Available online at www.sciencedirect.com
ScienceDirect
www.sciencedirect.com/science/journal/16720229
GENOMICS PROTEOMICS & BIOINFORMATICS

Review

Transcriptomics and Proteomics in the Study of H1N1 2009

Lijun Zhang[1,2*], Xiaojun Zhang[3], Qing Ma[4], Fang Ma[2], and Honghao Zhou[1*]

[1]*Department of Pharmacogenetics, Institute of Clinical Pharmacology, Central South University, Changsha 410078, China;*
[2]*Shanghai Public Health Clinical Center, Fudan University, Shanghai 201508, China;*
[3]*Department of Neurosurgery, Fuzhou General Hospital, Fuzhou 350025, China;*
[4]*School of Pharmacy and Pharmaceutical Sciences, University at Buffalo, NY 14260, USA.*

Genomics Proteomics Bioinformatics 2010 Sep; 8(3): 139-144 DOI: 10.1016/S1672-0229(10)60016-2

Abstract

Influenza A virus (H1N1) 2009, a new swine-origin influenza A virus, has been spread worldwidely and caused great public fear. High-throughput transcriptomics and proteomics methods are now being used to identify H1N1 and H1N1-host interaction. This article reviews recent transcriptomics and proteomics research in H1N1 diagnosis, treatment, and H1N1 virus-host interaction, to offer some help for further understanding the infection mechanism and controlling H1N1 transmission.

Key words: H1N1, proteomics, transcriptomics, host

Introduction

From March to early April in 2009, a new swine-origin influenza A virus (Influenza H1N1 2009) emerged in Mexico and the United States (*1*). This virus is unique in comparison to 2008 H1N1 influenza viruses with 27.2% difference in the amino acid sequence of hemagglutinin (HA) and 18.2% difference in that of neuraminidase (NA) (*2*). These differences are likely to be derived through reassortment of two or more viruses of swine origin (*3*). It can be transmitted from pig to human (*4*), human to human, and even from human back to pig (*5*). By May 11, 2009, the virus has spread worldwidely to 30 countries through human-to-human transmission, causing the World Health Organization (WHO) to raise its pandemic alert to level 5 of 6 during the first few weeks of surveillance (*6*). On June 11, 2009, the WHO raised the alert level to phase 6 and declared an H1N1 pandemic. So far, vaccination is still considered to be the best way to control infection. As reviewed by Baras *et al* (*7*), there are vaccinations preventing H1N1, H3N2 and B [Fluarix (against HA) and FluLaval (against HA)] and H5N1 [Prepandrix (against HA and NA)]. On September 8, 2009, China was reported to be the first country to use vaccine to control H1N1 2009 (http://www.tfol.com/10026/12696/12697/2009/9/8/10803926.shtml). However, at the same time, broader transmission of H1N1 was reported in China. Due to the high mutation rates of virus, the recurring emergence of influenza strains is resistant to available antiviral medications, and has the potential for a new influenza pandemic. So it is necessary and important to systematically surveille H1N1, develop new therapeutic approaches that can be rapidly deployed, and address the issue of recurring resistance.

*Corresponding authors.
E-mail: zhanglijun1221@163.com; hhzhou@public.cs.hn.cn

The development of high-throughput genomics (*8, 9*), transcriptomics (*3, 10, 11*) and proteomics (*12, 13*) techniques has facilitated the investigation of many complex issues of H1N1 and H1N1-host interactions. Although genomics offered great help to understand H1N1 and H1N1-host interaction (*8*), it is functional genome (*i.e.*, RNA and proteins) that carries the function of genes. For example, messenger RNA expression microarray technology that allows simultaneous measurement of tens of thousands of genes has greatly increased our ability to detect subtypes of influenza A (*3, 10*). Since viruses employ diverse strategies to control host cellular processes at the post-transcriptional level, studying the proteome of H1N1 or host can help us to better understand this virus and control its transmission (*12, 13*). Here we review the progress with transcriptomics and proteomics on H1N1, which may provide a better understanding of H1N1 infection and shed new lights on further research.

Transcriptomics Research in H1N1

Diagnosis

RT-PCR

PCR is widely used for the detection of H1N1. Poon *et al* (*14*) reported conventional and real-time RT-PCR protocols, and subsequently developed three real-time RT-PCR assays for the detection of pandemic H1N1 2009 virus using primers and hydrolysis probes targeting the HA and matrix (M) genes (*15*). These rapid, sensitive, and specific methods can detect the virus in a 10^6 dilution of 4×10^6 TCID50/mL using 5 μL as template, which facilitates the identification of new cases of pandemic H1N1 2009 virus, ensures optimal management to minimize transmission to vulnerable individuals, and aids surveillance activities to understand the full impact of this virus in the community.

