Models, Mechanisms and Experimental Therapies

65 SIN3A is required for epigenetic regulation of diaphragm and lung development

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109 Transcriptome Analysis of Umbilical Vein Endothelial Cells: A Patient-Derived Cellular Model of Studying CDH Endothelial Dysfunction

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SIN3A is required for epigenetic regulation of diaphragm and lung development

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A major barrier to the impact of genomic diagnosis in patients with congenital diaphragmatic hernia (CDH) is the lack of clarity regarding how an identified genetic variant causes abnormal development and how this information could be used to generate novel approaches for treatment. Our central hypothesis is that a core group of genes is required for diaphragm, lung, and pulmonary vascular development and that pathogenic variants in these genes are responsible for failure of diaphragm formation as well as defects in lung and pulmonary vascular development in patients. To better understand the genetic and developmental mechanisms responsible for CDH, we identified loss of function sequence variants in the SIN3A gene in patients with the disease. SIN3A acts as an epigenetic regulator of gene expression during development by directing the cell, timing, and genomic region-specific activity of histone deacetylase enzymes HDAC 1 and 2. We found that tissue-specific deletion of Sin3a in mice resulted in defects in diaphragm development, lung hypoplasia, and pulmonary hypertension, the major causes of mortality associated with CDH. Histological and gene expression analysis demonstrated that loss of SIN3A in the developing lungs resulted in reduced cellular differentiation, impaired cell cycling, and increased DNA damage caused by loss of histone deacetylase function. We found that the balance of histone acetylation could be restored by embryonic inhibition of histone acetyltransferase which increased cell differentiation and cell cycling while reducing DNA damage and improving lung development in SIN3A mutant mice. These results demonstrate the importance genetic analysis in complex congenital malformations such as CDH. In the case of SIN3A loss of function, impaired epigenetic regulation and defects in lung development can be rescued by restoring the balance of histone acetylation.

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In vivo administration of amniotic fluid stem cell extracellular vesicles rescues the transcriptomic profile of fetal lungs in rats with CDH

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Background

We recently reported that administration of extracellular vesicles from rat amniotic fluid stem cells (AFSC-EVs) restored impaired lung branching morphogenesis and promoted epithelial and mesenchymal differentiation in various models of CDH. To better understand AFSC-EV mechanism of action, we aimed to characterize the transcriptomic changes induced by intra-amniotically (IA) administered AFSC-EVs to fetal lung cell populations.

Methods

AFSC-EVs: isolated from AFSC conditioned medium (ultracentrifugation), characterized for size, morphology, and expression of canonical EV protein markers (CD63, TSG101, Flotilin), and fluorescently labeled (ExoGlow-Vivo™) for tracking experiments.

CDH model: at embryonic day (E)9.5, rat dams were gavaged with olive oil or nitrofen. At E19.5, fetuses received an IA injection of saline (control+saline, n=38; CDH+saline, n=64) or AFSC-EVs (CDH+AFSC-EVs, n=56) and sacrificed at E21.5.

IA validation studies: for EV tracking fetuses were imaged using bioluminescence (IVIS®); for lung growth/maturation, H&E was performed for branching morphogenesis and RT-qPCR for Fgf10, Sftpc, and Sox9 expression.

Single nucleus RNA-sequencing: lungs from control+saline (n=2), CDH+saline (n=3), and CDH+AFSC-EVs (n=3) groups were sequenced (10X genomics) and analyzed for cell-type specific gene expression profiles (Seurat/R).

