T-Box Genes Regulate Mesenchymal Cell Fate Specification in the Developing Lung

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Introduction

- *TBX4* mutations cause **diffuse lung diseases** (DLD) and neonatal **pulmonary arterial hypertension** (PAH). (Kerstjens-Frederikse et. al., 2013) (Szafranski et. al., 2016) (Suhrie et. al., 2019) (Austin et. al., 2020) (Haarman et. al., 2020) (Prapa et. al., 2022).
- **TBX4 Syndrome** is a rare condition characterized by DLD, PAH, lower limb abnormalities, and other systemic symptoms with variable severity; DLD is associated with significant neonatal morbidity and mortality.
  - Unknown: **how** *TBX4* variants cause lung disease.

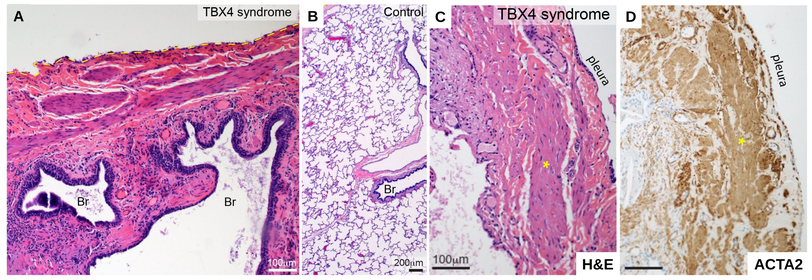


Figure 1. Lung architecture is severely disrupted in diffuse lung disease caused by TBX4 mutations. Hematoxylin & eosin-stained lung biopsy from a patient with TBX4 Syndrome exhibits airway abnormalities with proximal airways (Br) localizing near the pleura (A). Bronchi are normally found in the middle of the lung, far from the pleura (B). TBX4 Syndrome lungs also exhibit sub-pleural muscularization (C-D, yellow asterisk).

- We use a mouse model of TBX4 Syndrome lung phenotypes to study the developmental and postnatal basis of this disease. (Maldonado-Velez et. al., 2025) (Steffes, Chiles et. al., 2025)
  - In the mouse lung mesenchyme, *Tbx4* and *Tbx5* are homologs encoding important transcription factors for embryonic development.
  - Embryonically, *Tbx4/5* direct airway epithelial branching, but their molecular targets in fetal lung have not been identified. (Arora et. al., 2012)

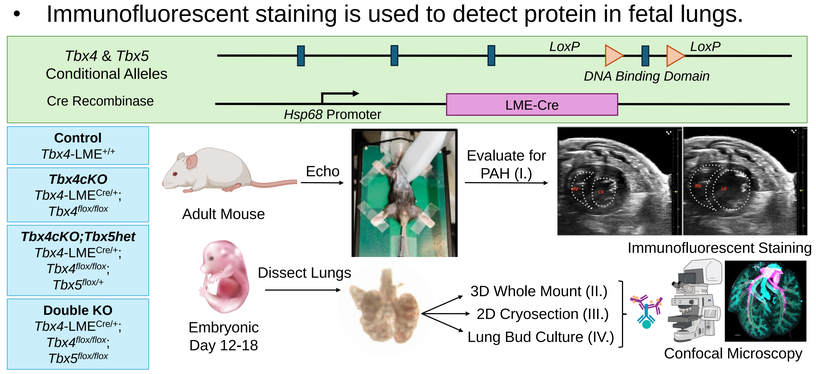
Objectives

Objectives: Determine the mechanism of *Tbx4/5* in mesenchymal cell fate specification; assess the developmental basis for TBX4-associated PAH.

Hypothesis: Lung-specific deletion of *Tbx4/5* will result in abnormal mesenchymal progenitor cell fate specification and differentiation through aberrant expression of developmental signaling pathways.

Methods

- *Tbx4*-Lung Mesenchyme-Specific Enhancer (LME) Cre recombinase conditionally deletes *Tbx4* and *Tbx5* in the fetal lung. (Kumar et. al., 2014)
- Adult mice underwent cardiac ultrasound (echo) to diagnose PAH.
- Immunofluorescent staining is used to detect protein in fetal lungs.



