READING BEYOND THE RED: What Fellows Are Reading in Other Journals

Developmental Origins of Chronic Lung Diseases Mechanical Stretch, Micro-RNAs, and Hydrogels

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Recommended Reading from the University of Colorado Fellows; Steven H. Abman, M.D., Program Director

Li *et al.* The Strength of Mechanical Forces Determines the Differentiation of Alveolar Epithelial Cells. *Dev Cell* (1)

Reviewed by Matthew D. McGraw

The primary function of the lungs is to exchange oxygen with carbon dioxide between the external environment and the internal cardiovascular system. Although seemingly straightforward, the development of a fully functional lung is fraught with challenges. To successfully undergo growth and maturation, the lungs develop through five well-orchestrated stages (2). Despite extensive work over the past decades, mechanisms through which mechanical forces contribute to epithelial differentiation and alveologenesis remain incompletely understood.

Using mouse genetics, live imaging, and quantitative cell biology, Li and colleagues (1) demonstrated that mechanical forces act synergistically with local growth factors to drive alveolar epithelial cell differentiation. By aspirating amniotic fluid from mouse embryo yolk sacs, creating a condition of “oligoamnios,” the authors found that mechanical forces generated by inhalation of amniotic fluid are essential for alveolar epithelial type 1 (AT1) cell differentiation. When compared with lungs from littermate controls at Embryonic Day 18.5, terminal sac opening and cell shape flattening of AT1 cells were impaired in oligoamnios-treated embryos as assessed by immunostaining and time-sequenced imaging. The lack of AT1 differentiation was accompanied by a compensatory increase in AT2 epithelial cells, suggesting that mechanical stretch has less impact on AT2 cell differentiation than on AT1 cells.

Secondarily, the authors explored cellular processes associated with sacular dilation and epithelial cell differentiation. Interestingly, the authors observed basal protrusions from cuboidal-shaped airway tip cells before alveolar formation. These cellular protrusions were

actin based and extended into the adjacent mesenchyme. Over time, epithelial cells with basal protrusions constricted, as assessed by phosphorylated nonmuscle myosin light chain II, remained cuboidal in shape, and ultimately differentiated into AT2. Conversely, cells without basal protrusions became thinner and flattened over time, and differentiated into AT1 cells. To examine the mechanism underlying the development of these basal protrusions, the investigators used genetic mice and chemical inhibitors of fibroblast growth factor (FGF) 10. In the heterozygous FGF10 embryos or after chemical FGF10 inhibition in wild-type embryos, the number of progenitor cells with protrusions decreased, suggesting that protrusion and its associated mesenchymal connection were FGF10 dependent.

These findings have significant clinical implications for children with developmental or chronic lung disease after preterm birth. Oligohydramnios and insufficient lung stretch contribute to pulmonary hypoplasia, especially in the setting of prolonged premature rupture of membranes before preterm birth and with diverse congenital malformations as omphaloceles and congenital diaphragmatic hernia, at least partly due to the lack of mechanical force–induced stimulation of AT1 cell differentiation (3, 4). For some fetuses with congenital diaphragmatic hernia, an *in utero* procedure, called fetoscopic endotracheal occlusion, is used to obstruct the trachea with a latex balloon (5). By occluding the trachea, airway-distending pressures are increased owing to ongoing epithelial production of lung liquid, which stretches the distal lung and stimulates AT1 and AT2 cell maturation (4). The fetoscopic endotracheal occlusion procedure likely improves survival through forced lung maturation by mechanical stretch (5), as suggested by this study.

Despite the significant advances made by Li and colleagues (1), interrogating the effect of mechanical forces on other essential cells in lung development, specifically stromal and endothelial cells

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(ECs), is still needed. Proper alveologenesis requires adjacent stromal cell alignment for proper support. When stromal cells are misaligned, elevated mechanical tension develops from stiffening of α -smooth muscle actin-containing interstitial myofibroblasts and loss of elastic fibers (6). This extracellular matrix remodeling further disrupts angiogenesis, another essential component of proper alveolar–capillary membrane formation (7). The resultant clinical entities associated with aberrant mesenchymal and endothelial development include bronchopulmonary dysplasia (BPD) and pulmonary hypertension. Thus, future work assessing the effect of mechanical strain on stromal cell and EC development will improve our understanding, management, and treatment of chronic lung diseases originating in the prenatal period.

