MicroRNA Expression in Tracheal Aspirates and Saliva Samples in Extreme Preterm Infants

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RATIONALE: Tracheal aspirate (TA) is non-invasive biofluid, easily obtained in intubated preterm infants. Its transcriptomic expression has been studied in complications of prematurity including bronchopulmonary dysplasia (BPD) (1). However, TAs can be difficult to obtain in non-intubated infants. This study examines saliva as non-invasive source for microRNAs (miRNA) analysis in the preterm infants at risk for BPD. METHODS: This cross-sectional study included six extremely preterm infants (<28 weeks gestation), who later were diagnosed with mild-to-moderate BPD. TAs were obtained at multiple time points along with saliva using DNA Genotek saliva collection swabs within 30 days of delivery. Exosomes were isolated from TAs samples using ExoQuick reagent and total RNAs were extracted, and all circulating RNAs were extracted from saliva samples using Quick-RNA Microprep (Zymo Research). Small RNA-sequencing libraries were prepared from using the QIAseq miRNA Library Kit (QIAGEN), per manufacturer instructions (2). The resulting multiplexed high-throughput sequencing data was mapped and UMI analysis conducted via the GeneGlobe Data Analysis Center (QIAGEN). The DESeq2 R package (REF) was used to identify miRNA features with similar expression patterns between paired TA and saliva samples (which might serve as non-invasive markers of BPD). Similarly expressed miRNAs were those with adjusted p-value > 0.1 (calculated by the Benjamini-Hochberg method) and fold change < 2. The list of similarly expressed small RNAs was analyzed with Ingenuity Pathway Analysis (IPA) for functional enrichment (3). RESULTS We isolated 939 miRNAs from TA exosomes, and 917 of these were also expressed in the salivary samples. We identified 161 miRNAs as similarly expressed in both TA and saliva samples with high correlation (r=0.97). IPA Bioinformatic analysis of the 161 similarly expressed exosomal miRNAs in TAs and salivary samples identified organismal injury and abnormalities as the top disease, cellular development as the top molecular function and organismal development as the top physiological system development and function. CONCLUSIONS Our study establishes feasibility of saliva collection in extreme preterm infants born < 28 weeks of gestation at less than 30 days of age. Although TA and saliva are distinct biofluids, arising from lower airway and upper airway microenvironments, we isolated 161 miRNAs similarly expressed in both biofluids. These miRNAs may represent non-coding RNA signals that regulate pathways of organismal growth, development and injury throughout the infant respiratory tract. Non-invasive measurement of these miRNAs in the saliva of extreme preterm infants with BPD may provide molecular information about pulmonary disease.

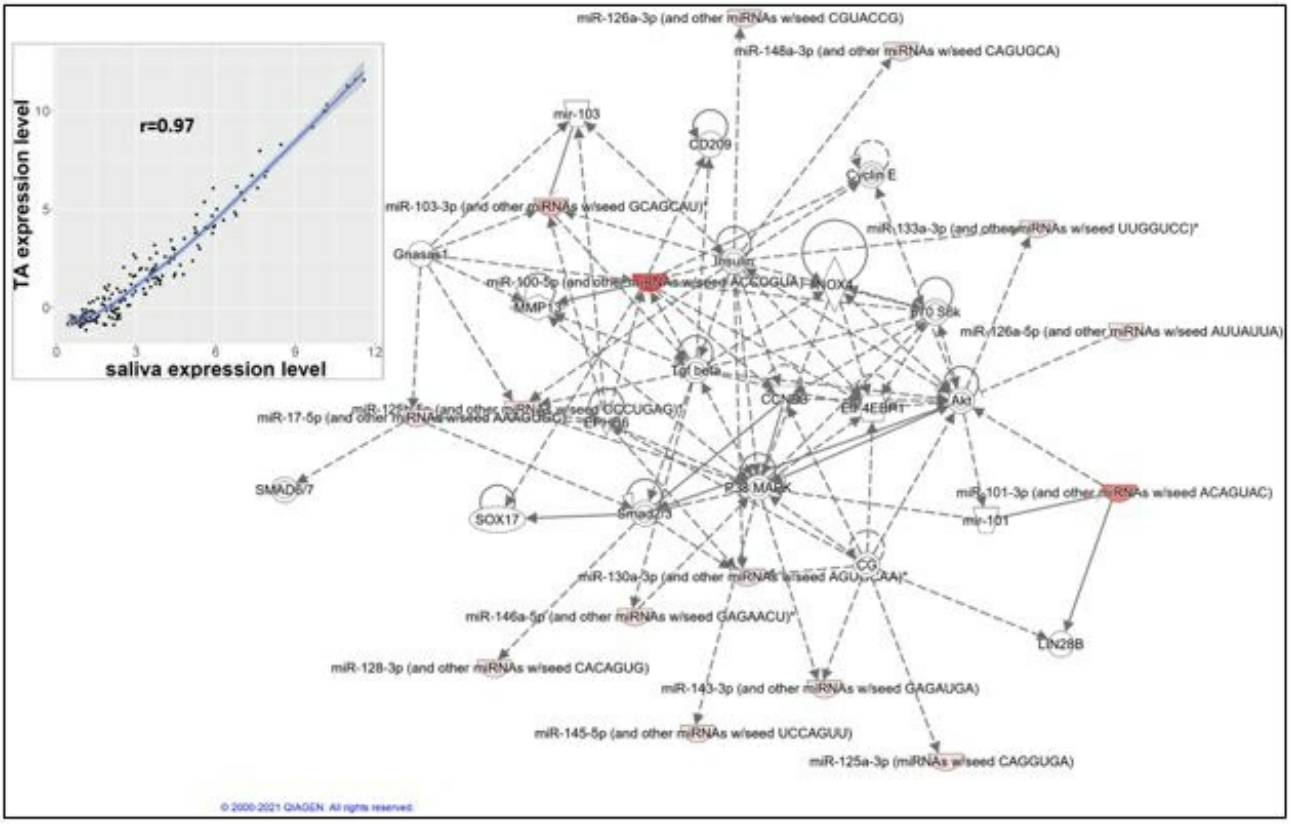

Figure 1 Correlation plot of the similarly expressed miRNAs in tracheal aspirate samples and saliva samples in 6 extreme preterm infants collected at 3, 7 and 28 days old and their predicted pathways analyzed via Ingenuity pathway Analysis

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