Pathophysiological Roles of ASK1-MAP Kinase Signaling Pathways

Hiroaki Nagai, Takuya Noguchi, Kohsuke Takeda and Hidenori Ichijo*

Laboratory of Cell Signaling, Graduate School of Pharmaceutical Sciences, The University of Tokyo, CREST, Japan Science and Technology Corporation, and Strategic Approach to Drug Discovery and Development in Pharmaceutical Sciences, Center of Excellence (COE) program, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

Received 14 November 2006

Apoptosis signal-regulating kinase 1 (ASK1) is a mitogen-activated protein kinase (MAPK) kinase kinase that activates JNK and p38 kinases. ASK1 is activated by various stresses, such as reactive oxygen species (ROS), endoplasmic reticulum (ER) stress, lipopolysaccharide (LPS) and calcium influx which are thought to be responsible for the pathogenesis or exacerbations of various human diseases. Recent studies revealed the involvement of ASK1 in ROS- or ER stress-related diseases, suggesting that ASK1 may be a potential therapeutic target of various human diseases. In this review, we focus on the current findings for the relationship between pathogenesis and ASK1-MAPK pathways.

Keywords: ASK1, ER stress, Innate immunity, MAPK, Oxidative stress

Introduction

Cells are exposed to various kinds of stresses, which are continuous and unavoidable. The adaptive responses to these stresses are essential for the maintenance of homeostasis. Mitogen-activated protein kinase (MAPK) pathways are one of the intracellular signaling systems to induce the optimal stress response (Kyriakis and Avruch, 2001). The importance of MAPK pathways is demonstrated by the fact that MAPK cascades are evolutionarily well conserved in all eukaryotic cells (Widmann et al., 1999). In mammals, three major MAPK pathways that converge on extracellular signal-regulated kinases (ERK, including ERK1 and ERK2 isoforms), c-Jun N-terminal kinases (JNK, including JNK1, JNK2 and JNK3 isoforms), and p38 MAPKs (including p38α, p38β, p38γ and p38δ isoforms) induce a variety of cellular functions such as gene expression, mitosis, and apoptosis through the phosphorylation of specific serine and/or threonine residues of target proteins. Each MAPK pathway typically includes central three-tiered core signaling modules comprising a MAPK kinase kinase (MAP3K), MAPK kinase (MAP2K), and MAPK (Fig. 1). MAP3K phosphorylates and thereby activates MAPKK, and activated MAPKK in turn phosphorylates and activates MAPK (Fig. 1). Because activation status of MAPKs is largely dependent on MAPK kinase kinases (MAP3Ks), it is important to understand how MAP3Ks are regulated.

Apoptosis signal-regulating kinase 1 (ASK1) is one of the MAP3Ks that is activated by various types of stress such as reactive oxygen species (ROS), tumor necrosis factor (TNF) α, lipopolysaccharide (LPS), endoplasmic reticulum (ER) stress and calcium influx, and selectively activates JNK and p38 MAPK pathways (Nishitoh et al., 1998; Saitoh et al., 1998; Takeda et al., 2004; Matsuzawa et al., 2005) (Fig. 2). ASK1 is one of the most extensively studied MAP3Ks. Here we introduce the up-dated studies of human diseases in which ASK1-MAPK pathways are reported to be involved (summarized in the table).

Oxidative stress-related diseases and ASK1

Whole aerobic organisms are continuously exposed to reactive oxygen species (ROS) generated by aerobic metabolism. Excessively generated ROS is generally counteracted by ubiquitously expressed antioxidant proteins represented by thioredoxin (Trx), glutaredoxin and glutathione. Once the generation of ROS exceeds the capacity of the antioxidant proteins, cells suffer from so-called “oxidative stress”, which is known to be a potential cause of many diseases such as ischemia-reperfusion injuries, neurodegenerative disorders, cardiovascular diseases, chronic hepatitis and diabetes mellitus (Finkel, 2003).