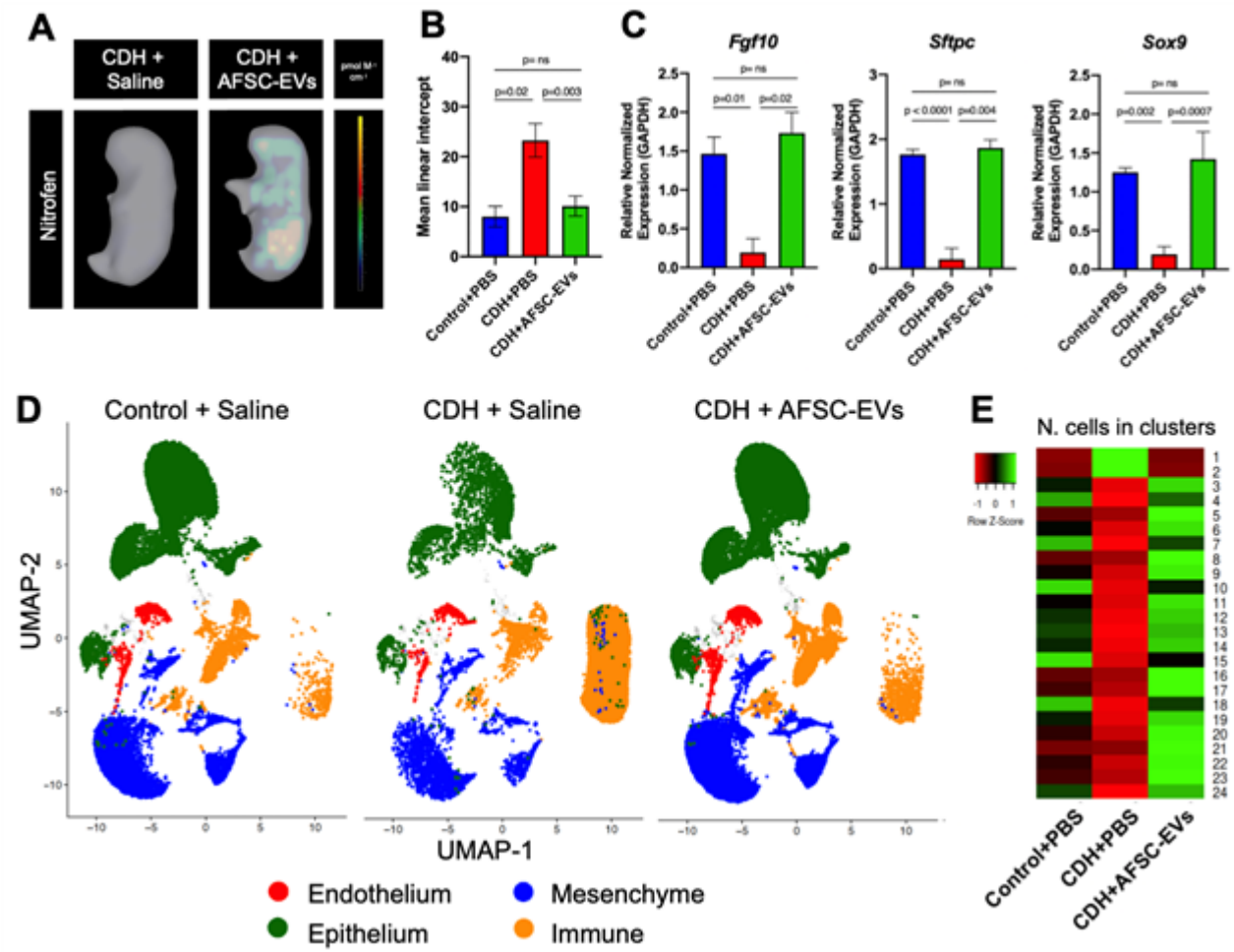
Results

IA-injected bioluminescent AFSC-EVs reached several organs, including lungs (Fig.A). Compared to control, CDH lungs had fewer branches and reduced Fgf10, Sftpc, and Sox9 expression, which were restored back to control levels with AFSC-EV administration (Fig.B-C). snRNA-seq analysis revealed 4 clusters representative of epithelial, endothelial, mesenchymal, and immune cells (Fig.D). CDH lungs have a profound population level shift in cell state that was reversed following AFSC-EV administration (Fig.E).

