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Analyzing the changes in the pulmonary vasculature associated with pulmonary hypertension in congenital diaphragmatic hernia (CDH) using a miR-200b knock out mouse model

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Background:

The high mortality and morbidity in congenital diaphragmatic hernia **(**CDH) are mainly caused by pulmonary hypertension (PH) resulting from changes in vasculature remodeling. We have shown that microRNA-200b (miR-200b) is an epigenetic factor linked to the pathogenesis of CDH. We hypothesized that changes in the pulmonary vasculature are associated with miR-200b knockdown.

Objective:

We aimed: 1) to evaluate the morphological changes of the lung vasculature in miR-200b$^{-/-}$ mice; 2) to determine the expression of vascular endothelial growth factor (VEGF) in lungs of miR-200b-/- mice.

Methods:

Morphometric vasculature changes were assessed non-invasively with echocardiography to measure pulmonary arterial pressure (n=6). We used micro-computed tomography to demonstrate the complexity of the pulmonary vasculature at the microlevel with high resolution, quantitative, three - dimensional images. Verhoeff-van Gieson (VVG) staining was used to assess vascular remodeling (n=3). VEGFR-1 expression was assessed using Western blotting and immunohistochemistry (IHC).

Results:

Echocardiography revealed PH in miR-200b$^{-/-}$ mice. MiR-200b$^{-/-}$ mice have 14% increased right ventricular outflow tract ($p=0.006$) and shortened pulmonary acceleration time by 24% ($p<0.0001$). Also, right ventricular internal diameter significantly increased during systole ($p=0.0383$) and diastole ($p=0.024$). Morphometric assessment showed that miR-200b$^{-/-}$ lungs have 29% increased medial wall thickness, 32% adventitial wall thickness, and 65% adventitial area in pulmonary arteries compared to wildtype lungs ($p<0.05$). IHC showed that VEGFR1 expression was higher in the vessels of miR-200b-/- lungs, particularly in the endothelial cells of arterioles. Western blot results indicated that the expression of VEGFR1 was higher in miR-200b-/- compared to WT (non significant).

Conclusion:

Changes in vascular morphology in miR-200b mice are associated with PH in CDH. Our results suggest that miR-200b is involved in the alteration of the VEGF signaling pathway and thus can contribute to abnormal pulmonary vasculature development.