Oxidative stress is one of the most potent activators of ASK1, furthermore, which is essential for oxidative stress-induced cell death (Tobiume et al., 2001). These findings easily raise the possibility that ASK1 is involved in oxidative...
stress-mediating diseases. ASK1-p38/JNK pathways regulate apoptosis of H_2O_2-stimulated human pulmonary vascular endothelial cells (EC), and play an important role in regulating left ventricular (LV) remodeling by promoting apoptosis (Machino et al., 2003; Yamaguchi et al., 2003). When subjected to myocardial ischemia-reperfusion injury, ASK1-/− mice showed decreased infarct size and a resistance to myocardial cell death (Watanabe et al., 2005), suggesting that ASK1-mediated myocardial cell death is at least in part responsible for ischemia-reperfusion injury. Furthermore, oxidative stress-mediated biphasic activation of ASK1-JNK pathway is involved in brain ischemia in hippocampus (Zhang et al., 2003). Recently, relevant examples that ASK1-mediated cell death is involved in pathogenesis of human diseases are further reported. Fanconi anemia (FA) is a genetic disorder typified by bone marrow hypoplasia and increased cancer risk with progression of bone marrow failure (BMF). Experimental data support the idea that the hypersensitivity of hematopoietic progenitors to oxidants and myelosuppressive cytokines such as TNFα and IFNγ contributes to pathogenesis of BMF in FA. Recent study using FA type C protein (FANCC: a responsive gene of FA)-deficient mice, which is a model mice of FA, revealed that TNFα-induced apoptosis in hematopoietic progenitors from FancC−/− mice is caused by ROS-dependent activation of ASK1-p38 MAPK pathway, suggesting that ASK1 mediated-cell death is also responsible for hematopoietic diseases (Bijangi-Vishhehsraei et al., 2005; Saadatzadeh et al., 2004). These studies indicate the close relationship between ASK1-induced cell death and various kinds of diseases mediated by oxidative stress.

In some cases, oxidative stress-dependent activation of ASK1 participates in pathogenesis through the induction of cell transformation but not of cell death. Angiotensin II, which plays an important role in cardiovascular diseases, is known to induce hyperension and hypertrophy. Analysis of ASK1−/− mice revealed that ASK1 is activated by angiotensin II in a ROS-dependent manner and thereby induces not only myocardial cell apoptosis but also cardiac remodeling including myocardial cell hypertrophy and fibrosis that are considered as one of the risk factors of heart failure (Izumiya et al., 2003). In addition, it is implicated that ASK1 activation may induce the proliferation and migration of vascular smooth muscle cells, the ischemia-induced angiogenesis and the airway hyperplasia which is a characteristic pathology of asthma (Izumi et al., 2003; Jibiki et al., 2003; Izumi et al., 2005; Kumatsuka et al., 2005; Tsujimoto et al., 2005). These findings, thus, indicate that ASK1 plays a role in a wide range of cardiovascular pathogenesis from hyperension to heart failure. Nevertheless, further studies are required for the full elucidation of the relationship between ASK1 and these diseases.

Neurodegenerative diseases and ASK1

Endoplasmic reticulum (ER) stress is triggered by accumulation of unfolded and/or misfolded proteins in the ER lumen.
Recently, it has been revealed that ER stress-induced cell death plays critical roles in the pathogenesis of neurodegenerative diseases such as polyglutamine (polyQ) diseases, Alzheimer’s disease, Parkinson’s disease, amyotrophic lateral sclerosis (ALS) and Prion diseases (Aridor and Balch, 1999; Kopito and Ron, 2000; Kaufman, 2002; Sekine et al., 2006). ASK1-MAPK pathways are activated by ER stress as well as by oxidative stress.

Polyglutamine (polyQ) diseases, such as Huntington’s disease (HD), spinobulbar muscular atrophy (SBMA), and several forms of spinocerebellar ataxia (SCA) including SCA3/Machado-Joseph disease (MJD), are inherited neurodegenerative disorders induced by the insoluble aggregations of pathogenic proteins with expanded polyQ repeats (Kakizuka, 1998; Soto, 2003). It has been demonstrated that the expanded polyQ repeats cause the dysfunction of ubiquitin-proteasome system and then evoke ER stress, resulting in neuronal cell death. Furthermore, analysis of primary neurons derived from ASK1-/- mice revealed that expanded polyQ repeats-induced neuronal cell death requires the activation of ASK1-JNK pathway via ER stress (Nishitoh et al., 2002).