Conclusions

This study is the first to evaluate the effects of CDH on fetal lungs using single nucleus RNA-sequencing. This analysis revealed that the transcriptomic profile of the main lung cell populations is altered in CDH lungs. AFSC-EV based treatment rescued the normal morphological and transcriptomic profile of CDH lungs.

Images


A
CDH + Saline
CDH + AFSC-EVs
Nitrofen
B
Mean linear intercept
p= ns
p=0.02
p=0.003
Control+PBS
CDH+PBS
CDH+AFSC-EVs
C
Fgf10
Sftpc
Sox9
Relative Normalized Expression (GAPDH)
p= ns
p=0.01
p=0.02
p < 0.0001
p=0.004
p=0.002
p=0.0007
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CDH + Saline
CDH + AFSC-EVs
UMAP-2
UMAP-1
Endothelium
Epithelium
Mesenchyme
Immune
E
N. cells in clusters
Row Z-Score
Control+PBS
CDH+PBS
CDH+AFSC-EVs

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Transcriptome Analysis of Umbilical Vein Endothelial Cells: A Patient-Derived Cellular Model of Studying CDH Endothelial Dysfunction

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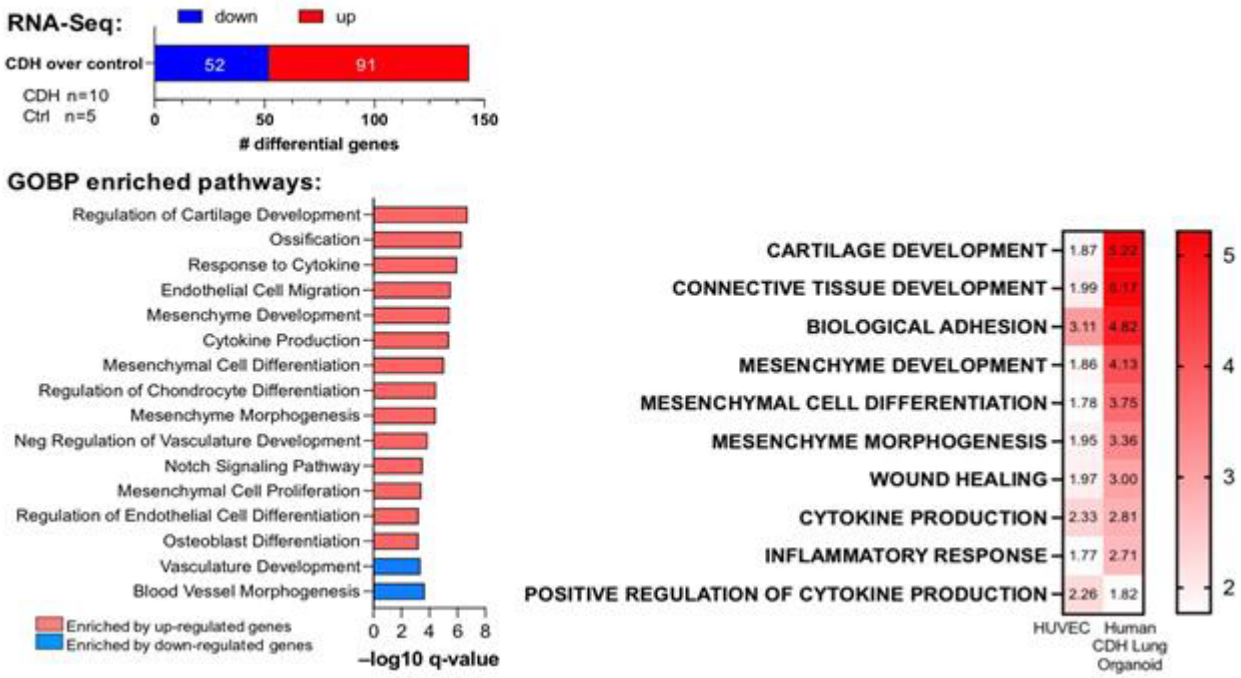
Background:
Congenital diaphragmatic hernia (CDH) is a complex anomaly characterized by pulmonary hypoplasia and severe pulmonary hypertension (PH). Aberrations in extracellular matrix (ECM) deposition and endothelial cell function have been implicated in the pathogenesis of CDH-PH. However, no useful ex-vivo human model currently exists to study these patterns of disease. Human umbilical vein endothelial cells (HUVECs) have been shown to be representative of the pulmonary vasculature and are a validated model for studying other neonatal diseases, such as bronchopulmonary dysplasia. We propose that HUVECs isolated from infants with CDH can be used as a model to investigate pathways involved in the pathogenesis of CDH-PH, allowing for the development of therapeutics to address these pathologies.

