Influenza virus induced novel miRNAs regulate the STAT pathway

Introductory Information

MicroRNAs are essential regulators of gene expression in humans and can control pathogenesis and host-virus interactions. Notably, the role of specific miRNAs during influenza virus infections are still ill-defined. The central goal of this study was to identify novel miRNAs and their target genes in response to influenza virus infections in airway epithelium. Human airway epithelial cells exposed to influenza virus induced several novel miRNAs that were identified using next generation sequencing (NGS) and their target genes by biochemical methods. NGS analysis predicted forty-two RNA sequences as possible miRNAs based on computational algorithms. Expression patterns of these putative miRNAs were further confirmed using RT-PCR in human bronchial epithelial cells (HBEpC) exposed to H1N1, H9N1(1P10) and H9N1 (1WF10) strains of influenza virus. A time course study showed significant downregulation of put-miR-34 in H1N1 and put-miR-35 in H9N1(1P10) infected cells, consistent with the NGS data. Additionally put-miR-34, and put-miR-35 showed a high fold enrichment in argonaute-immunoprecipitation compared to the controls, indicating their ability to form a complex with argonaute protein and RNA induced silencing complex (RISC), a typical mode of action found with miRNAs. Our earlier studies have shown that replication and survival of influenza virus is modulated by certain transcription factors, such as, NF-κB. To identify the target(s) of these putative miRNAs, we screened 84 transcription factors that have a role in viral pathogenesis. Cells transfected with mimic of the put-miR-34 showed significant decrease in expression of Signal Transducers and Activators of Transcription 3 (STAT3), and the inhibitor of put-miR-34 showed significant increase in STAT3 expression and its phosphorylation. In addition, put-miR-

34 had 76% homology to the untranslated region (UTR) of STAT3. NGS and PCR array data submitted to the Gene Ontology also predicted the role of transcription factors modulated by put-miR-34. Our data suggests that put-miR-34 could be a good target for the antiviral therapy as the hyperactivation or inactivation of STAT3 results in viral disease, as tightly regulated STAT3 function is central to health.

Methods Collection

1. **Cell culture**
    - **Primary human bronchial epithelial cells HBEp cells. exposed to influenza virus H1N1, H9N1 (1P10) and H9N1 (1WF10)**
    - **SAEp cells. exposed to influenza virus H1N1, H9N1 (1P10) and H9N1 (1WF10)**
    - **Madin Darby Canine Kidney (MDCK) cells were cultured in Eagle's Minimum Essential Medium (ATCC, Manassas, VA) with 10% fetal bovine serum, and appropriate antibiotics. MDCK cells were used for the propagation of influenza virus H1N1 (A/WSN/33), H9N1 (1P10), and H9N1 (1WF10). Influenza virus H1N1 (A/WSN/33)**
    - **Transfection of HBEp cells with mimic of miRNA putative 31, 34, and 35**
2. **MiRNA analysis**
    - **HBEpC were transfected with mimic of put-miR-22, 23, 31, and 34 for 24 h and the isolated RNA was used for Next generation sequencing (NGS) analysis**
    - **Quality of RNA was assessed by Agilent 2100 Bioanalyzer**
    - **RT-PCR analysis. miRCURY LNA™ Universal RT microRNA PCR; each microRNA was assayed once by qPCR on the microRNA Ready-to-Use PCR kit.**
    - **miRNA Custom Pick and Mix Panel using ExiLENT SYBR® Green master mix. LightCycler® 480 Real-Time PCR System (Roche).**
    - **Co-immunoprecipitation of Argonaute proteins with associated RNAs.**
    - **HBEpC were transfected with put-miR-34 inhibitor oligonucleotide or a put-miR-34 mimic oligonucleotide (Thermo Fisher Scientific, Carlsbad, CA) using the lipid-based Lipofectamine 2000.**
3. **Western immunoblotting**

- Protein collected from cells infected or uninfected using RIPA lysis buffer. Proteins were estimated by BCA method. Proteins were resolved using a 10% SDS-polyacrylamide gel electrophoresis (SDS-PAGE).

4. Viral Detection in cells

    - Real-time PCR (Applied Biosystems 7500 RT-PCR System) using matrix gene specific forward and reverse primers.

5. Transcription factor assay
    - Real-time PCR (Applied Biosystems 7500 RT-PCR System). 84 Genes investigated; list of genes studied provided in data dictionary with abbreviation.

Citations- Publications based on the dataset

Othumpangat S, Beezhold, D, Noti J. Influenza virus induced novel miRNAs regulate the STAT pathway. Viruses. 2021 May 23;13(6):967. doi: 10.3390/v13060967. PMID: 34071096; PMCID: PMC8224765.

Acknowledgements

This work was supported by NIOSH CAN 939ZUMK

Sreekumar Othumpangat seo8@cdc.gov

Donald H Beezhold zec1@cdc.gov

Christina Umbright cfo3@cdc.gov

John D. Noti ivr2@cdc.gov

Contact

For further information contact:

Allergy and Clinical Immunology Branch (ACIB), Health Effects Laboratory

Division (HELD), National Institute for Occupational Safety and Health (NIOSH),

Morgantown, WV

304.285.6024