AN ABSTRACT OF A THESIS

IDENTIFICATION AND CHARACTERIZATION OF THE HOST FACTORS REGULATING INFLUENZA B VIRUS RNA SYNTHESIS AND VIRUS LIFE CYCLE

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Abstract

Influenza virus RNA synthesis is an intricate multistep process that is regulated by a variety of mechanisms. Viral ribonucleoprotein complexes (RNPs), comprising heterotrimeric polymerase (composed of PB2, PB1 and PA), nucleoprotein (NP) and a single copy of segmented viral genomic RNA, perform viral gene transcription at the early stage of infection and genome replication at the later stage, thus lies at the core of the virus replication cycle. Viral RNPs interact with countless host factors at each step of their replication cycle which regulates the RNP function either positively or negatively. Being the core replication machinery of the virus, RNPs also provide an excellent target for antivirals. Therefore, developing a greater understanding of the role of host factors in regulating RNP activity and viral RNA could be helpful in developing more effective countermeasures against future influenza virus outbreaks.

Multiple host factors have been discovered and characterized for their role in regulating influenza A virus RNPs. However, the knowledge of influenza B RNP-host factor interaction is limited. One of the major reasons for this limitation is the lack of tools to study influenza B RNP activity. In the present work, a fast, sensitive and high throughput reporter assay system has been developed for monitoring influenza B virus RNP activity and viral RNA synthesis in an infection-free setting. Using the reporter assay system, we validated the role of viral nonstructural protein-1 (NS1) and host protein kinase C-δ (PKC-δ) as the critical modulators of influenza B RNP activity, as previously shown in the case of influenza A viruses. In addition, this assay system was found to be ideal for screening small molecules and drugs such as Ribavirin and Favipiravir, in inhibiting influenza B virus RNA synthesis and virus replication.

Next, an extensive survey of influenza A virus RNP interactome related literatures were performed in order to shortlist potential host factors that may associate with and hence regulate the activity of influenza B virus RNPs. Identification of common interacting partners from these interactome data sets, followed by gene ontology analysis and background study of each of the host factors from the highly enriched gene classes revealed that DEAD box helicases are one of the most important classes of host factors that regulate the functioning of viral RNPs. Subsequently, Dead box helicase 3X (DDX3X) was identified as one of the most potent host factors to investigate its role in modulating influenza B RNA synthesis. In experimentation with the newly developed influenza B virus reporter assay system, the DDX3X showed a prominent role in downregulating viral polymerase activity in a dose-dependent manner. The effect of DDX3X was found to be mediated through its catalytic activity and not dependent on the intracellular immune response. The DDX3X was observed to bind to the influenza B virus promoter RNA both in the transfection and infection setting. DDX3X also associated with influenza B nucleoprotein particles during viral infection. Viral RNPs immunoprecipitated from the infected cells were found to be associated with endogenous DDX3X protein. Finally, overexpression of DDX3X showed a negative impact on the overall progress of influenza B virus infection. Together, this study established DDX3X as an antiviral factor for the influenza B virus.

Keywords: Influenza virus, ribonucleoprotein particles, host-pathogen interaction, DEAD box helicases, polymerase activity assay