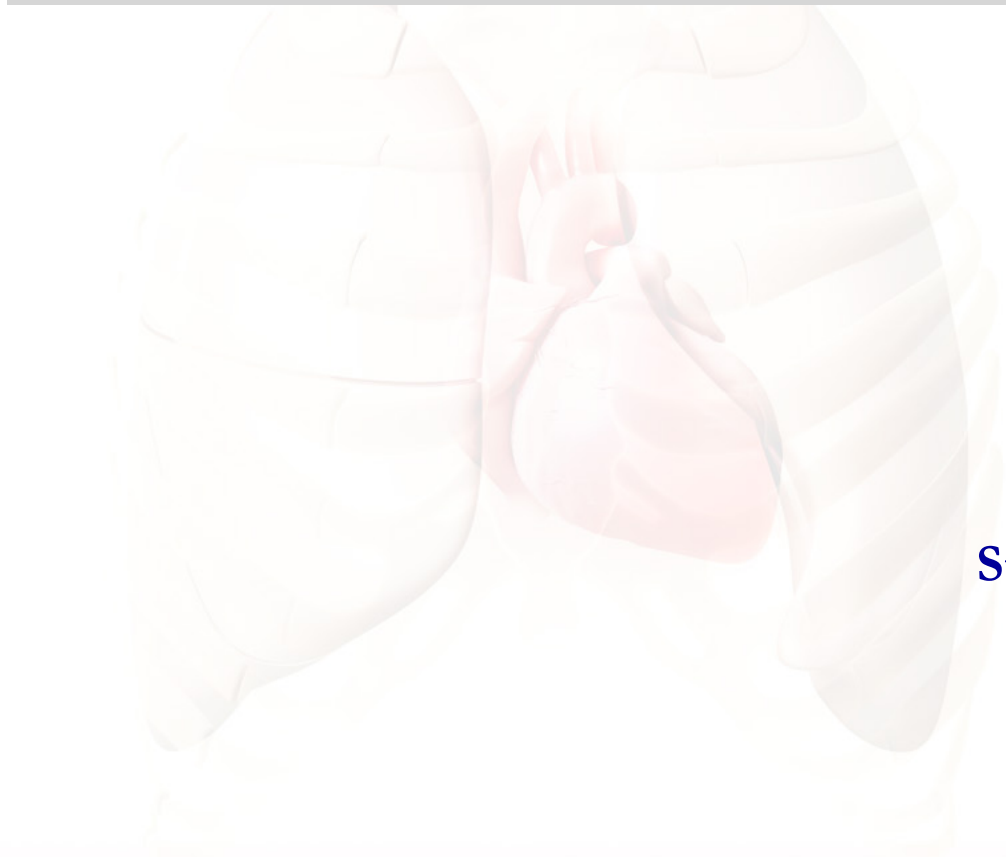
Circulating Exosomes isolated from human lung transplant recipients with respiratory viral infections contain nucleic acids and activate Antiviral Signaling pathways cGAS/STING and RIG-1



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Relevant Financial Relationship Disclosure Statement

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Nothing to disclose

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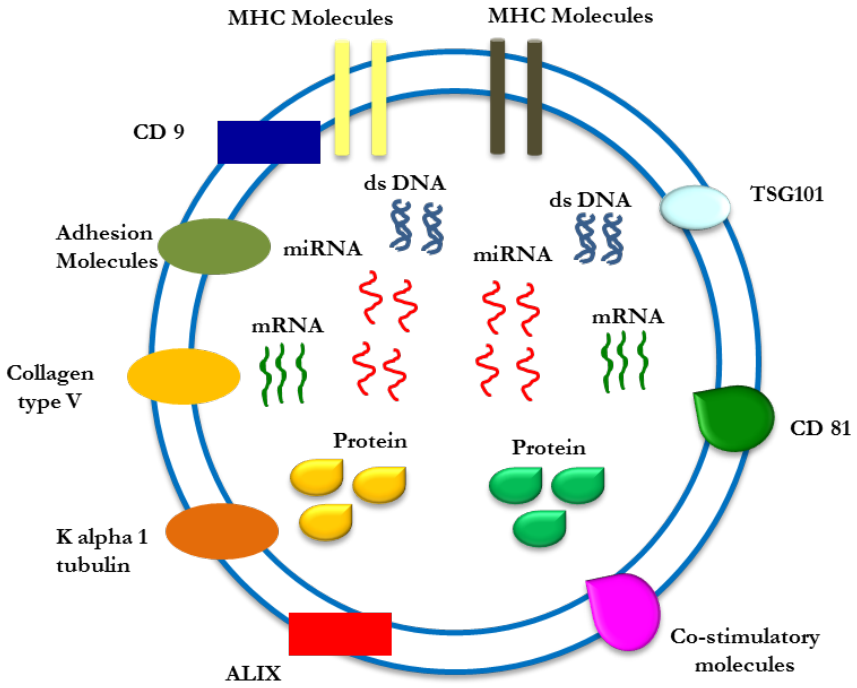
Introduction

Exosomes:

- Exosomes are small vesicles <200nm in size.
- Exosomes are released by cells, has a lipid bilayer, and surface proteins, antigens and specific markers (e. g, CD9, CD81, tetraspanins, Alix, CD63, tumor susceptibility gene 101, heat shock proteins, and specific markers) depending on the cells that release them.
- We demonstrated lung self antigens (Collagen V and K alpha 1 tubulin, MHC molecules, Co stimulatory molecules, different miRNA`s and viral antigens) in the Exosomes released from lung transplants recipients with rejection, primary graft dysfunction and respiratory viral infections (RVI).
- **In this presentation, I will provide evidence for nucleic acids within the exosomes from LTxR diagnosed with RVI and their function.**



Methods

Detection of DNA and RNA in exosomes:

- Plasma collected from Human Lung Transplant Recipients diagnosed with RVI and controls.
- Exosomes isolated from LTxR: control and Viral (ultracentrifugation)
  Followed by 0.22 micron filtration.
  Size verification <200nm by nanosight.

