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Influenza virus and cell signaling pathways

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Summary

Influenza viruses comprise a major class of human respiratory pathogens, responsible for causing morbidity and mortality worldwide. Influenza A virus, due to its segmented RNA genome, is highly subject to mutation, resulting in rapid formation of variants. During influenza infection, viral proteins interact with host proteins and exploit a variety of cellular pathways for their own benefit. Influenza virus inhibits the synthesis of these cellular proteins and facilitates expression of its own proteins for viral transcription and replication. Infected cell pathways are hijacked by an array of intracellular signaling cascades such as NF-κB signaling, PI3K/Akt pathway, MAPK pathway, PKC/PKR signaling and TLR/RIG-I signaling cascades. This review presents a research update on the subject and discusses the impact of influenza viral infection on these cell signaling pathways.

key words: **influenza virus • cellular signaling (NF-κB, PI3K/Akt, MAPK, PKC/PKR, TLR/RIG-I signaling)**

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Background

Infectious diseases such as AIDS, tuberculosis, malaria, meningitis and influenza remain the most important cause of death and disabilities in human populations worldwide. Influenza virus causes epidemics almost every year and occasionally pandemics, due to antigenic drift or antigenic shift [1]. Each year Influenza virus causes 65 million illnesses and 25,000 deaths in the USA alone. Globally, the World Health Organization (WHO) estimates the burden of influenza at ~3–5 million cases of severe illness and >300,000 deaths annually. Most recently the WHO declared a phase 6 global pandemic on 11th June 2009, and more than 209 countries and overseas communities have reported laboratory confirmed cases of pandemic influenza H1N1 2009, including at least 14,142 deaths [2]. Influenza virus belongs to the Orthomyxoviridae family. Orthomyxoviridae are enveloped viruses with negative sense ssRNA genome, split into 8 segments, encoding 11 proteins. Hemagglutinin (HA) and neuraminidase (NA) are 2 important proteins present on the surface of the viral envelope; mutation in these 2 proteins gives rise to the 16 HA and 9 NA, different subtypes of influenza virus [3]. The viral RNA polymerase complex, composed of the 3 largest gene segments – PB1, PB2, PA and nucleoprotein (NP) – is associated with viral RNA ribonucleoprotein complex. The 2 matrix proteins, M1 and M2, are the smallest RNA proteins and M2 forms ion channels in the viral membrane. The nonstructural gene (NS1) is a multifunctional protein, and NS2 protein is involved in the nuclear export of the viral RNPs to the cytoplasm. PB1-F2 protein is a product of the second gene product of PB1. To date it has been documented that Influenza viral proteins interact with 1023 host proteins [4]. Like other viruses, Influenza virus also takes advantage of the host cellular machinery for their efficient replication.

According to recent research reports, the following cellular signaling pathways are altered following influenza infection: NF-κB signaling, PI3K/Akt pathway, MAPK pathway, PKC/PKR signaling, and TLR/RIG-I signaling cascades [5–13]. These pathways are important for viral entry, viral replication, viral propagation and apoptosis, and are involved in antagonizing the host antiviral response. Influenza virus manipulates the molecular function of signaling molecules for efficient viral pathogenesis. This review addresses the impact of influenza infection on cellular signaling events.

NFκB/IκB Pathway

The NFκB/IκB pathway plays a vital role in mediating inflammation, immune response, proliferation and apoptosis [14,15]. NF-κB is a nuclear factor κB, a transcriptional factor which plays a central role in promoting the expression of more than 150 genes, which in turn governs the cellular status of genes encoding cytokine/chemokines, adhesion molecules and anti/pro-apoptotic genes [6,16]. NF-κB is present as a complex with its inhibitor IκB. For release from this complex, activation of IKK is required [17]. The IKK complex is composed of active IKK1/IKKα, IKK2/IKKβ and scaffold protein NEMO (NF-κB essential modulator)/IKKγ [15]. Upon activation, IKK phosphorylates and degrades the IκB protein [18], which leads to the release of transcriptionally active NF-κB subunits p65/p50 from the inhibitory complex which translocate into the nucleus, where they can 'turn on' the expression of specific genes that have DNA-binding sites for NF-κB in their promoters [18,19] (Figure 1(2)). IKK2 degrades the IκB and activates NF-κB, while IKK1 primarily phosphorylates the other factors of the NF-κB family, such as p100/p52 [15].

