# The Role of MicroRNAs in the Viral Pathogenesis of H5N1 Influenza Virus Infection


Owen S. T. Chan, Michael C. W. Chan, Kenrie P. Y. Hui

School of Public Health, Li Ka Shing Faculty of Medicine,
The University of Hong Kong, Pokfulam, Hong Kong SAR, China

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CHAN Sze Tan, Owen (MPhil)
Primary Supervisor: Professor Kenrie P.Y. Hui


## INTRODUCTION

Influenza A virus (IAV) is a significant global health concern especially the highly pathogenic avian influenza (HPAI) H5N1 virus which is associated with a high fatality rate and severe symptoms due to the hyper-induction of cytokines[1].

There are some studies in microRNAs (miRNAs) induced by H1N1 infection but there is a lack of information on the miRNAs in extracellular vesicles (EVs) released from HPAI H5N1-infected cells.

This study aims to investigate the role of miRNAs in the pathogenesis of HPAI H5N1 virus.

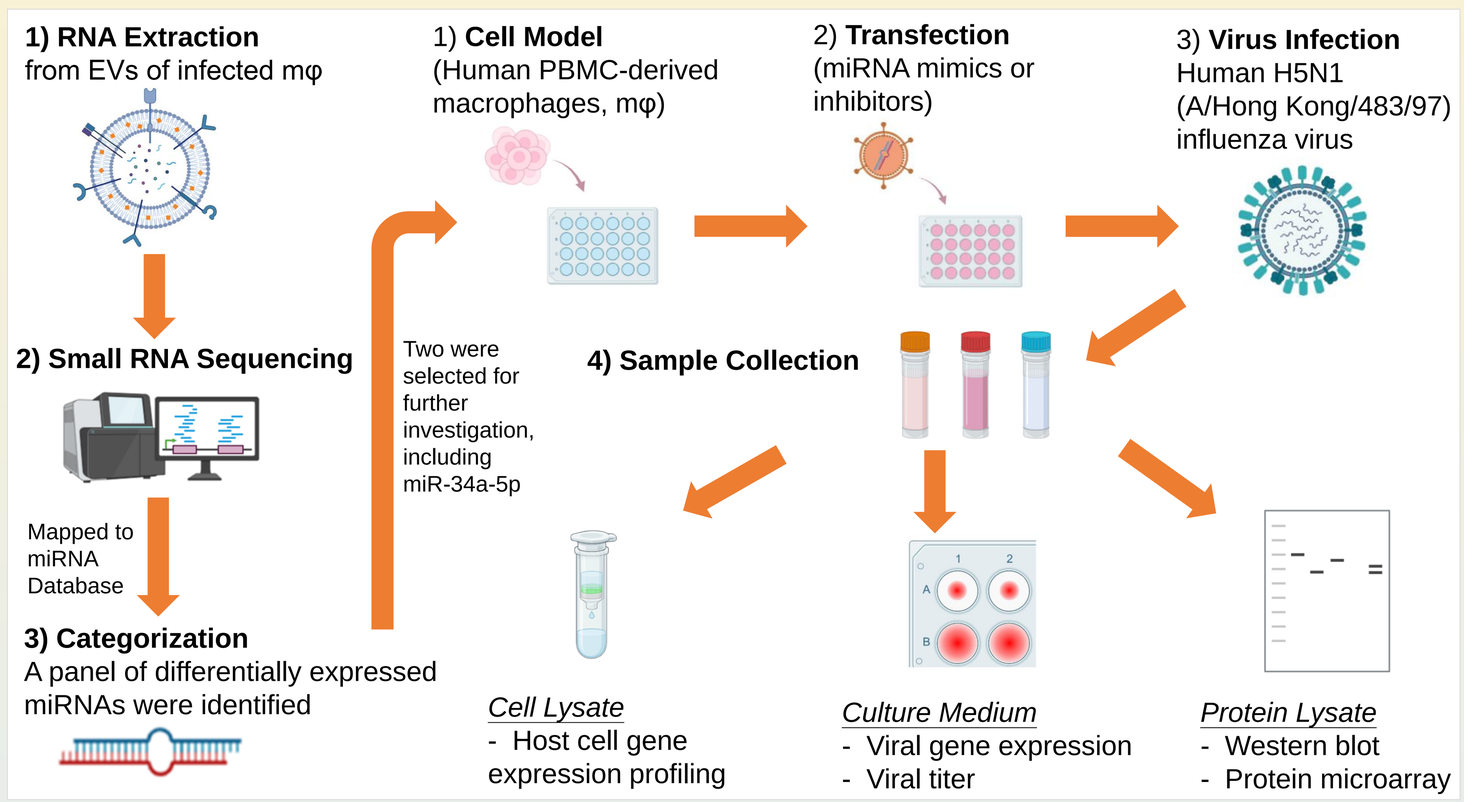
## OBJECTIVES

1: To identify the differentially expressed miRNAs in human peripheral blood monocyte (PBMC)-derived macrophage (mφ) after H5N1 IAV infection.

2: To characterize the selected miRNA activities on the induction of proinflammatory cytokines during IAV infection.

3: To identify the downstream signaling targets involved in immune response regulation by miRNAs.

## METHODOLOGY




## RESULTS

### Viral replication kinetics of H5N1 IAV in miRNA mimics- and inhibitors- transfected Mφ

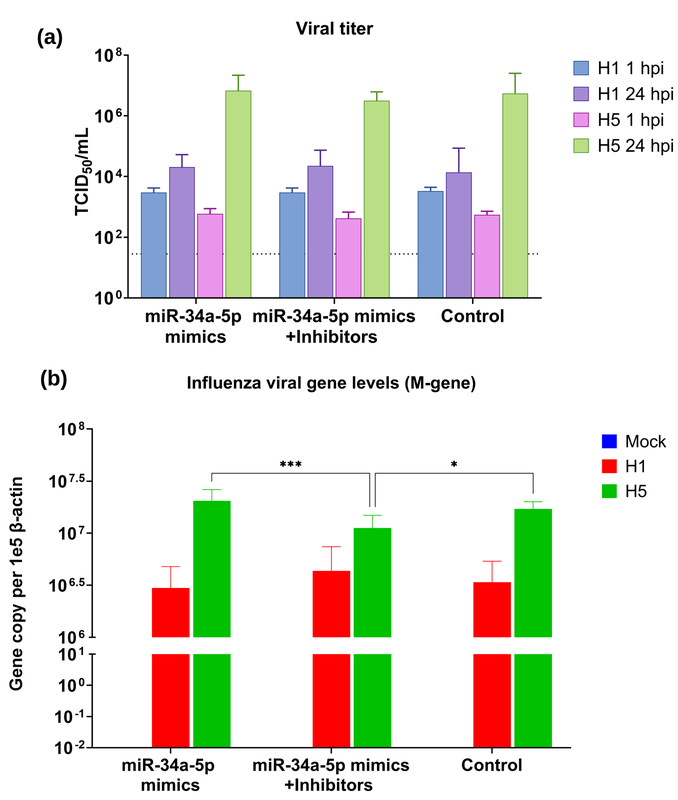



Figure 1. The effects of miR-34a-5p on influenza viral replication

miR-34a-5p mimics showed no significant effects on **(a)** viral titers and **(b)** M-gene expression, but the induction of **(b)** M-gene expression was attenuated by the miRNA inhibitors in H5N1 infection only.

