Validation of a Circular RNA Liquid Biopsy Technique for the Prognosis and Diagnosis of Congenital Anomalies

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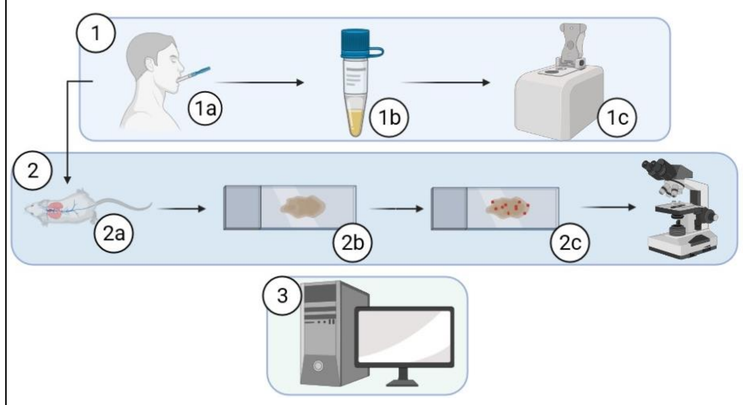
MiRACLA MicroRNAs • And • Congenital • Lung • Anomalies

BIOLOGY OF BREATHING Research Theme

Introduction

- Our lab has identified circular ribonucleic acids (circRNA) as a potential biomarker of congenital diaphragmatic hernia (CDH) via microarray analysis of amniotic fluid (AF).[1]
- We have developed a BaseScope™ *in situ* hybridization liquid biopsy to detect differentially expressed circRNAs, including rno_circRNA_007475 and mmu_circRNA_31436, in the amniotic fluid of nitrofen induced CDH rats.[2]
- The aim of this study is to refine the circRNA isolation and detection steps of our BaseScope™ liquid biopsy.

Methods



(1) Human saliva was collected and used for proof of concept (1a). RNA isolation was performed with TRIzol™ or the PureLink™ RNA mini kit (1b) and quality was assessed via spectrophotometry (1c).

(2) E21 control and nitrofen-induced CDH rat lungs were fixed in formalin and paraffin embedded (2a). Lungs were sectioned (2b) and assessed for the presence of rno_circRNA_007475 and mmu_circRNA_31436 via BaseScope™.

(3) CircRNA RT-qPCR primers were designed with PrimerQuest and NCBI Primer-BLAST.

Results

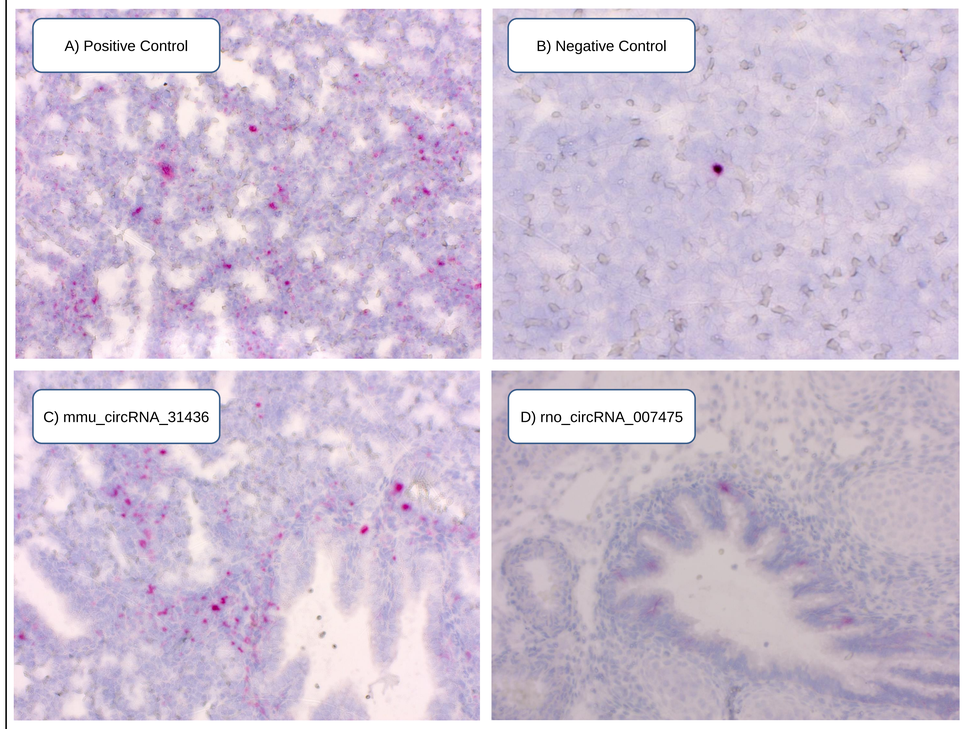


Figure 1: BaseScope™ assessment of circRNA expression in E21 control and nitrofen-induced CDH rat lungs. Tissue was treated with Protease IV and optimal permeabilization was achieved with 2 minutes at room temperature and 4 minutes at 40°C. Signal (punctate red dots) was detected in the positive control (A). Non-specific binding and/or signal amplification error was revealed by blurred purple-grey dots in the circRNA probes (B,C) as well as the negative control (D).

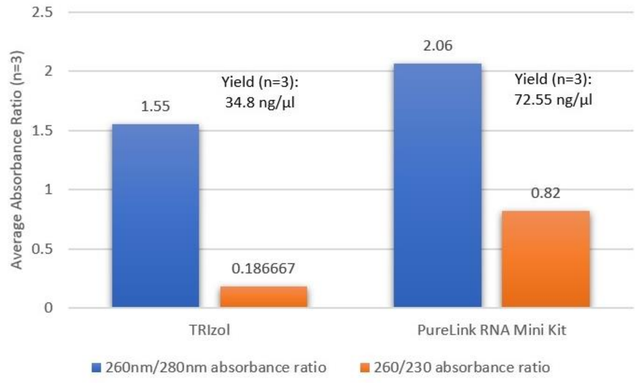


Figure 2: Comparison of salivary RNA isolation methods. Spectrophotometric analysis revealed TRIzol™ RNA isolation resulted in lower quality and yield of RNA, as compared to the PureLink™ RNA Mini Kit.

Conclusion

- Re-design of the *in situ* hybridization probes for rno_circRNA_007475 and mmu_circRNA_31436 will be considered to allow for higher specificity during the BaseScope™ assay.
- PureLink™ RNA mini kit provides higher quality RNA, as well as higher yield. Presence of chemical contaminants remains high, but may be mitigated with additional RNA washing steps.
- Considering the low yield and high amounts of chemical contaminants relative to RNA content, saliva may not be the best biofluid to test RNA isolation.

Future Directions

- Quantification and comparison of circRNA expression in control and nitrofen-induced E21 rat lungs, as well as human control and CDH lungs, with BaseScope™ (microarray validation).
- RT-qPCR to determine relative circRNA expression patterns in the amniotic fluid of control and nitrofen-induced CDH rats, as well as control and CDH human amniotic fluid (microarray validation).
- Presence or absence of circRNAs in DNA extracts will be examined via the PureLink™ DNA kit.
- Validation of our previous microarray results with our modified BaseScope™ liquid biopsy.

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CIHR IRSC

Contact & References

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1) Wagner, R. *et al*. European Respiratory Journal (2020)
2) Kirby, E. et al. Pediatric Surgery International (2019)