The Second International Symposium on Optimization and Systems Biology (OSB'08)
Lijiang, China, October 31– November 3, 2008

Regulation of cell signaling cascades by influenza A virus

Yu-Li Wan Hui Zhong Cheng-Jun Wu Yao-Wu Yang
Jian-Wei Wang* Tao Hong*

Institute of Pathogen Biology (IPB), Peking Union Medical College,
Chinese Academy of Medical Sciences (CAMS), Beijing 100730,PR China

Abstract There were a lot of studies related to global gene expression in cells infected with influenza A virus. However, few studies have focused on the systematic analysis of the regulation of cell signaling cascade by influenza A virus. To understand fully the regulation of cell signaling cascade associated with influenza virus infection, we used cDNA microarray to investigate the gene expression of A549 infected with influenza A virus A/PR/8/34. Then we used the KEGG pathway database to analyze the up-regulated genes. From the results, we can see that different pathways including Antigen processing and presentation, Toll-like receptor signaling pathway, Apoptosis, Jak-STAT signaling pathway, MAPK signaling pathway and Complement and coagulation cascades were effected by the infection of influenza A virus. Our results will provide a good platform to understand the host genes regulated by influenza virus and targets for drug design.

Keywords Influenza A virus; A549; cell signaling cascade

1 Introduction

Influenza A virus is still a major cause of morbidity and mortality worldwide. The rapid change in viral genetics allows the virus to infect new host species and quickly overcome protective immunity, which produces obstacles in controlling its spread in nature. Contrary to the virus, the genetics host is relatively stable. So research into the gene expression of host cells infected with influenza A virus will provide a good platform to understand targets for drug design. There are a few studies focused on the gene expression profile of cells infected by influenza virus. However, almost all the reports till now are revolved in single gene or single pathway [1,2,3,4,5] . Our research focuses on the cell signaling networks regulated by the influenza A virus, which is important for determining targets for drug design and develop new strategies for antivirals.

2 Materials and methods

2.1 Virus and Cell lines

A549 and MDCK were purchased from American Type Culture Collection (ATCC, Manassas, VA) and maintained in DMEM supplemented with 10% FBS, 2 mM glu-

*To whom correspondence should be addressed:wangjw28@vip.sina.com;hongt@cae.cn

tamine, 100 units/ml penicillin, and 100 units/ml streptomycin at 37°C. Wild-type influenza A/PR/8/34 was propagated in MDCK for three generations and then was used for the following infection.

2.2 Viral Infection, RNA Extraction

Infection with wild type influenza A viruses was performed in A549 cells grown on T-175 cultural flasks. The inoculum was removed, and cells were washed two times with Hanks before addition of serum-free DMEM with 5% BSA. The final TPCK-trypsin concentration was 1 ug/ml. In all cases, the multiplicity of infection (moi) was adjusted before each independent experiment to ensure that at least 80% of the cells were infected 24h post-infection as determined by immunofluorescence using nucleo- protein (NP)-specific antibody. Mock infection was performed with serum-free DMEM with 5% BSA instead of virus. At 24h after infection, cells were washed with Hanks for two times and the total cellular RNA was extracted using Trizol reagent (Invitrogen, USA) according to the manufacturer's instructions.

2.3 cDNA microarray

The cDNA microarray methods have been described in detail elsewhere[6,7,8] .In brief, a human genome oligonucleotide set (version 2.0) consisting of 5' amino acid-modified 70-mer probes and representing 21,329 well -characterized Homo sapiens genes was used for the detection of gene expression. Arrays were scanned with a confocal LuxScan^{TM}scanner and the images obtained were then analyzed using LuxScan^{TM} 3.0 software (Both from CapitalBio). For individual channel data extract, faint spots whose intensities were below 400 unit after background subtracted in both channels (Cy3 and Cy5) were removed.

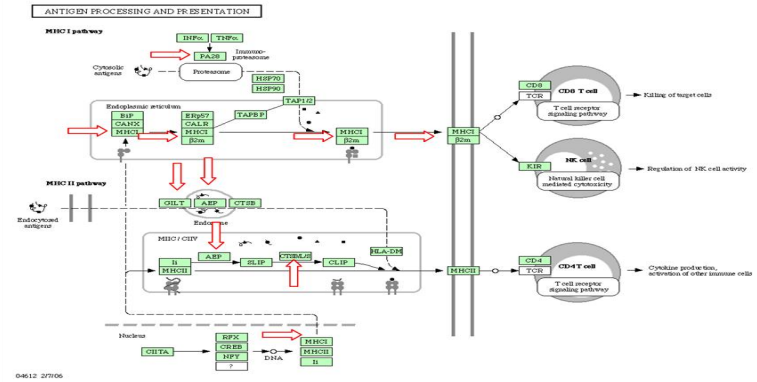
2.4 Array data analysis

The genes up-regulated by influenza A virus were analyzed with KEGG(Kyoto Encyclopedia of Genes and Genomes) pathway database.