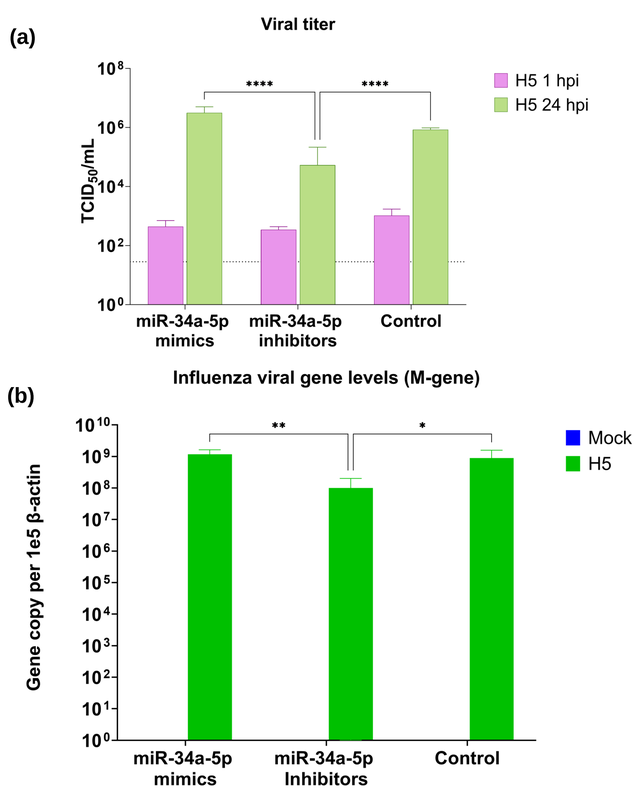



Figure 2. The effects of miR-34a on H5N1 viral replication

miR-34a-5p inhibitors significantly downregulated the **(a)** viral titers and **(b)** M-gene expression upon H5N1 infection

### The regulation of MCP-1 expression by miRNA mimics and inhibitors

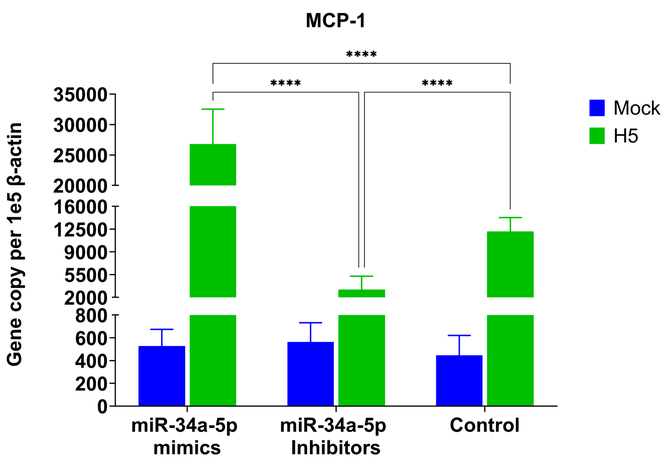



Figure 3. The expression levels of MCP-1

The expression levels of MCP-1 were further upregulated by miR-34a-5p mimics, while the corresponding miRNA inhibitors suppressed its levels in H5N1 infection.

### The semi-throughput gene expression profiles induced by miR-34a-5p mimics in H5N1-infected Mφ

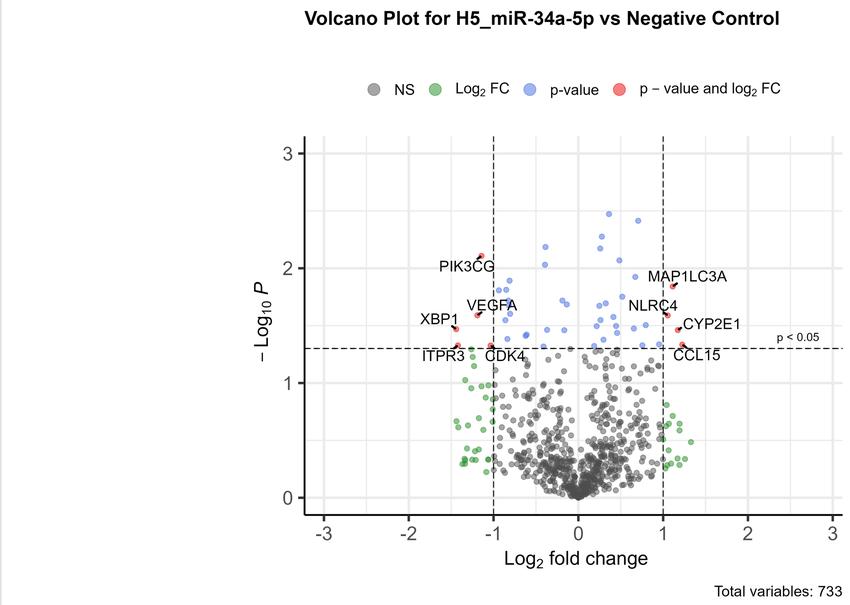



Figure 4. Volcano plot showing the expression levels of mRNAs upon H5N1 infection between miR-34a-5p mimics and negative control