- Isolation of ds DNA and RNA from exosomes
- Validation of Double Stranded DNA in exosomes
- Next Generation Sequencing (NGS) for DNA and RNA
- Bioinformatics Analysis of DNA and RNA (Human Genome and viral genome)

Electron Microscopy of exosomes Isolated from Stable, RSV and Corona LTxR:
Immunogold staining was done using mouse anti-RSV Ab (1:100) and mouse anti-Corona and secondary goat anti-mouse IgG Ab conjugated to colloidal gold.

In Vitro Experiments

- **Can nucleic acids inside exosomes induce Stress response in cells:**
  Cell lines were CO-Cultured with Exosomes up to 24 hours:
  Human Bronchial Epithelial cell line (BEAS2B), Human Airway Epithelial cells (KCC266), Human Embryonic Kidney Cells (HEK293) and Human epithelial type 2 (HEp-2) human laryngeal carcinoma cells.
- Analyzed for viral antigens (Respiratory Syncytial Virus, Corona virus, Influenza and Rhinovirus) by Western Blot.
- Stress markers (PERK, IRE1α, ATF4, ATF6, eIF2 α and BiP) by Western Blot.
- Viral signaling proteins (cGAS, STING, pSTING, IRF3, pIRF3, TBK, pTBK, IKKε, pIKKε, NFkB, RIG1, MAVS, MDA5, TLR`s, MyD88, IFNβ) by Western Blot.

Nucleic Acids (dsDNA/RNA) in Exosomes

- **We identified nucleic acids from exosomes of Lung transplant recipients diagnosed with respiratory viral infections (RVI) .**

- **Biological Significance of nucleic acids in exosomes isolated from Lung transplant recipients diagnosed with RVI :**

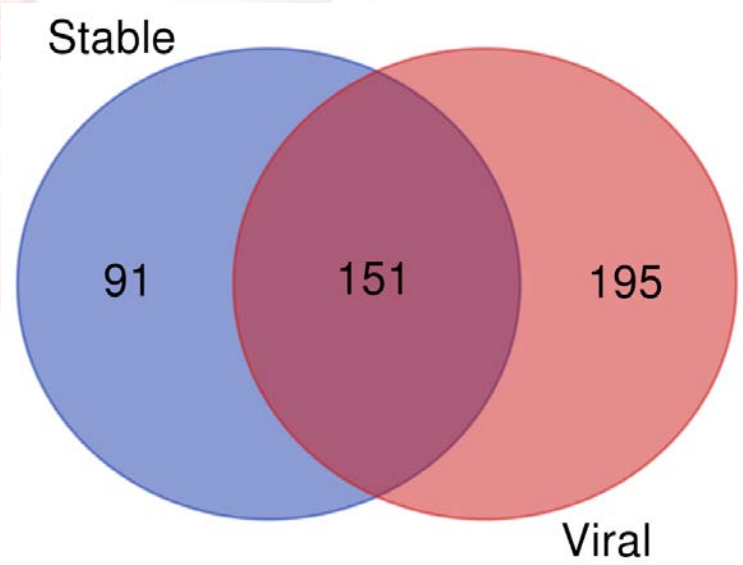
1. **Exosomes carry Viral nucleic acid sequences and viral antigens**

2. **Exosomes from RVI can induce immune signaling**

3. **Exosomes from RVI can induce cellular Stress**

Unique DNA Sequences in Circulating Exosomes from lung transplant recipients with Respiratory Viral Infections

- 195 unique DNA sequences were observed in exosomes from LTxR diagnosed with RVI Defensins and GTPase pathways genes were common in RVI exosomes.
- Nucleic acid Sequences related to respiratory viruses identified in RVI exosomes.
- Circulating exosomes from RVI LTxR contained sequences for Apoptotic Cleavage and NMDA Receptor Activation.



Sequences of ds DNA present in exosomes comparing to human genome.

