The Surprising Complexity of Virus-Host Cell Interaction Revealed by the Powerful Systems Biology Approaches of Genomics and Proteomics

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Abstract

Understanding interaction between viruses and host cells during infection process is the first step in discovering appropriate drugs and vaccines against viral diseases. Advance technologies based on genomics and proteomics approaches provide great tools to disclose the complexity of virus-host interaction. In this essay, the application of RNAi screens method and proteomics-based approaches on influenza virus will be elucidated as an example. Using those methods, the primary factors controlling viral replication pathway were discovered. These findings are useful for the development of potential strategies to overcome *viral diseases.*

Keywords : virus, host, pathogen, interaction, genomic, proteomic.

Abstrak

Memahami interaksi antara virus dan pejamu dalam proses infeksi merupakan langkah awal dalam upaya penemuan obat dan vaksin yang tepat untuk melawan penyakit yang disebabkan oleh virus. Pendekatan teknologi berbasis genomik dan proteomik merupakan terobosan yang dapat menjawab kompleksitas interaksi virus-pejamu. Dalam kajian ini, akan dibahas penerapan teknologi screening RNAi dan teknologi berbasis proteomik pada virus influenza. Dengan menggunakan metode ini, faktor-faktor utama yang mengontrol tahap replikasi virus akan dapat ditemukan. Penemuan ini sangat bermanfaat dalam pengembangan strategi pengobatan yang potensial untuk mengatasi penyakit bersumber virus.

Kata kunci: virus, pejamu, patogen, interaksi, genomik, proteomik.

Introduction

Recent advancement in genomics and proteomics approaches unravels the biological complexity of the virus-host interaction.[1] The development of genomics and proteomics technologies facilitate the finding of a powerful strategy in combating viral diseases.[2] With an emphasis on genomics and proteomics approaches, the application of new methods using genome-wide RNAi screen in the Drosophila model in revealing infuenza virus-host interaction will be explained in this essay. Another method using proteomics-based approached also will be elucidated. This essay will focus on the replication step of RNA virus. To illustrate the application of genomics and proteomics approaches, the finding in influenza virus replication factors will be described as an example.

Method

This study utilized secondary data gained from published articles. Data regarding findings in virus-host interaction using genomic and proteomic approaches were chosen then reviewed and analysed.

Results

Virus-host interaction is a complex pathway. Viruses strive to infect the host cells, while the host strives to defend from the viruses attack. The defensive effort may vary including interferon production or apoptotic cascade that involves the

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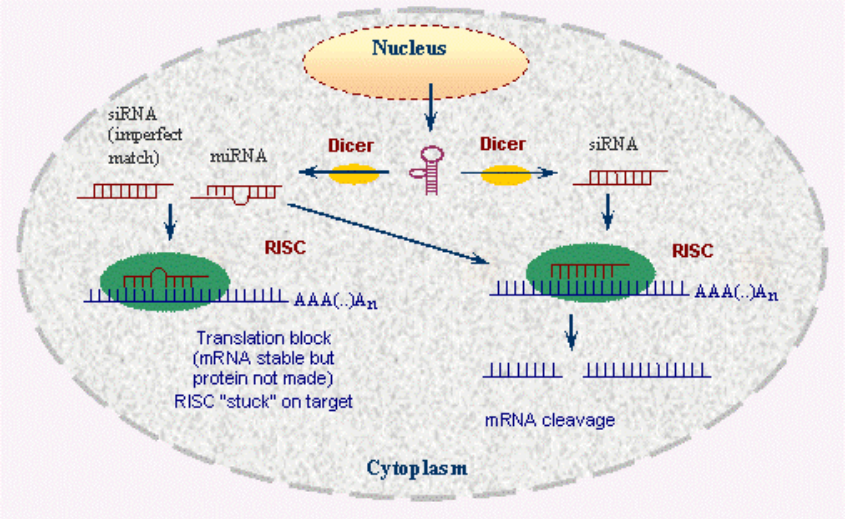
changes in gene expression. A virus infects the host by multi-step mechanism that allows the virus to bind, enter and replicate within the host cells.[3] The first step of virus infection is attachment to the host cell receptor. For this attachment, viruses require specific proteins such as neuraminidase in the influenza virus.[4] The next step is penetration that includes virion translocation across the cell membrane, endocytosis into the cytoplasm, and fusion the envelope to the cell membran. In this step, viral envelope proteins such as influenza hemaglutinin is needed for fusion.[5] Next, in the uncoating step, the viral capsid is stripped off before transferring genome into the nucleus. After that, the genetic material of viruses is replicated in the nucleus and transcribed into mRNA. Then, all of components required for virion forming are assembled followed by the budding of envelope adjacent to the virus release.[6] Because of the insufficiency of components for their independent survival, viruses need host cell for their replication process.[7]

