Gene Expression Profiles Comparison between 2009 Pandemic and Seasonal H1N1 Influenza Viruses in A549 Cells[1]

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Objective To perform gene expression profiles comparison so that to identify and understand the potential differences in pathogenesis between the pandemic and seasonal A (H1N1) influenza viruses. **Methods** A549 cells were infected with A/California/07/09 (H1N1) and A/GuangdongBaoan/51/08 (H1N1) respectively at the same MOI of 2 and collected at 2, 4, 8, and 24 h post infection (p.i.). Gene expression profiles of A549 cells were obtained using the 22 K Human Genome Oligo Array, and differentially expressed genes were analyzed at selected time points. **Results** Microarrays results indicated that both of the viruses suppressed host immune response related pathways including cytokine production while pandemic H1N1 virus displayed weaker suppression of host immune response than seasonal H1N1 virus. Observation on similar anti-apoptotic events such as activation of apoptosis inhibitor and down-regulation of key genes of apoptosis pathways in both infections showed that activities of promoting apoptosis were different in later stage of infection. **Conclusion** The immuno-suppression and anti-apoptosis events of pandemic H1N1 virus were similar to those seen by seasonal H1N1 virus. The pandemic H1N1 virus had an ability to inhibit biological pathways associated with cytokine responses, NK activation and macrophage recognition .

Key words: Influenza A virus; Pandemic H1N1; A549; Expression profiling array

INTRODUCTION

The 2009 pandemic influenza A (H1N1) virus emerged in humans in North America is genetically and antigenically distinct from other influenza viruses circulating in human populations[1]. Due to the lack of pre-existing immunity in the majority of the population，the new virus spread at an unprecedented speed. With the pandemic ongoing, it has been gradually realized that it is not as virulent as the Spanish Flu virus or the H5N1 highly pathogenic avian influenza virus (HPAIV), and the symptom is usually mild and similar with that caused by seasonal influenza viruses[2-3]. Interestingly, unlike seasonal H1N1 influenza viruses, the 2009 pandemic H1N1 influenza virus caused higher mortality and morbidity in the young adults[4-5] suggesting that there may be some pathogenetic differences between these two H1N1 influenza viruses. For the purpose of further understanding the pathogenesis of the 2009 pandemic H1N1 virus, its influence on gene expression of A549 cells using a seasonal H1N1 influenza virus as control was studied and a global view of the gene expression profiles of A549 cells infected with seasonal H1N1 influenza virus and 2009 pandemic (H1N1) influenza virus was provided.

MATERIALS AND METHODS

Viruses

A/California/07/09(H1N1) (CA/07), the repress entative strain of the 2009 pandemic (H1N1) influenza virus, was kindly provided by Centers for Disease Control and Prevention, USA; seasonal influenza virus A/GuangdongBaoan/51/08 (H1N1) (Baoan/51), which is antigenically similar to the seasonal H1N1 influenza vaccine strain A/Brisbane/59/2007 (H1N1) was isolated from Guangdong Provincial CDC and stored in the National Influenza Center. Viruses were propagated in MDCK cells followed the standard procedure, and stored in −80 ℃ freezer.

[1]This work was supported by the National Basic Research Program of China (973 program: 2010CB534001)

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0895-3988/2010
CN 11-2816/Q

Cells

A549 cell line (ATCC, no: CCL-185^{TM}), a carcinomatous human alveolar basal epithelial cell line, was selected to represent human respiratory epithelial cells. Cells were cultured in Dulbecco's Modified Eagle Medium (D-MEM) culture medium containing 10% FBS, 100 U/mL Penicillin, 100 μg/mL Streptomycin, and 25 mmol/L HEPES buffer solution as final concentration. FBS had been inactivated at 56 ℃ for 30 min prior to its addition to D-MEM. Cells were cultured in 37 ℃ incubator set at 5% CO_2.

Virus Infection

Cells were prepared in 6-well plates and inoculated with 2 multiplicity of infection (MOI) of A/GuangdongBaoan/51/2008 (H1N1) virus or A/California/07/2009 (H1N1) virus respectively. The inoculums were removed after 1 hour of virus adsorption by washing the monolayer three times with PBS. The monolayer cells were maintained in virus isolation medium (100 U/mL Penicillin, 100 μg/mL Streptomycin, 25 mmol/L HEPES buffer solution, 2 μg/mL TPCK-trypsin and 0.2% BSA as final concentration) and were incubated at 35 ℃.