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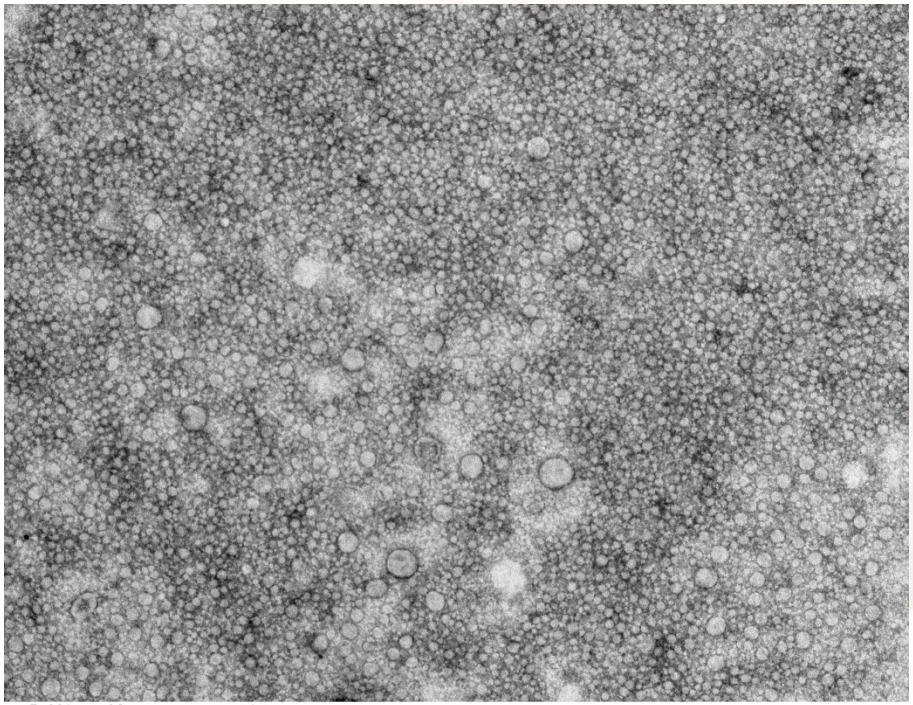
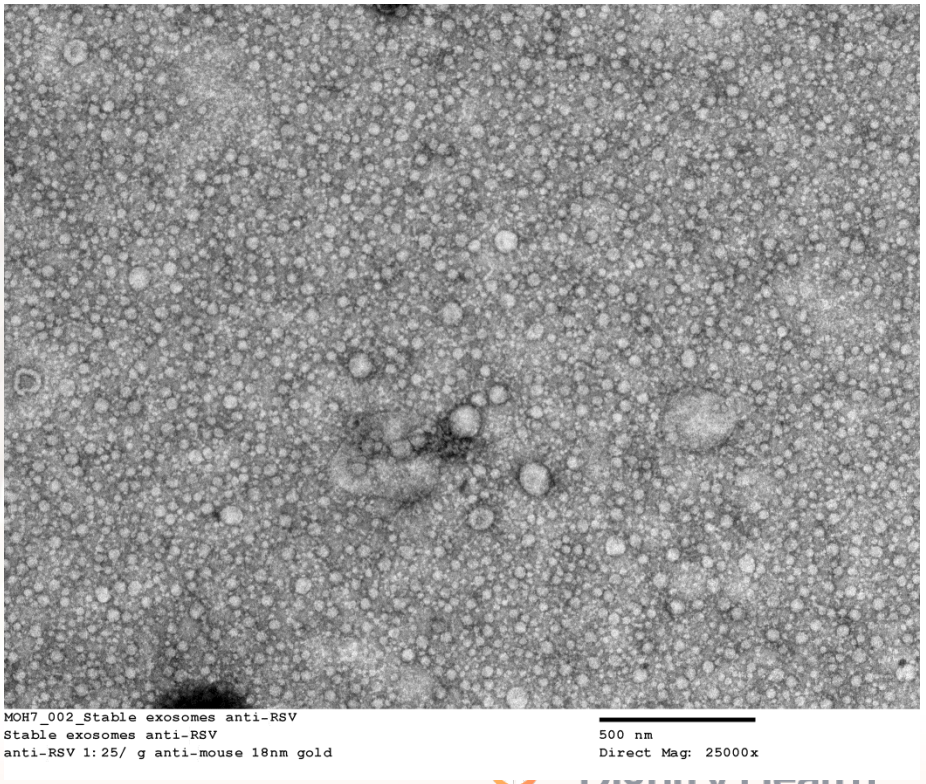
Electron Microscopy Images of Circulating Exosomes from lung transplant recipients with Respiratory Viral Infections: Stable Exosomes with Anti Corona and anti RSV antibody

