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

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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.

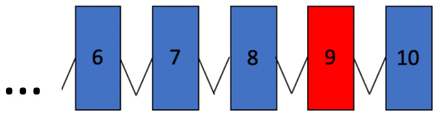

Figure 1: Whole genome sequencing in the DHREAMS study identified two CDH patients with mutations in the *WT1* gene in Exon 9 (highlighted in red). Exon 9 encodes the 3rd zinc finger that is required for DNA binding and regulation of gene transcription.

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**

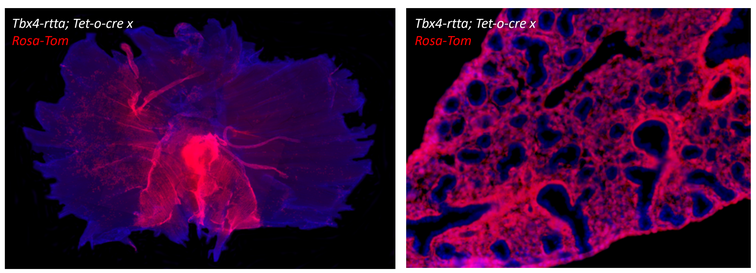

Figure 2: *Tbx4*-rtTA; *Tet-O-Cre* induces cell specific recombination in the diaphragm mesothelium(A) and the lung mesenchyme (B). The timing of recombination induction leads to different phenotypes giving us insight to spatial and temporal requirements of *Wt1*.

Wt1 deletion leads to CDH and lung hypoplasia

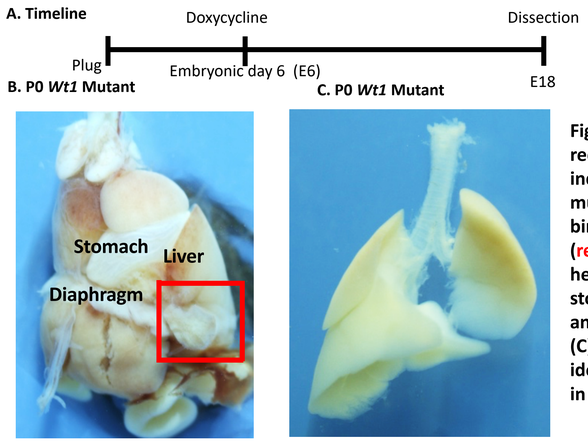

Figure 3: When recombination is induced at E6 (A), *Wt1* mutant mice die after birth with left-CDH (red box) and thoracic herniation of the stomach and liver (B) and lung hypoplasia (C). This phenotype is identical to that seen in CDH patients.

Wt1 is required in diaphragm development

Diaphragm Development

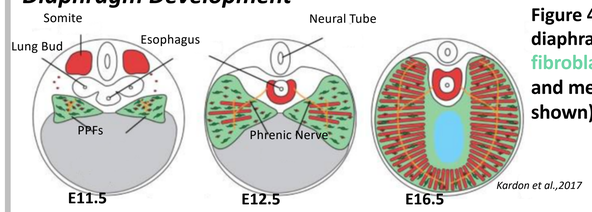

Figure 4: The developing diaphragm is composed of fibroblasts, skeletal muscle, and mesothelium (not shown).

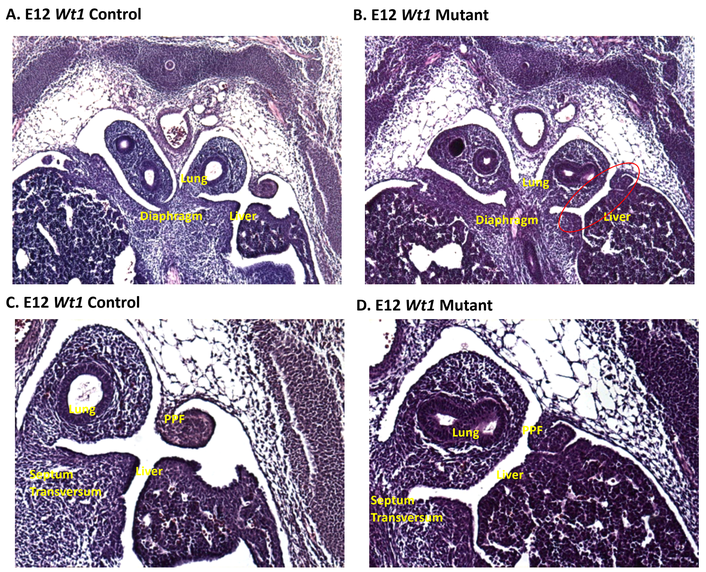

Figure 5: Compared to controls (A, C), deletion of *Wt1* at E6.5 results in failure of left diaphragm development at E12.5 (red circle) but normal right diaphragm (B). The left diaphragm in *Wt1* mutants shows increased distance between the septum transversum and the pleuroperitoneal fold (PPF, D).

Wt1 is required for early lung development

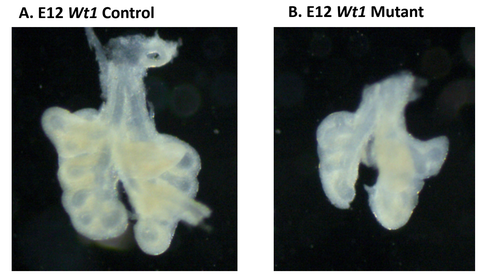

Figure 6: Compared to controls (A), *Wt1* mutant lungs have decreased branching and hypoplasia at E12 (B).

Wt1 is not required for late embryonic or postnatal lung development

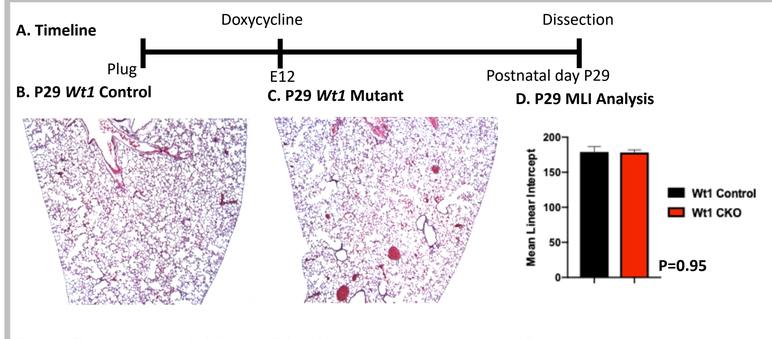

Figure 7: Deletion of *Wt1* after E12 (A) compared to controls (B) does not impair embryonic or postnatal lung development (C) quantified by mean linear intercept analysis (MLI, D).

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 mesothelium-specific mechanisms of diaphragm and lung development

Acknowledgements

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