Discusions

RNA interference (RNAi) is a process involving small non-coding RNAs integrated into cells to inhibit the expression of genes. There are three types of small RNA function in RNAi, short interfering RNA (siRNA), microRNA (miRNA) and PIWI-associated RNA (piRNA). miRNA biogenesis requires RNAse-III enzyme dicer, while siRNA forming depends on dicer only.[8] A small RNA in RNA silencing associates with Argonaute protein family in RNA-Induced Silencing Complex (RISC). This RISC complex may inhibit mRNA translation or degrade it. This way, the gene expression is inhibited (Figure 1).[8,9]

Genome-wide RNA interference (RNAi) analysis using Drosophila model has a powerful ability to screen various cellular pathways that control diseases. This method offers detection of gene functions related with phenotypes in organisms. Quantitative reproducibility of this method was proven by phenotypes analysis from independent RNAi against the genes target in the same screen.[10] Viruses are appropriate target for genome-wide analysis due to their small size and their fully genome sequence availability. Additionally, viruses are manageable to be genetically recombined.[11] Drosophila is a powerful model system to study cellular and development processes.[10] Of 14,600 genes in the Drosophila genome, ~3,600 are related with mutant phenotypes.[12] The experiment in gene functions found that 20% of 438 selected genes from Drosophila play a role in cell growth, cell cycle and anti apoptotic in cell survival.[10] In addition, Drosophila genes are highly conserved in vertebrates.



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Thus, Drosophila genome is also used as a model of mammalian cell study.[13] The most recent technology using RNAi can be targeted for the depletion of gene products by gene knockouts and silencing gene expressions. In this case, RNAi is used as a tool to detect the function of uncharacterized genes by identifying the loss of functional phenotypes in Drosophila.[12] RNAi is a potential tool to knockout genes and suppress the expression. RNAi mainly affects the post-transcriptional phase. The target of RNAi is endogenous mRNA containing similar sequences that will be degraded before translation.[14] Consequently, RNAi suppress the viral replication. Furthermore, RNAi is applied in the reverse genetic techniques to analyse the function of genes associated with infectious diseases.[15] Moreover, RNAi is promising in the development of antiviral against influenza A virus.[16]

The Drosophila RNAi screens method is used to uncover the essential factors of influenza virus replication during virus-host interaction.

A study done by Hao et al., in 2008 applied this method to demonstrate the host genes that participate in the virus replication. WSN/33 (H1N1) virus, H5N1 Indonesia 7 virus, and recombinant influenza viruses (FVG-G and FVG-R)

were used in this experiment. Drosophila cell culture was used to determine host genes required for virus infection. The recombinant viruses, in which the haemaglutinin (HA) and neuraminidase (NA) ORFs were replaced by vesicular stomatitis virus-G (VSV-G), Glycoprotein G and Renilla luciferase genes respectively were used because Drosophila D-Mel2 cells are unable to express the wild type influenza virus receptor. The infection to Drosophila was detected by GFP fluorescence and RNA virus replication were detected by real-time PCR. Drosophila cells support the replication phase of influenza virus during the virus life cycle. This process includes the release of RNA containing viral-ribonucleoprotein-complexes (vRNPs) into cytoplasm, the entry of vRNP into the nucleus, synthesis of mRNA, and the release of mRNA into cytoplasm continued with translation.[13]

