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Review

Role of mitogen-activated protein kinase signaling in the pathogenesis of dengue virus infection

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ABSTRACT

Dengue virus (DENV) infection is a disease that is endemic to many parts of the world, and its increasing prevalence ranks it among the diseases considered to be a significant threat to public health. The clinical manifestations of DENV infection range from mild dengue fever (DF) to more severe dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Increased proinflammatory cytokines and vascular permeability, both of which cause organ injury, are the hallmarks of severe dengue disease. Signs of liver injury were observed in studies using hepatic cell lines, mouse models, and autopsy specimens from DENV-infected patients, and these signs substantiated the effects of inflammatory responses and hepatic cell apoptosis. Mitogen-activated protein kinases (MAPK) are involved in inflammatory responses and cellular stress during viral infections. The roles of MAPK signaling in DENV infection were reviewed, and published data indicate MAPK signaling to be involved in inflammatory responses and hepatic cell apoptosis in both *in vitro* cultures and *in vivo* models. Modulation of MAPK signaling ameliorates the inflammatory responses and hepatic cell apoptosis in DENV infection. This accumulation of published data relative to the role of MAPK signaling in inflammatory responses and cell apoptosis in DENV infection is elucidatory, and may help to accelerate the development of novel or repositioned therapies to treat this unpredictable and often debilitating disease.

1. Introduction

Dengue virus (DENV) infection, which is most prevalent in tropical countries, is one of the most important arbo-viral diseases of the 21st century [1]. Four serotypes of DENV are recognized based on antigenicity, and each causes clinical disease [2]. However, multiple strains of each serotype have been identified among outbreaks that developed in endemic areas. DENV-infected patients show different levels of clinical severity, including mild fever (DF), dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS), the last of which is the most severe. The hemorrhagic form of DENV infection includes plasma leakage and hematologic disorders. Secondary DENV infection may lead to shock [3] and multiple organ injuries [4,5].

In 1964, Nelson and colleagues first noted the association between DENV infection and thrombocytopenia [6–8]. The hematologic aspects of dengue infections were well-reviewed in 1982 by Halsted, et al. [9]. Abnormalities in hematologic parameters were also observed in animal models, with thrombocytopenia being consistently and prominently reported [10,11]. Boonpucknavig, et al. detected antigens of DENV, and described them as irregular granules in reticuloendothelial cells of the liver, lymph nodes, and spleen of female mice infected with DENV [12]. Later, clinical manifestations of DENV infection that correlated with liver injury were investigated. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyl transpeptidase (GGT) levels were reported to be abnormal in DENV-infected patients, and AST was significantly higher than ALT [13].

Apoptotic cell death and phagocytic cell activation were preliminarily given as the explanations for DENV-induced liver injury, with the associated importance of viral replication being thereafter reported [14–16]. The pathologic highlights of liver injury in DHF patients were reported from an extensive pathologic study in DENV infection conducted in Myanmar [17]. In that study, livers of DENV-infected patients had the classical signs of cell death in varying degrees, with morphologic changes that included ballooning of hepatocytes, cytoplasmic vacuolization, dilated sinusoids with debris, and Kupffer cell hyperplasia. With clear signs of necrosis, the positive staining of cytoplasmic cleaved caspase-3 in the liver revealed apoptotic cell death. Splenomegaly with dilated splenic sinusoids that contained red blood cells and positive caspase-3 staining were observed [17].

DENV can induce inflammatory response and hepatic cell apoptosis

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by activation of the mitogen-activated protein kinase (MAPK) signaling pathway. The classical roles of MAPKs in various cellular events were previously reviewed [18], especially relative to inflammatory responses [19]. The involvement of MAPKs was previously reported in both *in vitro* cultures and animal models of viral infections [20–24]. The roles of MAPKs in the pathogenesis of DENV infection were further elucidated, to include intensive cytokine response and liver injury. Increased phosphorylation of MAPKs, including extracellular signal-regulated kinases (ERKs), p38 MAPK, and c-Jun N-terminal kinases (JNKs), in liver injury was evident. A specific inhibitor for each MAPK was used to examine how these signals respond to and associate with clinical symptoms and liver histopathology in animal models [25–27]. In this review, we set forth to summarize the published data relating to the roles of MAPK signaling in DENV infection, and the roles of various modulators of MAPK signaling in response to DENV infection.

2. MAPK signaling in DENV infection

MAPKs are serine/threonine-specific protein kinases that are prominent in eukaryotes, that are involved in various physiological conditions, and that regulate various cellular processes, including gene expression, differentiation, proliferation, mitosis, apoptosis, and cell survival [28]. MAPKs are catalytically inactive until phosphorylated within their individual activation loops [29,30]. The classical MAPKs are considered to be the most important to cell function, but little is known about atypical MAPKs [31]. In the early 1980s, Cooper and colleagues discovered the first classical MAPKs. ERK1/2 were identified as two structurally similar proteins that worked in combination to function as a mitogen-stimulated 42 kDa phosphorylated protein [32] that was later named ERK p44/42 [33,34]. Other proteins among the classical MAPKs include JNK 1/2 and p38 MAPK (four isoforms, including p38-α, p38-β, p38-γ, and p38-δ). The proteins in atypical MAPKs include ERK 3/4, ERK 5, ERK 7/8, and Nemo-like kinase (NLK). The functional roles of the proteins in atypical MAPKs have not been widely investigated.

