# CDH Gene *PBX1* is Required in the Mesenchyme for Postnatal Alveologenesis

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## Background

- Congenital diaphragmatic hernia (CDH) is a common and severe congenital malformation affecting 1 in 3,500 live births.
- The morbidity and mortality in patients with CDH is due to lung hypoplasia and pulmonary hypertension.
- *PBX1*: Pre-B-cell leukemia transcription factor 1
- *Pbx* deletion in fibroblasts causes CDH in mice

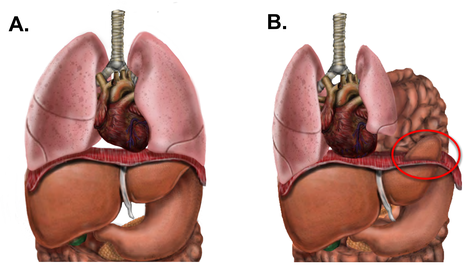



**Figure 1: Compared to normal newborns (A), infants with CDH (B) have a hole in the diaphragm (red circle) with herniation of abdominal organs and compression of the developing lungs.**

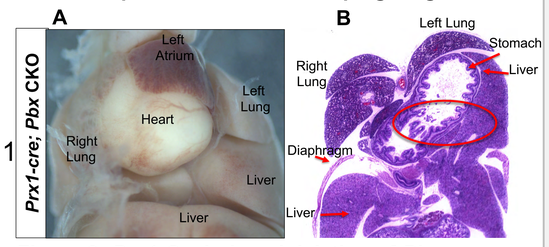


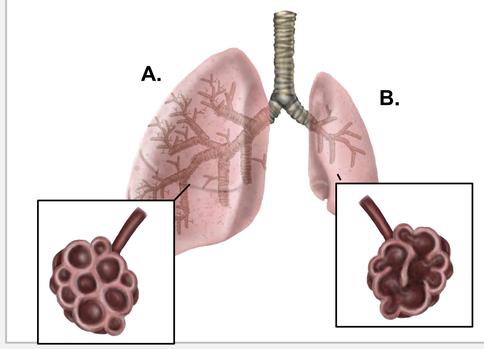

**Figure 2: *Prx1-Cre* induced deletion of *Pbx* causes left-sided CDH (red circle, A, B)**

## Hypothesis/ Objective

- Our **hypothesis** is that a core group of genes is required for both diaphragm formation and development of the lungs.
- Defects in alveologenesis are common in patients with CDH
- Mutation of the *PBX1* gene has been identified in patients with CDH.
- ***Does deletion of Pbx in mice cause defects in alveologenesis?***

## Alveologenesis in CDH

- Alveologenesis starts at 38 weeks in human fetuses (5-day-old mice) and ends during school age (36-day-old mice).




- Lung hypoplasia is a leading cause of mortality and morbidity in patients with CDH

**Figure 3: Compared to normal lungs (A) lungs in patients with CDH are underdeveloped and fail to undergo alveologenesis (B).**

## Methods: Tissue specific deletion of *Pbx* in the developing lung

### Lung mesenchyme specific deletion

**Figure 4: *Tbx4*-rtta; *Tet*-o-Cre directs inducible recombination throughout the lung mesenchyme**

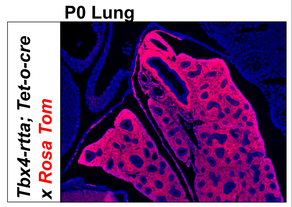
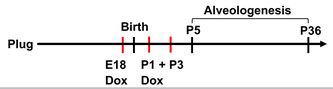



**Figure 5: To induce *Pbx* deletion in the last stages of development, doxycycline was administered via food given to the mothers at E18, and then injected into the pups at P1 and P3**