The differentially expressed mRNAs in H5N1 infection were identified using a threshold of log2 fold change > 1 or < -1 and a p-value < 0.05, based on the results from 733 variables in the gene.

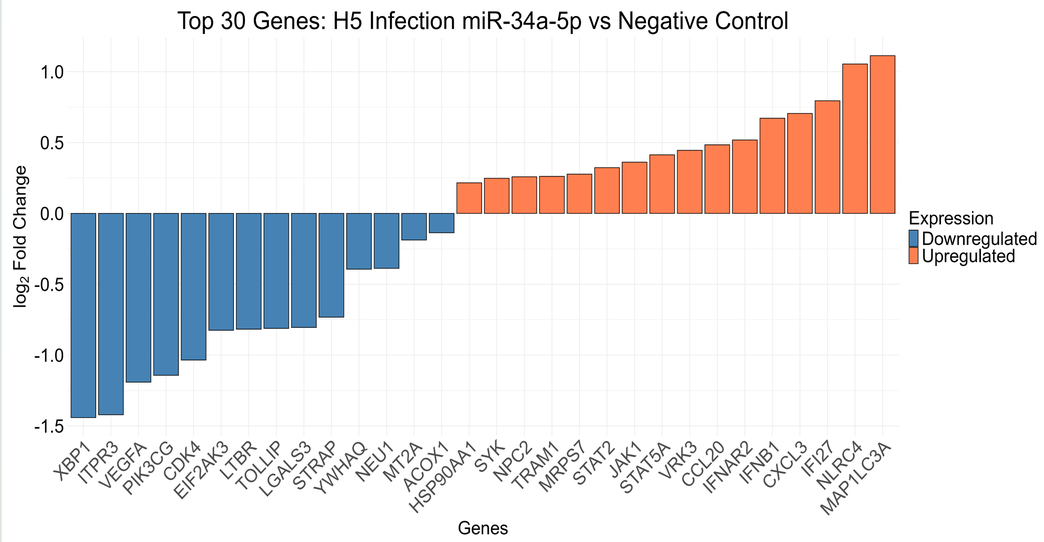



Figure 5. Bar chart showing the fold change of top 30 differentially expressed mRNAs from miR-34a-5p mimics-transfected samples upon H5N1 infection

MAP1LC3A showed the highest level of upregulation (log2 fold changes of 1.33) suggesting a robust response to miR-34a-5p mimics, while the XBP1 and IFNG were both downregulated with log2 fold changes of -1.44.

## CONCLUSION

Our findings showed that exogenous miR-34a-5p in human macrophages showed no antiviral effects in terms of viral titers and viral gene expression, but its corresponding inhibitor suppressed the viral replication. The miR-34a-5p also demonstrated immune-modulatory functions by further enhancing the expressions of several cytokines and chemokines, such as MCP-1 upon H5N1 IAV infections.

Besides, the differentially expressed mRNAs upon H5N1 infection varied in response to the miR-34a-5p were identified, of which some specific mRNA targets regulated can be further studied.

The findings of this study reveal the immuno-regulatory effects of miR-34a-5p, which may help the understanding of the pathogenesis of H5N1 infection and uncover potential therapeutic options for IAV-infected diseases.

## ON-GOING AND FUTURE WORK

Exploring the regulation of immune responses by analyzing protein expression through Western blotting or examining associated signaling pathways derived from the identified semi-throughput gene expression profiles

## REFERENCE


1. World Health Organization (2021). Avian Influenza Weekly Update Number 812, 1 October 2021.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