AMBIVALENT ROLE OF MIR-124-3P ON INFLUENZA A VIRUS IN VITRO

Enhancing replication by attenuating innate immunity while causing defects in virion production

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INTRODUCTION

miRNA & Therpaeutics

In hope of identifying new potent therapeutic targets, virus-host interactations are constantly being scrutinized to gain new insight in its mechanism and players involved. Recently miRNAs are

miRNA are small non-coding RNAs that act as a "rheostat" (Bartel & Chen, 2004) to control expression level of cellular RNA.Common target sites of miRNAs usually locate at 3' Untranslated Region (UTR) of the mRNA, but recent findings also suggest that target sites in the open reading frame (ORF) of the mRNA can also exert its silencing effect, just not as effective targets in the 3' UTR in human (Forman & Coller, 2010). However in the context of viral mRNA, majority of miRNA were found to target ORF of miRNA instead (Xu et al., 2022).

miRNA-124-3p & IAV

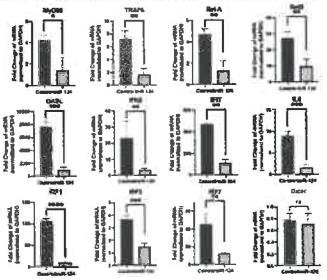
miR-124-3p is expressed across the human body and act as an omportant regulator in both the **nervous system** and the **immune system**. Its dysfunction in glial cells was found to be involve in neuroinflammatory diseases as it acts as part of the **negative feedback loop** in immune modulation. Yet, miR-124-3p's effect on epithelial cell's immunity were not yet sdoxifbgdirfubfd

Altough previous reports have demonstrated that miR-124-3p has **viral suppressive effect**, the mechanism requires further elucidation.

METHODS & RESULTS

Fig 1. miR-124-3p suppresses epithelial cells' innate immunity

There is significant decreased level of expression of IFNβ, IFIT, IL6, TRAF6, MyD88, OASL, RelA, RelB, IRF 1,3 and 7 in miR-124-3p transfected cells 24hpi by SeV compared to control. Level of Dicer has no significant changes Levels of cellular mRNAs were measured with specific primer by RT-qPCR. Data represent means plus standard errors of the means.



There is significant decreased level of release of IFNβ in miR-124-3p transfected cells 24hpi by SeV compared to control. Levels of IFNβ were measured with ELISA. Data represent means plus standard errors of the means.

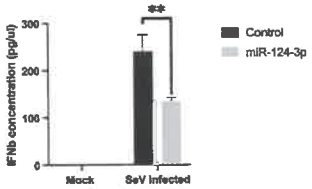
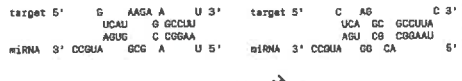


Fig 2. miR-124-3p affects the expression of viral Neuraminidase

Mode of hybridization between miR-124-3p and 2 sites of WT WSN NA ORF. The 2 sites were first identified by miRanda, and then the mode of hybridization was predicted by RNAHybrid. Parameters applied in RNAHybrid were set as follows: f 2,6 d 1.9,0.28.



miR-124-3p exerts different effect on different viral proteins. siGADH is added as a positive control for validating successful transfection. Cells were harvest from WSN IAV infected A549 control, siGAPDH transfected cells and miR-124-3p transfected cell at 8.h.p.i. and identified with specific antibody for NA, HA, GAPDH, β-Actin NS1 and NP by WB assay

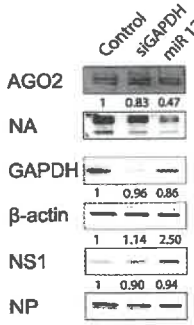


Fig 3. miR-124-3p targets open reading frame of NA viral mRNA

Control 293T cells and miR-124-3p transfected 293T cells were co-transfected with miRNA and Luciferase plasmid. Luciefrase plasmid was subcloned with dedired target site respectively. Supernatant was collected 48h.p.i. Luciferase assay was subsequently performed. There is significant decrease in Luciferase activity for those with predicted target in miR-124-3p group compared to that of contorl.

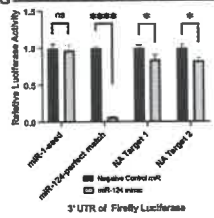
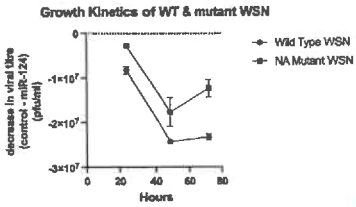


Fig 4. Mutation in target sites rescue viruses

Control A549 cells and miR-124-3p transfected A549 cells were infected with WT WSN and mWSN respectively. Supernatant was collected 24, 48 &72 h.p.i. Plaque assay was subsequently performed. The decrease in viral titer is less in mWSN group compared to WT WSN.



CONCLUSION

The existing knowledge gap of attenuated immunity and viral replication demonstrated by miR-124-3p were bridged in this study as miR-124-3p were discovered to have an ambivalent role during IAV infection. In this study, we first validated that in the context of viral infection, miR-124-3p negatively regulates innate immunity, affecting multiple pathways simultaneously. We demonstrated that besides known miR-124-3p targets, miR-124-3p also has significant effect on mRNA level of non-miR-124-3p target proteins such as OASL and IFNβ which are crucial anti-viral proteins.This suggest that miR-124-3p attenuates innate immunity by directly targeting the upstream proteins, causing a domino effect compromising the cellular innate immune response.. We thus probe into whether the miRNA has direct effect on the virus itself.

The decreased expression of the surface protein NA & HA between control group and miR-124-3p transfected group, together with identification 2 distinct non-canonical sites of miR-124-3p on the ORF of WSN IAV NA mRNA by in silico analysis is suggestive that miR-124-3p's antiviral effect is mediated by targeting the NA viral mRNA, which was later validated by luciferase assay. To further demonstrate that the target site has directy effect on viral replication, a mutant virus is generated and growth kinetics in miR-124-3p transfected cells demonstrated that the mutation is able to rescue viruses and normalised partially virus replication.

Our findings uncovered a larger picture of how miR-124-3p interacts with WSN IAV during infection, revealing that the miRNA has ambivalent role on WSN IAV: simultaneously promoting replication by attenuating epithelial cell's immune response, and affects production of infective virion via targeting viral surface protein NA directly. The broad potential coverage of miR-124-3p makes it a promising therapeutic agent against both pandemic and seasonal epidemic Influenza strains. Moreover, since miR-124-3p is simultaneously suppresses immune response and downregulates HA and NA expression, it could have great utility against severe cases of IAV infection.

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