Differential Gene Expression in Exosomes Infected with Respiratory Syncytial Virus (RSV) or Human Metapneumovirus (HMPV)

utmb Health

Esmeralda Hechavarria, Kyle T. Nguyen, Heidi Spratt, Tiziana Corsello-Gorgun, Roberto P. Garofalo

Internal Medicine

The University of Texas Medical Branch, Galveston, TX

Introduction

Respiratory syncytial virus (RSV) and Human metapneumovirus (HMPV) are two of the leading causes of respiratory tract infections in young children and other immunocompromised patients.[1] RSV is most likely to cause severe illness in infants younger than 6 months while HMPV causes severe illness between 6 and 12 months.[2] MicroRNA (miRNA) and Piwi-interacting RNA (piRNA) are two types of non-coding RNA (RNA that is not translated into protein) that regulate gene expression. Exosomes are vesicles that carry biologically active proteins, lipids, and RNAs from one cell to another. Exosomes from infected cells have been shown to shelter and deliver viral RNA and proteins, viral and cellular miRNA, and other genetic elements to other cells. Exosomes play a role in both limiting and spreading infections depending on the type of virus. They can be used in creating antiviral treatments and vaccines.[3] We aim to investigate the differential expression of genes that code for miRNAs and piRNAs between exosomes that originate from RSV infected cells, HMPV infected cells, or non-infected cells.

Methods

Primary cell cultures, RSV and hMPV infections. Primary small airway epithelial (SAE) cells (Lonza Inc., San Diego, CA, USA), derived from the terminal bronchioles of cadaveric donors, were grown in culture medium containing 7.5 mg/mL bovine pituitary extract (BPE), 0.5 mg/mL hydrocortisone, 0.5 µg/mL hEGF, 0.5 mg/mL epinephrine, 10 mg/mL transferrin, 5 mg/mL insulin, 0.1 µg/mL retinoic acid, 0.5 µg/mL triiodothyronine, 50 mg/mL gentamicin, and 50 mg/mL bovine serum albumin. SAE cells were switched to basal media (no supplemented added) four hours prior to RSV and hMPV infections. At 90 to 95% confluence, cell monolayers were infected with RSV at multiplicity of infection (MOI) of 1 and with hMPV in basal media with 1.0 µg trypsin/ml at MOI of 2. An equivalent amount of 30% sucrose solution was added to uninfected SAE cells as a control (mock cells). The culture media, from both mock, RSV- and hMPV -infected cells, were collected after 24 hours post infection (p.i.) and processed for the exosome isolation.

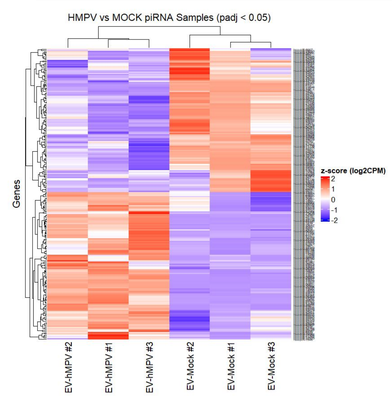
Exosome isolation and characterization. Exosomes were isolated from uninfected (control-Exosomes), RSV (RSV-Exosomes) and hMPV infected (hMPV-Exosomes) primary small airway epithelial (SAE) cells. Exosomes were isolated using a two-step enrichment procedure (ExoQuick reagent, and CD63 antibody immuno-magnetic purification. The isolated Exosomes were characterized by antibody blot array for the typical exosome markers. The size and amount of Exosomes was determined using the ZetaView PMX 110 instrument (Particle Metrix GmbH, Meerbusch, Germany).

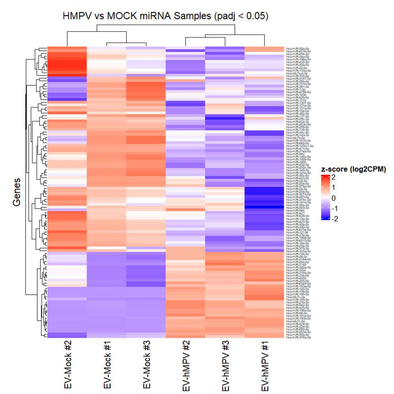
Total RNA extraction and Next generation sequencing (NGS). RNA was extracted from control, RSV-, and hMPV-exosome samples by column for next generation high-throughput sequencing (NGS) to identify sncRNAs in exosomes. RNA was isolated using the SeraMir exosome RNA purification column kit according to the manufacturer's instructions. cDNA library was constructed, size selected and analyzed for quality with Bioanalyzer. Exosomes NGS was performed using the Illumina platform. The RNA type abundance and changes in expression were determined.

