Nanoparticle Delivery of Angiogenic Gene Therapy

Save the Vessels, Save the Lung!

Significant growth of the lung occurs after birth during alveolarization, the final stage of lung development that markedly increases the gas exchange surface area (1). The completion of lung development postnatally renders the immature lung highly susceptibile to injuries that disrupt this final stage of development. This is particularly evident in the context of premature birth in which arrested alveolar and vascular development result in bronchopulmonary dysplasia (BPD), the most common complication of prematurity and significant cause of morbidity and mortality (2). Evidence suggests that disrupted pulmonary angiogenesis is key to the pathogenesis of BPD. Proangiogenic factors are decreased, and the pulmonary microvasculature is dysmorphic in infants dying from BPD (3). In experimental models, directly blocking angiogenesis impairs alveolarization and, conversely, enhancing angiogenesis; for example, by augmenting VEGF (vascular endothelial growth factor) signaling, can improve alveolarization in the setting of injury (4). Therefore, the development of strategies to enhance alveolarization by promoting pulmonary angiogenesis hold great promise as potential therapies to meaningfully impact the incidence and severity of BPD. In this issue of the *Journal*, Bolte and colleagues (pp. 100–111) demonstrate that nanoparticle delivery of the transcription factors *Foxf1* and *Foxm1* effectively enhances angiogenesis and preserves alveolarization in a murine model of BPD induced by chronic hyperoxia (5).

There is currently an unmet need for therapies that can stimulate reparative and regenerative responses in an injured or diseased lung. Although animal models and cell-based assays have highlighted molecules and pathways critical for the regenerative response, translating these findings into therapies remains a major challenge. Nanoparticle systems created from natural or synthetic polymers are good candidates as a gene or drug delivery system. As gene delivery agents, nanoparticles protect nucleic acid cargo from degradation and promote cellular uptake of the genetic material. Synthetic polymers composed of polyethyleneimine (PEI), which contains amine groups as its backbone (branched or linear), are effective gene transfection agents (6). Further inclusion of poly(ethylene glycol) or cholesterol can also greatly enhance the bioavailability of these nanoparticles *in vivo* (7). Recently, Dunn and colleagues and Pradhan and colleagues have shown that derivations of PEI-based nanoparticles effectively target the pulmonary endothelium when injected intravenously in mice (8, 9).

In their most recent study published in this issue, Bolte and colleagues test whether the neonatal pulmonary capillary endothelium can be targeted by nanoparticles carrying plasmid DNA encoding *Foxm1* and *Foxf1* to improve alveolarization in a mouse hyperoxia injury model (5). Newborn mice exposed to 75% O_2 (hyperoxia) for 1 week were injected intravenously on Day 2 of hyperoxia with PEI_{600}-polyethylenimine-(5) myristic acid/poly(ethylene glycol)-oleic acid/cholesterol nanoparticles harboring plasmids encoding cytomegalovirus-driven *Foxm1*, *Foxf1*, or an empty vector. In this model, the effects of the hyperoxia exposure are long-lasting, resulting in impaired lung function into adulthood (10, 11). Remarkably, the alveolar simplification and lung function were markedly improved in the groups receiving nanoparticles with either *Foxm1* or *Foxf1* plasmids versus the empty plasmids. Using different assays, including dye-labeled nanoparticles or plasmids encoding GFP, the authors show that endothelial cells take up the nanoparticles and actually begin to transcribe the genetic material. Although the hyperoxia exposure resulted in endothelial cell apoptosis regardless of treatment group, the recovery of the microvascular endothelium in mice receiving nanoparticle delivery of *Foxm1* or *Foxf1* plasmids was drastically improved. The authors show that nanoparticle delivery of *Foxm1* or *Foxf1* increases proliferative endothelial cells compared with the empty plasmid control group.

A key finding of the study was that the beneficial effects of the nanoparticle therapy appear primarily due to modulation of the reparative response, rather than mitigation of the acute detrimental effects of hyperoxia on the endothelium. At the end of exposure, all the hyperoxia-exposed mice demonstrated similar impairments in alveolarization and degrees of endothelial death. However, two days later, mice receiving the *Foxf1*-containing and *Foxm1*-containing nanoparticles exhibited increased endothelial proliferation that translated into a dramatic improvement in parenchymal and vascular growth at 28 days, corresponding to the end of the alveolarization. Moreover, although novel therapies to enhance pulmonary angiogenesis often do not translate into beneficial effects on lung structure (12, 13), in this study the *Foxf1* and *Foxm1* nanoparticle therapy was associated with a normalization of lung compliance and TLC. Taken together, these data highlight the importance and putative power of focusing therapies not only to limit injury but also to harness the regenerative capacity of the still developing lung. This has tremendous implications for infants with severe BPD, many of whom require mechanical ventilation for over two years (14).

Although the findings presented in this study are exciting, a number of questions remain. Although a greater percentage of endothelial cells were targeted by the nanoparticles than other cell types, the nanoparticles also targeted the transient alveolar myofibroblasts, as well as $NG2^+$/$PDGFRb^+$ pericytes. How much of an effect *Foxf1* and *Foxm1* delivery to these other populations may have played in promoting lung growth is not known. This would be an important question to answer given that the authors

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found that this therapy also improved the aberrant elastin deposition and pathologic pulmonary vascular remodeling induced by hyperoxia, two findings not as easily attributable to enhanced endothelial cell proliferation. Further work may also dissect whether specific subsets of endothelial cells are sensitive to nanoparticle targeting, such as the *Vegfa*-sensitive Car4^{+} alveolar endothelial cell described in mice (15, 16), and define the specific genetic cargo that would be optimal to restore function of distinct endothelial subsets.

In addition, questions remain regarding the timing, durability, and the generalizability of this specific nanoparticle therapy. The authors used a relatively short exposure of hyperoxia, and the majority of the beneficial effects were likely occurring in the absence of further injury. In the clinical setting, the most severely affected infants will continue to require supplemental oxygen and mechanical injury during the window of potential recovery. Many may also develop postnatal infections, representing an additional lung injury with distinct molecular mechanisms. It would be important to explore whether this strategy is effective in the setting of ongoing injury or in experimental models of BPD induced by stimuli other than hyperoxia (e.g., infection, mechanical ventilation). Similarly, would a personalized approach be required in patients? As the authors acknowledge, patients with BPD are heterogenous, each exhibiting varying degrees of parenchymal, vascular, and airway disease. Would strategies to enhance endothelial proliferation be helpful in all groups or should similar gene therapy strategies be used to target other cell types in the lung?

Despite these remaining questions, the observations reported by Bolte and colleagues, affirm the utility of nanoparticles to target the pulmonary vasculature (5). After the transition to air-breathing life, the microvasculature of the perinatal lung is still actively growing and maturing. The outcomes of nanoparticle targeting during this time period provide provocative evidence to suggest that perturbations of the developing endothelium can have dramatic consequences for the alveolar architecture. This underscores the role of the endothelium in responding and/or signaling to the surrounding tissue niche. Given that current therapy for BPD is entirely supportive, the study by Bolte and colleagues represents a significant step forward in the development of targeted, curative therapies to treat this vulnerable population by augmenting the reparative power of the endothelium (5). ■

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