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Review

Pathophysiological Roles of ASK1-MAP Kinase Signaling Pathways

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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 NH_2-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

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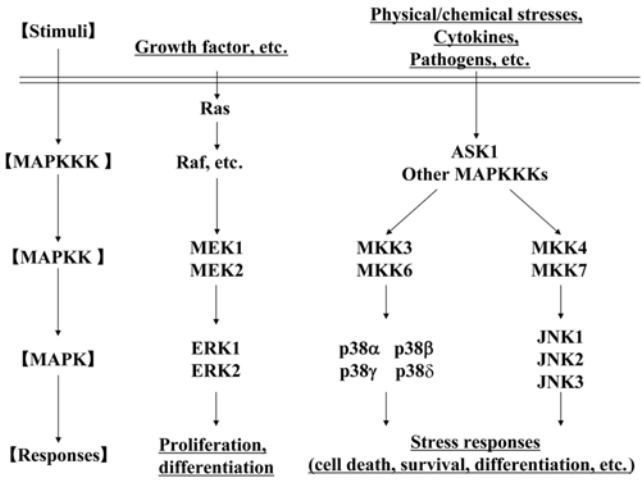

Fig. 1. Mammalian MAPK cascades. Each MAPK pathway is composed of three kinases that establish a sequential activation pathway comprising MAP3K (MAPKKK), MAP2K (MAPKK), and MAPK. In mammals, three major subgroups of MAPK (ERK, JNK, and p38) have been identified, which are structurally similar but functionally distinct. Whereas ERK is generally involved in cell growth and differentiation, JNK and p38 are preferentially activated by various types of environmental stress. ASK1 is a MAP3K that activates the JNK and p38 pathways by directly activating respective MAP2Ks, MKK4 (SEK1)/MKK7 and MKK3/MKK6. Only representative signaling molecules are shown.

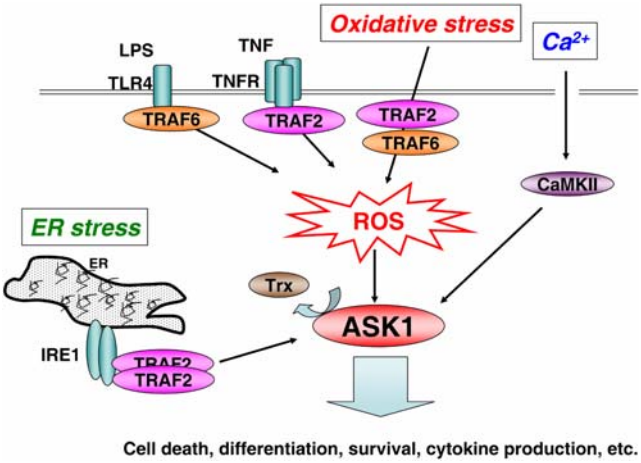

Fig. 2. ASK1 in cellular stress responses. ASK1 is activated by various stresses such as oxidative stress (including TNF and LPS stimuli), ER stress and calcium influx. In response to oxidative stress, Trx (thioredoxin), a negative regulator of the ASK1-JNK/p38 pathway, is dissociated from ASK1, and TRAF2 and TRAF6 are reciprocally recruited and thereby ASK1 is activated. In response to ER stress, TRAF2 is recruited to ASK1 and activates ASK1. In response to calcium influx, CaMKII activates ASK1 presumably by phosphorylation.

stress-mediated 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 resposible for hemapoietic diseases (Bijangi-Vishehsaraei *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 hypertension 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; Kumasawa *et al.,* 2005; Tsujimoto *et al.,* 2005). These findings, thus, indicate that ASK1 plays a role in a wide range of cardiovascular pathogenesis from hypertension 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.

Table 1. ASK1-related diseases and pathologies

Organ/tissue	related diseases	ASK1 activators	related pathologies	References
Nervous system	polyQ disease	ER stress	neuronal death	Nishitoh *et al*., 2002
	ALS	ER stress?	neuronal death?	Holasek *et al*., 2005
	Alzheimer's disease	Oxidative stress ER stress?	neuronal death	Kadowaki *et al*., 2005, Song *et al*., 2003
	Parkinson's disease	?	neuronal death	Bonifati *et al*., 2003, Taira *et al*., 2004
	brain ischemia	Oxidative stress	cell death?	Zhang *et al*., 2003
Heart	ischemic heart disease	Oxidative stress, Ca^{2+} influx	cardiomyocyte death LV remodeling	Watanabe *et al*., 2005, Yamaguchi *et al*., 2003
	Hypertension	Oxidative stress	LV remodeling	Izumiya *et al*., 2003, Yamaguchi *et al*., 2003, Tsujimoto *et al*., 2005
Vessel	vessel injury	Growth factors?	neoplasia	Izumi *et al*., 2003
	peripheral vascular disease?	Oxidative sterss?	angiogenesis	Izumi *et al*., 2005
Hemapoietic system	Fanconi anemia	Oxidative stress (TNFα)	cell death	Bijangi-Vishehsaraei *et al*., 2005 Saadatzadeh *et al*., 2004
Lung	asthma	NO?, leukotriene D_4?	airway remodeling	Kumasawa *et al*., 2005, Jibiki *et al*., 2003
	pulmonary edema	Oxidative stress?	EC death	Machino *et al*., 2003
	influenza virus infection	?	cell death	Maruoka *et al*., 2003
Immune system	infection	Oxidative stress	septic shock	Matsuzawa *et al*., 2005
Skin	infection	?	production of antibiotic peptides	Sayama *et al*., 2005

Summarized the reports that suggest the involvement of ASK1 in pathgenesis.

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 through its own neurotoxicity (Song *et al.,* 2003). Although the precise mechanism by which Aβ induces cell death is controversial, at least two major factors are proposed: ER stress and ROS. The involvement of ER stress in Aβ-induced neuronal cell death has been suggested by the finding that Aβ-induced neuronal cell death was impaired in the cells derived from caspase-12, which is specifically involved in ER stress-induced apoptosis (Nakagawa *et al.,* 2000). On the other hand, it has been reported that Aβ impairs mitochondrial redox activity and increases the generation of ROS, which leads to apoptotic neuronal death in an antioxidants-sensitive manner (Behl *et al.,* 1994; Hensley *et al.,* 1994; Shearman *et al.,* 1994). Furthermore, it has been reported that Aβ activates ASK1 mainly through the generation of ROS but not through ER stress in cultured neuronal cells and that ASK1-deficient

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 motoneurons in spinal cord, brainstem and cerebral cortex (Cleveland and Rothstein, 2001). Mutation in the gene encoding Cu/Zn superoxide dismutase 1 (SOD1) is thought to be responsible for pathogenesis of certain familial ALS (FALS). Compared with non-transgenic littermates, the FALS model mice, which is a transgenic mice expressing ALS-linked SOD1 mutants, showed significant increase in the numbers of motoneurons 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 (Maruoka *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 (Geleziunas *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 vivo* 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-oligomarization 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.

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