Effects of Stimulating Human Bronchial Epithelial Cells With House Dust Mite on RNA Secretion

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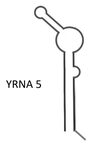
Introduction

- Asthma is a chronic inflammatory pulmonary disease that affects 8% of adults
- Human bronchial epithelial (HBE) cells make up our allergic airway epithelium, our first line of defense against allergens, pathogens, etc.
- Extracellular RNA (exRNA) is hypothesized to be a biomarker within the immune system
  - MircoRNA (miRNA) – small, noncoding, regulatory functions such as inhibiting gene expression , ~22 nucleotides long
  - YRNA – regulate DNA replication, participate in RNA quality control, ~110 nucleotides long, cleave into 2 fragments



- Extracellular vesicles (EVs) are a form of packaging for exRNAs that consist of a lipid bilayer

Methodology

Goal

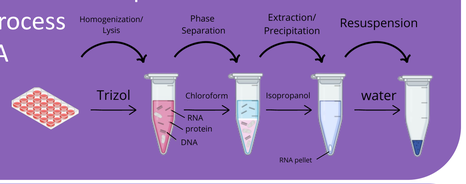
To identify miRNA and YRNA secretion patterns in HBE cells upon the stimulation of different concentrations of house dust mite (HDM).

Plating and Stimulating Cells

- Plated ~400k HBE cells/well
- Round 6 – IL-13 was also tested (10 ng/mL & 1 ng/mL)

Sample Collection, RNA Extraction, & RT-PCR

- Culture media containing extracellular RNA was collected
- Cells were lysed and intracellular RNA was extracted
- A known amount of calibrator was spiked in to account for RNA loss during extraction process
- Converted RNA → cDNA using RT-PCR



qPCR

- qPCR quantifies the amount of RNA in a sample by measuring the fluorescence that accumulates as the cDNA is amplified
- The RNA in Calibrator 2 , Mir-21, Mir-200, YRNA 5 5’ fragment, YRNA 5 3’ fragment, YRNA 5 full strand were quantified

EV Counting

- 8 million cells were plated in a 10 cm Petri dish
- Cells were stimulated with 100 µg/mL HDM
- EVs were counted using a ZetaView Nanoparticle Tracking instrument
- The ZetaVIew uses a camera to detect particle displacement which allows the device to quantify the amount of particles present

