Apoptosis Signal-Regulating Kinase 1 in Stress and Immune Response

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Key Words

oxidative stress, ER stress, innate immunity, ROS, MAP kinase

Abstract

Apoptosis signal-regulating kinase 1 (ASK1) is a mitogen-activated protein kinase (MAPK) kinase kinase of the c-Jun N-terminal kinase (JNK) and p38 MAPK pathways. ASK1 is preferentially activated by various cytotoxic stressors and plays pivotal roles in a wide variety of cellular response to them. Recent analyses of ASK1 orthologs in *Caenorhabditis elegans* and *Drosophila melanogaster* have revealed that ASK1 is an evolutionarily conserved signaling intermediate in stress responses and appears to constitute a primitive but efficient defense system against stimuli harmful to organisms. Consistent with this notion, ASK1 has been shown to be required for the innate immune response, which is essential for host defense against a wide range of pathogens. In this review, we focus on the molecular mechanisms by which ASK1 functions in stress and immune responses and discuss the possible involvement of ASK1 in human diseases.

INTRODUCTION

MAPK: mitogen-activated protein kinase

JNK: c-Jun N-terminal kinase

TNF: tumor necrosis factor

Eukaryotic cells are equipped with multiple intracellular signal transduction systems, which sense various stressors from the external and internal environments and elicit a wide variety of cellular responses, including cell cycle arrest, DNA repair, cytokine production, and apoptosis. The mitogen-activated protein kinase (MAPK) cascades are among such signaling systems, which are evolutionarily conserved in all eukaryotic cells (1, 2). Four main MAPK cascades that converge on extracellular signal-regulated kinases 1 and 2 (ERK1/2), ERK5, c-Jun N-terminal kinases (JNKs), and p38 MAPKs have been well characterized. Each cascade consists of three classes of protein kinases: MAPK, MAPK kinase (MAP2K), and MAP2K kinase (MAP3K). MAP3K phosphorylates and thereby activates MAP2K, and activated MAP2K in turn phosphorylates and activates MAPK.

Among these cascades, the JNK and p38 cascades serve as potent regulators of cellular functions in response to a wide variety of environmental stressors, such as ultraviolet (UV) radiation, X-irradiation, heat shock, and osmotic shock, as well as to proinflammatory cytokines, such as tumor necrosis factor (TNF) and interleukin (IL)-1 (3). As shown in **Figure 1**, signaling components comprising these stress-activated MAPKs are well conserved among species. Recent genetic analyses using *Caenorhabditis elegans* and *Drosophila melanogaster* have revealed that signaling through the JNK

