Mutations in *SIN3A* cause diaphragmatic hernia, lung hypoplasia, and pulmonary hypertension in humans and mice

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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 lung hypoplasia and pulmonary hypertension.
- Our **hypothesis** is that a core group of genes is responsible for both diaphragm formation and development of the lungs and pulmonary vasculature.
- Whole exome sequencing in patients with CDH identified mutations in the *SIN3A* gene.
- *SIN3A* encodes a transcription factor required for cell cycle regulation, histone modification, muscle cell development, and lung epithelial cell development.

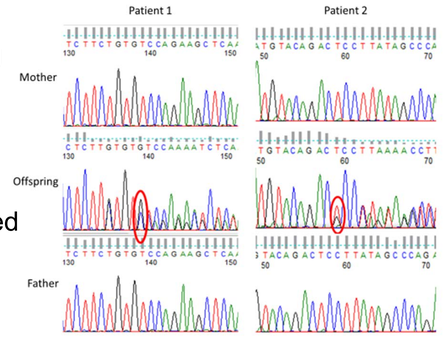

Figure 1: Whole exome sequencing identified two CDH patients with mutations in the *SIN3A* gene.

Objectives

1. Determine the role of *Sin3a* in the developing diaphragm and lung mesenchyme
2. Identify the mechanisms responsible for lung hypoplasia and pulmonary hypertension due to *Sin3a* loss of function

Methods: Tissue-specific conditional deletion of *Sin3a* in diaphragm or lung mesenchyme

Diaphragm and lung mesenchyme cell specific deletion

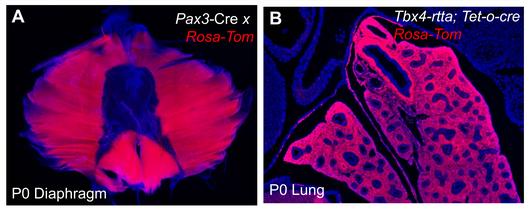

Figure 2: *Pax3*-Cre directs recombination in skeletal muscle (A). *Tbx4*-rtta; *Tet*-o-Cre directs inducible recombination in the lung mesenchyme.

Results: *Sin3a* deletion in skeletal muscle results in defective diaphragm development

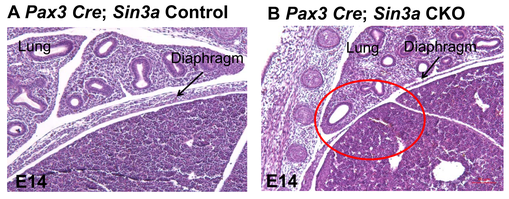

Figure 3: Compared to controls (A), skeletal muscle deletion using *Pax3*cre results in diaphragm malformation (red circle, B).

Results: Early *Sin3a* deletion causes CDH and lung hypoplasia

***Sin3a* Deletion at Embryonic day 9 (E9)**

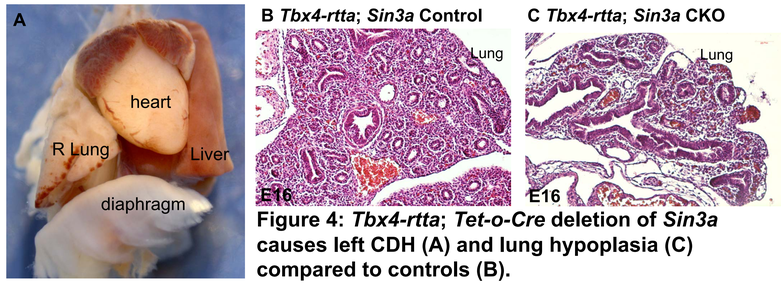

Figure 4: *Tbx4-rtta*; *Tet-o-Cre* deletion of *Sin3a* causes left CDH (A) and lung hypoplasia (C) compared to controls (B).

Results: *Sin3a* mutant mice have lung defects present at birth

***Sin3a* Deletion at E12**

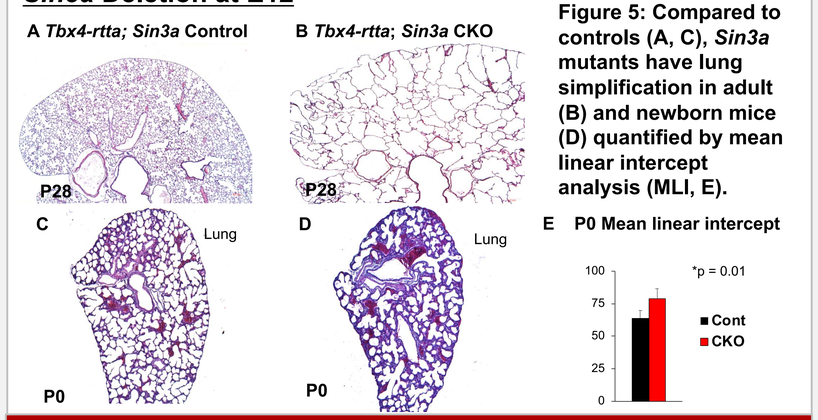

Figure 5: Compared to controls (A, C), *Sin3a* mutants have lung simplification in adult (B) and newborn mice (D) quantified by mean linear intercept analysis (MLI, E).