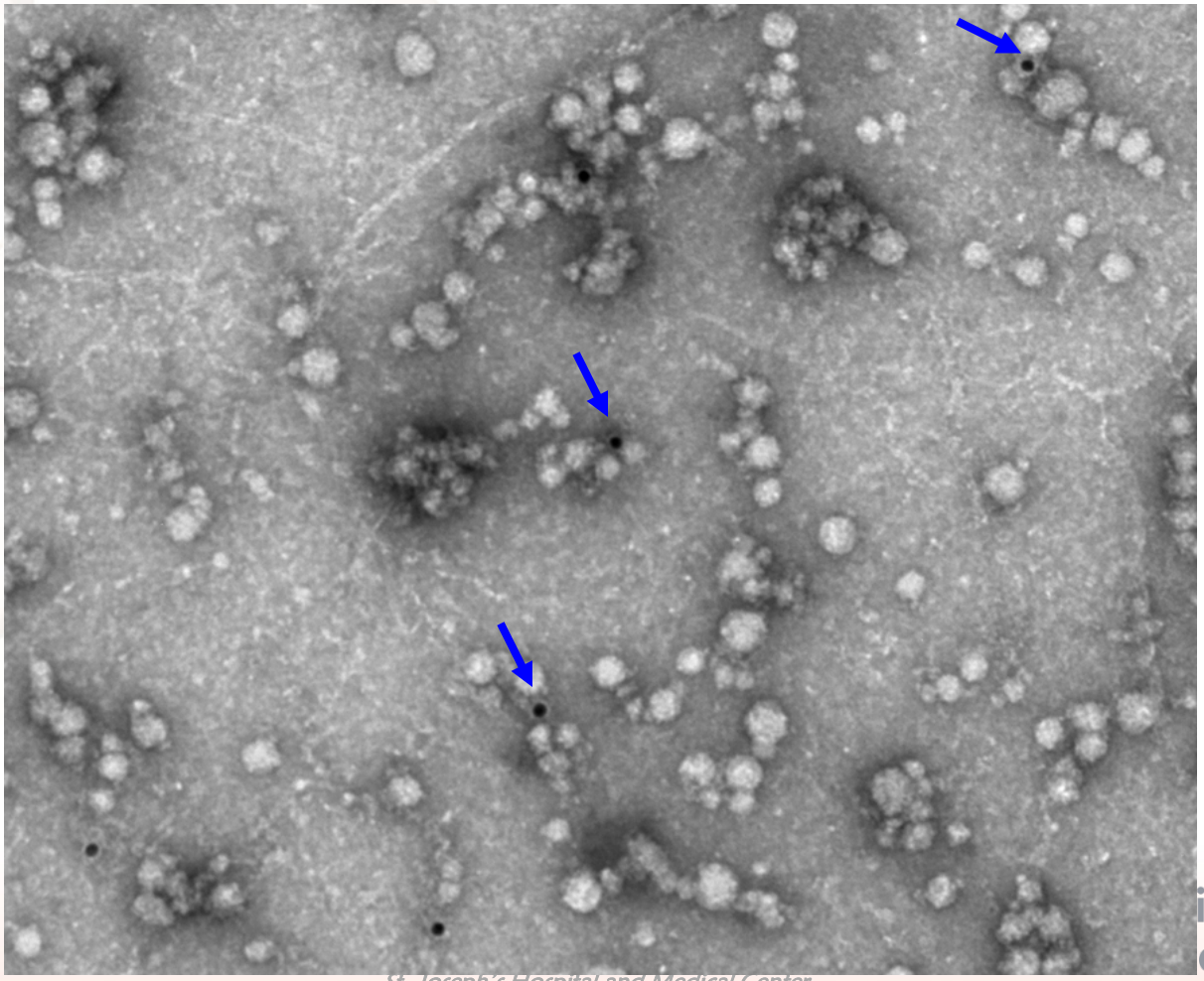
- **Exosomes from Stable LTxR Stained negative with Anti Corona and anti RSV antibodies**





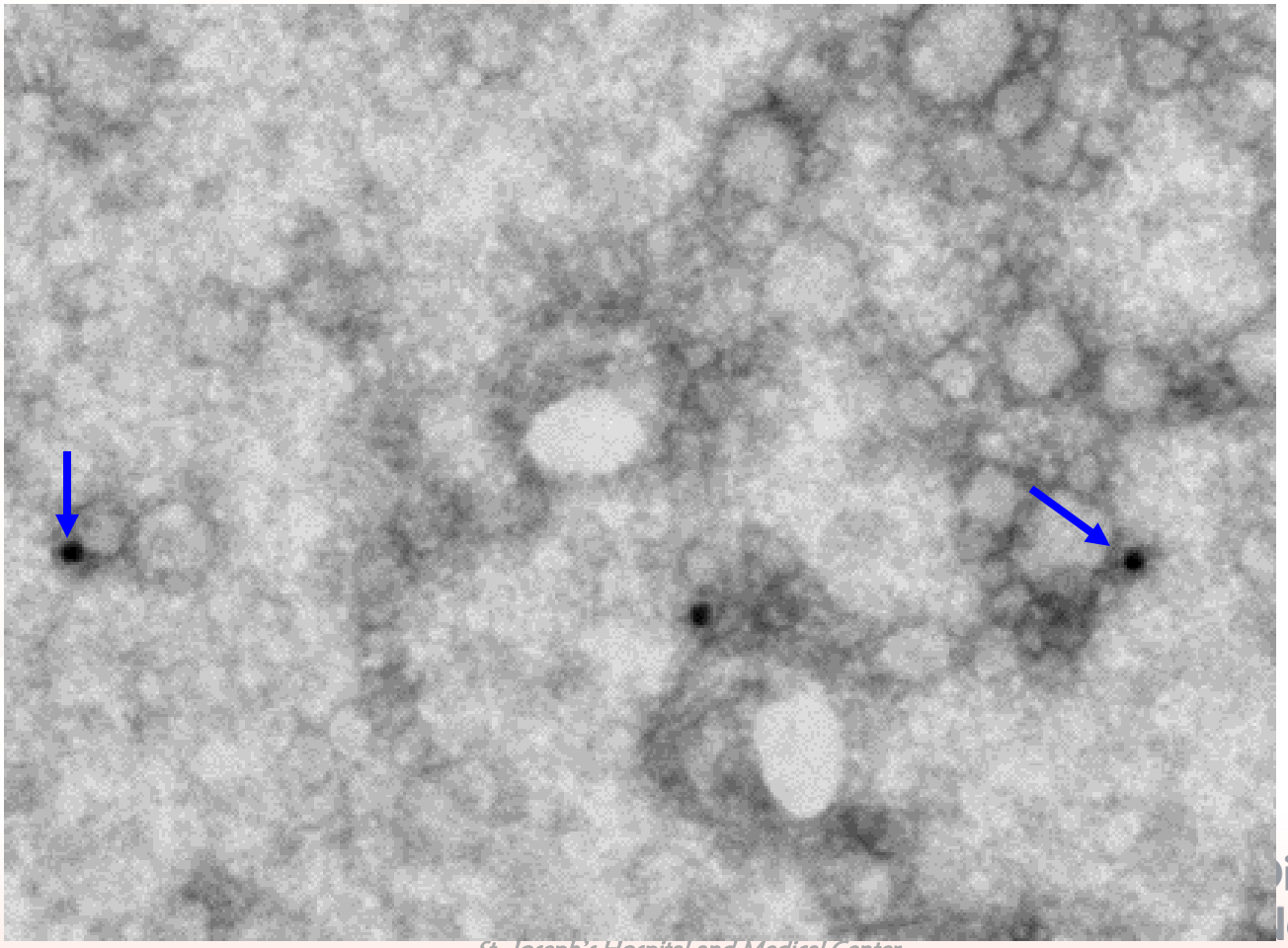
Electron Microscopy Images of Circulating Exosomes from lung transplant recipients with RSV Infection Stained Positive with RSV Antibody