Array Hybridization and Data Analysis

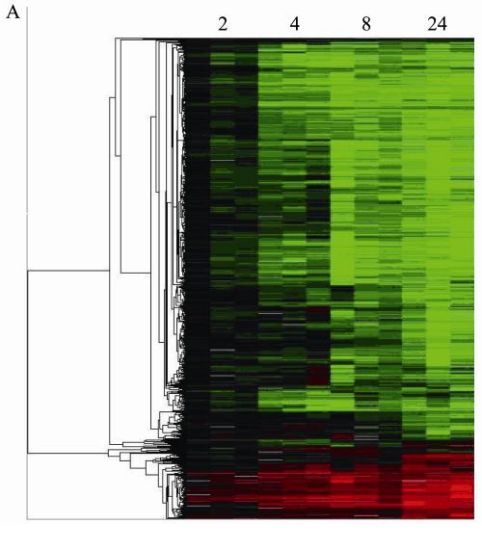
Virus-infected cells and uninfected control cells (mock) were collected at 2, 4, 8, and 24 h p.i. and were lysed with TRIzol Reagent. Total RNAs were extracted, quantified and analyzed for integrity. The array experiments were accomplished by CapitalBio Corporation. RNA samples were reverse-transcribed to synthesize cDNA. As cDNAs were labeled with fluorescent dyes Cy3 and Cy5, the concentration, fluorescent intensity and labeling efficiency of the labeled cDNA sample were determined, and test-reference sample normalization was conducted before applying onto arrays. 22 K Human Genome Oligo Arrays were hybridized according to CapitalBio ś submicro protocols. Array experiments included three biological replicates.

Gene expression was observed and preliminarily analyzed. Clusetr3.0 software was used for cluster analysis; SAM (significance analysis of microarrays) was performed in screening differentially expressed genes. The gene expression of virus-infected cells was first compared with mock, and then differentially expressed genes of CA/07 and Baoan/51 infected cells were screened. The *q*-value (%) ⩽ 5 and ⩾2-fold change was used as the standard when screening differentially expressed genes. Gene ontology and related biological pathway analyses were performed on the differentially expressed genes at different time points with Molecule Annotation System V4.0 supported by CapitalBio®, the KEGG database, and the OMIM database of PUBMED.

RESULTS

Overview of Gene Expression Profiles

In order to identify host cell responses specific to the 2009 pandemic H1N1 influenza virus, A549 cells was infected with either CA/07 or Baoan/51 virus and the global gene expression profiles of A549 cells were compared at 2, 4, 8, and 24 h p.i.. A ±2-fold change in gene expression was used as the cut-off value to identify genes significantly differentially expressed. It was showed that gene expression alterations in A549 were induced by both viruses after infection with the similar tendency, but the gene expression profiles were different during the early infection time points. As shown in Fig. 1, compared with mock-infected cells, the majority of changes in gene expression occurred at 8 h and 24 h p.i. in response to both viruses. Marked alteration in gene expression could be observed in Baoan/51 infection at 2-4 h p.i.(Fig.1 A), more than 800 genes expression level significantly changed (at least 2- folds changing compared with Mock cells). On the contrary, as shown in Fig.1 B, few significant changes were observed in gene expression of A549 cells infected with CA/07 virus (only 50 genes expression level significantly changed) at the early stage of infection. By 24 h p.i., both of the infection groups showed marked activation and repression of gene expression, and the quantity of influenced genes by both viruses reached similar level with almost 2 000 genes expression significantly changed.



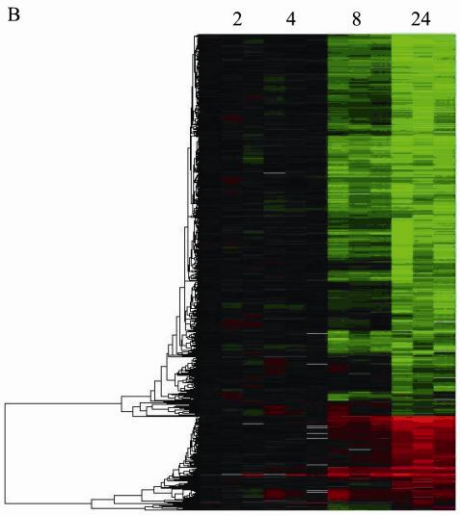


FIG. 1. Overview of the gene expression profiles of A549 cells in response to Baoan/51 and CA/07 influenza viral infection. Genes shown in red are up-regulated, while genes shown in green are down-regulated in virus-infected cells compared to mock-infected A549 cells.

