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Upregulation of Endothelin Receptor A Through Decreased miR-200b May Contribute to Pulmonary Hypertension in Congenital Diaphragmatic Hernia

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Background:

Pulmonary hypertension (PH) is a leading cause of death in babies born with congenital diaphragmatic hernia (CDH). Higher expression of microRNA-200b (miR-200b) in the lungs of babies with CDH has been linked to better patient outcomes. Previously, we demonstrated that miR-200b knockout (KO) mice develop PH. Endothelin receptor A (ETA), a known vasoconstrictor, possesses a potential miR-200b binding site which may regulate ETA protein expression.

Objective:

The objective of this study was to determine the relationship between miR-200b and ETA in PH.

Methods:

8-week-old miR-200b KO and wild type (WT) mice were compared for PH and ETA expression (n=6). Pulmonary acceleration time (PAT) and cardiac output was observed through echocardiography. Lung ETA expression was studied through immunohistochemistry and western blotting. Lung ETA mRNA was measured by RT-qPCR.

Results:

Echocardiography confirmed PH in miR-200b KO mice. MiR-200b KO mice displayed decreased PAT as compared to WT mice (14.8 ms and 19.3 ms, respectively; $P<0.0001$). Cardiac output was increased in miR-200b KO mice as compared to WT mice (24.8 ml/min and 14.8 ml/min, respectively; $P<0.0057$). Immunohistochemistry showed ETA expression in both miR-200b KO and WT endothelial cells, though higher expression was seen in the arteries of miR-200b KO mice. Qualitative western blot analysis showed higher overall expression of ETA in miR-200b KO mouse lungs. There was no significant difference in normalized ETA mRNA expression between miR-200b KO and WT mouse lungs (1.46 and 1.17, respectively; $P=0.1109$).

Conclusion:

The development of PH in miR-200b KO mice suggests that miR-200b is an important factor in the development of PH in CDH. Higher protein expression of ETA in the lungs of miR-200b KO mice may be a factor in the development of PH. This, along with unchanged ETA mRNA, suggests a possible regulatory role of miR-200b on ETA protein expression, which could contribute to better CDH patient outcomes.