Results

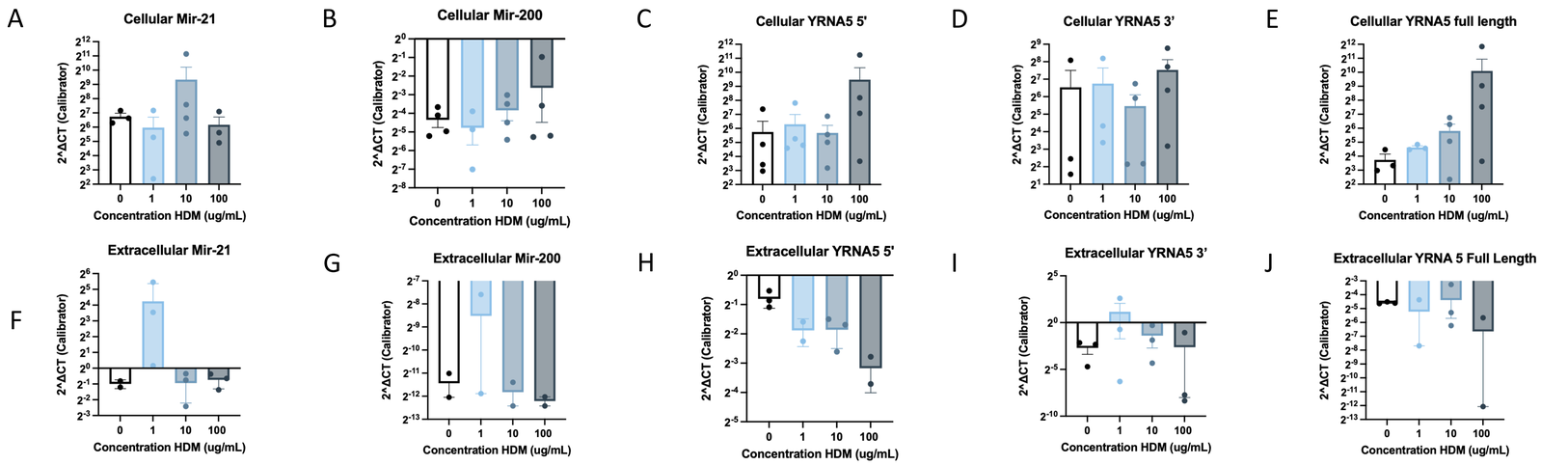


Figure 1: RNA Secretion Patterns

(A-E) The RNA contents within the cells after different concentrations of HDM stimulation are shown. (F-J) The RNA contents in the extracellular region after different concentrations of HDM stimulation are shown. All data is normalized to the amount of Calibrator 2 present.

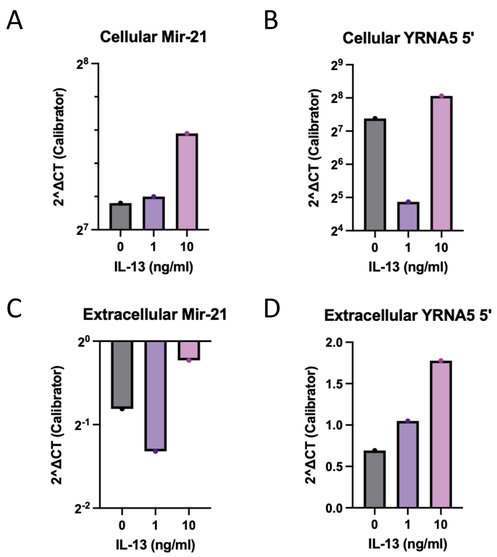


Figure 2: IL-13 Stimulation

(A & C) The RNA contents of the intracellular and extracellular regions of Mir-21 respectively. (B & D) The RNA contents of the intracellular and extracellular regions of YRNA5 5’ respectively. All data is normalized to the amount of Calibrator 2 present.

IL-13, known to induce an allergic response, acts to demonstrate the behavior of cells undergoing allergic response. Many of the trends are inconsistent to those treated with HDM.

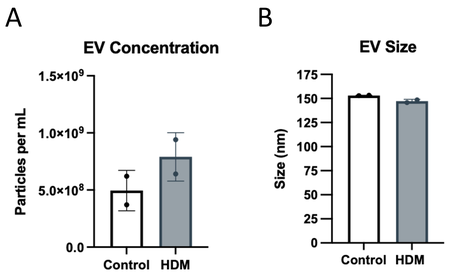


Figure 3: EV Analysis

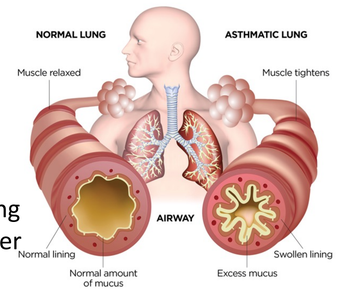
(A) The concentrations of EVs before and after HDM stimulation are shown. The number of EVs increased slightly after HDM stim in this round of testing. (B) The size of EVs present before and after HDM stimulation are shown. The sizes remained similar.

Discussion

- While some patterns in RNA secretion were evident, further testing needs to be done to verify them
- Detecting patterns in RNA secretion could suggest that RNAs are communicating after the stimulation
  → RNA communication plays a role in our body’s inflammatory response to asthma

Future Directions

- Repeat IL-13 stimulations and EV counting
- Conduct experiment in air liquid interface cultures
- Test different miRNAs and YRNAs
- Test for a gene that responds during inflammation to determine whether stimulation is occurring (ITGB4)



References

1. Secret messengers: Extracellular RNA communication in the immune system, Nation, GK, DOI: 10.1111/imr.13027
2. Airway Epithelial cGAS Is Critical for Induction of Experimental Allergic Airway Inflammation, Meng P, DOI: 10.1111/imcb.12223
3. YRNAs and YRNA-Derived Fragments as New Players in Cancer Research and Their Potential Role in Diagnostics, Guglas K, DOI:10.3390/ijms/ 21165682
4. www.addgene.org/protocols/kit-free-rna-extraction
5. https://community.aafa.org/