- **Exosomes from RSV LTxR Stained positive anti RSV antibodies**



Electron Microscopy Images of Circulating Exosomes from lung transplant recipients with RSV Infection Stained Positive with RSV Antibody

- **Exosomes from Corona LTxR Stained positive anti RSV antibodies**



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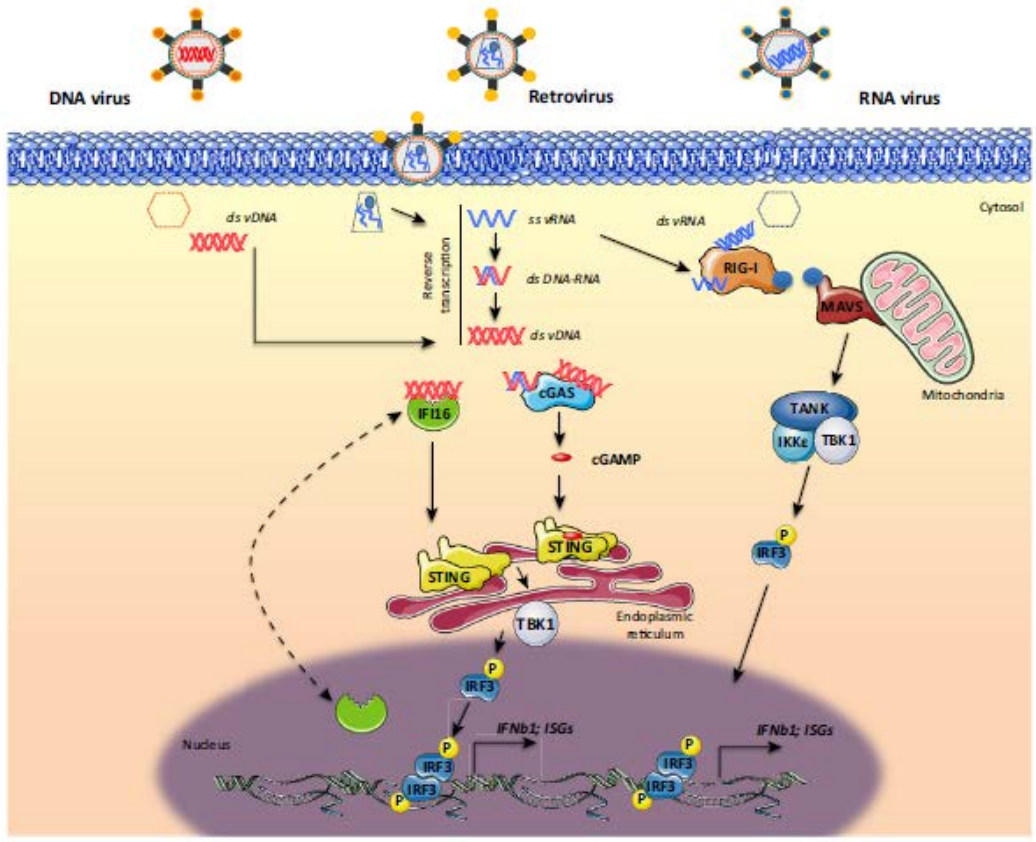
- *Can nucleic acids inside exosomes induce Viral Signaling response in cells?*

- **Cells were Co-Cultured with Exosomes up to 24 hours:**
- **Viral activation pathway proteins: cGAS, STING, pSTING, IRF3, pIRF3, TBK, pTBK, IKKε, pIKKε, NFkB, RIG1, MAVS, MDA5, TLR`s, MyD88, IFNβ were analyzed using Western blot.**