In a preliminary study, we compared gene expression profiles of CA/07 or Baoan/51-infected cells versus mock-infected cells, and most of the genes were significantly down-regulated (fold-change$\geqslant$2) over time after infection. Results of gene ontology and pathway analysis revealed that both virus infections resulted in regulation of MAPK signaling, cell cycle, ubiquitin mediated proteolysis, oxidative phosphorylation, fatty acid metabolism, amino acid metabolism, ErbB signaling, Wnt signaling, B cell receptor signaling, antigen processing, and presentation, T cell receptor signaling, Toll-like receptor signaling, cell adhesion molecules, p53 signaling, Jak-STAT signaling pathway, apoptosis, and other pathways. Collectively, these findings demonstrated that infection with both viruses resulted in the expression of genes associated with virtually the same functions and pathways, and only a few differences were observed.

Analysis of Differentially Expressed Genes

To further inspect the differences observed in the gene expression analysis, the expressed genes in response to CA/07 and Baoan/51 infection was screened differentially at each time point using SAM analysis. To compare the cellular response to pandemic and seasonal H1N1 virus infection, gene ontology and pathway analyses were performed on the genes that displayed more than 2-fold change and q-value$\leqslant$0.05 in CA/07 and Baoan/51 infected A549 cells.

The pathway analysis revealed that significantly differentially expressed genes included members from pathways of metabolism, cell junction, cell cycle and cell surface receptor signal transduction, and mainly enriched in N-glycan biosynthesis, cyclin, adherens junction, tight junction, apoptosis, cell adhesion molecules (CAMs), antigen processing, and presentation, T cell and B cell receptor signaling, Toll-like receptor signaling and other immune and inflammation-related pathways (Fig. 2).

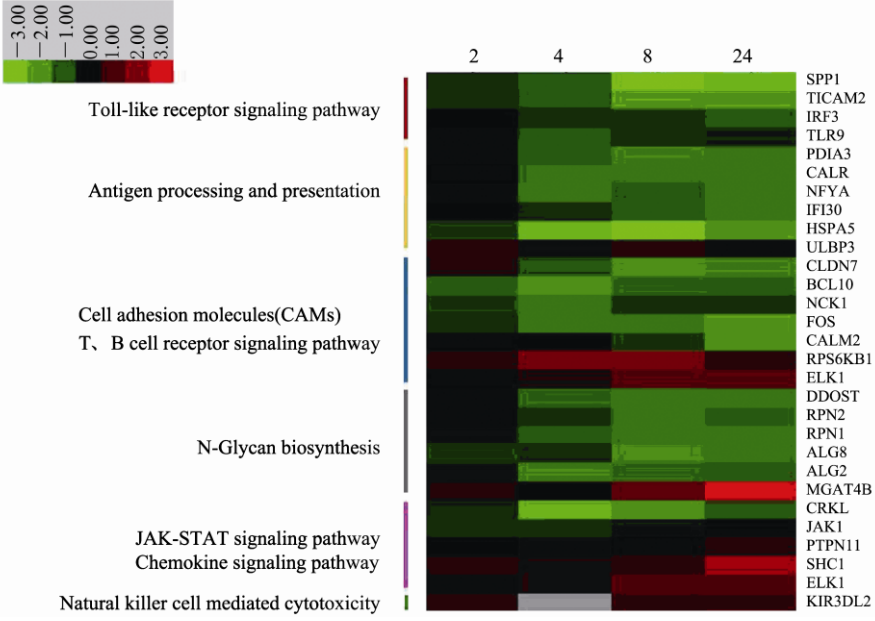


FIG. 2. Host immune response-related genes preferentially regulated during CA/07 and Baoan/51 infections. Fold change (ratio of Baoan:CA) is displayed with brightness of colors. Genes shown in green indicate that the expression level in Baoan/51 infected cells is lower than that in CA/07, while red indicates the expression level in Baoan/51 infected cells which is higher than in CA/07.

Differentially Expressed Genes of Immune-related Pathways

The result from expression microarray analysis showed that the most dramatic differences between CA/07 and Baoan/51 infection were observed at 8 h and 24 h p.i.. In order to compare the prominent functional clusters induced differentially by the two viruses, gene expression at 8 h and 24 h p.i. were analyzed. The expression of these genes in most instances had the same trend of expression. A significant down-regulation of genes associated with immune response pathways was observed in both H1N1 viruses infected cells at 8 h and 24 h p.i. (Table. 1 and Table. 2), and both H1N1 viruses displayed similar immunity suppression in down-regulating related genes of Toll-like receptor signaling pathway, antigen processing and presentation, T/B cell signaling pathway, Jak-STAT signaling pathway, cytokine receptors, and cytokine interaction etc. However, it seemed that the alteration of most immune related genes in CA/07 infection was not as remarkable as that of seasonal influenza Baoan/51.