It is well established that NF-κB is a major target of most viral pathogens [20,21]. During viral infection there is activation of the NF-κB signaling pathway and an increase in the gene expression levels of IFN-β/TNFα/IL8 [22,23], which suggests that IKK-mediated NF-κB signaling is essential for the host innate immune response [24]. For instance, vaccinia virus expresses the TIR domain to suppress the TLR/IL-1 receptor- induced NF-κB activation [25,26]. Oncogenic viruses such as Epstein Barr virus (EBV) and Kaposi's sarcoma-associated herpes virus (KSHV), activate NF-κB during

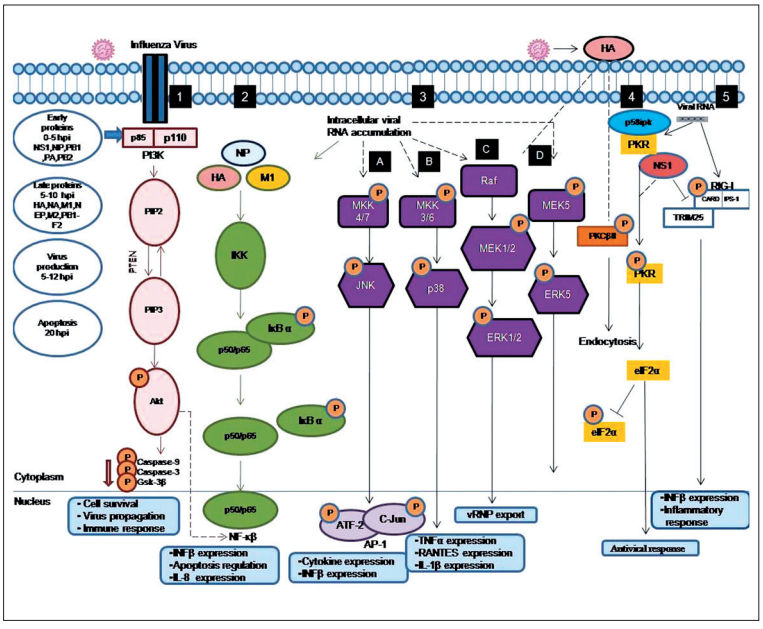

Figure 1. Host cell machinery gets activated during influenza infection and leads to trigger Signaling pathways. Influenza is know to produce early and late viral proteins post infections. NS1, NP, PB1, PB2 are early protein and synthesize in 0-5 h.p.i. HA, NA, M1, NEP, M2, PB1-F2 are late proteins and synthesize in 5-10 h.p.i. This process leads to the virus production in between 5-12 h.p.i. and followed apoptosis of the cells in 20 h.p.i. During this whole processes some of the pathways are know to induce: 1, PI3-Akt pathway; 2, NFkB/IkB pathway; 3, MAPK pathway (A, JNK pathway; B, p38 pathway; C, ERK1/2 patyhway; D, ERK5 pathway); 4, protein kinase C (PKC)/PKR signaling; 5, TLR/RIG-I signaling.
Symbols used in the Figure: ⊣ inhibition; ··› unknown mechanism; ⟶ direct activation; ⇆ reversible process.

latent infection, which subsequently causes tumorogenesis [27]. Thus, viruses have evolved mechanisms to manipulate and thereby evade/modulate host immune responses [28].

Several lines of evidence suggest that activation of the NF-κB pathway is a primary requirement for influenza virus infection and its efficient replication [6,29–31]. NF-κB activation is biphasic after the influenza infection. Early activation of NF-κB has been observed 1 hour post-infection and the later activation was associated with viral replication [32]. Some studies also indicate that viral protein overexpression, specifically HA, NP and M1 (Figure 1(2)), may be responsible for activation of the NF-κB pathway [5,20,29,31,33]. These viral proteins are sufficient to transcriptionally activate NF-κB by involving the generation of oxidative radicals which activates IKK as a signal transduction intermediate, a kinase which phosphorylate IκB, thereby regulating NF-κB activity [5]. As a result of activation of this pathway, the host immune response is elicited, to combat which, non-structural protein NS1 of influenza virus comes to its rescue by serving as an antagonist of host IFN response [34].

PI3K – Akt Pathway

Influenza A virus infection also activates the PI3K/Akt pathway. It has been recently reported that the PI3K/Akt signaling pathway is induced by the viral NS1 protein to support its efficient replication [7,35,36].

