcription factor, encoded by the gene *NKX2-1*, is a critical regulator of lung development and its expression marks the first recognizable step in lung differentiation [2]. Absence of the thyroid transcription factor results in absence of lung development in mice, while heterozygous mutations in this gene result in severe respiratory distress

syndrome (RDS) in newborns and interstitial lung disease in older children [3–5]. Several other transcription factors, including members of the forkhead box (FOX) family, Sry-related high mobility group box (SOX) family, and Sam pointed domain Ets-like factor (SPDEF), among others, are necessary for lung development and respiratory epithelial cell differentiation, as demonstrated in murine lineages. However, only a few of these factors have been linked to neonatal respiratory disease or lung malformations in humans. Mutations in the genes encoding the transcription factors FOXF1 and FOXC2 have been implicated in the genesis of alveolar capillary dysplasia, a developmental disorder of lung vascular development [6, 7]. Deletions of a cluster of genes around the *FOXF1* locus have also been associated with abnormalities in tracheoesophageal development [8]. Aberrant lung growth and development associated with congenital diaphragmatic hernia continues to be an enigma. Although commonly attributed to a mass effect from herniated abdominal contents, some animal models as well as clinical experience suggest a more global disruption of lung airspace, and vascular development contributes to the cases in which there appears to be discordance between the clinically assessed lung volume and actual gas exchanging capacity. In murine lineages, the transcription factors FOG-2 and GATA-4 result in diaphragmatic defects and pulmonary hypoplasia, but mutations in the genes encoding these factors in humans have been identified only infrequently in children with congenital diaphragmatic hernia [9–12].

Aside from genetic factors, mechanical factors also play an important role in structural lung development. Pulmonary hypoplasia is seen in neuromuscular disorders associated with absence of fetal breathing, suggesting that transduction of mechanical stretch into biochemical signals is a critical stimulus for lung development [13, 14]. Conditions with low or absent amniotic fluid, such as prolonged rupture of fetal membranes or disorders of renal development, are also associated with disrupted lung development [15]. However, recent observations suggest that in some cases, the mechanisms of pulmonary maldevelopment may be linked to the renal maldevelopment through a common underlying mechanism. For example, the genes responsible for autosomal dominant polycystic kidney disease (*PKD1*, encoding polycystin) and autosomal recessive polycystic kidney disease (*ARPKHD1*, encoding fibrocystin) are also expressed in primary cilia, and bronchiectasis has been identified in individuals with mutations in *PKD1* [16, 17]. Further, in murine lineages, *Cux1* is expressed in lung

and the developing genitourinary tract and may provide a link between cystic kidney disease and lung development [18].

Disorders of Functional Lung Development

Functional disorders of the lung result from incomplete expression of the biochemical factors necessary for pulmonary cellular homeostasis and gas exchange. The most common and well-known is RDS, which is typically due to a developmentally regulated quantitative deficiency of pulmonary surfactant. The pulmonary surfactant is a unique phospholipid-protein complex that is synthesized, packaged, and secreted by alveolar type II cells with a primary function of lowering surface tension and preventing atelectasis at end-expiration. A regulated cycle of synthesis, intracellular trafficking, secretion, and recycling involves both the phospholipid and protein components of pulmonary surfactant [19, 20]. The development of surfactant replacement therapy has been the single most important therapeutic advance in the care of infants with RDS and has decreased the severity of the disease as well as mortality [21]. However, not all infants respond to surfactant replacement therapy, which has led to the identification of mutations in genes encoding surfactant-associated molecules, including surfactant proteins B and C (*SFTPB* and *SFTPC*), the ATP-binding cassette member A3 (*ABCA3*), and the thyroid transcription factor (*NKX2-1*), that result in severe neonatal RDS as well as chronic interstitial lung disease in children (table 1) [22–26]. The syndrome of retained amniotic fluid, more commonly known as transient tachypnea of the newborn, results from a complex developmental interaction between pulmonary neuroendocrine signals and epithelial ion and water transporters, including the amiloride-sensitive epithelial sodium channels and aquaporins, in the pulmonary epithelium and lack of fluid reabsorption near birth [27–29]. Another occasionally overlooked mechanism of RDS in newborns is primary ciliary dyskinesia in which ciliary dysfunction due to mutations in genes encoding inner and outer dynein arms and other ciliary elements (*DNAH5*, *DNAI1*, and *DNAH11*, to name a few) results in varying forms of thoracic and abdominal isomerism, recurrent pulmonary infections, and RDS [30–32]. The developmental and molecular processes that determine antioxidant capacity, inflammation, and immune regulation are less well characterized and their role in the ability to respond to environmental stress and pulmonary dysfunction remains an active area of investigation [33–36].