Most of the genes associated with T/B cell receptor signaling and Toll-like receptor signaling pathway were down-regulated at 8 h and 24 h p.i. in both viruses infected cells, as shown in Table.1 and Table.2. Expression of FOS, BCL10, TICAM2, and MAP2K4 had an obvious down-regulation in response to Baoan/51 infection, however, interestingly, CA/07 induced a slight up-regulation of FOS at 8 h p.i., and the expression down to the level as mock-infected cells by 24 h p.i.. A marked down-regulation in the expression of CALR, PDIA3, HSPA5, and IFI30 genes which were associated with antigen processing and presentation in response to Baoan/51 infection was identified, but not so significant in CA/07 infection. Moreover, activation of NK inhibitory receptor gene KIR3DL2 was observed in both viral infections, but it was showed that the pandemic H1N1 virus had a weaker ability to induce its expression.

In addition, genes that were down-regulated unique to CA/07 virus response were observed. As shown in Fig. 2, the expression of SHC1 was down-regulated significantly in CA/07 infected A549 cells at 24 h p.i.. Furthermore, the down-regulation of MGAT4B gene of the N-glycan biosynthesis pathway at 8 h following CA/07 infection, and by 24 h p.i.was observed and its expression was 7.59-fold lower in the response to pandemic H1N1 virus versus seasonal H1N1 virus.

Differentially Expressed Genes Associated with Anti-apoptotic Activities and Pro-apoptotic Effects

Another cluster of differentially expressed genes which were associated with apoptosis was analyzed and it was found that both of the viral infections displayed obvious anti-apoptotic activities, including activation of anti-apoptotic regulators and regulation of key genes in various apoptosis pathways. Seasonal influenza virus Baoan/51 displayed a stronger anti-apoptotic effect compared to that of CA/07 virus.

The data on gene expression indicated an increased expression of apoptosis inhibitor, such as SFRP5, BCL2L1, and RPS6KB1 at time points throughout Baoan/51 infection, but in CA/07 infected A549, they displayed a slight down-regulation at 8 h p.i., and then an up-regulation at 24 h p.i.. Anti-apoptotic activities of both viruses were also reflected in the inhibition of apoptosis pathway related genes. Significant reduction in the expression of genes associated with apoptosis pathways was observed in Baoan/51 infection, including DDIT3, FOS, CRKL, MYC, MAP2K4, SRF, TNFRSF1A, MAP4K3 of MAPK pathway; CCND1, CCNB1, CCNE1, CDC2, MDM4 of p53 pathway, and PORCN of Wnt pathway. However, unlike seasonal H1N1 virus, the pandemic H1N1 virus displayed weaker inhibition of some of the genes mentioned above.

Pro-apoptotic effects were observed in both H1N1 viral infections at later stage of infection. ELK gene was significantly down regulated at 24 h p.i. in CA/07 infected cells. Simultaneously, inhibition of key genes in cell cycle such as CCNE1, CDC2, and CCNA2 at 8-24 h p.i. was observed in Baoan/51 infected cells.

DISCUSSIONS

A variety of epidemiological and clinical data have indicated that the pandemic H1N1 virus has spread all over the world faster than previous influenza viruses with its efficient transmission, but its symptoms and disease courses are similar to seasonal influenza. Studies demonstrate that no major cytokine storm, as seen in H5N1 infection, is associated with 2009 H1N1 infection of cell lines. Recent evidence suggests that an increased release of cytokines is not the main features of the body response to the 2009 influenza infection and the 2009 H1N1 viruses exhibited mild to moderate virulence in mice[6], which is very different from highly

TABLE 1

Differentially Expressed Genes Associated with Immune Response and Apoptosis at 8 h p.i.