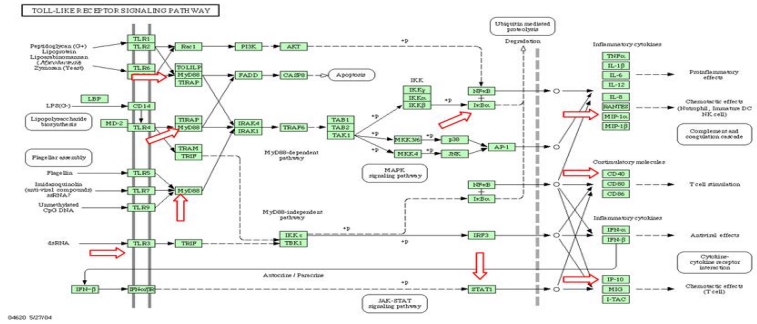
3 Results

There were above 300 genes up-regulated after infection. The genes were analyzed with KEGG Orthology database. Here only those genes related to the different signaling pathways were shown. The genes signed by red arrows represented up-regulated genes. Different signal cascades effected by influenza A virus were as follows: Antigen processing and presentation, Toll-like receptor signaling pathway, Apoptosis, Jak-STAT signaling pathway, MAPK signaling pathway and Complement and coagulation cascades:

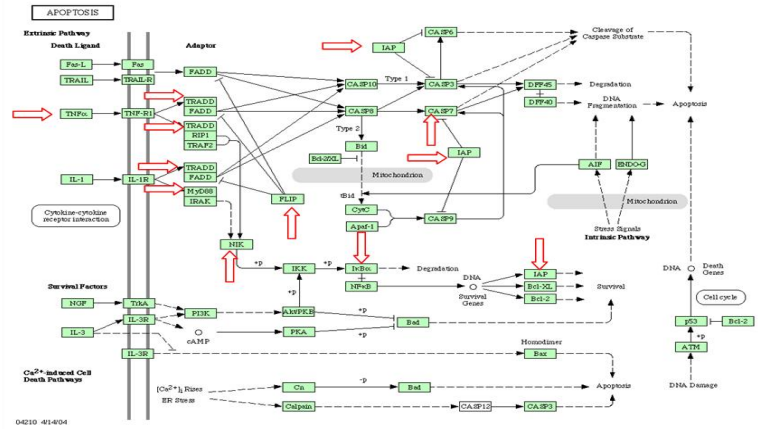
3.1 Antigen processing and presentation



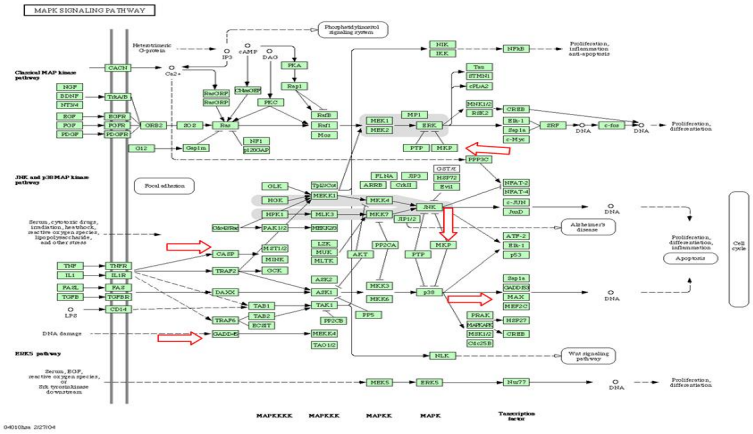
3.2 Toll-like receptor signaling pathway



