mesenchymal cells. The loss of Nfib in all cells decreases the expression of differentiation markers of alveolar epithelial cells (Aqp5 and SftpC), Clara cells (Scgb1a1) and ciliated cells (Foxj1) in E18.5 lungs. To test for a specific role of Nfib in lung mesenchyme we generated and analyzed Nfiblox/flox, Dermol1-Cre mice. Loss of Nfib only in mesenchyme results in decreased Aqp5 and SftpC expression, increased cell proliferation, and a defect in sacculation similar to that seen in Nfib−/− mice. In contrast, mesenchyme specific loss of Nfib had no effect on the expression of Scgb1a1 in the airway. Microarray and QPCR analyses indicate that the loss of Nfib in lung mesenchyme affects the expression of genes associated with extracellular matrix, cell adhesion and FGF signaling which could affect distal lung maturation. Our data indicate that mesenchymal Nfib regulates both mesenchymal and epithelial cell proliferation through multiple pathways and that mesenchymal NFI-B-mediated signals are essential for the maturation of distal lung epithelium.

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Program/Abstract # 192
Tbx4 and Tbx5 are important for lung growth and branching and tracheal/bronchial cartilage ring development
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Tbx4 and Tbx5 are expressed in the developing lung and trachea mesenchyme. Complete loss of Tbx4 or Tbx5 leads to embryonic lethality before lung branching begins. We used conditional mutant alleles for Tbx4 and Tbx5 along with two different cre recombinase driver lines to understand the time and tissue specific roles of Tbx4 and Tbx5 in lung and trachea development. Mutants that are Tbx4 null;Tbx5 heterozygote or Tbx4 heterozygote;Tbx5 null are severely delayed in lung growth and branching at E13.5. Concordant with this phenotype, the expression of important mesenchymal markers Fgf10, Wnt2a and Bmp4 is downregulated in lung mesenchyme. Mutant pups that are lung-specific Tbx4 heterozygote;Tbx5 null are born but die soon after owing to respiratory distress. Tracheal and bronchial cartilage development is severely disrupted in these pups. We show that Sox9 is expressed in these mutants at E12.5 but mesenchymal cells expressing Sox9 fail to condense at E13.5 and fail to develop cartilage normally at birth. When Tbx4 and Tbx5 are completely excised in vitro, lung branching is arrested. In summary, Tbx4 and Tbx5 are absolutely required to drive lung growth and branching and are also important for tracheal/bronchial cartilage development.

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Program/Abstract # 193
Hyperoxia down-regulates periostin protein during a critical period of lung alveolar development
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Bronchopulmonary Dysplasia (BPD) is a chronic lung disease affecting preterm infants, characterized by alveolar simplification due to an arrest of secondary alveolar septation. Up-regulation of Transforming Growth Factor Beta (TGFβ) signaling has been implicated as a possible mechanism, and currently therapeutic efforts are being developed to suppress TGFβ signaling in postnatal lungs to achieve rescue of the BPD phenotype. Because TGFβ signaling is mediated via three ligands, TGFβ1, 2 and 3, that serve overlapping yet specific functions in normal lung development, we hypothesized that they would be differentially affected in a neonatal mouse model of arrested alveolarization. Continuous exposure to 21 days of hyperoxia (85% O2) significantly arrested alveolar development with enlarged, simplified distal airspaces as early as postnatal (P) day 7 when compared to age-matched littermate controls exposed to room-air (RA). Moreover, alveolar simplification correlated with an early suppression of cell proliferation at P4, rather than elevation of cell death. However, this was surprisingly not associated with increased Smad 2/3 phosphorylation until P21. In situ hybridization revealed a notable decrease in Tgfb2 ligand mRNA in the O2-exposed lungs at P7, while Tgfb1 and b3 were unaltered. To further delineate TGFβ signaling responses, we examined the expression of the Tgfb-inducible downstream extracellular matrix protein target, periostin. Significantly, periostin mRNA and protein levels were down-regulated 40% in the P4 alveolar seatae of O2-exposed pups during the critical period of alveolarization. In order to evaluate whether periostin suppression was directly linked with arrested alveolarization, we examined periostin knockout mice lungs during postnatal morphogenesis. Notably, loss of periostin results in delayed secondary alveolar septation. Combined, these data demonstrate that hyperoxia down-regulates Tgfb2 gene expression as well as the TGFβ-responsive periostin ECM target gene, during a critical period of secondary alveolar septation. Further, this study highlights the complexity of TGFβ signaling during postnatal lung remodeling and suggests that downstream effectors of TGFβ signaling may be better-quality targets for therapeutic intervention.

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Program/Abstract # 194
Wntless promotes pulmonary differentiation and growth
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Wnt ligands are secreted hydrophobic growth factors that reach their target cells in a hydrophilic medium. Although the molecular mechanism underlying Wnt secretion and spreading is largely unknown, recent studies have identified Wntless (Wls) as a gene that mediates Wnt ligand secretion and whose cellular function is conserved across the animal kingdom. Wnt signaling is required for lung formation. Deletion or mutation of Wnt ligands and related transcription factors impairs lung morphogenesis, cell differentiation and growth. However, the role of Wls in lung organogenesis is not known. We hypothesize that Wls plays a critical role in lung development and that its ablation leads to pulmonary congenital malformations. We generated conditional Wls mutant mice utilizing Shh-Cre and Dermo1-Cre drivers to delete Wls in embryonic respiratory epithelium and mesenchyme, respectively. Epithelial deletion of Wls disrupted lung branching morphogenesis, proximal/distal patterning, mesenchymal growth and differentiation—manifested by altered smooth muscle cell differentiation and diminished pulmonary microvasculature—and patterning of tracheal cartilaginous rings. Epithelial Wls mutant mice died at birth due to respiratory failure caused by lung hypoplasia and pulmonary hemorrhage. Mesenchymal deletion of Wls in the developing lung affected neither branching morphogenesis nor early mesenchymal differentiation, as determined by expression of endothelial and mesenchymal markers Sox17 and Foxf1. We conclude that Wls acts in autocrine and paracrine fashion to promote branching morphogenesis, peripheral lung development and mesenchymal differentiation by controlling secretion of epithelially expressed Wnt ligands.

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