The Wnt Signaling Pathway and the Development of Bronchopulmonary Dysplasia

Extremely premature neonates born at the canalicular to saccular stage of lung development (22–28 wk of gestation) are at very high risk of developing bronchopulmonary dysplasia (BPD). The premature lung, now having to complete lung development in the extrauterine environment, is subjected to many adverse exposures, including hyperoxia, that promote the development of BPD. Many developmental pathways are precisely orchestrated for optimal lung development and in lung diseases throughout the lifespan (1). The Wnt family of proteins includes a large number of members that control a variety of developmental processes, including cell fate, proliferation, polarity, and migration. Wnt signaling consists of canonical, β-catenin–dependent signaling and two noncanonical pathways, including planar cell polarity and calcium-calmodulin–dependent protein kinase II/protein kinase C signaling. The canonical signaling pathway involves a number of proteins, including the transmembrane receptor Frizzled, coreceptors, and a variety of proteins that make up a complex that control degradation versus nuclear translocation of β-catenin. On translocation to the nucleus, β-catenin activates planar cell polarity and calcium-dependent protein kinase II/protein kinase C signaling. The canonical signaling pathway involves a number of proteins, including the transmembrane receptor Frizzled, coreceptors, and a variety of proteins that make up a complex that control degradation versus nuclear translocation of β-catenin. On translocation to the nucleus, β-catenin activates planar cell polarity and calcium-dependent protein kinase II/protein kinase C signaling. The canonical signaling pathway involves a number of proteins, including the transmembrane receptor Frizzled, coreceptors, and a variety of proteins that make up a complex that control degradation versus nuclear translocation of β-catenin. On translocation to the nucleus, β-catenin activates planar cell polarity and calcium-dependent protein kinase II/protein kinase C signaling. The canonical signaling pathway involves a number of proteins, including the transmembrane receptor Frizzled, coreceptors, and a variety of proteins that make up a complex that control degradation versus nuclear translocation of β-catenin. 

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Wnt5a mice die immediately after birth and show abnormal functional coupling of capillaries and the developing alveoli and thickening of the intersaccular interstitium (10). On the other hand, Wnt5a overexpression in distal lung epithelium using the SPC promoter reduced epithelial branching and dilated distal airways (11).

In the current studies, the critical involvement of NFκB (nuclear factor-κB) in the expression of Wnt5a and the mechanistic role of Wnt5a in the changes noted because of hyperoxia exposure during lung development was studied using organotypic cocultures, ex vivo precision-cut lung slices (PCLS), and in vivo mouse models. Hyperoxia exposure of organotypic cocultures resulted in increased expression of the fibrotic genes ACTA2, COL1A1, and ELN and decreased expression of the alveogenesis genes FOXM1, MYB, and MCM2. In examining the Wnt signaling pathway, hyperoxia was associated with increased nuclear accumulation of phosphorylated β-catenin and expression of AXIN2. Hyperoxia increased the expression of Wnt2b, Wnt5a, Wnt9a, and Wnt16 and decreased the expression of Wnt4, Wnt10a, and Wnt11. The increased Wnt5a expression was in mesenchymal cells. Addition of Wnt5a to cultures in normoxia demonstrated the same gene expression changes as observed with hyperoxia, and blockade of Wnt5a using a neutralizing antibody reversed the changes in gene expression observed in hyperoxia-exposed cultures. Alveolarization was decreased in PCLS exposed to hyperoxia, and this was abrogated in the presence of anti-Wnt5a antibody. In the in vivo mouse model of BPD (85% oxygen exposure from PN2 to PN14), increased expression of Wnt5a was noted in hyperoxia-exposed mouse lungs. Human samples from patients with BPD confirmed the increase in Wnt5a expression as compared with samples from babies who had succumbed to nonrespiratory causes. Next, pharmacologic or genetic inhibition of NFκB in PCLS exposed to hyperoxia showed decreased expression of Wnt5a and normal alveolarization. Thus, these exciting studies open the possibility of using Wnt5a as a potential therapeutic target to prevent or limit the severity of BPD.

The interplay between the developing lung (exposed to various postnatal stresses including hyperoxia) and other physiological factors (circulation and the immune system) is critical in the pathogenesis of injury. The three-dimensional organotypic coculture model with type 2 AECs and mesenchymal cells (from canalicular stage of lung development) used in these studies was able to localize the expression of Wnt5a to the mesenchymal compartment. Although this model has several advantages over two-dimensional culture systems (12) and preserves the spatial context and the lung microenvironment, it is a static system that does not have the ability to incorporate the contribution of systemic immune cells that may be recruited to the injured lung (13). Wnt5a may also play a role in the developing endothelium (14), which was not studied by Sucre and colleagues (7). For example, in a recent study by Yuan and colleagues, loss of endothelial Wnt5a led to small vessel loss in pulmonary arterial hypertension (15).

The authors show that the blockade of NFκB resulted in decreased Wnt5a and improved alveolarization. However, NFκB also drives the transcription of pro-IL1β, and the NLRP3 inflammasome controls the formation of mature IL1β, a system that is critical for the inflammatory process and the pathogenesis of BPD (16). Interestingly, IL1β increases the expression of Wnt5a in myofibroblasts (17), endothelial cells (18), and chondrocytes (19–21). Ge and colleagues showed induction of Wnt5a by IL1 and IL1ra secondary to increased Wnt signaling in the pulmonary mesenchyme and type 2 alveolar epithelial cells still need to be elucidated.

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NFκB-mediated increase in Wnt5a expression impaired alveolarization in this study. However, other studies have reported beneficial effects of NFκB signaling in alveolarization (24), specifically protecting the saccular and alveolar lung from hyperoxia-induced injury (25). Even though the upstream regulation of Wnt5a may be through NFκB, therapeutic strategies focused on Wnt5a may alleviate hyperoxic lung injury while preserving the other beneficial aspects of NFκB signaling in the developing lung. The mechanisms leading to impaired alveolar development secondary to increased Wnt signaling in the

**Figure 1.** NFκB (nuclear factor-κB) drives the transcription of pro-IL1β, and the NLRP3 inflammasome controls the formation of mature IL1β. IL1β increases the expression of Wnt5a in the mesenchyme, leading to increased Wnt signaling in type 2 alveolar epithelial cells. Wnt5a expression can also be increased by several other signaling pathways such as Hedgehog, TGFβ, and Notch. The mechanisms leading to impaired alveolar development secondary to increased Wnt signaling in the pulmonary mesenchyme and type 2 alveolar epithelial cells still need to be elucidated.
pulmonary mesenchyme and type 2 AECs need to be elucidated. We look forward to future studies that will build on the ex vivo results in the in vivo model and delineate the potential mechanisms accounting for Wnt5a-mediated effects.

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