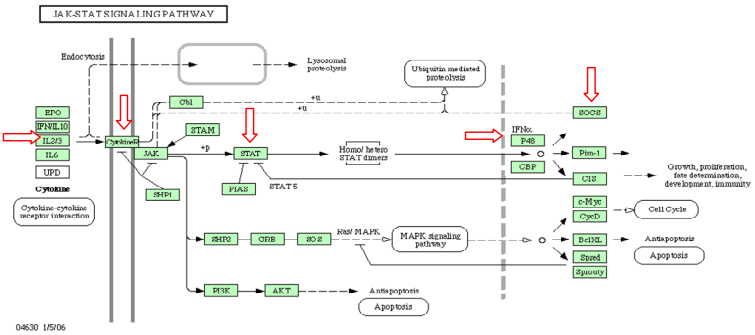
3.3 Apoptosis



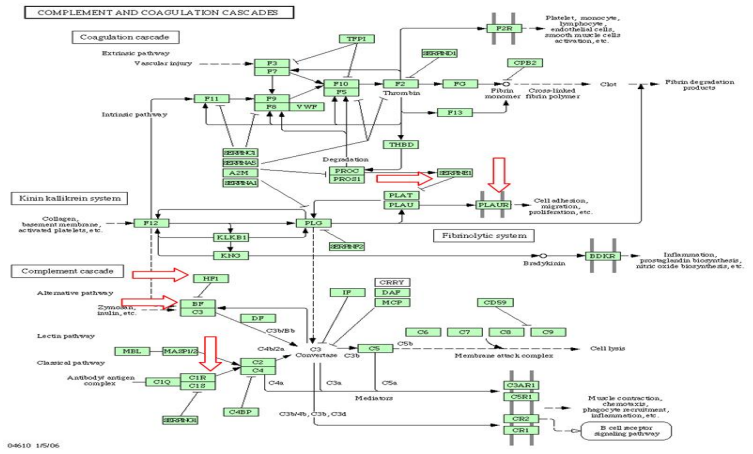
3.4 MAPK signaling pathway



3.5 Jak-STAT signaling pathway



3.6 Complement and coagulation cascades



4 Discussion and conclusion

In this study, we have explored cDNA microarray to analyze the gene profiling of A549 cells infected with influenza A virus. The results include some well-known genes that must be up-regulated such as RIG-1, MDA-5, IRF3 and IFN-related genes, *et al*(data not shown) which are consistent with the previous related studies [9,10,11,12,13,14]. Then we analyzed these genes up-regulated by influenza virus with KEGG pathway database and found some signaling pathways such as MAPK signaling pathway have been investigated deeply, and some drugs such as inhibitor of ERK have been developed for antivirals[15,16,17]. However, present drugs were designed to target only to one or two pathway and can't completely control spread of influenza. So we should design drugs targeting multi-pathways on the system biology view. Our results supply multi-pathways related with the infection and we may develop drugs related to the different targets and develop cocktail drugs for preventing spread of influenza.

In summary, our study provides a platform to understand the infection of influenza and targets for developing anti-influenza drugs.

Acknowledgements

We acknowledge Kanehisa Laboratories for developing KEGG pathway Database. We thank Liang Zhang and Jian-qin Zhao for providing excellent technical support. We thank CapitalBio Corporation for providing microarray services.

References

[1] Cellular transcriptional profiling in influenza A virus-infected lung epithelial cells: the role of the nonstructural NS1 protein in the evasion of the host innate defense and its potential contribution to pandemic influenza. Proc Natl Acad Sci U S A. 2002 Aug 6;99(16): 10736-41.

[2] Influenza A virus NS1 protein prevents activation of NF-kappaB and induction of alpha/beta interferon. J Virol. 2000 Dec;74(24): 11566 -73.

[3] Restraint stress alters lung gene expression in an experimental influenza A viral infection. J Neuroimmunol. 2005 May;162(1-2): 103-11.

[4] Comparative transcriptional profiling of the lung reveals shared and distinct features of Streptococcus pneumoniae and influenza A virus infection. Immunology. 2007 Mar;120(3):380-91.

[5] mRNA expression in mouse hypothalamus and basal forebrain during influenza infection: a novel model for sleep regulation. Physiol Genomics. 2006 Feb 14;24(3):225-34.

[6] The MicroArray Quality Control (MAQC) project shows inter- and intraplatform reproducibility of gene expression measurements. Nat Biotechnol. 2006 Sep;24(9):1151-61.

[7] Performance comparison of one-color and two-color platforms within the microarray quality control (MAQC) project. Nat Biotechnol. 2006 Sep;24(9):1140-50.

[8] Transcriptome profiling, molecular biological, and physiological studies reveal a major role for ethylene in cotton fiber cell elongation. Plant Cell. 2006 Mar;18(3):651-64.

[9] RIG-I-mediated antiviral responses to single-stranded RNA bearing 5'-phosphates. Science. 2006 Nov 10;314(5801):997-1001.

[10] Cutting Edge: Influenza A virus activates TLR3-dependent inflammatory and RIG-I-dependent antiviral responses in human lung epithelial cells. J Immunol. 2007 Mar 15;178(6):3368-72.

[11] Cutting edge: innate immune response triggered by influenza A virus is negatively regulated by SOCS1 and SOCS3 through a RIG-I/IFNAR1-dependent pathway. J Immunol. 2008 Feb 15;180(4): 2034-8.

[12] Retinoic acid-inducible gene I mediates early antiviral response and Toll-like receptor 3 expression in respiratory syncytial virus-infected airway epithelial cells. J Virol. 2007 Feb;81(3): 1401 -11.

[13] Interferon-lambda contributes to innate immunity of mice against influenza A virus but not against hepatotropic viruses. PLoS Pathog. 2008 Sep 12;4(9):e1000151.

[14] Influenza A virus abrogates IFN-gamma response in respiratory epithelial cells by disruption of the Jak/Stat pathway. Eur J Immunol. 2008 Jun;38(6):1559-73.

[15] Higher polymerase activity of a human influenza virus enhances activation of the hemagglutinin-induced Raf/MEK/ERK signal cascade. Virol J. 2007 Dec 5;4:134.

[16] Membrane accumulation of influenza A virus hemagglutinin triggers nuclear export of the viral genome via protein kinase Calpha-mediated activation of ERK signaling. J Biol Chem. 2006 Jun 16;281(24):16707-15.

[17] Influenza virus propagation is impaired by inhibition of the Raf/MEK/ERK signalling cascade. Nat Cell Biol. 2001 Mar;3(3): 301-5.