Table 1. Surfactant dysfunction disorders that present as neonatal respiratory distress syndrome

	ABCA3	SFTPB	SFTPC	NKX2-1
Clinical presentation	severe neonatal RDS; ILD	severe neonatal RDS	severe neonatal RDS; ILD	severe neonatal RDS; ILD
Inheritance	recessive	recessive	dominant/sporadic	dominant/sporadic
Mutation carrier frequency	~3%	0.1%	<0.1%	unknown
Mechanism	surfactant dysfunction	surfactant dysfunction	aggregation of misfolded proSP-C	surfactant dysfunction; growth arrest
Diagnosis	gene sequencing	gene sequencing	gene sequencing	gene sequencing; copy number analysis
Outcome	neonatal: lethal ILD: variable	lethal	variable	variable

ILD = Interstitial lung disease; ABCA3 = ATP-binding cassette subfamily A No. 3; NKX2-1 = NK2 homeobox 1.

Environmental Influences on Lung Development

Aside from acquired bacterial, viral, or mycoplasma infection and meconium aspiration as common and well-known ‘environmental’ factors that can disrupt lung development, emerging data suggest that exposure to nicotine metabolites, or ‘third-hand’ smoke, may affect alveolar epithelial differentiation and cellular signaling resulting in disruption of pulmonary functional and structural homeostasis [37].

Bronchopulmonary dysplasia represents a combination of disrupted structural and functional lung development resulting from a combination of genetic, environmental and developmental factors. Although the many mechanisms that result in bronchopulmonary dysplasia remain elusive, intrauterine and postnatal inflammation, preterm birth, and need for gas exchange across a structurally and biochemically immature lung combine to further disrupt lung development, prolong the need for respiratory support, and result in long-term morbidity [38].

Technology for Diagnosis of Lung Disorders

The procedures, approach, and technology to diagnose these developmental lung disorders in the newborn period, although more refined and responsive to clinical needs, have not changed dramatically for quite some time. A combination of insights gained from standard diagnostic tests along with knowledge of the natural history of the disease often permits retrospective application of a diagnosis, but this approach does not permit mech-

anism-specific treatment. However, exciting new tools that have been limited to research are now finding their way into clinical use and will provide the next step in understanding the mechanisms of these disorders, permit more sophisticated diagnosis, and ultimately lead to personalized mechanism-based treatment strategies.

Imaging

Chest radiography, despite digital technology permitting decreased radiation exposure and immediate visualization, has not enhanced our ability to specifically diagnose or understand newborn lung disease. High-resolution computed tomography of the chest has provided greater definition and spatial localization of lung pathology; however, only now are quantitative means and more refined descriptions being developed to more accurately provide insights into pulmonary pathology [39, 40].

Functional magnetic resonance imaging (MRI) with spectroscopy is starting to provide insights into brain metabolism and has the potential to be applied to the lung. Hyperpolarized helium MRI has the capability to clearly assess alveolar structural development and gas exchange surface area in a longitudinal fashion [41, 42].

Echocardiography has provided some ability to understand the contribution of the pulmonary vasculature to disorders of gas exchange, but the ability to accurately measure pulmonary arterial pressure in newborns is unreliable, at best [43]. Novel methods that take advantage of the physical properties of ultrasound have the potential to provide more sensitive measures of elevated pulmonary vascular resistance, including backscatter analysis to

characterize the collagen content in myocardium as a marker of tissue remodeling and speckle-tracking to measure regional myocardial strain [44–48]. While validated for measurements of left ventricular structure and function in adults, studies are underway to determine the reliability of these approaches for the neonatal right ventricle.

