Impaired Infant Lung Growth in Mice with Genetic Deletion of Endothelial Cell-Specific Vitamin D Receptor Expression

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Background

• Maternal Vitamin D deficiency (VDD) during pregnancy is increasingly recognized and associated with several maternal and fetal morbidities.
  • High risk for asthma and wheezing in childhood (Litonjua AA, 2012, 2014).
  • Maternal VDD disrupts normal lung airspace and vascular development in infant rats (Mandell EW, 2020).
  • Mechanisms through which decreased VD alters lung structure and function during development are incompletely understood.
  • Past studies have suggested that lung endothelial cell (LEC) from the offspring of mothers with VD deficiency demonstrate reduced growth and tube formation in vitro, suggesting that impaired VD signaling may contribute to persistent LEC dysfunction with altered infant lung growth (Gonzalez T, unpublished).
  • However, the direct effects of EC-specific VD receptor (VDR) deletion on lung development is unknown.

Hypothesis

We hypothesize that endothelial cell-specific deletion of the VDR gene disrupts normal lung alveolar and vascular growth in neonatal mice.

Study Questions

Does endothelial cell-specific vitamin D receptor knockout in 2 week mice: 1. Alter lung development through alveolarization and distal lung growth? 2. Alter pulmonary vessel density? 3. Alter right ventricular hypertrophy?

Methods

Animal Model and Study Design:
• Endothelial cell-specific vitamin D receptor knockout mouse (VDR-ECKO) were generated using Cre-loxP technology (figure 1).
• LoxP alleles flag the vitamin D receptor (VDR) gene
• Cre recombinase identifies loxP sites and deletes VDR.
• Cre expression is regulated by Tie2, an endothelial-cell-specific promoter.
• Cre + and Cre – mice with VDR LoxP were mated, generating VDR-ECKO mice and control littermates (figure 2).

Study Measurements:
• At DOL 14, lungs and heart were fixed in 4% paraformaldehyde and pulmonary vessel density was measured by counting the number of stained vessels per field of view.
• Right ventricular hypertrophy was assessed by calculating the weight ratio of right ventricle to left ventricle and septum.

Results

VDR-ECKO mice demonstrated reduced lung alveolarization compared to controls:

- Mean linear intercepts
- Radial alveolar counts

VDR-ECKO mice demonstrate impaired distal lung development compared to controls:

- Pulmonary vessel density
- Right ventricular hypertrophy

Summary

• In comparison to control mice, VDR ECKO mice demonstrate:
  • Impaired distal lung airspace growth:
    • Increased mean linear intercepts
    • Decreased radial alveolar counts
    • Decreased pulmonary vessel density
  • Increased right ventricular hypertrophy

Conclusion

We found that decreased vitamin D signaling in a VDR-ECKO model impairs lung alveolar and vascular growth and caused right ventricular hypertrophy in newborn mice.

Speculation

• We speculate that endothelial cell-specific vitamin D signaling plays an important role in endothelial cell function, which contributes to normal lung growth and development.
• Vitamin D signaling may be a therapeutic target for the treatment and management of bronchopulmonary dysplasia in infants.