Results

I. TBX4/5-deficient mice develop PAH and ectopic smooth muscle.

a. Adult *Tbx4cKO*, *Tbx4cKO*;*Tbx5* heterozygous, and Double KO lungs exhibit ectopic smooth muscle cells (SMCs). *Tbx4cKO* and *Tbx4cKO;Tbx5het* mice develop PAH with right heart pathology.

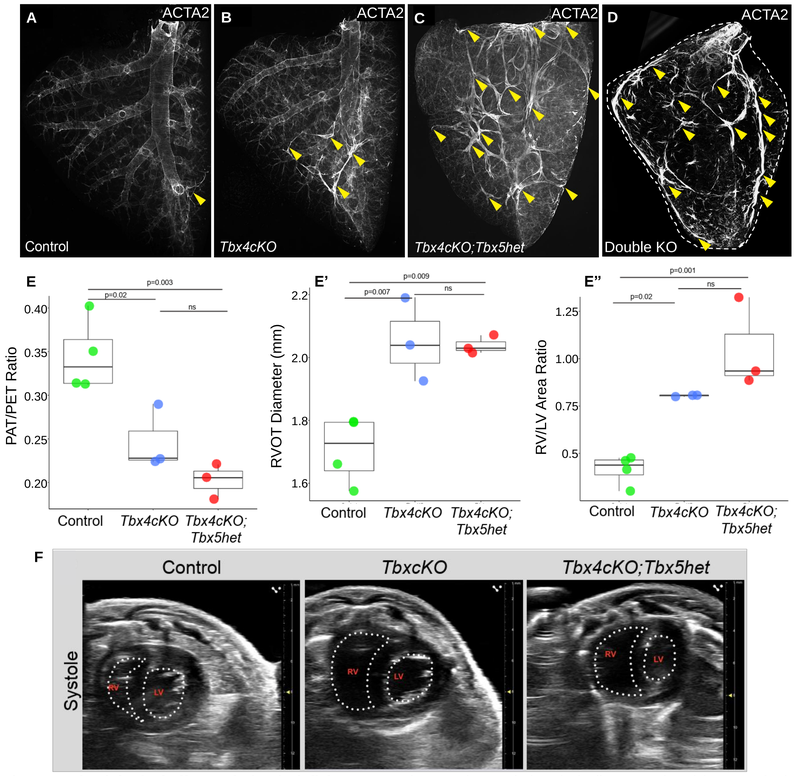


Figure 2. Physiological effects of *Tbx4/5* deficiency. Ectopic smooth muscle bands (yellow arrowheads) appear in the lungs of all adult mice (A-D); progressive loss of *Tbx4* and *Tbx5* alleles correlates with severity of banding and right ventricular dilation (F). Conditional deletion of *Tbx4* alone is sufficient for PAH pathogenesis (E-E"), but PAH is not significantly worse in *Tbx4cKO;Tbx5het* than *Tbx4cKO* mice (E-E").

II. Embryonic *Tbx4/5* double KO lungs are hypoplastic with reduced airway branching and ectopic smooth muscle resembling adult.

a. Deletion of all *Tbx4* and *Tbx5* alleles causes diminished airway branching at E14.5 and ectopic smooth muscle bands at E16.5.

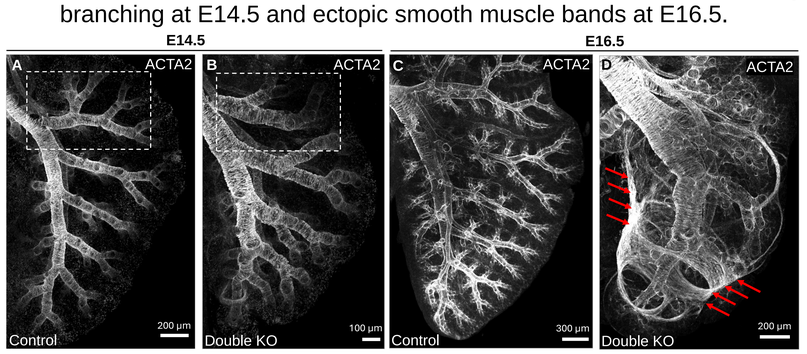


Figure 3. *Tbx4/5* double KO lungs are smaller than control lungs with aberrant airway and smooth muscle morphology. At embryonic day (E) 14.5, double KO lungs exhibit reduced branching of proximal muscularized airways (B). The dashed box highlights the lack of branches in the double KO compared to control (A-B). At E16.5, double KO lungs exhibit ectopic SMCs forming bands at the lobe edge (D, red arrows); these bands are absent in control (C).

