HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS AS MODEL FOR EXAMINING ENDOTHELIAL DYSFUNCTION AND SEX-BASED DIFFERENCES IN CDH

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Background: The main source of morbidity and mortality in congenital diaphragmatic hernia (CDH) is pulmonary hypoplasia and hypertension (PH) mediated by endothelial dysfunction, fibrosis, and mesenchymal differentiation. Preliminary data suggests that males have a predisposition toward CDH-PH; however, there are no human models to study the pathogenesis of CDH. Human umbilical vein endothelial cells (HUVECs) have been used in other disease models to represent disease-based vascular changes. We hypothesize that HUVECs from CDH patients will display pathologic features of PH found in CDH and will help decipher the role of sex as a biological variable.

Materials/Methods: HUVECs were harvested from umbilical cords of 10 CDH patients (7 male, 3 female) and 5 human age- and sex-matched controls (3 male, 2 female). CDH HUVECs and control HUVECs were treated with recombinant TGF-β1 for 48 hours. Expression of α-SMA and Col-1a1 was measured using RT-qPCR. RNA sequencing was performed to determine the differential regulation of genes and biological pathways between CDH HUVECs and HUVEC controls and HUVECs of different sexes. These data were then compared to a published CDH lung organoid database. Continuous data were analyzed with paired t-tests.

Results: Following TGF-ß1 treatment, CDH HUVECs demonstrated higher expression of α-SMA (4.9 ± 1.1 vs. 0.9± 0.1, $p<0.05$) and Col-1a1 (6.1±1.0 vs. 0.8± 0.2, $p <0.01$) than controls. RNA sequencing on CDH HUVECs and CDH lung organoids highlighted overlapping pathways consistent with endothelial dysfunction, fibrosis, and mesenchymal development and differentiation (Figure 1A). Male and female CDH HUVECs shared only 5 of 166 upregulated and 2 of 251 downregulated genes (Figure 1B).

Conclusions: CDH HUVECs demonstrated sex-based gene expression differences, which may suggest an underlying cause for the sex discrepancy in CDH-PH. Our data confirms that CDH HUVECs demonstrate gene expression and pathway regulation changes consistent with other known models of CDH. Furthermore, males show upregulation of the ECM organization and mesenchymal cell development pathways that may explain the elevated incidence of CDH-PH in males versus females. These findings have the potential to alter the way we manage sex-specific differences in CDH-PH.

Images / Graph / Table

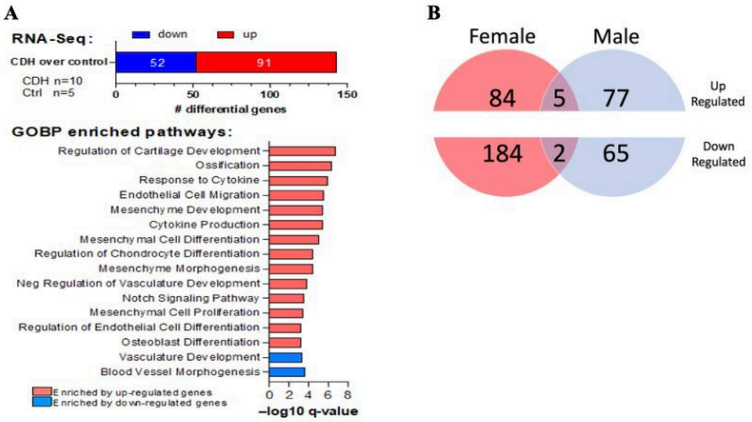

Figure 1: A: RNA Sequencing Results of All CDH HUVECs. B: RNA Sequencing Differential Gene Comparison of CDH HUVEC Male vs. Female