Viral Sensing pathway



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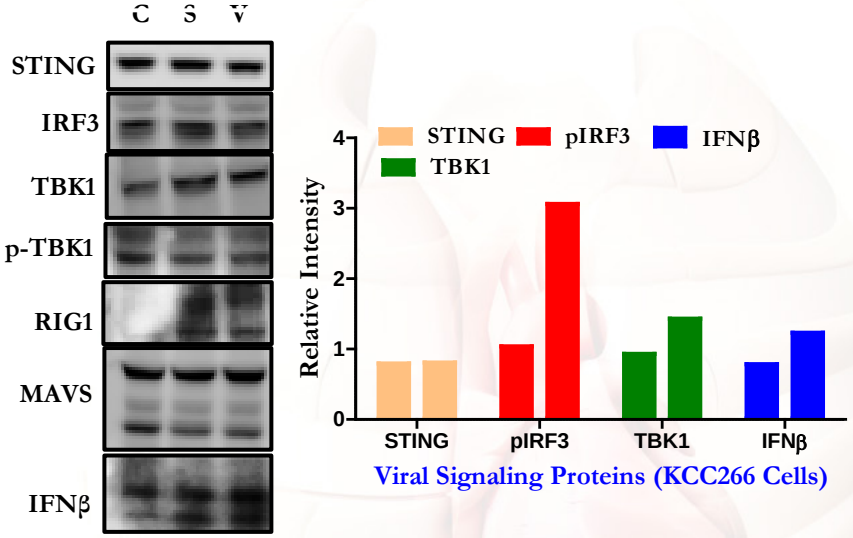
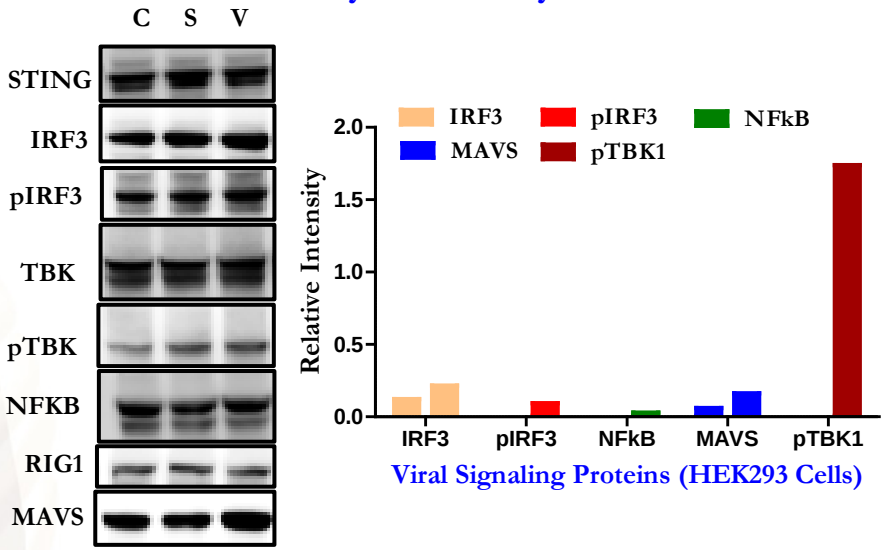
Viral Signaling Proteins (cGAS, STING, pSTING, IRF3, pIRF3, TBK, pTBK, IKKε, pIKKε, NFkB, RIG1, MAVS, MDA5, TLR`s, MyD88, IFNβ) were induced following co culture of Human cell lines with Exosomes From LTxR with RVI