Results: Loss of *Sin3a* causes defects in cell cycling and increased cell death

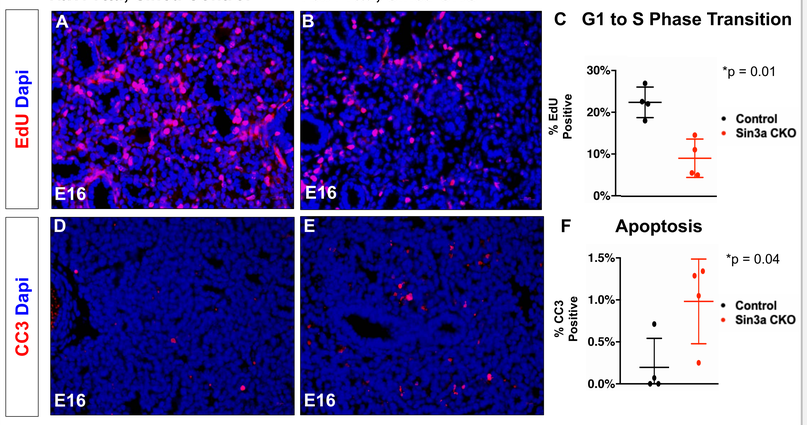

Figure 6: Compared to controls (A, D), *Sin3a* mutants have fewer cells undergoing G1 to S phase transition (B, C) and increased apoptosis (E, F).

Results: Loss of *SIN3A* transcriptional regulation causes multiple gene expression changes

- RNA-seq analysis of recombined lung mesenchymal cells at E16 demonstrated mis-regulation of 6026 genes.

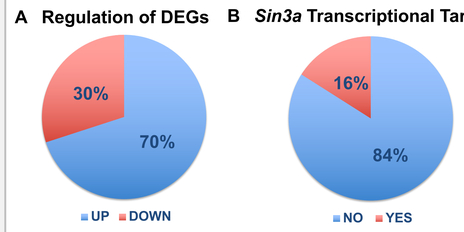

Figure 7: RNA-seq analysis demonstrated that of the top differentially expressed genes (DEGs) the majority are upregulated (A) and 16% are known transcriptional targets of *SIN3A* (B).

- Many of the top DEGs are involved in regulation of cell cycling, inflammation, and rRNA processing.

Results: *Sin3a* deletion in lung mesenchyme causes pulmonary hypertension

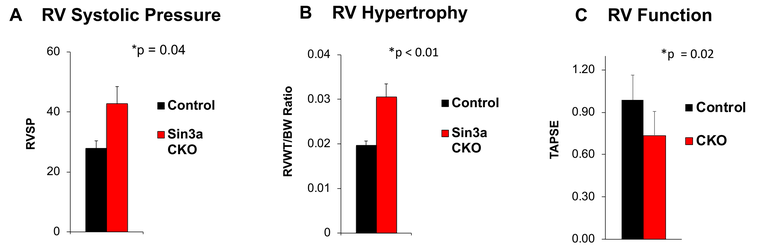

Figure 8: Compared to controls, *Sin3a* mutant mice have elevated right ventricular systolic pressure (RVSP, A), right ventricular hypertrophy (B), and decreased right ventricular function indicated by decreased tricuspid valve annular plane excursion (TAPSE, C).

Conclusions

- Mutations in the *SIN3A* gene were identified in patients with CDH.
- *Sin3a* deletion in mice causes CDH, lung hypoplasia, defects in cell cycling, and pulmonary hypertension.

Future Directions

- Use *Sin3a* genetic targets as a guide to improve lung and pulmonary vascular defects in mice as a path toward future therapy for patients with CDH.

Acknowledgements

Projected supported by funding from the University of Wisconsin-Madison School of Medicine and Public Health, Department of Pediatrics, UW-ICTR, NICHD, and NHLBI

Many thanks to the members of the McCulley laboratory and collaborators

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