Alzheimer’s disease (AD) is a progressive neurodegenerative disorder characterized pathologically by cerebral neuritic plaques of amyloid-β (Aβ) peptide and neurofibrillary tangles, and clinically by progressive loss of memory and cognitive impairment. Fibrillar Aβ peptides produced abnormally by mutant pathogenic genes such as amyloid precursor protein (APP), presenilin (PS) 1 and PS2 induce neuronal cell death (Yankner, 1996; Selkoe, 2001). Aβ also activates ASK1 mainly through the generation of ROS but not through ER stress in cultured neuronal cells and that ASK1-deficient

### Table 1. ASK1-related diseases and pathologies

<table>
<thead>
<tr>
<th>Organ/tissue</th>
<th>related diseases</th>
<th>ASK1 activators</th>
<th>related pathologies</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nervous system</td>
<td>polyQ disease</td>
<td>ER stress</td>
<td>neuronal death</td>
<td>Nishitoh et al., 2002</td>
</tr>
<tr>
<td></td>
<td>ALS</td>
<td>ER stress</td>
<td>neuronal death</td>
<td>Holasek et al., 2005</td>
</tr>
<tr>
<td></td>
<td>Alzheimer’s disease</td>
<td>Oxidative stress</td>
<td>neuronal death</td>
<td>Kadowaki et al., 2005, Song et al., 2003</td>
</tr>
<tr>
<td></td>
<td>Parkinson’s disease</td>
<td>ER stress?</td>
<td>neuronal death</td>
<td>Bonifati et al., 2003, Tain et al., 2004, Zhang et al., 2003</td>
</tr>
<tr>
<td>Brain ischemia</td>
<td>Oxidative stress</td>
<td>cell death?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>ischemic heart disease</td>
<td>Oxidative stress, Ca^2+ influx</td>
<td>cardiomyocyte death</td>
<td>Watanabe et al., 2005, Yamaguchi et al., 2003</td>
</tr>
<tr>
<td></td>
<td>Hypertension</td>
<td>Oxidative stress</td>
<td>LV remodeling</td>
<td>Izumiya et al., 2003, Yamaguchi et al., 2003, Tsujimoto et al., 2005</td>
</tr>
<tr>
<td>Vessel</td>
<td>vessel injury</td>
<td>Growth factors?</td>
<td>neoplasia</td>
<td>Izumi et al., 2003</td>
</tr>
<tr>
<td></td>
<td>peripheral vascular disease?</td>
<td>Oxidative stress?</td>
<td>angiogenesis</td>
<td>Izumi et al., 2005</td>
</tr>
<tr>
<td>Hematopoietic system</td>
<td>Fanconi anemia</td>
<td>Oxidative stress (TNFα)</td>
<td>cell death</td>
<td>Bijangi-Vishelsimani et al., 2005, Saadatzadeh et al., 2004</td>
</tr>
<tr>
<td>Lung</td>
<td>asthma</td>
<td>NO?, leukotriene D4?</td>
<td>airway remodeling</td>
<td>Kumasa et al., 2005, Jibiki et al., 2003</td>
</tr>
<tr>
<td></td>
<td>pulmonary edema</td>
<td>Oxidative stress?</td>
<td>EC death</td>
<td>Machino et al., 2003</td>
</tr>
<tr>
<td></td>
<td>influenza virus infection</td>
<td>?</td>
<td>cell death</td>
<td>Mankova et al., 2003</td>
</tr>
<tr>
<td>Immune system</td>
<td>infection</td>
<td>Oxidative stress</td>
<td>septic shock</td>
<td>Matsuzawa et al., 2005</td>
</tr>
<tr>
<td>Skin</td>
<td>infection</td>
<td>?</td>
<td>production of antibiotic peptides</td>
<td>Sayama et al., 2005</td>
</tr>
</tbody>
</table>

Summarized the reports that suggest the involvement of ASK1 in pathogenesis.
neurons are refractory to Aβ-induced JNK activation and cell death, suggesting that ROS-induced ASK1 activation by Aβ is an important step in the pathogenesis of AD (Kadowaki et al., 2005).

Furthermore, possible involvement of ASK1 in the pathogenesis of Parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS) is also reported. PD is a common neurodegenerative disorder characterized by the progressive loss of dopaminergic neurons in the substantia nigra pars compacta with subsequent defects in movements (Lang and Lozano, 1998a; Lang and Lozano, 1998b). It is known that the dysfunction of Parkin or DJ-1 is responsible for autosomal recessive juvenile Parkinsonism (AR-JP) (Kitada et al., 1998; Shimura et al., 2000; Bonifati et al., 2003; Taira et al., 2004). In particular, it is reported that AR-JP-linked DJ-1 mutation failed to protect ASK1-induced cell death in dopaminergic neuroblastoma cells whereas normal DJ-1 protected (Junn et al., 2005). These findings suggest that DJ-1 mutation results in neuronal cell death in part through the activation of ASK1.