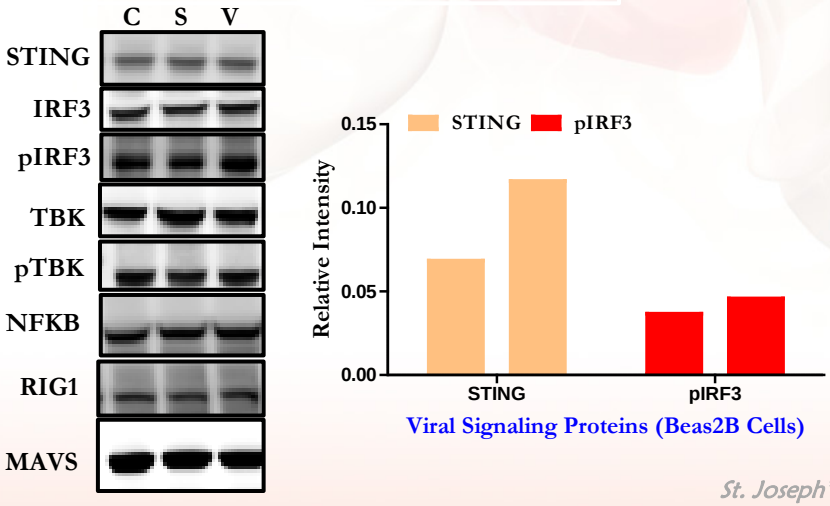
Airway Epithelial Cells: KCC266



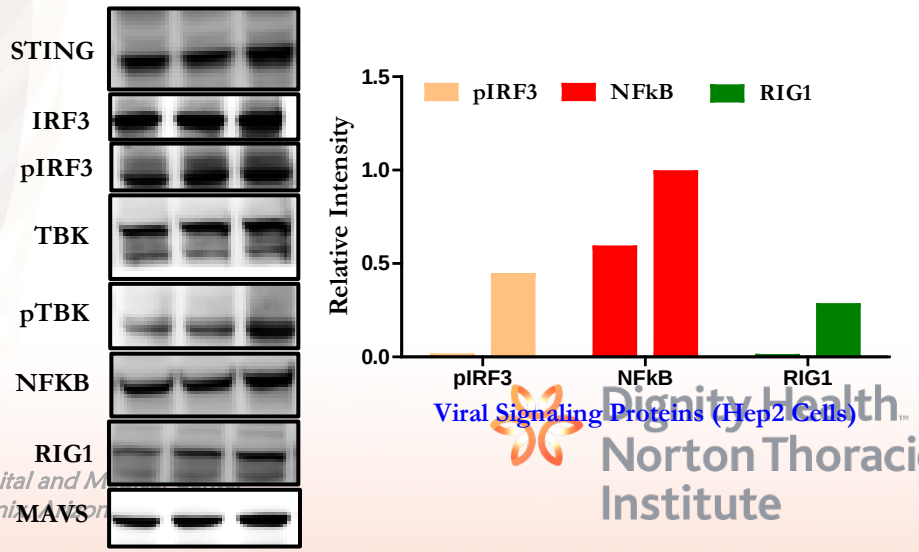
Human Embryonic Kidney Cells : HEK293



Bronchial Epithelial Cells : Beas2B



Human laryngeal carcinoma Cells : Hep2



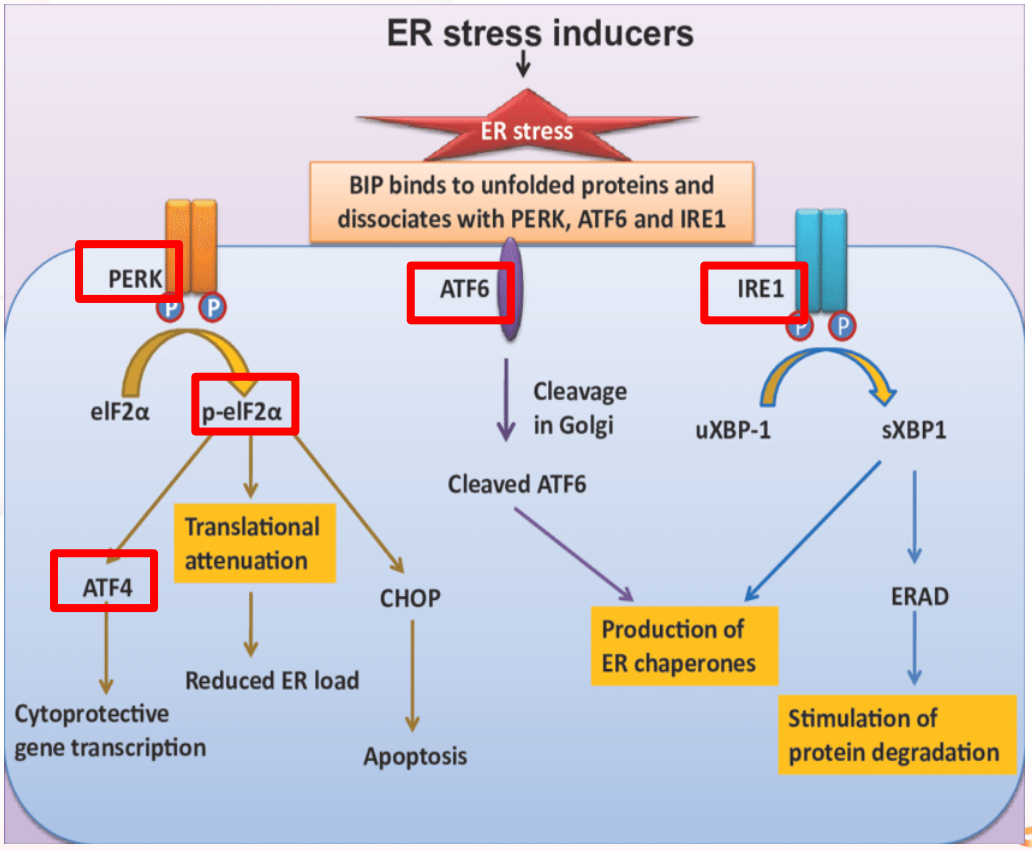
Summary

Viral sequences present in circulating exosomes from Human LTxR with RVI are capable of inducing Stress response proteins (PERK, IRE1α, peIF2α, BiP, ATF4 and ATF6) in Human cell lines.

Can nucleic acids inside exosomes induce Stress response in cells?

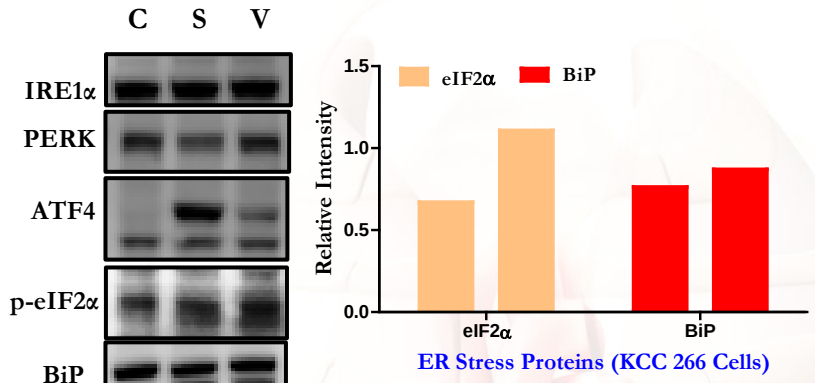
Cells were Co-Cultured with Exosomes up to 24 hours:
Stress proteins: PERK, IRE1α, ATF4, ATF6, eIF2 α and BiP were analyzed using Western blot.