RNA microarray

To enable a quick response to a potential outbreak, it is desirable to have a fast, accurate, and comprehendsive diagnostic method capable of simultaneously typing and subtyping influenza viruses. Currently, the diagnostic methods available for identifying influenza viruses include viral culture, direct fluorescent antibody testing, rapid point-of-care immunoassays, RT-PCR, sequencing, and multiplex RT-PCR. Although viral culture is the "gold standard" for typing and subtyping of influenza viruses, it usually takes three to seven days to culture the virus (*16*). Both rapid point-of-care immunoassays (*17*) and real-time RT-PCR (*15*) can provide results within 30 min to 1 h, but they do not provide subtype information. Recently, different types of microarrays in combination with multiplex amplification have been reported for the typing and subtyping of influenza viruses (*3, 18, 19*). A recent study showed that a microarray with 46 short virus-specific oligonucleotides can be utilized to detect influenza A virus of 5 subtypes, including H1N1, H1N2, H3N2, H5N1 and H9N2 (*3*). The assay correctly and specifically detected and subtyped 11 different influenza A viruses isolated from human, avian, and swine species belonging to the 5 subtypes. When tested with 225 clinical samples, 20 were detected to be positive using the microarray-based assay, whereas only 10 were found positive by the conventional culture method. The entire analysis was completed within 7 h. A more high-throughput microarray reported by Huang *et al* (*18*) could accurately type and subtype 15 different influenza virus isolates, including two influenza B, five A/H1N1, six A/H3N2, and two A/H5N1 isolates with an analytical sensitivity of 10^2 to 10^3 copies of transcripts per reaction for each of the genes. The assay showed a clinical sensitivity of 96% and a clinical specificity of 100% through a study of 146 human clinical specimens.

A carbohydrate microarray, based on the neoglycolipid technology, was developed and used for analyzing the receptor-binding specificity of pandemic influenza A 2009 virus (*19*). A clear distinction was found when comparing the receptor-binding repertoire of the pandemic H1N1 viruses Cal/09 and Ham/09 with the seasonal virus Mem/96. The Cal/09 and Ham/09 viruses bound not only to the majority of α2-6-linked sialyl sequences, but also to a considerable range of α2-3-linked sialyl sequences. In contrast, Mem/96 bound exclusively to α2-6-linked sialyl sequences, even at a high virus concentration, not to the α2-3-linked sialyl sequences (*19*).

Infection mechanism

Mammalian cells have developed complex systems to detect and eliminate viral pathogens, while viruses have evolved mechanisms to co-opt host processes and suppress host defenses. During the course of a viral infection, viral proteins interact with an array of host proteins. Shapira *et al* (*11*) combined genomics and transcripteomics to study the physical and regulatory map of host-influenza interactions, and presented the potential roles for some unanticipated host and viral proteins in viral infection and the host response, including a network of RNA-binding proteins, components of WNT signaling, and viral polymerase subunits. Karlas *et al* (*20*) carried a genome-wide RNA interference (RNAi) screen and discovered 287 human host cell genes influencing influenza A virus replication. For example, SON DNA binding protein was found to be important for normal trafficking of influenza virions to late endosomes early in infection. This genome-wide RNAi screen identified many human host factors crucial for influenza virus replication, and offered some help for the dissection of virus-host interactions and the identification of drug targets for a broad range of influenza viruses.

Treatment

RNAi is a powerful tool to silence gene expression. Small interfering RNA (siRNA)-induced RNA degradation has been recently used as an antivirus agent to inhibit specific virus replication (*3, 21-23*).

The siRNA targeting influenza M2 gene (siM2) reported by Sui *et al* (*22*) potently inhibits viral replication, including H1N1 virus and highly pathogenic avian influenza virus H5N1. Similarly, Zhou *et al* (*23*) used siRNA technology to interrupt the expression of five target sequences (M-48, M-754, M-949, NP-749 and NP-1383), and found that siRNA treatment targeting conserved regions of influenza virus matrix (M2) and nucleocapsid protein (NP) genes could specifically inhibit influenza A virus replication in MDCK cells. The delivery of pS-M48 and pS-NP1383 significantly reduced lung virus titers in the infected mice, and partially protected the mice from lethal influenza virus challenge. Moreover, the treatment of pS-M48 and pS-NP1383 suppressed the replication of different subtypes of influenza A viruses, including a highly pathogenic avian isolate strain of H5N1.