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Syed et al. Hyperoxia Causes miR-34a-Mediated Injury via Angiopoietin-1 in Neonatal Lungs. *Nat Commun* (8)

Reviewed by Laurie Sherlock

Although oxygen therapy is a life-saving intervention for premature infants, hyperoxia can have toxic side effects, including inflammation, oxidative stress, and lung injury (9). Clinical studies demonstrate that early cumulative oxygen exposure increases an infant's risk for BPD, the chronic lung disease of prematurity (10). Recent multicenter, randomized trials comparing oxygen saturation goals of 85–89% versus 91–95% suggest, however, that lower oxygen saturation targets are associated with increased mortality (9). This may drive clinicians to aim for higher oxygen saturations, exposing neonates to more oxygen and related toxicities (9). Despite extensive study, safe and effective therapeutic options to prevent BPD and its long-term associated late respiratory morbidities are limited (11).

Micro-RNAs (miRs) are short single strands of noncoding RNA, which can cause RNA cleavage, destabilization, or decreased transcription, and play important roles in lung development and disease (12). Using a murine model of hyperoxia-induced BPD, Syed and colleagues (8) identify increased lung miR-34a after exposure to hyperoxia when compared with room air by miRNA arrays. The investigators validate increased miR-34a expression and transcription in hyperoxia-exposed lungs and, importantly, report that hyperoxia increases miR-34a in isolated AT2 epithelial cells, but not in ECs or macrophages. In addition, miR-34a treatment by intranasal administration inhibits alveolar development in room air. The authors further demonstrate that genetic mouse models with either global or AT2-specific miR-34a deficiency are protected from hyperoxia-induced injury, as reflected by decreased inflammatory markers in epithelial lining fluid, alveolar simplification, and cellular apoptosis. Importantly, intranasal delivery of a miR-34a inhibitor prevented these adverse effects of hyperoxia.

To determine how miR-34a contributes to hyperoxia-induced BPD, the investigators demonstrate that hyperoxia and miR-34a overexpression decrease tyrosine kinases with immunoglobulin and epidermal growth factor homology domain 2 (Tie2), phosphorylated Tie2, and angiopoietin1 (Ang1) expression, suggesting that a role for disruption of postnatal angiogenesis. These studies provide *in vivo* corroboration, as this axis also decreases after miR-34a overexpression in neonatal AT2 cells and mouse alveolar epithelial (MLE12) cells

Strong clinical correlative data from three cohorts of human infants are presented, which show aberrations in the miR-34a/Ang1/Tie2 axis. First, miR-34a expression is higher in tracheal aspirate cell pellets from infants who develop BPD or died when compared with surviving infants without BPD. Second, *in situ* hybridization of lung tissue demonstrates increased miR-34a expression, primarily in AT2 cells, in infants with respiratory distress syndrome at 3–7 days compared with term. Third, when compared with age-matched samples from infants without lung disease, lung tissue content of Ang1 and Tie2 proteins is decreased in infants with established BPD.

The human genome contains at least 1,500 miRNA genes, thought to target more than 400 individual mRNAs (13). Preclinical research implicates miRNAs as essential for alveolar development; however, translating these findings into clinical applications is a new and evolving field. As miRNAs can be detected in body fluids, such as blood, sputum, saliva, and breast milk, their potential as predictive biomarkers for BPD is promising (14, 15). In addition to the work by Syed and colleagues, Lal and colleagues (16) demonstrate exosomal miR-876-3p from tracheal aspirates are predictive and protective of severe BPD. It is also compelling to then consider miRNAs as possible therapeutics for BPD. Although efficacious in rodents, miRNA activity and safety in humans are currently limited by drug delivery and tissue-targeting technology (17). Before miRNAs can become clinical tools, greater validation in human clinical trials is required with close regulatory oversight. Until then, miRNA research provides an innovative and promising area for future investigation.

