TGF-β and NFκB Cross Talk: Unexpected Encounters in the Developing Lung

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Abnormalities in vascular architecture and altered angiogenesis have been recognized for more than two decades as pathological underpinnings contributing to the development of bronchopulmonary dysplasia (BPD) (1). Key pathways that direct normal pulmonary alveolar growth and function have provided potential pathogenic avenues and beneficial replacement therapies for the treatment of BPD in experimental models of disease. Yet, protective therapeutic strategies have thus far been ineffective in improving clinical outcomes. With BPD being now recognized as a neonatal pulmonary vascular disease with persistent pulmonary hypertension phenotype (2), finding new molecular and cellular targets that promote vascular growth in infants born prematurely remains critical.

The local microenvironment shapes alveolar and vascular development of the lung through secreted signals. Among those, transforming growth factor (TGF)-β ligands and downstream mediators have been shown to influence mesenchymal homeostasis and lung development (3), but the biology of TGF-β in the lung is complex and its effects often seem contradictory (4,5). Here, Liu and colleagues describe a new mechanism that activates pro-angiogenic NFκB signaling in the developing pulmonary vasculature (6). They show that during early alveolarization, myofibroblasts secrete transforming growth factor beta induced protein (TGFBI) to promote the activation of NFκB and to enhance endothelial cell migration via integrin signaling. Activation of NFκB by TGFBI increases the expression of colony-stimulating factor 3 (CSF3) and the production of nitric oxide (NO) in endothelial cells. Moreover, they report that absence or dysregulation of TGFBI in two animal models of BPD leads to impaired alveolarization, disrupted septation and importantly, poor vascular growth.

To test the hypothesis that unique factors secreted during early alveolar development induce temporal-specific activation of NFκB in the pulmonary epithelium, the authors first profile the

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lung secretome during different stages of development. They identify TGFBI as a factor that activates NFκB and promotes endothelial migration. They also confirm previous reports of the spatiotemporal expression of TGFBI in the developing lung that show intensified accumulation in septal tips (7) at a stage of maximal alveolar septation (8). This is important because it challenges previous assumptions suggesting that excessive TGF-β activation contributes to BPD pathogenesis and it adds to the notion that investigating TGF-β ligands and effectors in the developing lung may require a cell-specific and dose-dependent approach.

Since the addition of recombinant TGFBI to early alveolar pulmonary endothelial cells (PEC) increased NFκB activity, the authors then investigated the NFκB dependency of TGFBI-mediated PEC migration. Sustained NFκB activity is required for normal angiogenesis and alveolarization (9,10), and inhibition of endothelial NFκB signaling with the pharmacological inhibitor BAY11-0782 or genetic deletion of *Ikkβ* completely abrogated TGFBI-mediated migration. This is significant, as these results strongly put forth TGFBI as a critical factor aimed at maintaining endothelial NFκB activity during early alveolarization and suggest a mechanistic link between TGF-β and NFκB signaling pathways not previously described. Furthemore, by blocking endothelial αvβ3 binding sites with the use of neutralizing antibodies and siRNA, Liu and colleagues go on to demonstrate that TGFBI-mediated NFκB activation and PEC migration depend on its ability to bind integrins. Importantly, these findings emphasize the role of TGFBI in mesenchymal-endothelial interactions within the extracellular matrix (ECM) niche. Integrins regulate vessel morphogenesis by stimulating endothelial proliferation and migration while interacting with ECM proteins (11), and the possibility of TGFBI stimulating this interaction is intriguing and suggests an important contribution, maybe equally as important as VEGF, in the development of the vasculature.

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Although the work by Liu and colleagues demonstrate an interaction between TGF-β and NFκB signaling pathways in promoting PEC migration, some questions remain unanswered. The authors argue that TGFBI is necessary and sufficient to promote endothelial migration. However, while TGFBI could be necessary to activate NFκB it does not appear to be sufficient, as nuclear p65 subunit localization and NFκB activation by early lung conditioned media is only partially inhibited by neutralizing TGFBI antibodies. This incomplete effect raises mechanistic questions that could be unraveled with the use of early lung conditioned media of *tgfbi*-deficient mice. In addition, the effect of TGFBI deficiency on NFκB activation and CSF3/NO signaling remains to be fully explored *in vivo*. Evidence of increased NFκB activity and induction of CSF3/NO expression in the septal tips of neonatal lungs examined here would have confirmed the signaling interactions described in their *in vitro* studies and improved our knowledge of the spatiotemporal mechanisms that govern early alveolarization and angiogenesis. Importantly, inhaled NO therapy does not prevent BPD in premature newborns despite its beneficial effects on experimental models (12) and identifying the precise temporal induction of NO in the lung would provide a new therapeutic and more efficient window of opportunity for premature newborns.

One of the most intriguing conclusions derived from the work of Liu and colleagues is that it allows us to speculate about the mechanisms operating during early angiogenesis. As TGFBI binds to integrins and interacts with components of the ECM (13), its role may be that of a regulator of vascular morphogenic processes occurring during early vascular generation. At a time when angiogenesis has to follow the process of newly formed septum, vascular morphogenesis has to dominate over vascular stabilization. By promoting endothelial activation, invasion and proliferation, TGFBI seems to stimulate lumen formation and early tubular morphogenesis. These processes not only require binding to αvβ3, but also sequestration of interstitial ECM proteins (13).

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While the authors have not analyzed the influence of TGFBI on tube formation *in vitro*, evidence of its role in tumor development seems to suggest that TGFBI effects in the developing lung may be similar and highlights the need to further investigate its transient interactions in the vascular niche. Finally, it will be important to understand how TGFBI dysregulation interferes with the production of myofibroblast-derived α-smooth muscle actin and matrix deposition in the context of vascular remodeling and lung fibrosis and whether the disruption TGFBI-NFκB axis affects other cell-interactions in the developing lung.

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