PI3K is a family of enzymes that play a pivotal role in regulation of essential cellular functions (cell survival, proliferation, differentiation, etc.) [37,38]. PI3K family is divided into 3 classes: class I, II and III. Class I PI3K are heterodimeric enzymes composed of a catalytic subunit, p110, and a regulatory subunit, p85 [39,40]. They catalyze the generation of phosphatidylinositol-3-phosphate (PIP), phosphatidylinositol-3,4-bisphosphate (PIP2) and phosphatidylinositol-3,4,5-triphosphate (PIP3) [8]. The regulatory subunit of PI3K p85 contains 2 Src homology domains, SH2 and SH3. Through its SH2 domain, the p85 subunit binds to autophosphorylated receptor tyrosine kinase, in the process activating PI3K. Following activation, the p110 subunit converts PIP2 to PIP3 [41], which in turn leads to phosphorylation and activation of a number of kinases, including Akt/PKB. Activation of Akt through phosphorylation at Thr 308 and Ser 473 residues [42] plays a major role in modulating diverse downstream signaling pathways, including cell survival, proliferation, migration, differentiation and inhibition of proapoptotic factors such as BAD and caspase-9 by their phosphorylation (Figure 1(1)).

The class II PI3K is composed of 3 catalytic subunits (C2α, C2β, and C2γ) but no regulatory subunit. This is involved in the production of PIP and PIP2 from PI. Class III consists of catalytic (Vps34) and regulatory (Vps15/p150) subunits that catalyze the generation of PIP from PI.

Many viruses have been reported to induce the PI3K pathway, such as DNA tumor viruses and RNA viruses, promote cell survival or suppress cell death. For example, LMP1 protein of Epstein-Barr virus induces B-cell survival. Furthermore, E5 protein of papillomavirus and HBx protein of Hepatitis B virus have been reported to induce PI3K signaling [43]. Hepatitis C virus, an RNA virus, can cause chronic infection [39]. The NS5A protein of HCV directly binds to the SH3 domain of p85 and induces PI3K/Akt-mTOR signaling to control cell survival [44]. Additionally, viruses which cause acute infection such as respiratory syncytial virus (RSV) manipulate PI3K activity for efficient viral replication.

Other viruses such as SARS coronavirus, poliovirus, dengue virus and influenza virus also utilize the PI3K signaling pathway to their advantage [8,45–47]. Influenza A virus has devised diverse mechanisms to activate PI3K, which in turn leads to activation of several other downstream kinases at different time points during viral replication. Though early activation of PI3K has also been reported in influenza B virus infection, late activation only occurs in type A virus [48]. During the later stages of replication, the NS1 protein binds to the SH2 domain of the p85 subunit and activates PI3K, which leads to suppression of cell death [37,38]. A recently proposed model for activation of PI3K by NS1 suggests formation of an active heterotrimeric complex of p110/p85-NS1 wherein NS1 disrupts the inhibitory interaction interface between p110 and p85 [49,50]. The activated PI3K/Akt pathway is further involved in efficient virus replication and propagation [35,36] and may also regulate antiviral function, although these processes remain largely unclear.

MAPK Pathway

Mitogen activated protein kinase (MAPK) cascades are involved in the conversion of various extracellular signals into cellular responses as diverse as proliferation, differentiation, immune response and cell death [51,52].

Four distinct subgroups of the MAPK family have been well studied [9,51]: (a) Extracellular signal-regulated kinases (ERKs), (Figure 1(3 C)), (b) The p38 MAPK, (Figure 1(3 B)) (c) c-jun N-terminal or stress-activated protein kinases (JNK/SAPK) (Figure 1(3 A)), and (d) ERK5/Big MAP kinase 1(BMK1) (20) (Figure 1(3 D)).

In mammals 3 major pathways have been identified: MAPK/ERK, SAPK/JNK and p38 MAPK. MAPK signaling promotes cell survival by a dual phosphorylation event on threonine and tyrosine residues [10]. The upstream MAPKK regulates these 4 enzyme activities. The 2 MAPKK (MKK3/6, MKK4/7) are responsible for activation of p38 and JNK, respectively. These enzymes are involved in apoptosis and cytokine expression [53], and can be activated by environmental stress conditions. The upstream Raf controls the phosphorylation of MAPK/ERK kinase (MEK) ½, which regulates the activation of ERK ½, which plays a regulatory role in cell proliferation and differentiation. Lastly, but importantly, enzyme ERK5 is activated by MEK5 [10] (Figure 1(3 D)).