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Li *et al.* Hydrogels with Precisely Controlled Integrin Activation Dictate Vascular Patterning and Permeability. *Nat Mater* (18)

Reviewed by Kolene E. Bailey

Improving techniques to repair diseased or damaged blood vessels is important in diverse settings of human cardiovascular and respiratory disorders (18). The design of novel therapeutics to promote restorative angiogenesis engages bioengineering techniques to exploit biological pathways. Hydrogels are a class of hydrated, polymeric material that are widely used as artificial three-dimensional scaffolds and as vehicles for drug delivery (19, 20). Integrins are heterodimeric transmembrane receptors, composed of α and β subunits, which facilitate cellular adhesion between the extracellular matrix and cellular cytoskeleton. These receptors provide mechanical and biochemical signals critical in the regulation of tissue morphogenesis, homeostasis, repair, and, ultimately, survival (21, 22).

Li and colleagues explore the synergy between integrin ligands and growth factors to develop hydrogel scaffolds for targeted angiogenesis (18). In the first part of the study, these investigators hypothesized that alterations of integrin binding can impact EC behavior. Human umbilical vein ECs (HUVECs) were cultured on hydrogels with recombinant fibronectin fragments that were customized to bind only specific integrins ($\alpha 3/\alpha 5\beta 1$ or $\alpha \nu \beta 3$). The investigators compared the integrity of the actin cytoskeleton, which is necessary for the maintenance of cell shape and organization of intracellular parts, in HUVECs cultured on $\alpha 3/\alpha 5\beta 1$ - or $\alpha \nu \beta 3$ -specific fibronectin surfaces. HUVECs cultured on $\alpha 3/\alpha 5\beta 1$ surfaces had longer and more organized fibers, and responded to exogenous vascular endothelial growth factor (VEGF) with greater

migration, suggesting that cellular behavior depends on the integrin-binding specificity of the attachment surface.

Next, Li and colleagues assessed the impact of integrin binding on branching during angiogenesis. The authors cultured ECs on full-length fibronectin with either $\alpha 3/\alpha 5\beta 1$ -specific or $\alpha \nu \beta 3$ -specific conditions. The number of branch points or total network length of ECs showed no differences; however, ECs grown on $\alpha \nu \beta 3$ -specific fibronectin revealed aberrant and pathologic vascular network formation with increased vascular permeability compared with the other conditions.

Lastly, Li and colleagues used hyaluronic acid (HA) hydrogels with $\alpha 3/\alpha 5\beta 1$ -specific and $\alpha \nu \beta 3$ fibronectin fragments and VEGF to assess angiogenesis in mice. HA hydrogels were implanted subcutaneously in mice for 14 days. The $\alpha 3/\alpha 5\beta 1$ -specific HA gels displayed nontortuous vessels, similar to normal mouse vasculature, whereas $\alpha \nu \beta 3$ -specific HA gels displayed tortuous and unorganized vessel development. Using a murine model of stroke, HA hydrogels were injected directly into the stroke cavity in brain tissue 5 days after the stroke. $\alpha 3/\alpha 5\beta 1$ -specific HA hydrogels with VEGF significantly increased the vascular area growth and reduced vascular permeability in the stroke cavity.

Segura's laboratory demonstrates the novel use of customized hydrogels with specific integrin activation modified by growth factors for angiogenic modulation after acute vascular injury. These customized hydrogels show promise as improved models of study to elucidate unknown pathophysiology of angiogenesis, as well as novel technology for drug delivery (20). Various types of hydrogels with different chemical and physical properties have been developed previously to improve localized delivery of growth factors for therapeutic angiogenesis (23). The optimal modification and delivery of these hydrogels to properly display growth factors to specific tissues are the holy grail of future therapeutic development. Properties contributing to optimal hydrogel modification include geometry, ultrastructure, degradation, and stiffness (24). In addition to integrin activation, further investigation into these other hydrogel properties is required for therapeutic optimization. In addition, the optimal delivery of hydrogels to the lung has yet to be determined. For myocardial ischemia, two minimally invasive procedures, epicardial and intracoronary injection, have been suggested. Both delivery techniques reduce the degree of damage caused by delivery while limiting off-target deposition. Similar minimally invasive techniques will need to be adapted for delivery of hydrogels to the lung vasculature. Consideration of these variables in hydrogel development are essential for a wide spectrum of pulmonary diseases, such as chronic obstructive pulmonary disease, idiopathic pulmonary fibrosis, interstitial lung disease, and pulmonary hypertension, which represent settings in which microvascular injury has been considered nonreparable and irreversible (25–27). ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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