Characterization of Extracellular RNA from Bronchoalveolar Lavage Fluid

Ravi Rajendra[1], Mark N. Gillespie[2], and Glen M. Borchert[1,2]

[1]Department of Biology, University of South Alabama, Mobile, AL

[2]Department of Pharmacology, University of South Alabama, Mobile, AL

Abstract

Until recently, all types of ribonucleic acid (RNA) were thought to exist only within the cell. Excitingly, evidence now indicates that RNA can at times be secreted outside of the cell within extracellular vesicles (EVs). This RNA called extracellular RNA (exRNA) has been shown to play a role in aiding intercellular communication and regulating cellular processes. In a clinical setting, exRNA has the potential to either serve as a biomarker indicating the presence of a disease or as a targeted tool used to treat a disease. Our project marks the first time that exRNA has been isolated at the University of South Alabama. Our study aims to characterize the exRNA composition found in bronchoalveolar lavage fluid (BALF) from the lungs of rats and how the exRNA composition found in BALF varies based on stressors. BALF from three experimental groups was used: 1) control 2) the effect of a high pressure environment and 3) the effect of *Pseudomonas* bacteria. In our study, exRNA was successfully isolated from all three samples of BALF with the addition of buffers and series centrifugation. A method for analyzing the composition of exRNA was developed through the use of a publicly available exRNA dataset. In the future, we will receive results from commercial next generation sequencing and utilize the Basic Local Alignment Search Tool (BLAST) algorithm to analyze the exRNAs isolated. Through this analysis, we can determine what types of RNAs are released by cells in the lungs, how exRNAs released might vary with stressors, and what role exRNAs play within the respiratory system.

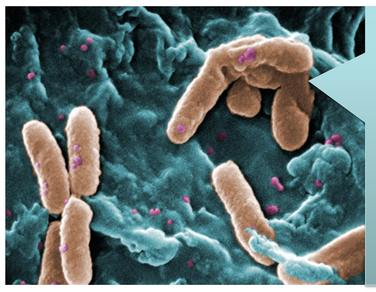

FIGURE A: *Psudomonas aeruginosa* bacteria. ExRNA could potentially serve as an anti-microbial agent in the immunological response to infections caused by bacteria like *Pseudomonas*, which is responsible for causing the second most common infection in hospitalized patients. Conversely, exRNA could be released from *Pseudomonas* as part of the bacteria's pathogenesis.

Hypothesis

- ExRNA can be isolated from the EVs contained within BALF.
- The overall composition and types of exRNA found in BALF will vary based on the stress that the rats experienced.
- ExRNA composition can be analyzed using BLAST.

Acknowledgements

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Methods

Acquisition of BALF: Samples of BALF were acquired from rats that experienced no stressor (control), rats that were pressure challenged, and rats that were exposed to a virulent strain of *Pseudomonas* bacteria.

Removal of Large Impurities from BALF: All three samples were centrifuged at 10 minutes at 16,000 x g at 4°C to precipitate large impurities contained in BALF.

Isolation of EVs from BALF: The exoRNeasy Serum/Plasma Midi Kit (QIAGEN, Hilden, Germany) was used to isolate EVs from BALF. Standard manufacturer's protocol was used. A series of buffers from the kit was used along with serial centrifugation at room temperature.

Extraction of exRNAs from EVs: The exoRNeasy Serum/Plasma Midi Kit was then used to lyse the EVs isolated in the previous step and extract the exRNA. Standard manufacturer's protocol was used. After addition of 14 µl RNase-free water, the spin column was centrifuged for 1 minute at full speed to elute the RNA.

Confirmation of Presence of exRNA: One microliter of each sample was pipetted onto the optical surface of the Nanodrop Lite (Thermo Scientific, Waltham, MA, USA) spectrophotometer. ExRNA concentration was recorded.

Sequencing of exRNAs: All three samples containing the isolated exRNA were sent to Otogenetics (http://www.otogenetics.com) for commercial next generation sequencing of small RNAs. Approximately 8 million RNA reads will be provided and used for further analysis.

Analysis of existing data sets using BLAST: In anticipation of receiving our sequencing data for the exRNA we isolated, the BLAST algorithm was applied to a publicly available exRNA dataset. The results were downloaded and cross-referenced with the NCBI's Nucleotide database to characterize the composition of the exRNA.

Results

ExRNA was successfully isolated from all three samples of BALF. The reads from commercial next generation sequencing have not yet been received. ExRNA concentration was significantly higher in the sample of BALF from rats that were exposed to a high pressure environment.

Sample	exRNA concentration
No stressor (control)	11.2 ng/µL
High pressure	97.1 ng/µL
	89.0 ng/µL
Pseudomonas bacteria treated	8.5 ng/µL

FIGURE B: The concentration of exRNA contained within each sample as measured through spectrophotometric analysis is reported. The total volume of each sample was less than 14µL

Analysis

Using BLAST and the NCBI's nucleotide database, approximately 90% of the publicly available exRNA dataset was characterized.

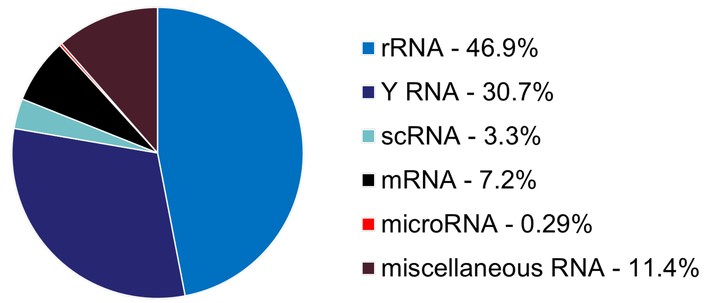

FIGURE C: The composition of RNA in the publicly available exRNA dataset resembles that of a regular cell.

Conclusions

- BALF contained exRNA that can be isolated and sequenced.
- ExRNA concentration varied based on the stressor.
- Analysis of a publically available dataset revealed a scenario in which exRNA composition did not vary significantly from normal cellular RNA composition.
- ExRNA isolated in this study can be analyzed using BLAST once the results from sequencing are received.

Future Directions

- Apply BLAST to sequencing results from the exRNA that we isolated to determine the exRNA composition
- Describe the variance based on stress (if any) in the types of exRNA released
- Determine if exRNAs play a role in immune response within the respiratory system
- If exRNA composition is consistently similar with cellular RNA composition, determine if exosomes function as "decoy cells" that reduce the pathogenicity of viruses
- Compare exRNA sequences generated from the *Pseudomonas* treated sample with the known genome of *Pseudomonas* bacteria to determine whether the bacteria releases exRNA as part of its pathogenesis

Literature Cited

Levänen B, Bhakta NR, Paredes PT, *et al.* 2013. Altered microRNA profiles in bronchoalveolar lavage fluid exosomes in asthmatic patients. *J Allergy Clin Immunol* **131:** 894-903.

Takahashi K, Yan I, Kim C, Kim J, Patel T. 2014. Analysis of extracellular RNA by digital PCR. *Front. Oncol.* **4:** 129.

Torregrosa Paredes P, Esser J, Admyre C, Nord M, Rahman QK, Lukic A, *et al.* 2012. Bronchoalveolar lavage fluid exosomes contribute to cytokine and leukotriene production in allergic asthma. *Allergy.* **67:** 911–19.

Witwer K, Buzas E, Bemis L, Bora A, Lasser C, Lotvall J, *et al.* 2014. Standardization of sample collection, isolation and analysis methods in extracellular vesicle research. *Journal of Extracellular Vesicles.* **2:** 20360.