CHRD 2021: Abstract & Poster Submission Form

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Research Category:

- ◉ Basic Science
- ○ Clinical
- ○ Community Health / Policy

What was your role in the project?

- ☑ Design
- ☑ Perform Experiments
- ☑ Analyze Data
- ☑ Write Abstract
- ☐

Presenter Status:

- ○ Undergraduate Students
- ◉ Masters Student
- ○ PhD Student
- ○ Post-Doctoral Fellows
- ○ Residents
- ○ Non-Trainee

Title

Validation of a circular RNA liquid biopsy technique for the prognosis and diagnosis of congenital anomalies

Background

Every 10 minutes, a baby is born with Congenital Diaphragmatic Hernia (CDH); a devastating birth defect presenting as a hole in the diaphragm, pulmonary hypoplasia, and pulmonary hypertension. Recently, we've identified circular ribonucleic acids (circRNAs) as a potential biomarker of CDH. Microarray analysis of human amniotic fluid and rat lung tissues demonstrated dysregulated expression of hsa_circRNA_001653, as well as rno_circRNA_007475 and mmu_circRNA_31436, respectively. Considering the 70% overlap between circRNAs expressed in lungs and amniotic fluid, we developed a BaseScope ™ liquid biopsy (BSLB) to validate the previous microarrays.

Objective

We hypothesize that the expression profiles of circRNAs in amniotic fluid can predict abnormal lung development in CDH.

Methods

This study was designed to refine the BSLB. Amniotic fluid and lungs were extracted from control and nitrofen-induced CDH rats on embryonic day 21 (E21). BaseScope™ was optimized on formalin-fixed, paraffin-embedded (FFPE) E21 rat lung tissues to detect rno_circRNA_007475 and circRNA_31436. RNA extraction was performed on human saliva as proof of concept with TRIzol, then optimized with the PureLink™ RNA Mini Kit. CircRNA primers were designed with PrimerQuest and NCBI Primer-BLAST.

Results

The optimal BaseScope™ protease treatment time on FFPE E21 rat lungs (2 minutes at room temperature and 4 minutes at 40°C) was determined empirically. Signal was detected in the positive control; however, background signal remains a problem with the circRNA and negative control probes. RNA extracted from human saliva via TRIzol was highly contaminated (absorbance 260/280 > 1.0; absorbance 260/230 > 0.2). RNA quality drastically improved using the PureLink™ RNA Mini Kit (absorbance 260/280 ~ 2.0; absorbance 260/230 ~ 2.0).

Conclusion

Re-design of the BaseScope™ circRNA probes may be necessary to increase specificity. The PureLink™ kits will now be used in the following stages of nucleic acid extraction for RT-qPCR and the BSLB. Further studies are required to defend or support the hypothesis.

Authors

- For each author, please click "**[+] Add Item**" and provide the author's information

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