Wilms Tumor 1 (WT1) is Required in Diaphragm and Early Lung Development

**Background**
- Congenital diaphragmatic hernia (CDH) is a common and severe congenital malformation affecting 1 in 3500 live births.
- The high mortality in CDH patients is due to a combination of lung hypoplasia and pulmonary hypertension.
- **Our hypothesis** is that a core group of genes is responsible for both diaphragm formation and pulmonary development.

- Whole genome sequencing identified mutations in the WT1 gene in two patients with CDH.
- WT1 encodes a transcription factor that is expressed specifically in the mesothelium and required for organ development and injury recovery.

**Objectives**
1. Identify role of WT1 in diaphragm development
2. Identify the role of WT1 in lung development

**Methods: Tissue-specific conditional deletion of WT1 in diaphragm or lung**

**Diaphragm and lung mesenchyme Specific Deletion**
- A. P0.5 Diaphragm
- B. E16.5 Lung

**WT1 deletion leads to CDH and lung hypoplasia**

- Figure 1: Whole genome sequencing in the DREAMS study identified two CDH patients with mutations in the WT1 gene in Exon 9 (highlighted in red). Exon 9 encodes the 3rd zinc finger that is required for DNA binding and regulation of gene transcription.

**WT1 is required in diaphragm development**

- Figure 2: Twist1 (T55) or Wt1-Cre induces cell-specific recombination in the diaphragm mesothelium(A) and the lung mesenchyme (B). The timing of recombination induction leads to different phenotypes giving us insight to spatial and temporal requirements of WT1.

- Figure 3: When recombination is induced at E6.5 (A), WT1 mutant mice die after birth with left CDH (red box) and thoracic herniation of the stomach and liver (B) and lung hypoplasia (C). This phenotype is identical to that seen in CDH patients.

**WT1 is required for early lung development**

- Figure 4: The developing diaphragm is composed of fibroblasts, skeletal muscle, and mesothelium (not shown).

**WT1 is not required for late embryonic or postnatal lung development**

- Figure 5: Compared to controls (A, C), deletion of WT1 at E5.5 results in failure of left diaphragm development at E12.5 (red circle) but normal right diaphragm (B). The left diaphragm in WT1 mutants shows increased distance between the septum transversum and the pleuroperitoneal fold (PPF, D).

**Conclusions**
- Mutations in WT1 were identified in patients with CDH.
- In mice, WT1 is required for diaphragm development.
- WT1 is also required for early lung development but not late embryonic or postnatal lung development.

**Future Directions**
- Identify the transcriptional targets of WT1 that are required for diaphragm and lung development.
- Identify the mesothelial-specific mechanisms of diaphragm and lung development.

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