Zhou *et al* (*21*) created transgenic tobacco plants that produce siRNAs targeting the mRNA of the non-structural protein NS1 of H1N1. They found that agroinfiltration of transgenic tobacco with an Agrobacterium strain harboring a 5mNS1-expressing binary vector causes a reduction of 5mNS1 transcripts in the siRNA-accumulating transgenic plants. Furthermore, H1N1 infection of siRNA-transfected mammalian cells notably suppressed viral replication. These results demonstrate that plant-derived siRNAs can inhibit viral propagation through RNAi and could potentially be applied in the control of viral-borne diseases.

An siRNA-targeting host protein, promyelocytic leukemia protein (PML), was studied by Li *et al* (*24*). They concluded that the antiviral effect of PML on influenza A viruses is viral subtype/strain specific according to their findings: (1) The depletion of pan-PML by siRNA rendered A549 cells more susceptible to influenza A virus strains PR8 (H1N1) and ST364 (H3N2), but not to strains ST1233 (H1N1), Qa199 (H9N2) and Ph2246 (H9N2); (2) Overexpression of PML-VI in MDCK cells conferred potent resistance to PR8 (H1N1) infection, while maintaining their sensitivity to ST1233 (H1N1), ST364 (H3N2), Qa199 (H9N2) and Ph2246 (H9N2).

Prediction of prognosis

Early identification of the mutation of H1N1 gene and prediction of the treatment are very important. Oseltamivir-resistant influenza A (H1N1) viruses with neuraminidase gene H274Y amino acid substitution were proven to be transmitted and retain significant pathogenicity and lethality in high-risk patients (*25*).

Proteomics in H1N1

As reviewed above, transcription research is very useful in H1N1 diagnosis and treatment. However, it is proteins that are ultimately responsible for the function of cells. During infection, the influenza virus modifies the host cell proteome by triggering host

anti-viral responses, hijacking host processes, and inhibiting host mRNA processing. Studying proteins using the technology based on mass spectrometry (MS) should provide additional information for H1N1 research (*13, 15, 26-28*).

Diagnosis

Although the diagnosis of H1N1 based on RNA is commonly used, there are still two proteomic reports based on the high-throughput capacity and high sensitivity of proteomics technology. Morrissey and Downard (*13*) used gel electrophoresis and MS to identify protein-protein interactions and the nature of the interaction interface with high-sample throughput and sensitivity. Studies on the protein antigens of the influenza virus have demonstrated that this approach can be successfully employed to detect determinants within the hemagglutinin antigen of two divergent type A forms of the virus in circulation. The determinants are localized to residues 206-224 following tryptic digestion of the hemagglutinin antigen. Specific peptide-antibody complexes formed following the treatment of gel-recovered antigen are preserved on the MALDI target array. Another study is a host peptide research reported by Wahl *et al* (*29*). They compared peptides eluted from the HLA of naïve and infected cells by MS to understand the host-encoded peptides presented by class I molecules following influenza infection, and identified 20 peptide ligands unique to infected cells and 347 peptides with increased presentation following infection. Infection with different influenza strains demonstrated that proteome changes are predominantly strain-specific, with few individual cellular interactions observed in multiple viral strains.

Infection mechanism

Quantitative proteome-wide profiling of virus infection can provide insights into complexity and dynamics of virus-host cell interactions and may accelerate antiviral research and support optimization of vaccine manufacturing processes. Vester *et al* (*27*) used 2-D differential in-gel electrophoresis (DIGE) and nanoHPLC-nanoESI-MS/MS analysis to qualitatively and quantitatively determine the dynamic cellular proteome responses. Proteins from a cell line used for vaccine production (MDCK) and a human lung carcinoma cell line (A549) were separated by 2-D DIGE, and 16 quantitatively altered protein spots were identified in both cell lines. Most significant changes were found for keratins, major components of the cytoskeleton system, and for Mx proteins, interferon-induced key components of the host cell defense. Most likely, these proteins are required for supporting functions during influenza viral life cycle or host cell stress response.