In 2001, Liao and colleagues suspected the involvement of p38 MAPK in the regulation of DENV replication [35]. Three years later, Hilgard, et al. preliminarily elucidated the JNK signaling cascade in DENV-infected HepG2 cells [36]. DENV-induced JNK phosphorylation was also described in macrophages to transactivate the phosphorylation of cJun, which in turn translocated into the nucleus for use in the transcriptional activity of activator protein 1 (AP-1) [37]. Similarly, DENV-induced interleukin-8 production could activate AP-1 in HepG2 cells *via* modulation of p38 MAPK signaling [38]. ERK1/2 phosphorylation was observed in DENV-infected HepG2 cells [39]; however, ERK activation was reported to be blocked in DENV-infected A549 cells [40]. Recently, Chun-Kuang, et al. found that cyclooxygenase-2 (COX-2) is required for DENV replication in Huh7 cells, and that JNK1/2 and NF-κB are essential for the expression of COX-2 and viral replication [41], which suggests that inhibition of COX-2 may reduce infectivity *via* MAPK signaling.

MAPKs are involved in DENV infection-related apoptosis [28]. DENV-infected human endothelial cells displayed apoptotic cell death with extreme cytokine responses, which may relate to vascular leakage [42]. Cellular apoptosis was induced by antibodies against NS1 protein, with declined expression of Bcl-2 and Bcl-xL, and increased expression of p53 and Bax [43]. Moreover, DENV-induced apoptosis was found to be associated with activation of the c-Jun and NF-κB pathways in human neuroblastoma cell line (SK-N-SH) [44]. The upregulation of galectin-9 was observed in DENV-infected human dendritic cells, and was found to be involved in MAPK signaling by activation of the NF-κB pathway [45]. Hepatocyte apoptosis associated with the activation of transcription factor NF-κB was evident by the presence of Councilman bodies in liver biopsies from patients with severe DENV infection [46]. The induction of protein kinase CK2α expression in Huh7 hepatoma cell line infected with DENV was mediated by proapoptotic JNK signaling cascade [36]. The lipid kinase sphingosine kinase 2 (SPHK2) was recently spotted as an efficient contributor of DENV- mediated apoptosis [47]. In an *in vitro* apoptotic gene expression profiler, the receptor-interacting protein (RIP) kinase family of serine/threonine protein kinase RIPK2 was also identified in contributing to DENV-mediated apoptosis. Interestingly, the inhibition of RIPK2 and p38 MAPK by the p38 MAPK inhibitor SB203580 reduced DENV-induced apoptosis in HepG2 cells [48,49].

3. MAPK signaling modulators in DENV infection

The impact of MAPK signaling was investigated in various viral diseases [21,50–55] including flavivirus infections [56–58]. In the fetal endothelial cells infected with Zika virus (ZIKV), inhibitors of both p38 MAPK and JNK were effective to reduce ZIKV replication [57]. Huh7 cells infected with hepatitis C virus (HCV), those were treated with MAPK-ERK inhibitor, U0126 curbed infectivity [59] *via* the partial modulation of interferon-stimulated genes (ISGs) [60]. In cells infected with Yellow fever virus (YFV), U0126 reduced not only the virus replication but also the ERK1/2 phosphorylation [56]. MAPK inhibitors efficiently modulated the pro-inflammatory cytokines and chemokines coupled with the MAPK signaling during West Nile virus (WNV) infection [58]. These studies suggest the impact of MAPK inhibitors in flavivirus infection.

The modulation of MAPK signaling by various agents ameliorates inflammatory responses and hepatic cell apoptosis in DENV infection. A number of antiviral agents have been tested against DENV infection in both *in vitro* cultures and *in vivo* models of DENV infection to elucidate the pathogenesis of dengue in an attempt to discover or develop a novel therapeutic approach to treat dengue.

During DENV infection in human peripheral blood mononuclear cells (PBMCs), THP-1 cell line, and KU812 cell line, the p38 MAPK inhibitor SB203580 curbed several proinflammatory cytokine responses, including TNF-α, IL-8, and RANTES [61]. SB203580 treatment reduced RIPK-2 [48], which is an important modulator of stress responses and apoptotic signals. SB203580 treatment in DENV-infected HepG2 cells reduced TNF-α expression and apoptosis; however, how SB203580 modulated these responses was not thoroughly investigated [49]. DENV-infected AG129 mice that received oral administration of SB203580 still had detectable viral particles, but they had improved clinical manifestations and prolonged survival [61]. That study was the first to report on the efficacy of SB203580 in DENV-infected animal model; however, the molecular signaling mechanism by which SB203580 modulates these parameters in DENV-infected mice was not investigated. Later, a Balb/c mouse model was used to investigate the molecular mechanism by which SB203580 modulates DENV-induced liver injury. DENV infection induced phosphorylation of p38 MAPK and reduced the phosphorylation of its MAP kinase-activated protein kinase 2 (MAPKAPK2), heat shock protein 27 (HSP27), and activating transcription factor 2 (ATF-2) downstream kinases to minimize liver injury in DENV-infected mice [26]. However, no reduction in viral copies was observed in DENV-infected SB203580-treated mice when compared to DENV-infected control mice, which suggests the role of host immune response modulated by SB203580. It was also found that increased expressions of cytokines (TNF-α, IL-6, and IL-10) and chemokines (RANTES and IP-10) in DENV infection were reduced by SB203580 treatment. Additionally, caspase-9, caspase-8, and caspase-3 proteins were significantly lower in SB203580-treated DENV-infected mice than in DENV-infected control mice [26].

In DENV-infected macrophages, JNK phosphorylation is induced and the presence of cholesterol is required for viral replication [37]. Interestingly, the inhibition of JNK with its inhibitor (SP600125) reduced virus production. An investigation of the proapoptotic role of CK2α in DENV-infected Huh7 cells revealed that SP600125 treatment reduced apoptosis, which initially suggested the importance of JNK signaling-mediated apoptosis in DENV infection [36]. DENV-induced