Results

III. Pulmonary myofibroblast marker PDGFRA is aberrantly expressed in embryonic double KO mesenchyme but not in ectopic SMCs.

a. Double KO lungs exhibit excess PDGFRA expression at E14.5 and aberrant distal airway architecture at E16.5.

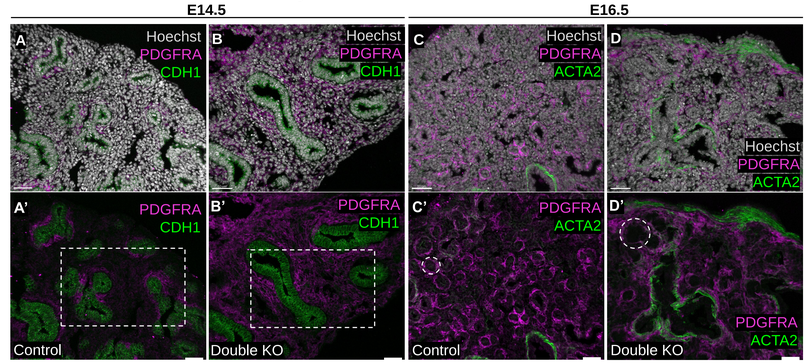


Figure 4. Embryonic double KO lungs have aberrant PDGFRA expression. Double KO lungs exhibit increased mesenchymal PDGFRA expression at E14.5 (A'-B', dashed box), with dilated distal airways (C'-D', dashed circle) seen at E16.5. Ectopic SMCs do not express PDGFRA, indicating that they are not myofibroblasts. Scale bar = 50 μm.

IV. Excess and ectopic SMC phenotypes are recapitulated in vitro.

a. In vitro, double KO lung bud explants exhibit excess airway SMCs and ectopic SMC bands on lobe edges in culture, recapitulating SMC phenotypes seen in adult and embryonic lungs.

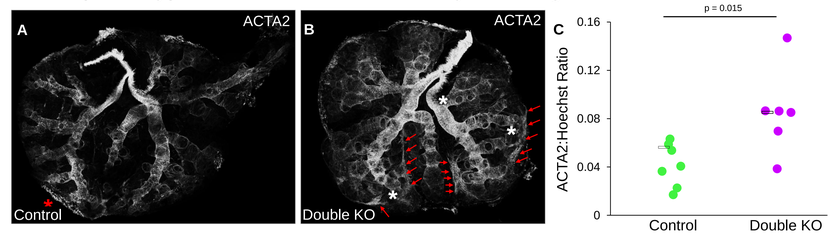


Figure 5. Double KO lung explants develop excess and ectopic smooth muscle in 96-hour culture. Double KO lungs develop excess (B, white asterisks) and ectopic (B, red arrows) smooth muscle when dissected at E12.5 and cultured in air-liquid interface for 96 hours. Ectopic smooth muscle bands are present in left and right lobes. Control lungs do not develop excess smooth muscle or SMC bands; however, small patches of smooth muscle cells were observed on the edge of some control lungs (A, red asterisk). Volumetric quantitation of the proportion of smooth muscle-to-total volume reveals a significantly greater ratio of SMCs in double KO lungs than control (C, n=6).

Conclusions

Interpretation of findings:

- Ectopic smooth muscle bands arise at E16.5 and resemble those seen in adults, indicating a prenatal origin for postnatal SMC phenotypes.
- Morphological changes (reduced branching, excess and ectopic SMCs, abnormal lobe shape, dilated distal airways) associated with *Tbx4/5* loss result in poorly developed, hypoplastic lungs.
- We hypothesize that ectopic SMCs arise through abnormal cell fate specification (i.e., towards SMC or myofibroblast-like fate) and excess and ectopic SMC accumulation in double KO lungs contributes to PAH.

Translational applications:

- Clarifying the function of *Tbx4/5* in lung mesenchymal differentiation will provide clinically relevant insights into the role of T-box genes in PAH due to DLD and the developmental origins of these diseases.
- Identifying dysregulated pathways involved in DLD and PAH pathogenesis may elucidate therapeutic targets for TBX4 patients.