Viruses such as dengue, HIV and hepatitis activate RANTES expression, indicating a wide range of control at the transcription level. In the case of HIV infection, RANTES gets upregulated by ERK activation. In the case of dengue virus, RANTES gets induced by bonding of NF-IL-6 [54]. Borna disease virus (BDV) and Visna virus replication are also decreased by MEK inhibition [55,56]. The E2 envelope protein of hepatitis C virus up regulates the p38 MAPK signaling.

Interestingly, influenza virus infection also activates the all 4 members of the MAPK family [9,54], which has reportedly

been shown to promote vRNP (viral ribonucleoprotein capsids) traffic and virus production. In the previous study, using specific kinase inhibitors, p38 and JNK have been linked to virus-induced expression of RANTES (a chemokine involved in the attraction of eosinophils during an inflammatory response) [54].Another study suggests that ERK and JNK are involved in the expression of inflammatory mediator cytooxygenase and phosphorylation of cytosolic phospholipase A2 in bronchial epithelial cells [57]. It has been shown that there is activation of the activator protein-1 (AP-1) during early stages of infection. JNK activation is induced by accumulation of RNA produced by viral polymerase. AP-1 is a transcription factor that includes c-Jun and activating-transcription-factor-2 (ATF-2), whose transcriptional activity is enhanced by JNKs [53,58], a part of the MAPK signalling pathway [23]. AP-1 is also crucial for the expression of interferon-β (IFN-β) and antiviral cytokines [59]. Furthermore, inhibition of JNK by dominant negative mutants of MKK7/JNK/c-Jun results in impaired transcription from IFN-β promoter during influenza virus infection, thereby increasing virus production [53]. Thus, this pathway is important as a mediator of inflammatory response to an influenza infection by co-regulating IFN-β expression (Figure 1(3 A)).

P38 MAPK activation regulates the expression of RANTES production [47,60] and chemokines by influenza virus infection [54]. In highly pathogenic H5N1-infected cells, p38 induces tumor necrosis factor (TNF) cytokine [47]. According to a recently published report, IL-1β stimulates activation of p38 MAPK with prostaglandin E2 production. The p38 inhibitor decreases the release of prostaglandin E2 [57] and also reduces the virus titer, which suggests that p38 MAPK activation is essential for inflammatory responses and contributes to the viral replication process. Influenza virus infection induces TNF-α in a p38-dependent manner [47] (Figure 1(3 B)).

Raf/MEK/ERK pathway is the best proven MAPK signaling pathway [51,61]. Many RNA viruses induce cellular signaling through MAPK cascades. The mechanism of this pathway is initiated by G protein-coupled receptors, which leads to the phosphorylation of downstream molecules and activates the serine threonine kinase Raf (dual specificity kinase MEK and MAPK/ERK). ERK phosphorylates various substrates, transforms the signals and followes different functions in cells [62]. Influenza infection also upregulates this signaling, which is important for efficient export of nuclear RNPs. The inhibition of MEK blocks this pathway [9], shown to impair the growth of viruses and decrease the nuclear RNP export [9]. Interestingly, it does not affect viral RNA or protein synthesis. This suggests that the nuclear RNP export appears in the inducible phase and correlates well with the hypothesis that ERK activation occurs in the late phase of infection. The ERK ½ are vital for the expression of pro-inflammatory IL1β, IL-6 and IL-8 [63]. ERK can regulate the expression of TNF-α, IL-12, IL-1β, and inhibition of ERK by U0126 inhibitor [64] can also reduce the rate of influenza replication by nuclear retention of vRNPs (Figure 1(3 C)).

A recent study suggests that Raf/MEK/ERK signaling is activated by proper accumulation of HA/lipid-raft association within the cellular membrane [65]. If there is inadequate transport of HA from cytoplasm to cell surface, this could be a possible reason for the low activation of ERK [65]. The viral polymerase complex is responsible for HA accumulation in connection with MAPK signaling, supporting the idea that more ERK activation follows more efficient nuclear RNP export and increased formation of infectious progeny virions [4].

Protein Kinase C (PKC)/*PKR* Signaling

Influenza viral infection induces antiviral responses in the host cell [66], which include increase in the levels of interferons, primarily of 3 types: IFN α, IFN β and IFN γ [67]. IFN activates a number of cellular genes, one of the most prominent being *PKR* encoding double-stranded RNA activated protein kinase [68]. Following interaction with dsRNA [69], *PKR* gets activated and undergoes autophosphorylation. This activated form of *PKR* phosphorylates the alpha subunit of eukaryotic initiation factor 2 (eIF2α), which in turn leads to translational arrest. Indeed, reports have suggested a critical role for *PKR* in mediating ds-RNA-induced apoptosis in cells [70].