Due to the complexity of proteins in cells and tissues, it is difficult to identify proteins with low abundance. Therefore, some researches focused on subcellular proteome (*30*). Ohman *et al* (*30*) used cytosolic and mitochondrial proteomics to reveal the host response to influenza A infection at the protein level in human macrophages. Through 2-DE for protein separation and MS for protein identification, several heat shock proteins and fragments of cytoskeletal proteins were detected to be up-regulated in cytosolic proteomes during influenza A virus infection. In mitochondrial proteomes, simultaneously with the expression of viral proteins, the levels of intact actin and tubulin were highly up-regulated. This was followed by the translocation of the components of antiviral RNA recognition machinery, including RIG-I (retinoic acid-inducible protein I), TRADD (TNFR1-associated death domain protein), TRIM25 (tripartite motif protein 25), and IKKepsilon (inducible IkappaB kinase), onto the mitochondria. Cytochalasin D, a potent inhibitor of actin polymerization, inhibited influenza A virus-induced expression of IFN-beta, IL-29, and TNF-alpha, suggesting that intact actin cytoskeleton structure is crucial for proper activation of antiviral response. At late phases of infection, mitochondrial fragmentation of actin was observed, indicating that actin fragments are involved in disruption of mitochondrial membranes during apoptosis of virus-infected cells. These results showed that actin and RIG-I/MAVS signaling components translocate to mitochondria upon influenza A virus infection of human primary macrophages (*30*).

Furthermore, recent advances in biotechnologies allow the detection of a complete cell proteome. Many differentially expressed proteins were detected. It is usually difficult to select proteins for further re-

search and interpret them in the biological context. A protein-protein interaction map might be useful and has been used for virus-host research (*28, 31*).

Conclusion

H1N1 continues to spread globally. The great transmissions occurred in 2009 have caused great social fear and economic losses. It is important to increase and coordinate preventive activities at a global level to control virus transmission. Fortunately, the vaccines for preventing H1N1 2009 were reported in August and September 2009. However, it should be noted that, this vaccine is not suit for children and people who are allergic to eggs. Understanding the molecular mechanisms of virulence and pathogenesis is critical for developing new antiviral therapies. This article has reviewed how global transcriptomic and proteomic studies have been used to diagnose and treat H1N1 as well as study the infection mechanism. Due to its high-throughput capacity, RNA microarray may be more suitable for rapid screening and controlling the transmission of H1N1 in the future. siRNA has been widely used for functional research of genes and might bring new methods to control H1N1 transmission. Proteomics has been used for H1N1-host interaction research, and might offer new theories to the understanding of H1N1. Discovery-based proteomic approaches can aid in identifying the host response pathways associated with immunopathology and better understanding of how global host responses are regulated during viral infection, which is critical for the development of novel antiviral treatments. The strength of transcriptomics and proteomics-based approaches lies in their ability to characterize the global effects of infection and to serve as a discovery-based tool to identify new host response components and pathways (*8*) involved in H1N1 infection for further study, which may ultimately lead to novel targets of therapeutic intervention.

Acknowledgements

We thank grants from Shanghai Natural Science Foundation (09ZR1426300), China Postdoctoral Science Foundation (20100471001), National Scientific Foundation of China (No. 30801421), Huge Project to Boost Chinese Drug Development (No. 2009ZX09501-032), and the Postdoctoral Science Foundation of Central South University.

References

1 Centers for Disease Control and Prevention (CDC). 2009. Swine influenza A (H1N1) infection in two children—Southern California, March-April 2009. *Morb. Mortal. Wkly. Rep.* 58: 400-402.
2 Gallaher, W.R. 2009. Towards a sane and rational approach to management of Influenza H1N1 2009. *Virol. J.* 6: 51.
3 Novel Swine-Origin Influenza A (H1N1) Virus Investigation Team. 2009. Emergence of a novel swine-origin influenza A (H1N1) virus in humans. *N. Engl. J. Med.* 360: 2605-2615.
4 Cohen, J. 2009. Pandemic influenza. Straight from the pig's mouth: swine research with swine influenzas. *Science* 325: 140-141.
5 Peiris, J.S., *et al*. 2009. Emergence of a novel swine-origin influenza A virus (S-OIV) H1N1 virus in humans. *J. Clin. Virol.* 45: 169-173.
6 ECDC Technical Emergency Team. 2009. Initial epidemiological findings in the European Union following the declaration of pandemic alert level 5 due to influenza A (H1N1). *Euro Surveill.* 14: 19204.
7 Baras, B., *et al*. 2008. A vaccine manufacturer's approach to address medical needs related to seasonal and pandemic influenza viruses. *Influenza Other Respi. Viruses* 2: 251-260.
8 Kash, J.C. 2009. Applications of high-throughput genomics to antiviral research: evasion of antiviral responses and activation of inflammation during fulminant RNA virus infection. *Antiviral Res.* 83: 10-20.
9 Fornek, J.L., *et al*. 2007. Use of functional genomics to understand influenza-host interactions. *Adv. Virus Res.* 70: 81-100.
10 Lu, Q., *et al*. 2009. Detection in 2009 of the swine origin influenza A (H1N1) virus by a subtyping microarray. *J. Clin. Microbiol.* 47: 3060-3061.
11 Shapira, S.D., *et al*. 2009. A physical and regulatory map of host-influenza interactions reveals pathways in H1N1 infection. *Cell* 139: 1255-1267.
12 Liu, N., *et al*. 2009. Mutations in influenza virus replication and transcription: detection of amino acid substitutions in hemagglutinin of an avian influenza virus (H1N1). *FASEB J.* 23: 3377-3382.
13 Morrissey, B. and Downard, K.M. 2006. A proteomics approach to survey the antigenicity of the influenza virus