Gene and Pathways	Gene Expression		Fold Change	Description
	Baoan/51-8h	CA/07-8h		
Cell cycle				
BUB1B	0.1153	0.3066	0.3760	Mitotic checkpoint serine/threonine-protein kinase BUB1 beta
ORC6L	0.4411	1.0553	0.4180	Origin recognition complex subunit 6
BUB1	0.3159	0.7304	0.4324	Mitotic checkpoint serine/threonine-protein kinase BUB1
MCM5	0.2806	0.5818	0.4823	DNA replication licensing factor MCM5 (CDC46 homolog)
PCNA	0.1712	0.6670	0.2566	Proliferating cell nuclear antigen (PCNA) (Cyclin)
CCNA2	0.2564	0.5631	0.4553	Cyclin A2 (Cyclin A)
p53 signaling pathway				
CCND1	0.2089	0.6767	0.3086	G1/S-specific cyclin D1 (PRAD1 oncogene) (BCL-1 oncogene)
CCNE1	0.3085	0.7177	0.4299	G1/S-specific cyclin E1
CCNB1	0.1123	0.4066	0.2761	G2/mitotic-specific cyclin B1
CDC2	0.2800	0.7493	0.3737	Cell division control protein 2 homolog (EC 2.7.1.37) (p34 protein kinase)
N-Glycan biosynthesis				
RPN1	0.2950	0.7936	0.3717	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase 67 kDa subunit precursor
DDOST	0.3374	1.0202	0.3307	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase 48 kDa subunit precursor
ALG2	0.2223	0.5437	0.4090	Alpha-1,3-mannosyltransferase ALG2
RPN2	0.3461	0.9249	0.3742	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase 63 kDa subunit precursor
MGAT2	0.2557	0.5885	0.4344	Alpha-1,6-mannosyl-glycoprotein 2-beta-N-acetylglucosaminyltransferase
ALG8	0.2531	0.8407	0.3011	Probable dolichyl pyrophosphate Glc1Man9GlcNAc2 alpha-1,3-glucosyltransferase
MGAT4B	1.6177	0.5784	2.7970	mannosyl (alpha-1,3-)-glycoprotein beta-1,4-N-acetylglucosaminyltransferase
Antigen processing and presentation				
NFYA	0.3464	0.7101	0.4878	CCAAT-binding transcription factor subunit B
IFI30	0.5957	1.3268	0.4490	Gamma-interferon inducible lysosomal thiol reductase precursor
CALR	0.3384	0.8827	0.3834	Calreticulin precursor (CRP55) (Calregulin) (HACBP) (ERp60) (grp60)
HSPA5	0.0904	1.0124	0.0893	78 kDa glucose-regulated protein precursor
PDIA3	0.1961	0.6361	0.3082	Protein disulfide-isomerase A3 precursor
HSPA4	0.2256	0.5546	0.4067	Heat shock 70 kDa protein 4
MAPK signaling pathway				
CRKL	0.1173	0.4250	0.2759	Crk-like protein [Source:Uniprot/SWISSPROT;Acc:P46109]
MYC	0.2046	0.4772	0.4287	Myc proto-oncogene protein (c-myc)
SRF	0.1555	0.6330	0.2456	Serum response factor (SRF)
MAP4K3	0.1124	0.3683	0.3052	Mitogen-activated protein kinase kinase kinase kinase 3
DDIT3	0.2581	1.0264	0.2723	Growth arrest and DNA-damage-inducible protein GADD153
MAP2K4	0.2075	0.4285	0.4843	Dual specificity mitogen-activated protein kinase kinase 4
Toll-like receptor signaling pathway/T B cell receptor signaling pathway				
FOS	0.5699	1.7150	0.3323	Proto-oncogene protein c-fos (cellular oncogene fos) (G0/G1 switch regulatory protein 7)
SPP1	0.3200	2.2385	0.1430	Osteopontin precursor
TICAM2	0.1561	0.9010	0.2463	TIR domain-containing adaptor molecule2
BCL10	0.1385	0.3392	0.4083	B cell lymphoma/leukemia 10 (B-cell CLL/lymphoma 10)
Apoptosis related pathways				
TNFRSF1A	0.1658	0.3788	0.4376	Tumor necrosis factor receptor superfamily member 1A precursor
BCL2L1	1.4820	0.7770	1.8230	Apoptosis regulator Bcl-X (Bcl-2-like 1 protein)
RPS6KB1	2.1770	0.6215	3.5023	Ribosomal protein S6 kinase (EC 2.7.1.37) (S6K) (p70-S6K)
PORCN	0.3127	0.8289	0.3772	porcupine isoform A, 2410004O13Rik [Homo sapiens]
ELK	1.1101	0.3461	3.2077	ETS-domain protein Elk-1

TABLE 2

Differentially Expressed Genes Associated with Immune Response and Apoptosis at 24 h p.i.