Drosophila genes encoding important component for influenza viral infection in host machineries such as ATP6VOD1, COX6A1, and NXF1/TAP were selected to assess mammalian cells assay. ATP6VOD1 encodes V-ATPase that pumps a proton required in endocytosis pathway.[17] COX6A1 encodes cytochrome c-oxydase that catalyzes the electron transport chain from cytochrome c to oxygen in the mitochondria.[18] NXF1/TAP encodes mRNA nuclear export factor that is responsible for exporting mRNA from the nucleus.[19] Mammalian cells (HEK 293) were treated with siRNAs against those human homologue genes and infected with FVG-R, H1N1 and H5N1 viruses respectively. Then, Renilla luciferase activity was observed to assess the efficiency of virus replication and gene expression.[13] Renilla luciferase is an enzyme used as a bioluminesense reporter that can visualize gene expression by producing blue light. Transcription and translation of the gene encoding Renilla luciferase will be detected by camera.[20] The decrease of Renilla luciferase activity in the ATP6VOD1, COX6A1 and NCF1 groups indicates that the knockout of those genes by siRNA obstructs the genes expression that leads to the inhibition of influenza virus replication pathway. Thus, ATP6VOD1, COX6A1 and NCF1 are important genes for influenza virus replication. These results suggest that Drosophila identified genes can be correlated with host factors required in influenza virus replication pathway in mammalian cells. Because Drosophila RNAi screens provides more than 100 potential candidate genes, this method could be applied as well to other viruses.[13]

Another study demonstrates the advancement of Drosophila RNAi screens using long dsRNA. Compared to short dsRNA, long dsRNA gives higher effectivity in gene expression targeting. This finding leads to the identification of many unknown genes using viability hits. In addition, this method reduces the tolerance of mismatches and gaps in the alignment with target sequences and also minimizes the effect of siRNA to many other mRNAs besides the mRNA target.[21] Therefore, this method is promising for the study of virus-host interaction in order to get the most appropriate gene target for drug discovery effectively.

In spite of that, proteomics-based approaches have also been used as a powerful tool to understand cellular factors of viral infection. Another study of influenza A virus carried out by Mayer et al., in 2007 applied proteomics-based screens to determine essential cellular factors in the viral life cycle. Using Strep-purification and TAP-purification method continued with mass spectroscopy (MS), viral ribonucleoprotein (vRNP) and viral polymerase complexes were indentified. Activity measurement of those proteins was done by co-immunoprecipitation, immunoflu-eresence and minireplicon assays respectively. With application of those techniques, vRNP and viral

polymerase complexes were identified. vRNP is a genomic component which is transformed into the nucleus in the viral infection process, while polymerase is an enzyme needed for translation initiation. Thus, vRNP and viral polymerase are essential factors in the viral replication pathway.[7]

Subsequently, Shapira *et al.* combined a multi-layer approach to reveal dynamic interaction between H1N1 influenza virus and the human host. The study utilized protein-protein interaction (yeast two-hybrid), genome-wide expression profiling, functional genomic and network modelling. This study found a comprehensive map of physical and regulatory host-pathogen interactions. A number of 1745 candidate genes were discovered. Functional contribution of the selected genes to viral replication and IFN was evaluated by siRNA knockdown to gauge the specialized roles of host-pathogen network of each candidate gene product.[23]

Conclusion

In conclusion, studies in virus-host interaction required genomics and proteomics approaches to help understanding the complexity of infection pathway. The recent advance methods based on genomics and proteomics is desirable in determining the main factors required in viral infection processes. Those factors could be used as excellent targets in drug discovery. Application of those methods will be useful to uncover the main factors influence host-virus interaction including cellular functions on viral life cycles. However, those findings are not the end point but a starting point for the next innovation regarding the discovery of the viral diagnostics and antiviral drugs as well as vaccine candidates.[9] The finding of those essential factors support the effort in controlling viral diseases. Next findings regarding virus-host interaction are required to gain the most appropriate way to combat viral diseases.

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