Therefore, in order to counteract the effects of host IFN response and *PKR* activation, viruses have developed multiple mechanisms to suppress *PKR* activation [71]. Several lines of evidence support the fact that viral genes (vaccinia virus, adenovirus and hepatitis C virus) encode proteins that inhibit the IFN pathway by targeting *PKR* [72,73]. For instance, non-structural 5A protein of hepatitis C virus (HCV) causes repression of *PKR* activation, eventually leading to suppression of host IFN response. During influenza infection, *PKR* activation is inhibited by 2 processes: (1) IAV facilitates binding of p58IPK to *PKR*, causing inhibition of kinase activity [11,74]; And (2) non-structural 1 protein (NS1) of influenza virus blocks activation of *PKR*. Studies carried out *in vitro* using reticulocyte lysates have suggested that NS1 binds to dsRNA causing inhibition of *PKR* activity and phosphorylation of eIF2α, thus inhibiting PKR-induced translational arrest [75] (Figure 1(4)). However, direct interaction of *PKR* and NS1 has not yet been described.

The protein kinase C (PKC) is an upstream molecule of Raf, which transmits signals to the downstream molecules for the activation of the Raf/MEK/ERK pathway [65]. The PKC superfamily consists of 12 different isoforms which plays various roles in cells by activating several downstream signaling pathways. PKC is known to play a role in virus entry of enveloped viruses [76]. The viral HA acts as a signaling activator, both inside the cells and at the cell surface. Binding of influenza virus HA protein to host cell surface receptor activates PKC [12,77,78], and overexpression of HA inside the cells induces ERK signaling. Use of a PKC inhibitor, bisindolylmalimide I, demonstrated the inhibition of influenza virus entry, which shows that PKC plays a crucial role in influenza virus entry. It is likely that the PKCβII (PKC isoform) acts in this function. Furthermore, there is evidence suggesting that PKC phosphorylates the viral M1 protein and helps in viral replication [20]. The mechanism of this process remains unknown.

TLR/RIG-I Signaling

Viral infection elicits antiviral response via activation of a variety of pattern recognition receptors (PRRs) such as

toll-like-receptors (TLR) and RIG-I like receptors (RLRs) [17,79]. While ssRNA viruses are known to recognize by Toll-like receptor (TLR) 7/8 [80], dsRNA viruses recognize TLR3 and retinoic-acid-inducible protein (RIG-I), and a cytoplasmic RNA helicase plays a crucial role in detecting ssRNA during influenza A virus infection [81]. RIG-I can also recognize dsRNA generated during viral replication. During viral infection, RIG-I and MDA5 play an essential role in initiating antiviral response [82]. RIG-I recognizes viral RNA in a 5'-triphosphate-dependent manner [83], following which its N-terminal caspase recruitment domain (CARD) interacts with a downstream partner, MAVS (VISA/IPS-I/Cardif), and activates the antiviral signaling [84,85]. It has recently been shown that the TRIM25 (tripartite motif) protein interacts with CARD of RIG-I, which is important for initiating the antiviral cascade [86].

Like other viruses, influenza virus also has evolved strategies to antagonize host antiviral responses. TRIM25 is an ubiquitin ligase required for RIG-I activation. RIG-I activation leads to the association with the IPS-I, which phosphorylates IRF3 and follows the activation of IFN-β [13,87]. The NS1 protein of influenza virus is known to interfere with IFN production by binding to TRIM25. This process suppresses the RIG-I signaling and IFNβ production in infected cells [13,88,89]. This inhibitory activity was shown to depend on the NS1 RNA binding domain. It is believed that NS1 sequesters intracellular dsRNA like nucleic acids produced during viral replication, thereby keeping these molecules away from cellular dsRNA-sensor proteins, as TLR3/7 or RIG-I [88] (Figure 1(5)).

Conclusions

Influenza virus infection alters many cell signaling pathways involved in important physiological functions of the cell.

The virus takes over the host cell machinery to manipulate it for its own benefit. Influenza virus affects NF-κB signaling, PI3K/Akt pathway, MAPK pathway, PKC/PKR signaling and TLR/RIG-I signaling cascades by using various mechanisms. Most importantly, NS1 protein reduces the antiviral response by activating NF-κB signaling and also activates PI3K/Akt pathway for efficient viral replication. The virus misuses these (MAPK pathway, PKC/PKR signaling and TLR/RIG-I signaling) signaling pathways for inhibition of antiviral effects of cytokines and increasing formation of infectious progeny virions.

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