## Results: Late embryonic *Pbx* deletion causes failure of alveologenesis

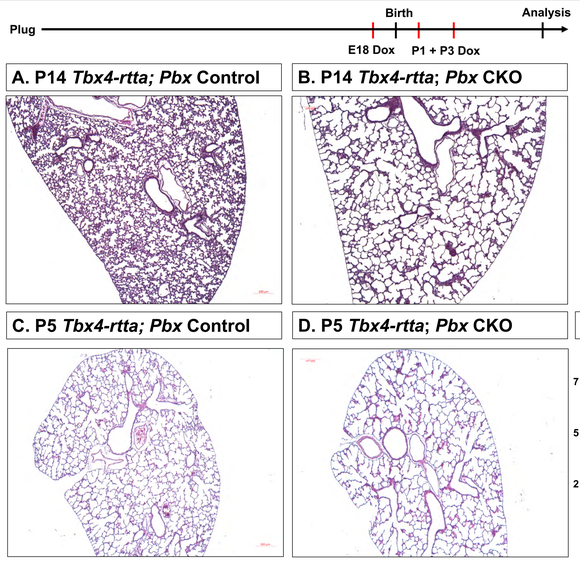



**Figure 6: Compared to controls (A,C), early postnatal deletion of *Pbx* causes failure of alveologenesis (B) that is first detected at P5 (D) quantified by mean linear intercept (MLI, E).**

## Results: Late embryonic *Pbx* deletion increases *α-SMA* myofibroblasts

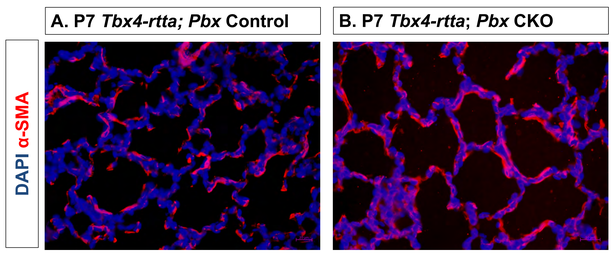



**Figure 7: Compared to the controls (A), the mutants have increased *α-SMA* myofibroblasts in a more diffuse organization (B).**

## Results: Late embryonic *Pbx* deletion does not affect *Pdgfrα*+ myofibroblasts

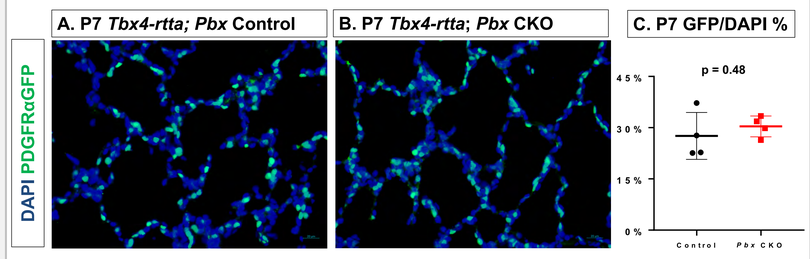



**Figure 8: Compared to the controls (A), there is no difference in the mutant *Pdgfra*+ cell population (B) as quantified by a ratio over total live cells (C).**

## Results: Late embryonic *Pbx* deletion affects extra cellular matrix production

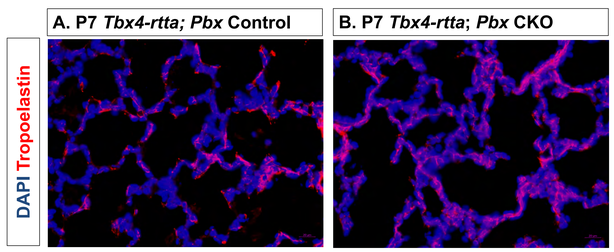



**Figure 9: Compared to the controls (A), the mutants have increased extra cellular matrix that is more diffusely organized (B).**

## Conclusions

- Postnatal deletion of *Pbx* in lung mesenchyme results in failure of alveologenesis
- *Pbx* deletion results in increased and more disorganized *α-SMA* myofibroblasts and extra cellular matrix production

## Future Directions

- Determine the cellular and molecular mechanisms responsible for failure of alveologenesis caused by the deletion of *Pbx*
- Conduct lung mesenchyme specific gene expression analysis to identify the genetic defects responsible for failure of alveologenesis following loss of *PBX* function

## Acknowledgements

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