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⊕ The Wnt Signaling Pathway and the Development of Bronchopulmonary Dysplasia

Extremely premature neonates born at the canalicular to sacular 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 maturation. Decreased or sustained activation of these pathways may contribute to the pathogenesis of this disease or may impair recovery of the lung from injury. Identification of these novel pathways and their mediators is crucial for the establishment of mechanisms leading to BPD and the development of novel therapeutic strategies.

The Wnt signaling pathway is critical both during embryonic 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 “destruction complex” that control degradation versus nuclear translocation of β -catenin. On translocation to the nucleus, β -catenin activates several Wnt target genes (1). In distal lung development, Wnts provide spatiotemporal cues to coordinate an intricate crosstalk between the lung epithelium and mesenchymal cells (2). Frank and colleagues showed that Wnt signaling is reactivated during alveologenesis and leads to proliferation of type 2 alveolar epithelial cells (AECs), whereas inhibition of Wnt signaling decreased proliferation and promoted transdifferentiation of type 2 AECs to type 1 AECs (3). Increased Wnt/ β -catenin activity occurs in patients with BPD, whereas inhibition of WNT/ β -catenin signaling attenuates hyperoxia-induced lung injury in neonatal rodent models (4–6).

In this issue of the *Journal*, studies by Sucre and colleagues (pp. 1249–1262) focus on the role of Wnt5a, a noncanonical Wnt that is required for normal distal lung morphogenesis (7). The authors chose to study Wnt5a because of previously published reports of its role in lung diseases such as idiopathic pulmonary

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fibrosis (8) and chronic obstructive pulmonary disease (9). It is known that *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 stressors 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 IL1β in chondrocytes (22), and this was blocked by NFκB inhibition. They further showed recruitment of NFκB p65 to the *Wnt5a* promoter after IL1β treatment. Thus, the decrease in *Wnt5a* expression secondary to NFκB inhibition could have been secondary to decreased IL1β *in vivo*. Therefore, whether NFκB acts directly on *Wnt5a* in this model remains to be clarified. Use of NLRP3 inflammasome inhibitors such as glyburide and IL1β blockers such as IL1 receptor antagonist in these models would help define this pathway. In addition, multiple other signaling pathways such as Hedgehog, TGFβ, and Notch can also regulate *Wnt5a* expression (23). Figure 1 shows the potential pathways that could regulate the expression of mesenchymal *Wnt5a* in the development of BPD.

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

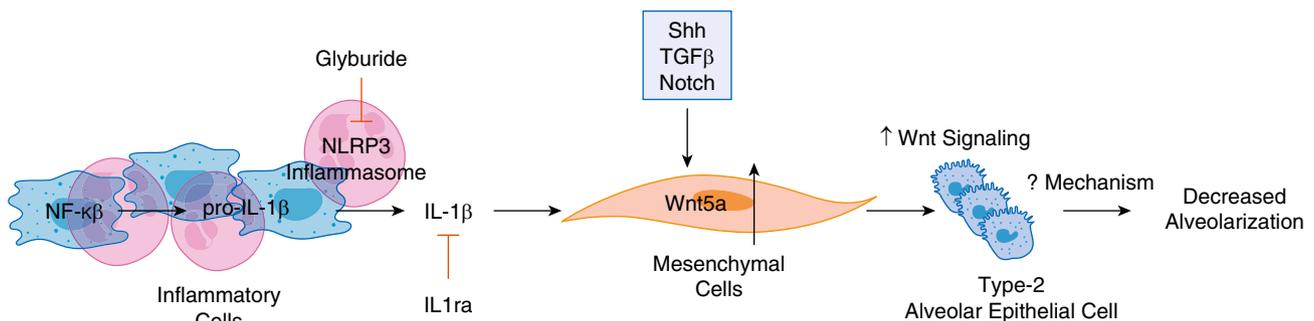


Figure 1. NFκB (nuclear factor-κB) drives the transcription of pro-IL1β, and the NLRP3 inflammasome controls the formation of mature IL1β. IL1β increases the expression of *Wnt5a* in the mesenchyme, leading to increased Wnt signaling in type 2 alveolar epithelial cells. *Wnt5a* expression can also be 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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Krithika Lingappan, M.D., M.S., Ph.D.
Division of Neonatal-Perinatal Medicine
Baylor College of Medicine
Houston, Texas

Rashmin C. Savani, M.D.
Division of Neonatal-Perinatal Medicine
University of Texas Southwestern Medical Center
Dallas, Texas

ORCID ID: 0000-0003-0821-2289 (K.L.).

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