Influenza viruses and MAP kinase cascades – Novel targets for an antiviral intervention?

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Viruses and Signalling

Influenza viruses only have a quite limited coding capacity. Thus, these viruses have to employ functions of their host cell for efficient replication. This of course creates dependencies that might be useful to design novel antiviral strategies by targeting host cell functions. One of the major advantages for such an approach would be that the virus can not easily replace the missing cellular function. Thus there should be no or only a reduced tendency for inhibitors of cellular functions to induce resistant virus variants. First indications that this assumption appears to hold true came from pioneering work from Christoph Scholtissek and colleagues in the early 90's [1]. In these studies it has been demonstrated that inhibitors of methyltransferases or broad range kinase inhibitors such as H7 blocked influenza virus replication without the emergence of resistant variants.

Cell fate decisions in response to extracellular agents, including pathogenic invaders are commonly mediated by intracellular signalling cascades that transduce signals into stimulus specific actions, e.g. changes in gene expression patterns, alterations in the metabolic state of the cell or induction of programmed cell death (apoptosis). In most cases the signal transduction within the cell is governed by kinases organized in different kinase cascades. These signalling enzymes are at the bottleneck of the control of cellular responses. Thus the signalling profile allows to predict the fate that a cell is committed to. It becomes more and more evident that viruses also misuse cellular signalling responses to support its replication. Here we will focus on the recent advances in the analysis of influenza virus induced MAPK signalling pathways and first attempts to use these mediators as targets for antiviral intervention.

MAP Kinase Cascades – Key Regulators of Cellular Signalling

Mitogen activated protein kinase (MAPK) cascades have gained much attention as being critical transducers to convert a variety of extracellular signals into a multitude of responses [2–4]. Thereby, these pathways regulate numerous cellular decision processes, such as proliferation and differentiation, but also cell activation and immune responses [5]. Four different members of the MAPK family that are organized in separate cascades have been identified so far: ERK (extracellular signal regulated kinase), JNK (Jun-N-terminal kinase), p38 and BMK-1/ERK5 (Big MAP kinase) [6, 4]. These MAP kinases are activated by a dual phosphorylation event on threonine and tyrosine mediated by MAP kinase kinases (MEKs or MKKs). The MAP kinase ERK is activated by the dual-specific kinase MEK that itself is activated by the serine threonine kinase Raf. Raf, MEK and ERK form the prototype module of a MAP kinase pathway and are also known as the classical mitogenic cascade. The MAP kinases p38 and JNK are activated by MKK3/6 and MKK4/7, respectively, and are predominantly induced by proinflammatory cytokines and certain environmental stress conditions. The MEK5/ERK5 module is both activated by mitogens and certain stress inducers. There is evidence that the different MAPK cascades are also activated upon infection with RNA viruses, including influenza viruses. Thus, these signalling cascades may serve different functions in viral replication and host cell response.

MAP Kinase Cascades and Influenza Virus Infection: Opposite Roles of the JNK and ERK Pathways

Interestingly all four so far defined MAPK family members are activated upon an influenza virus infection [7–9]. (Virginia Korte and S.L., unpublished). Recent work has helped to get a clearer picture of the function of these four signalling pathways in the infected cell. By the use of specific kinase inhibitors p38 and JNK but not ERK have been linked to virus-induced expression of RANTES, a chemokine involved in the attraction of eosinophils during an inflammatory response [7]. In a more recent study using the same set of inhibitors the ERK and JNK, but not p38 pathway were shown to be involved in the expression of the inflammatory mediator cyclooxygenase (COX) and phosphorylation of cytosolic phospholipase A2 (cPLA2) in bronchial epithelial cells [10]. Further, the inhibitors of all three MAPK pathways were effective to dose dependently-block prostaglandin E2 release by various extents [10], indicating that viral MAPK activation

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contributes to the onset of anti-inflammatory response. JNK and p38 activation has also been demonstrated in vivo in mice infected with a neurovirulent influenza A virus that caused lethal acute encephalitis, although it is not clear whether this activity is directly virus-induced or mediated by immunological or inflammatory responses [11]. In this study JNK but not p38 activity had been linked to the onset of apoptosis in the infected brain [11]. In embryonic fibroblasts from mice genetically deficient for apoptosis-signal-regulated kinase (ASK-1) virus-induced p38 and JNK activation was blunted concomitant with an inhibition of caspase 3 activation and virus-induced apoptosis [11]. ASK-1 is a ubiquitously expressed MAPK kinase kinase that activates the MAPK kinase 4/JNK and the MAPK kinase 6/p38 cascade. These findings not only identified a novel upstream component of virus induced-MAPK signalling but also linked activity of certain MAPKs to apoptosis induction [12].

The JNK subgroup of MAPKs further came into focus in the context of an influenza virus infection since a very early activation of activator-protein 1 (AP-1) transcription factors [13] was observed in productively infected cells [8]. AP-1 factors include c-Jun and ATF-2 that are phosphorylated by JNKs to potentiate their transcriptional activity [13]. Both factors are phosphorylated upon influenza virus infection [8, 14]. Accordingly, activation of JNK was observed with different virus strains in a variety of permissive cell lines [7, 8]. JNK activation required productive replication and was induced by the accumulating RNA produced by the viral polymerase. As upstream activators in the viral context the MAPK kinases MKK4 and MKK7 have been identified. The AP-1 factors c-Jun and ATF-2 are critical for the expression of IFNß, a most potent antiviral cytokine [15]. Accordingly, inhibition of the cascade by dominant-negative mutants of MKK7, JNK or c-Jun during a virus infection resulted in impaired transcription from the IFNß promoter and an enhanced virus production. Thus, the JNK pathway appears to be a crucial mediator of the antiviral response to an influenza virus infection by co-regulating IFNß expression [8]. Inhibition of such a pathway for an antiviral intervention is not recommended since this would rather interfere with the antiviral interferon response.