ALS is a late-onset neurodegenerative disease characterized by the selective loss of motorneurons in spinal cord, brainstem and cerebral cortex (Cleveland and Rothstein, 2001). Mutation in the gene encoding Cu/Zn superoxide dismutase 1 (SOD1) is known as responsible for pathogenesis of certain familial ALS (FALS). Compared with nonsymptomatic intermates, the FALS model mice, which is a transgenic mice expressing ALS-linked SOD1 mutants, showed significant increase in the numbers of motorneurons immunopositive for the activated ASK1 and p38, suggesting that ASK1-p38 pathway may be involved in neuronal cell death in FALS (Wengenack et al., 2004; Holasek et al., 2005). Thus, ASK1-MAPK pathways appear to contribute to the pathogenesis of these neurodegenerative diseases. However, in the case of PD and ALS, it is unclear what is the direct activator of ASK1: ER stress, ROS or others.

**Immune response and ASK1**

As mentioned above, the enhancement of ASK1 activity is responsible for various pathogenic events or disease progression. Similar results are demonstrated against virus infections. In the case of influenza virus (IV) infection, apoptosis in IV-infected cells is mediated through ASK1-dependent pathways (Murukka et al., 2003). Interestingly, in the case of human immunodeficiency virus type 1 (HIV-1) infection, Nef protein of human HIV-1 promotes the apoptosis of bystander cells through the induction of death signals, while simultaneously protecting the HIV-1-infected host cells from those signals through its interference with ASK1 function, suggesting that ASK1 function is used as a strategy by which HIV-1 avoid the host defense (Gelezianis et al., 2001).

On the other hand, the defensive role of ASK1 is also reported in immune system. In *Caenorhabditis elegans*, orthologs of the components of mammalian ASK1-p38 cascade are essential for the defense system against pathogenic bacteria. Furthermore, the importance of ASK1 in the mammalian innate immunity, which is the important mechanism for elimination of the pathogens such as bacteria and viruses at early stages of infections, has also been demonstrated (Matsuzawa et al., 2005; Sayama et al., 2005; Hayakawa et al., 2006). Lipopolysaccharide (LPS) is a cell wall component of Gram-negative bacteria and is specifically recognized by Toll-like receptor 4. LPS-induced activation of p38 MAPK was specifically diminished in splenocytes and bone marrow-derived dendritic cells (BMDCs) derived from ASK1-deficient mice. Concomitantly, production of inflammatory cytokines such as TNFα, IL-6, and IL-1β was diminished in ASK1-deficient splenocytes and BMDCs. In *vitro* study using ASK1−/− mice also supports the role of ASK1 in innate immunity; ASK1−/− mice were more resistant to LPS-induced septic shock than wild-type mice. The serum levels of TNFα and nitric oxide, which are the principal factors responsible for septic shock, were also reduced in ASK1-deficient mice (Matsuzawa et al., 2005). These results indicate the requirement of ASK1-p38 pathway against the bacterial infection in mammal as well as in *Caenorhabditis elegans*. Interestingly, LPS-induced activation of ASK1-p38 pathway was diminished by the pre-treatment with antioxidants such as N-acetyl-L-cysteine (NAC) and propyl gallate (PG), suggesting that LPS-dependent activation of ASK1 is mediated by ROS (Matsuzawa et al., 2005).

**Conclusion and perspectives**

As described throughout this review, recent studies have revealed the involvement of ASK1 in pathogenesis of various human diseases. ASK1 may be an attractive therapeutic target to overcome these diseases. However, in order to establish the novel therapeutic strategy targeting ASK1, it is necessary to fully elucidate the regulatory mechanisms and biological significance of ASK1.

Steady-state signaling complex of ASK1 forms a mega complex with its own homo-oligomerization and other components including unidentified factors and reduced-form of thioredoxin (Trx). Reduced-form of Trx is able to associate with ASK1 and inhibits the kinase activity of ASK1 by its direct binding to the N-terminal region of ASK1. Oxidative stress induces the oxidized-form of Trx, which is unable to associate with ASK1 any more, and allows the access of activating factors such as TRAF2 and TRAF6 to ASK1, thereby ASK1 is activated (Noguchi et al., 2005) (Fig. 2). Elucidation of the precise regulatory mechanisms of ASK1 by Trx or TRAF2/TRAF6 and their respective roles in stress signaling is required for our full understandings of stress responses through the ASK1-MAPK pathways. Furthermore, uncovering the unidentified components in the ASK1 signaling complex may reveal precise mechanisms of the ASK1-dependent signaling in ER stress and calcium overload.
which may be a promising strategy to understand and overcome ASK1-related human diseases.

Acknowledgments We are grateful to all the members of the Laboratory of Cell Signaling for valuable discussion.

References