Molecular Microbiology

For those infants whose respiratory dysfunction may have an infectious basis, molecular microbiological techniques now permit rapid identification of bacterial and viral genomes and provide the ability to limit antibiotic use, but are still limited by access to the best compartment to sample without invasive procedures. The Human Microbiome Project is identifying interactions between a host's genetic background and commensal organisms in the gastrointestinal tract, airway, skin, and genitourinary tract that influence expression of disease [49]. Enteric commensal microorganisms promote development of innate and adaptive immunity through a balance of tolerance to luminal antigens and recognition of pathogens [50]. Dendritic cells in the gastrointestinal tract epithelium monitor the intestinal environment for pathogens and, following recognition of non-commensal organisms, elicit a complex series of immunoregulatory and signaling molecules that maintain gut homeostasis [51, 52]. Disruption of the commensal intestinal community and balance between immune tolerance and activation has the potential to elicit systemic metabolic and inflammatory responses that may manifest as diabetes, obesity, necrotizing enterocolitis, atopy, and asthma [53–56]. The 'hygiene hypothesis' suggests that antibiotic use in early childhood results in delayed acquisition of normal enteric flora and is associated with increased risk for allergic disease [57, 58]. Studies to understand the development of the neonatal microbiome and the relationship to lung disease are underway.

Genomic Medicine

Advances in genomic medicine are currently being applied in many aspects of neonatal care and will contribute immensely to our understanding of neonatal disorders. For example, microarray analyses can interrogate 1.8 million or more probes of common nucleotide and copy number variation across the human genome and are used in routine clinical evaluation to detect genetic aberrations that may be responsible for structural defects of multiple organ systems. The continuing evolution and increasing capacity of next-generation DNA sequencing technology has permitted rapid detection of rare muta-

tions in candidate genes and the application of this technology for clinical decision-making [59, 60]. Furthermore, as the capacity increases and cost decreases, sequencing the entire protein-coding regions of the genome, the exome, will soon be within reach to identify the genetic basis of many Mendelian disorders [61]. As the role of non-coding and regulatory regions of the genome, microRNAs, and epigenetic regulation are more thoroughly understood, sequencing the entire genome holds promise for identifying the complex interactions among networks of genes that account for disease. The next major hurdle will be to translate this mechanistic knowledge into preventative strategies or therapeutic interventions [62].

Other 'Omics: Proteomics/Lipidomics/Metabolomics

Enhanced development of powerful and sensitive mass spectrometry techniques has led to the identification of novel protein, lipid, and metabolic biomarkers that can provide insights into mechanisms of disease and inform potential therapeutic interventions [63–66]. For example, proteomic analysis of serum in infants with late-onset sepsis or necrotizing enterocolitis led to the identification of proapolipoprotein CII and a des-arginine variant of serum amyloid A that permitted risk differentiation and informed decision making for antibiotic administration [67]. Elevated serum levels of KL-6, a glycoprotein that is expressed in type II and bronchiolar cells, may help differentiate children with interstitial lung disease due to surfactant dysfunction mutations from children with neuroendocrine cell hyperplasia of infancy (NEHI) [68]. Targeted lipidomic analysis of surfactant phospholipids has permitted further understanding of in vivo surfactant metabolism [69, 70]. As shotgun methods to identify these biomarkers become more clinically accessible, more refined diagnosis, prognosis, and assessment of response to treatment will become possible.

Induced Pluripotent Stem Cells

An exciting new area of investigation for understanding patient-specific mechanisms of disease has been the discovery that transfection of transcription factors *Klf4*, *Nanog*, *Oct4*, and *Sox2* into somatic cells, such as skin-derived fibroblasts, can result in reprogramming into pluripotent stem cells [71, 72]. These induced pluripotent stem cells can then be directed to differentiate into cells from the organ of interest, such as heart or lung, in which patient-specific mechanisms of disease and patient-specific therapeutic interventions can then be studied [73–

76]. In addition, in vitro correction of mutations using zinc finger nucleases in a cell along the pathway from fibroblast to differentiated cell with subsequent transplantation of these corrected cells offers another promise of therapeutic interventions for patients with diseases of genetic origin [77–79]. Obviously, many anticipated and unanticipated hurdles remain before these possibilities are realized; however, this burgeoning field of investigation will provide novel insights into mechanisms of development and disease.

Conclusions

These and other new approaches will gain more widespread utility in the near future and will provide an exciting new array of diagnostic and therapeutic tools for the

care of newborns and patients in general, permit insights into patient-specific mechanisms of disease, enhance our understanding of fetal and childhood determinants of adult disease, and ultimately provide the ability to develop patient-specific therapeutic interventions as we move toward the goal of ‘personalized’ medicine.

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Disclosure Statement

Dr. Hamvas has no financial conflicts of interest to report.

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