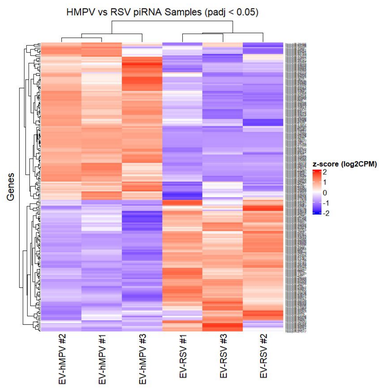
Differential gene expression analysis was done in R using the DESeq2 package. The DESeq2 analysis compared the level of gene expression across two different conditions (e.g. Mock vs RSV). Genes that showed the most significant differences in expression levels between the two groups (with adjusted p-values below an alpha level of 0.05) were selected.

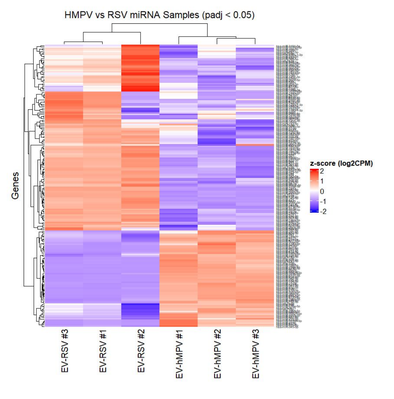
The R package ComplexHeatmap was used to illustrate these selected genes via heat maps. RNA counts were transformed using log base 2, standardized, and mapped. Red colors signify higher levels of expression and blue colors signify lower levels of expression. The Y-axis lists the gene names, and the X-axis labels the group names (e.g. RSV and Mock).

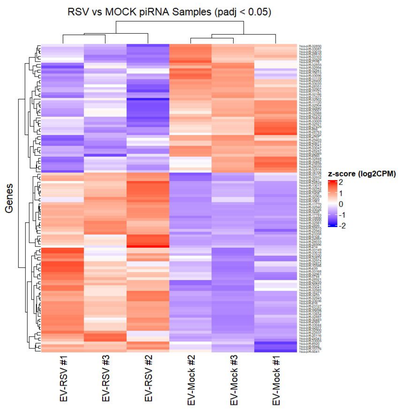
Results

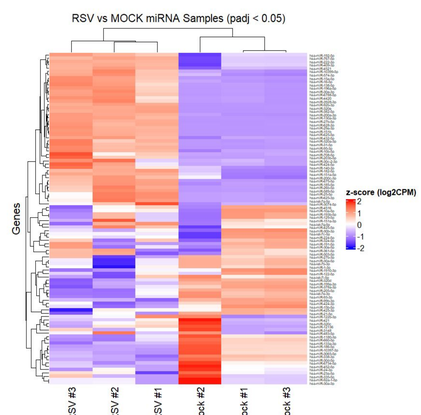












Total Amount of Significant Genes

	sig piRNA	sig miRNA
HMPV vs MOCK	227	108
HMPV vs RSV	204	193
RSV vs MOCK	115	100

Conclusion and Future Work

RSV and HMPV infection affect miRNA and piRNA gene expression levels in exosomes.

An analysis using the EdgeR package was previously done to compare the gene expression between the same groups compared in our analysis (e.g. Mock, HMPV, and RSV). The significant genes—those with statistically significant differences in expression between groups— found in our current analysis using the DeSEQ2 package can now be used to confirm the genes that were previously found to be significant. This cross-validation between the two methodologies strengthens the reliability of our results and provides robust evidence for the differential expression of these genes across the groups.

Identifying these significant genes may reveal potential targets for therapeutic intervention. For instance, if RSV causes a gene to be upregulated, meaning there is an increased amount of RNA from that gene in exosomes, targeting the pathway associated with that gene could help reduce the severity of the disease.

In the future, we plan to use a mixed model framework to analyze the three groups (mock, RSV, and hMPV) simultaneously. This approach will enable us to compare gene expression levels across all groups at once, providing a more comprehensive understanding of differences and accounting for potential random effects.

References

1 Kinder JT, Moncman CL, Barrett C, Jin H, Kallewaard N, Dutch RE. Respiratory Syncytial Virus and Human Metapneumovirus Infections in Three-Dimensional Human Airway Tissues Expose an Interesting Dichotomy in Viral Replication, Spread, and Inhibition by Neutralizing Antibodies. J Virol. 2020 Sep 29;94(20):e01068-20. doi: 10.1128/JVI.01068-20. PMID: 32759319; PMCID: PMC7527037.

2 Profession++al, C. C. M. 2023 Jun 20. Human metapneumovirus (HMPV). Cleveland Clinic. https://my.clevelandclinic.org/health/diseases/22443-human-metapneumovirus-hmpv

3 Chahar HS, Bao X, Casola A. Exosomes and Their Role in the Life Cycle and Pathogenesis of RNA Viruses. Viruses. 2015 Jun 19;7(6):3204-25. doi: 10.3390/v7062770. PMID: 26102580; PMCID: PMC4488737.

Acknowledgements

This Research was supported and funded by the NIH/NIAID grant (R25HL161715). Dr. Garofalo was supported by P01 AI062885. Dr. Corsello was supported by the National Institute of Environmental Health Sciences Award (P30ES030285), the Catalyst Award, American Lung Association (ALA), and the Parker B. Francis Fellowship Award.