Gene and Pathways	Gene Expression		Fold Change	Description
	Baoan/51-24h	CA/07-24h		
N-Glycan biosynthesis				
MGAT4B	1.0727	0.1412	7.5986	mannosyl (alpha-1,3-)-glycoprotein beta-1,4-N-acetylglucosaminyltransferase, isoenzyme B
DDOST	0.1951	0.5412	0.3605	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase 48 kDa subunit precursor
RPN2	0.2316	0.5606	0.4131	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase 63 kDa subunit precursor
ALG8	0.1579	0.4131	0.3821	Probable dolichyl pyrophosphate Glc1Man9GlcNAc2 alpha-1,3- glucosyltransferase
Antigen processing and presentation				
IFI30	0.3214	0.9227	0.3484	Gamma-interferon inducible lysosomal thiol reductase precursor
CALR	0.3845	1.1112	0.3461	Calreticulin precursor (CRP55) (Calregulin) (HACBP) (ERp60) (grp60)
HSPA5	0.0951	0.3610	0.2633	78 kDa glucose-regulated protein precursor
PDIA3	0.1462	0.4457	0.3280	Protein disulfide-isomerase A3 precursor
Cell cycle				
FOS	0.2426	1.0057	0.2412	Proto-oncogene protein c-fos
IRF3	0.2494	0.5179	0.4815	Interferon regulatory factor 3
TICAM2	0.2428	0.9768	0.2485	TIR domain-containing adaptor molecule2
PCNA	0.0615	0.2297	0.2677	Proliferating cell nuclear antigen (PCNA) (Cyclin)
CCNA2	0.0564	0.3757	0.1500	Cyclin A2
p53 signaling pathway				
CCNB1	0.0841	0.1773	0.4743	G2/mitotic-specific cyclin B1
MDM4	0.2933	0.8440	0.3791	Mdm4 protein (p53-binding protein Mdm4)
CDC2	0.1011	0.6339	0.1595	Cell division control protein 2 homolog
CALM2	0.1226	0.4950	0.2799	calmodulin 2, phosphorylase kinase delta
Toll-like receptor signaling pathway/T B cell receptor signaling pathway				
FOS	0.2426	1.0057	0.2412	Proto-oncogene protein c-fos
SPP1	0.1129	0.6599	0.1711	Osteopontin precursor
MAPK signaling pathway				
ELK1	0.9737	0.3777	2.4321	ETS-domain protein Elk-1
DDIT3	0.1377	0.5029	0.2738	Growth arrest and DNA-damage-inducible protein GADD153
Other immune or apoptosis-related pathways				
CLDN7	0.1031	0.3289	0.3136	Claudin-7
PPP2CA	0.1931	0.4687	0.4120	Serine/threonine protein phosphatase 2A, catalytic subunit, alpha isoform
SHC1	1.0020	0.2209	4.5359	SHC transforming protein 1

pathogenic avian influenza H5N1 and 1918 H1N1 virus. It was shown previously in animal experiments that the 2009 H1N1 influenza virus caused release of some cytokines such as IL-10, IFN-γ, IL-4, IL-12, and MCP-1 in mouse lungs[7]; Osterlund *et al* studied infected human macrophages and dendritic cells with the 2009 H1N1 virus and found a slight increase in cytokines[8] which was however negligible as compared to the 1918 H1N1 influenza virus and highly pathogenic H5N1 avian influenza[9-11]. Dysregulation of inflammatory cytokines and enhancement of apoptosis caused by the 1918 H1N1

virus and H5N1 avian influenza virus have already been reported and it was found that they inhibit cell proliferation-related gene expression, but activate genes expression of IFN signaling pathway[12-13]; in macrophages, the high expression of the IRF1 activates JAK-STAT signaling pathway and produces large amounts of IFN-β and other inflammatory factors causing a strong inflammatory dysregulation and induction of apoptosis[14-15]. So far, it has been assumed that increased production of pro-inflammatory cytokines may be a key factor contributing to immune injury in virulent influenza viral infections[16-17].

In the present study, it was found that the pandemic H1N1 virus inhibit pathways related to immune response including SHC1 related chemokine signaling, JAK-STAT signaling, antigen processing, innate immune cells activation, and cytokine production. The suppression of SHC1 gene and JAK-STAT signaling in response to CA/07 infection may contribute to the immunosuppression effect. Protein encoded by SHC1 can be bound to various receptorssuch as growth factor receptor, cytokine receptors, G protein receptorand involved in a variety of signal transduction including MAPK activation, cell proliferation, apoptosis, oxidative stress, IL-2 IL-6 pathway and chemokine signaling pathway[18]. JAK-STAT is considered as the main signaling cascade approach of interferon signaling pathway. Studies have demonstrated that lacking of JAK1 leads to completely defective interferon response while complementation of this mutant restores the response[19-20].

Our data have indicated that the pandemic H1N1 virus partially evades the host innate immune response by interfering with the cytokine production pathways in A549 cells, however, the immunosuppression is not as significant as the seasonal H1N1 virus, and some of the cytokines production related genes, such as FOS gene are slightly up-regulated, which may contribute to the weak cytokine release in host infection.