The ERK5 MAPK pathway is also activated upon productive virus infection and stimulation with double-stranded RNA as a mimic for viral RNA accumulation. However, interference with virus-induced activation of ERK5 or its upstream kinase MEK5 by expression of dominant negative mutants or antisense constructs does neither affect viral replication efficiencies nor antiviral responses to virus infection. Thus the ERK5 pathway is an example of an influenza virus-induced signalling process, which does not interfere with the outcome of virus propagation (V. Korte and S.L., unpublished data).

The MAP kinase ERK is also activated upon productive influenza virus infection [7]. However, in contrast to JNK and ERK5 it appears to serve a mechanism that is beneficial for the virus [9]. Strikingly, blockade of the pathway by specific inhibitors of the upstream kinase MEK, or dominant-negative mutants of ERK or the MEK activator Raf resulted in a strongly impaired growth of both, influenza A and B type viruses [9]. Conversely, virus titers are enhanced in cells expressing active mutants of Raf or MEK [16, 17]. This has not only been demonstrated in cell culture but also in vivo in infected mice expressing a constitutively active form of the Raf kinase in the alveolar epithelial cells of the lung [17]. While in the wt situation influenza viruses primarily infect bronchiolar epithelial cells, there is efficient replication in the alveolar layer most exclusively in the cells carrying the transgene. As a consequence this results in an earlier death of the transgenic animals [17]. This indicates that activation of the Raf/MEK/ERK pathway, in contrast to the JNK cascade, is required for efficient virus growth. Strikingly, inhibition of the pathway did not affect viral RNA or protein synthesis [9]. The pathway rather appears to control the active nuclear export of the viral RNP complexes. RNPs are readily retained in the nucleus upon blockade of the signalling pathway. Most likely this is due to an impaired activity of the viral nuclear export protein NEP [9]. This indicates that active RNP export is an induced rather than a constitutive event, a hypothesis supported by a late activation of ERK in the viral life cycle. So far the detailed mechanism of how ERK regulates export of the RNPs is unsolved. There are two likely scenarios: Either the process occurs directly via phosphorylation of a viral protein involved in transport or by control of a certain cellular export factor. It has been demonstrated quite early on that influenza virus proteins, especially the NP protein are phosphorylated and that the phosphorylation pattern changes throughout the replication cycle or in the presence of brad range kinase inhibitors [18, 19]. Although in the initial studies no alteration of the overall phosphorylation status of the NP, M and NS2 proteins was observed in the presence of MEK inhibitors [9] there are now first indications that certain phosphorylation sites of the NP indeed are affected by MEK inhibition (S.P., unpublished data). It remains to be shown whether this is of functional relevance for the RNP export process. It is striking that MEK inhibitors are not toxic for the cell while more general blockers of the active transport machinery, such as

leptomycin B exert a high toxicity even in quite low concentrations. This may indicate that MEK inhibitors are no general export blockers but only block a distinct nuclear export pathway. Indeed there are first evidences that the classical mitogenic cascade specifically regulates nuclear export of certain cellular RNA-protein complexes. In LPS treated mouse macrophages MEK-inhibition results in a specific retention of the TNF mRNA in the nucleus [20]. This is also observed in cells deficient for Tpl-2, an activator of MEK and ERK. In these cells the failure to activate MEK and ERK by LPS again correlated with TNF mRNA retention while other cytokines are normally expressed [20]. Thus the ERK-pathway may regulate a specific cellular export process but leaves other export mechanisms unaffected. It is likely that such a specific export pathway is employed by influenza A and B viruses.

The finding of an antiviral action of MEK inhibitors prompted further research showing that replication of other viruses, such as Borna disease virus [21], Visna virus [22] or Coxsackie B3 virus [23] is also impaired upon MEK inhibition.

Requirement of Raf/MEK/ERK activation for efficient influenza virus replication may suggests that this pathway may be a cellular target for antiviral approaches. Besides the antiviral action against both, A and B type viruses [16], MEK inhibitors meet two further criteria which are a prerequisite for a potential clinical use. Although targeting an important signalling pathway in the cell the inhibitors showed a surprisingly little toxicity (a) in cell culture [16, 21, 9] (b) in an in vivo mouse model [24] and (c) in clinical trials for the use as anti-cancer agent [25]. In the light of these findings it was hypothesized that the mitogenic pathway may only be of major importance during early development of an organism and may be dispensable in adult tissues [25]. Another very important feature of MEK inhibitors is that they showed no tendency to induce formation of resistant virus variants [16].

Although targeting of a cellular factor may still raise the concern about side effects of a drug, it appears likely that local administration of an agent such as a MEK inhibitor to the primary site of influenza virus infection, the lung, is well tolerated. Here the drug primarily affects differentiated lung epithelial cells for which a proliferative signalling cascade like the Raf/MEK/ERK cascade may be dispensable. Following this approach it was recently demonstrated that the MEK inhibitor U0126 is effective in reducing virus titers in the lung of infected mice after local administration (O.P., S.P. and S.L., unpublished).

Thus, the Raf/MEK/ERK cascade may be taken as an example for a cellular target which may be used for anti-flu intervention without side effects or the tendency to induce resistant virus variants.

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Influenza Viruses
Facts and Perspectives

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