by mass spectrometry. *Proteomics* 6: 2034-2041.

14 Poon, L.L., *et al*. 2009. Molecular detection of a novel human influenza (H1N1) of pandemic potential by conventional and real-time quantitative RT-PCR assays. *Clin. Chem.* 55: 1555-1558.

15 Pabbaraju, K., *et al*. 2009. Design and validation of real-time reverse transcription-PCR assays for detection of pandemic (H1N1) 2009 virus. *J. Clin. Microbiol.* 47: 3454-3460.

16 Ellis, J.S. and Zambon, M.C. 2002. Molecular diagnosis of influenza. *Rev. Med. Virol.* 12: 375-389.

17 Weinberg, A. 2005. Evaluation of three influenza A and B rapid antigen detection kits—update. *Clin. Diagn. Lab. Immunol.* 12: 1010.

18 Huang, Y., *et al*. 2009. Multiplex assay for simultaneously typing and subtyping influenza viruses by use of an electronic microarray. *J. Clin. Microbiol.* 47: 390-396.

19 Childs, R.A., *et al*. 2009. Receptor-binding specificity of pandemic influenza A (H1N1) 2009 virus determined by carbohydrate microarray. *Nat. Biotechnol.* 27: 797-799.

20 Karlas, A., *et al*. 2010. Genome-wide RNAi screen identifies human host factors crucial for influenza virus replication. *Nature* 463: 818-822.

21 Zhou, Y., *et al*. 2004. Transgenic plant-derived siRNAs can suppress propagation of influenza virus in mammalian cells. *FEBS Lett.* 577: 345-350.

22 Sui, H.Y., *et al*. 2009. Small interfering RNA targeting m2 gene induces effective and long term inhibition of influenza A virus replication. *PLoS One* 4: e5671.

23 Zhou, H., *et al*. 2007. Effective small interfering RNAs targeting matrix and nucleocapsid protein gene inhibit influenza A virus replication in cells and mice. *Antiviral Res.* 76: 186-193.

24 Li, W., *et al*. 2009. Differential suppressive effect of promyelocytic leukemia protein on the replication of different subtypes/strains of influenza A virus. *Biochem. Biophys. Res. Commun.* 389: 84-89.

25 Gooskens, J., *et al*. 2009. Morbidity and mortality associated with nosocomial transmission of oseltamivir-resistant influenza A (H1N1) virus. *JAMA* 301: 1042-1046.

26 Patriarca, P.A. 1999. New options for prevention and control of influenza. *JAMA* 282: 75-77.

27 Vester, D., *et al*. 2009. Quantitative analysis of cellular proteome alterations in human influenza A virus-infected mammalian cell lines. *Proteomics* 9: 3316-3327.

28 Antonov, A.V., *et al*. 2009. PPI spider: a tool for the interpretation of proteomics data in the context of protein-protein interaction networks. *Proteomics* 9: 2740-2749.

29 Wahl, A., *et al*. 2010. HLA class I molecules reflect an altered host proteome after influenza virus infection. *Hum. Immunol.* 71: 14-22.

30 Ohman, T., *et al*. 2009. Actin and RIG-I/MAVS signaling components translocate to mitochondria upon influenza A virus infection of human primary macrophages. *J. Immunol.* 182: 5682-5692.

31 Jensen, L.J., *et al*. 2009. STRING 8—a global view on proteins and their functional interactions in 630 organisms. *Nucleic Acids Res.* 37: D412-416.