The significant down-regulation of MGAT4B gene in CA/07 infection may have important practical significance. This gene encodes Alpha-1, 3-mannosyl-glycoprotein 4-beta-N-acetylglucosamin yltransferase B, a key glycosyltransferase in N-glycan biosynthesis that catalyzes the transfer of N-acetylglucosamine to mannose residues (Fig. 3). Polysaccharides frequently found on the surface of viruses come from the host cell, and the mannose residues structure of glycan can be recognized by MR of macrophages, which play a role in phagocytosis[21]. CA/07's suppression of glycosyltransferase gene MGAT4B may result in inhibition of N-glycan biosynthesis and further reduction of mannose residues; the reduction of ligand of pattern-recognition receptors may reduce macrophage recognition and contribute to immune evasion. In addition, complement system can be initiated through MB-lectin pathway by binding of the serum protein MBL (mannose binding lectin) to mannose-containing carbohydrates on viruses. The suppression of MGAT4B gene is also impeding the activation of the complement system which is also an important part of innate immune response. In vitro and in vivo experiments are needed to verify the possible way of immune evasion inferred from the gene expression data.

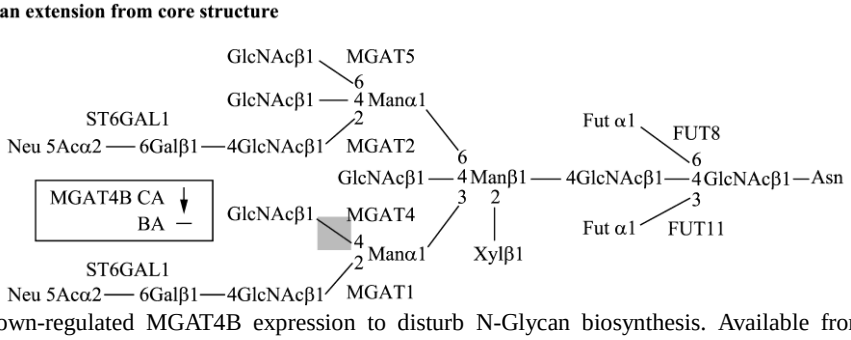


FIG. 3. CA/07 down-regulated MGAT4B expression to disturb N-Glycan biosynthesis. Available from Molecule Annotation System V4.0 and KEGG database.

Anti-apoptotic events are similar in both H1N1 viral infections. Highly expression of apoptosis inhibitor might result in anti-apoptotic effects. Previous studies have revealed that SFRP5 could block Wnt signaling pathway in start-up phase [22-23]; the proteins encoded by BCL2L1 gene are located at the outer mitochondrial membrane, and are shown to regulate outer mitochondrial membrane channel (VDAC) opening. VDAC regulates mitochondrial membrane potential, and thus controls the production of reactive oxygen species and release of cytochrome C by mitochondria, both of which are the potent inducers of cell apoptosis. Moreover, RPS6KB1 encoded protein P70-S6 is a protein kinase that can lead to an increase in protein synthesis and cell proliferation. On the other hand, the down-regulation of key genes of the apoptosis pathways, for example, TNFRSF1A, also plays an important role in

anti-apoptotic effect. TNF-α receptor can activate the transcription factor NF-kB and mediate apoptosis, and the down regulation of TNFRSF1A which encoding TNF-α receptor, thereby inhibits apoptosis.

In addition to viral anti-apoptosis effect, events of apoptosis are also underway. Inhibition of ELK in response to CA/07 infection may have an effect on apoptosis, as ELK involved in MAPK and other pathways, and have shown the function to promote cell growth and proliferation. Furthermore, down-regulation of anti-apoptosis genes BCL2L1 and RPS6KB1 at 8 h p.i. may also contribute to the apoptosis events. Unlike CA/07, in Baoan/51 infection the inhibited expression of cyclins and protein kinase encoded by CCNE1, CCNA2, and CDC2 may result in the cell cycle arrest in G1/S or G2/M phase and lead to cell death through p53 signaling pathway[24-25]. These differences observed in pro-apoptotic events suggest that the viruses may have different ways of promoting apoptosis.

Taken together, the gene expression characteristics of A549 cells induced by the pandemic H1N1 virus are similar to those induced by the seasonal H1N1 virus. Being consistent with the previous studies, our results have revealed that in spite of the large genetic and antigenic distance of the pandemic H1N1 virus from the seasonal H1N1 virus, the immunosuppression and anti-apoptosis events of the pandemic virus were still similar to those of the seasonal H1N1 virus. Since the pandemic H1N1 virus has shown immune evasion and inhibition of innate immune response while infecting A549 cells, additional work is needed to verify the effect of the observed phenomenon.

ACKNOWLEDGEMENTS

This work was granted by the National Basic Research Program of China (973 program: 2010CB534001). We thank Liang ZHANG, Hong-Lin ZHU, Xiao-Yu ZHANG, Jian-Qing ZHAO, and Guang-Wei FAN, CapitalBio Corporation for their assistance and support.