Methods:
Following institutional and parental approval, HUVEC samples were isolated from patients with CDH (n=10) and age- and sex-matched controls (n=5) at birth. Transcriptome analysis was performed on HUVECs via RNA-sequencing (RNA-seq) and compared to gene expression in CDH lung organoid samples for overlap. Heat maps were developed to assess enriched pathways of interest.

Results:
Compared to age- and sex-matched controls, RNA-seq of CDH HUVECs demonstrated enrichment in pathways related to endothelial dysfunction such as endothelial cell migration, mesenchymal cell differentiation, regulation of endothelial differentiation, and blood vessel morphogenesis (Figure 1). Genes involved in ECM remodeling, endothelial function, and lung development overlapped in the transcriptome of CDH HUVECs when compared to CDH lung organoid gene expression.

Conclusion:
There was marked overlap in up- and down-regulated genes expected to be involved in pulmonary vascular endothelial regulation, when comparing the transcriptome from CDH HUVECs to lung organoid samples. These findings support that HUVECs derived from CDH patients can be used as a model to examine endothelial dysfunction in CDH.

Images



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Background:

The abnormal vascular development in hypoplastic lungs of fetuses with congenital diaphragmatic hernia (CDH) causes postnatal pulmonary hypertension, which remains a primary determinant of outcome. We have recently shown that administration of extracellular vesicles derived from rat amniotic fluid stem cells (rAFSC-EVs) restores vascular development in fetal rat hypoplastic lungs. To take the next steps towards clinical translation, herein, we investigated the ability of human AFSC-EVs (hAFSC-EVs) to rescue pulmonary vascular development in human fetal hypoplastic lung explants.

Methods:

hAFSC-EVs were isolated by ultracentrifugation from the conditioned medium of hAFSC obtained under good manufacturing practice guidelines. hAFSC-EVs were characterized for size, morphology, and canonical EV protein markers (CD63, FLOT1, TGS101).

Human fetal lung explants were obtained from healthy terminations (18-19 weeks of gestation; REB#10-0128-E). 1-2mm3 lung specimens were cultured as explants with medium alone (control group) or medium + NSC23766 (RAC-1 inhibitor) at 0h and 24h to induce pulmonary hypoplasia. The latter specimens were treated with medium alone (hypoplasia group) or hAFSC-EVs at 48h and 72h (hAFSC-EV group). Explants from all groups were harvested at 96h and assessed for vascular density (CD31; immunofluorescence) and endothelial markers critical for pulmonary vascular development (VEGFA, VEGFR1, VEGFR2, PECAM1) by RT-qPCR. Statistical analysis was performed using Kruskal-Wallis test; $p<0.05$ was considered significant.

Results:

Compared to control, hypoplastic explants had significantly decreased vascular density (Figure 1A) and lower levels of endothelial markers (Figure 1B). hAFSC-EV treatment rescued vascular density and gene expression levels of endothelial markers back to control levels (Figure 1A-B).

Conclusions:

This study reports for the first time that administration of hAFSC-EVs has a regenerative effect on the fetal pulmonary vasculature in a novel human model of pulmonary hypoplasia. This EV-based therapy holds great

potential for reversing pulmonary vascular remodeling and possibly preventing postnatal pulmonary hypertension in babies with CDH.