

CHRD 2024: Abstract Submission Form

Presenter Name

Nolan De Leon

Presenter Status

PhD Student

Role in the project

Design
Perform Experiments
Analyze Data
Write Abstract

Research Category

Basic Science

Title

Characterization and differential expression analysis of circAZ2 in nitrofen-induced CDH fetal rat lungs

Background

Circular RNAs (circRNAs) are a class of long non-coding RNAs that have been identified as potential biomarkers and epigenetic regulators of disease. The effects of circRNAs and their respective parental genes in congenital diaphragmatic hernia (CDH) and have not yet been identified.

Objective

Here, we investigate the expression profile of a circular RNA labelled as CircAZ2, as well as its respective parental gene, in fetal rat lungs to identify potential links to abnormal lung development in nitrofen-induced CDH.

Methods

Nitrofen was given to pregnant rats at embryonic day (E) 9 to induce CDH in the offspring. E21 rat lungs were collected, flash-frozen, and processed for total RNA isolation. Total RNA isolates were converted to cDNA for divergent primer PCR assays and junction point (JP) sequencing after subcloning. Custom divergent primers were employed to validate E21 expression levels in addition to cellular detection in situ. To optimize primers for detection, conventional PCR and JP sequence alignment using Sanger Sequencing was applied. Optimized primers were utilized for qPCR assays to validate the differential expression of circAZ2 in rat lung cDNA libraries of E21. CircAZ2 parental gene expression was also studied with RT-qPCR.

Results

Custom circAZ2 exonic primers were established and confirmed by Sanger sequencing. CircAZ2 exon-specific JP amplicons were also obtained with target size-specific products. Sex-specific expression of the circAZ2 parental gene showed no significant difference between CDH and control lungs, however, a trend of higher expression in female CDH and lower expression in male CDH was identified.

Conclusion

CircAZ2 exonic and JP sequences have been characterized. CircAZ2 parental gene trends towards sex-specific expression in nitrofen-induced CDH. Further studies will characterize circAZ2 splice isoforms using exonic JP-specific sequencing, as well as optimize divergent primers and RT-qPCR conditions.

Do you have a table/figure to upload?

No

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