REFERENCES

1. Garten R J, Davis C T, Russell C A, *et al*. (2009). Antigenic and genetic characteristics of swine-origin 2009 A(H1N1) influenza viruses circulating in humans. *Science* **325**, 197-201.
2. Davies A R, Webb S A, Seppelt I M, *et al*. (2010). Comparison of adult patients hospitalized with pandemic (H1N1)2009 influenza and seasonal influenza during the "PROTECT" phase of the pandemic response. *Med J Aust* **192**, 356-358.
3. Cunha B A (2010). Swine influenza (H1N1) pneumonia: clinical considerations. *Infect Dis Clin North Am* **24**(1), 203-228.
4. WHO (2009). Human infection with new influenza A (H1N1) virus: clinical observations from Mexico and other affected countries, May 2009. *Wkly Epidemiol Rec* **84**(21), 185-189.
5. 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**(25), 2605-2615.
6. Belser J A, Wadford D A, Pappas C, *et al*. (2010). Pathogenesis of pandemic Influenza A (H1N1) and triple-reassortant swine influenza A(H1) viruses in mice. *J Virol* **84**, 4194-4203.
7. Itoh Y, Shinya K, Kiso M, *et al*. (2009). In vitro and in vivo characterization of new swine-origin H1N1 influenza viruses. *Nature* **460**, 1021-1025.
8. Osterlund P, Pirhonen J, Ikonen N, *et al*. (2010). Pandemic H1N1 2009 influenza A virus induces weak cytokine response in human macrophages and dendritic cells and is highly sensitive to antiviral actions of interferons. *J Virol* **84**, 1414-1422.
9. Kash J C, Tumpey T M, Proll S C, *et al*. (2006). Genomic analysis of increased host immune and cell death responses induced by 1918 influenza virus. *Nature* **443**, 578-581.
10. Perrone L A, Plowden J K, Garc á-Sastre A, *et al*. (2008). H5N1 and 1918 pandemic influenza virus infection results in early and excessive infiltration of macrophages and neutrophils in the lungs of mice. *PLoS Pathog* **4,** e1000115.
11. Korteweg C, Gu J (2008). Pathology, molecular biology, and pathogenesis of avian influenza A (H5N1) infection in humans. *Am J Pathol* **172**, 1155-1170
12. Cameron C M, Cameron M J, Bermejo-Martin J F, *et al*.(2008). Gene expression analysis of host Innate immune responses during lethal H5N1 infection in ferrets. *J Virol* **82**, 11308-11317
13. Li Y, Chan EY, Li J, *et al*.(2010). MicroRNA expression and virulence in pandemic influenza virus infected mice. *J Virol* **84**, 3023-3032.
14. Lee S M, Gardy J L, Cheung C Y, *et al*. (2009). Systems-Level comparison of host-responses elicited by avian H5N1 and seasonal H1N1 influenza viruses in primary human macrophages. *PLoS One* **4**, e8072.
15. Zhang C, Yang Y, Zhou X, *et al*. (2010). Highly pathogenic avian influenza A virus H5N1 NS1 protein induces caspase-dependent apoptosis in human alveolar basal epithelial cells. *Virol J* (In Press)
16. Chan M C, Cheung C Y, Chui W H, *et al*. (2005). Proinflammatory cytokine responses induced by influenza A (H5N1) viruses in primary human alveolar and bronchial epithelial cells. *Respir Res* **6**, 135.
17. Maines T R, Szretter K J, Perrone L, *et al*. (2008). Pathogenesis of emerging avian influenza viruses in mammals and the host innate immune response. *Immunol Rev* **225**, 68-84.
18. Ravichandran K S (2001). Signaling via Shc family adapter proteins. *Oncogene* **20**, 6322-6330.
19. Schindler C, Levy D E, Decker T (2007). JAK-STAT signaling: from interferons to cytokines. *J Biol Chem*. **282**, 20059-20063.
20. Muller M, Briscoe J, Laxton C, *et al*. (1993). The protein tyrosine kinase JAK1 complements defects in interferon-alpha/beta and-gamma signal transduction. *Nature* **366**, 129-135.
21. Gazi U, Martinez-Pomares L (2009). Influence of the mannose receptor in host immune responses. *Immunobiology* **214**, 554-561.
22. Database online. Available from: http://www.genome.jp/kegg-bin/show pathway? map04310.
23. Kawano Y, Kypta R (2003). Secreted antagonists of the Wnt signaling pathway. *J Cell Sci* **116**, 2627-2634.
24. B åint E E, Vousden K H (2001). Activation and activities of the p53 tumor suppressor protein. *Br J Cancer* **85**, 1813-1823.
25. Pietenpol J A, Stewart Z A (2002). Cell cycle checkpoint signaling: cell cycle arrest versus apoptosis. *Toxicolog* **181-182**, 475-481.

(*Received June* 1, 2010 *Accepted August* 12, 2010)