Cellular Stress Pathway

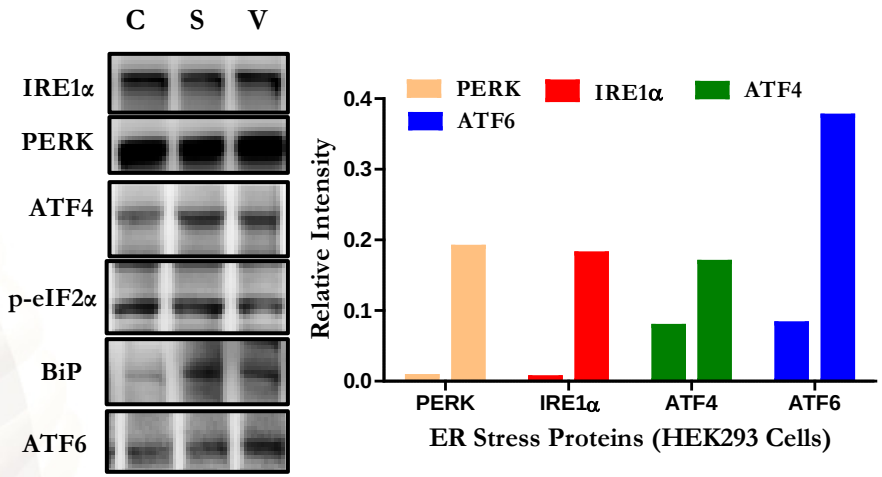


ER Stress Markers (PERK, IRE1α, ATF4, ATF6, eIF2 α and BiP) induction following co-culture of Exosomes From LTxR diagnosed with RVI with cell lines

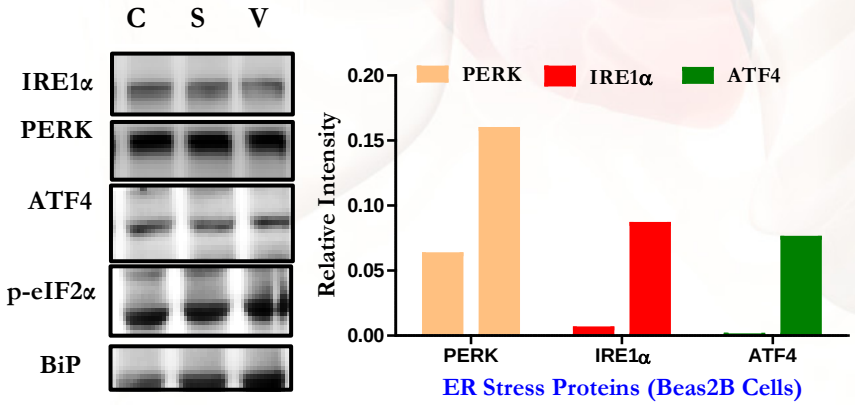
Airway Epithelial Cells: KCC266



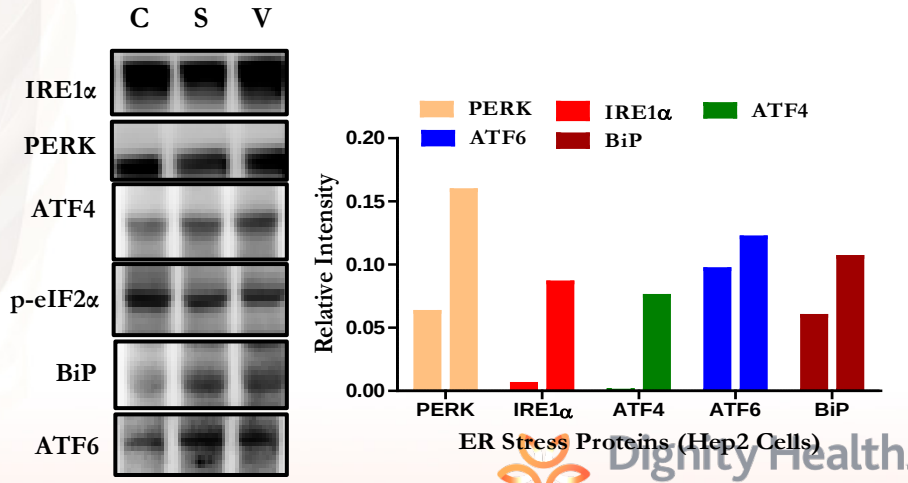
Human Embryonic Kidney Cells : HEK293



Bronchial Epithelial Cells : Beas2B



Human laryngeal carcinoma Cells : Hep2



Summary

- **Viral sequences in exosomes from LTxR with RVI** can induce **signaling proteins.**
- **cGAS/STING and RIG-1 Pathway proteins were upregulated *i. e* (cGAS, STING, pSTING, IRF3, pIRF3, TBK1, pTBK1, IKKε, pIKKε, INFβ, RIG-1, MAVS, MDA5, TLR`s, NFkB, MyD88).**

- **Signaling proteins in cells varied based on the origin of cell lines.**

Summary

- **Viral Sequences are present in circulating exosomes from LTxR with RVI.**
- **Respiratory Syncytial Virus, Corona, Influenza and Rhino.**
- **Viral sequences in exosomes from LTxR with RVI induced Stress Proteins (PERK, IRE1α, ATF4, ATF6, eIF2 α and BiP).**
- **Circulating exosomes from LTxR with RVI also induced Signaling Proteins: cGAS/STING and RIG-1 pathways (cGAS, STING, pSTING, IRF3, pIRF3, TBK1, pTBK1, IKKε, pIKKε, INFβ, RIG-1, MAVS, MDA5, TLR`s, NFkB, MyD88).**

Conclusions

Sequences related to respiratory viruses are present in circulating exosomes Therefore, Circulating Exosomes from RVI LTxR can activate /augment immune responses leading to rejection following Human Lung Transplantation.