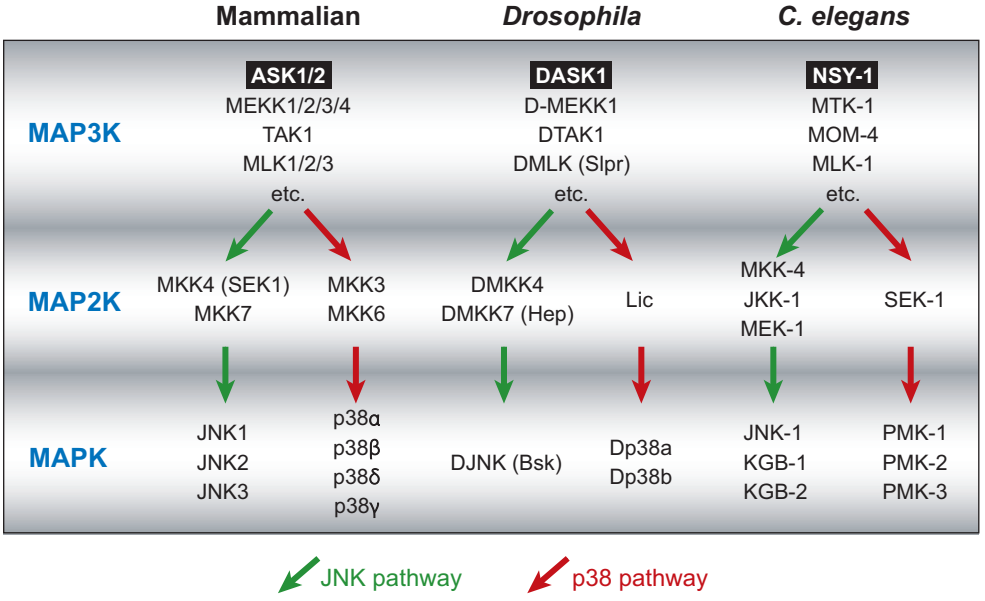

Figure 1

The signaling components comprising major stress-activated MAPK pathways are evolutionarily conserved. The two MAPK pathways that converge on JNKs and p38 MAPKs in mammals, *Drosophila*, and *C. elegans* are shown. Each pathway consists of three classes of protein kinases: MAP3K, MAP2K, and MAPK.

and p38 pathways in response to stress and immune responses is also functionally conserved (4–6).

Apoptosis signal-regulating kinase 1 (ASK1, also designated as MAPKKK5 or MAP3K5) is one of a growing number of MAP3Ks identified in the JNK and p38 pathways (7, 8). ASK1 is activated by various stressors, including oxidative stress, endoplasmic reticulum (ER) stress, and calcium overload, as well as by receptor-mediated inflammatory signals, such as TNF and lipopolysaccharide (LPS) (9–12). Exogenous expression of wild-type ASK1 or constitutively active ASK1 induces death of various cells mainly through mitochondria-dependent caspase activation (9, 13, 14). ASK1-deficient cells are resistant to the cell death induced by oxidative stress, TNF, and ER stress, suggesting that ASK1 is a pivotal component in stress-induced cell death (10, 15). Previous studies have also suggested that ASK1 mediates signaling for determination of cell fate, such as differentiation and survival, probably in a cellular context-dependent manner (16–18). ASK1 thus also appears to be a pivotal component in a broad range of biological activities. Consistent with this, recent analysis of ASK1-deficient mice has demonstrated that the ASK1-p38 pathway is required for the innate immune response (12). Importantly, NSY-1 and PMK-1, *C. elegans* orthologs of ASK1 and p38, respectively, have been shown to play critically important roles in the defense system against pathogenic bacteria (19), suggesting that ASK1 serves as an evolutionarily conserved regulator of this innate immune response. In this review, we focus on the molecular mechanisms by which ASK1 and closely related kinases comprising the ASK family function in response to stress and immune responses.

ER: endoplasmic reticulum

Lipopolysaccharide (LPS): a chief pathogen-associated molecular pattern for gram-negative bacteria

Innate immune response: a first line of defense against pathogens based on the recognition of pathogen-associated molecular patterns (PAMPs) by host pathogen-recognition receptors (PRRs)

THE ASK FAMILY

Overall structural features of ASK1 are depicted in **Figure 2**. The human and mouse ASK1 molecules consist of 1374 and 1380 amino acids, respectively, and each possesses a serine/threonine kinase domain in its midportion flanked by long N- and C-terminal regulatory domains (21). ASK2 (also designated MAPKKK6 or MAP3K6) is another member of the ASK family in mammals. The human and mouse ASK2 molecules consist of 1288 and 1291 amino acids, respectively, and each possesses an overall structure similar to that of ASK1 (22). NSY-1 and *Drosophila* ASK1 (DASK1) are the single orthologs of mammalian ASK1 in *C. elegans* and *Drosophila*, respectively, and are considered prototypic molecules of ASK1 and ASK2 (23, 24).

Whereas the primary structures of the N- and C-terminal regulatory domains exhibit a fair degree of divergence in the ASK family, those of the kinase domain are highly conserved. It has been reported that activation of ASK1 is tightly regulated by phosphorylation of a threonine residue within the activation segment in the kinase domain of ASK1 (Thr838 and Thr845 of human and mouse ASK1, respectively) and that the phospho-specific (phospho-ASK) antibody, which recognizes phosphorylation of this threonine residue, monitors the state of activation of ASK1 (25). Because most of the amino acids in the activation segments of the ASK family members are identical, kinase activities of other members of the family appear to be regulated by phosphorylation of the threonine residues corresponding to Thr838 in human ASK1 (Thr806, Thr747, and Thr825 of human ASK2, DASK1, and NSY-1, respectively). It

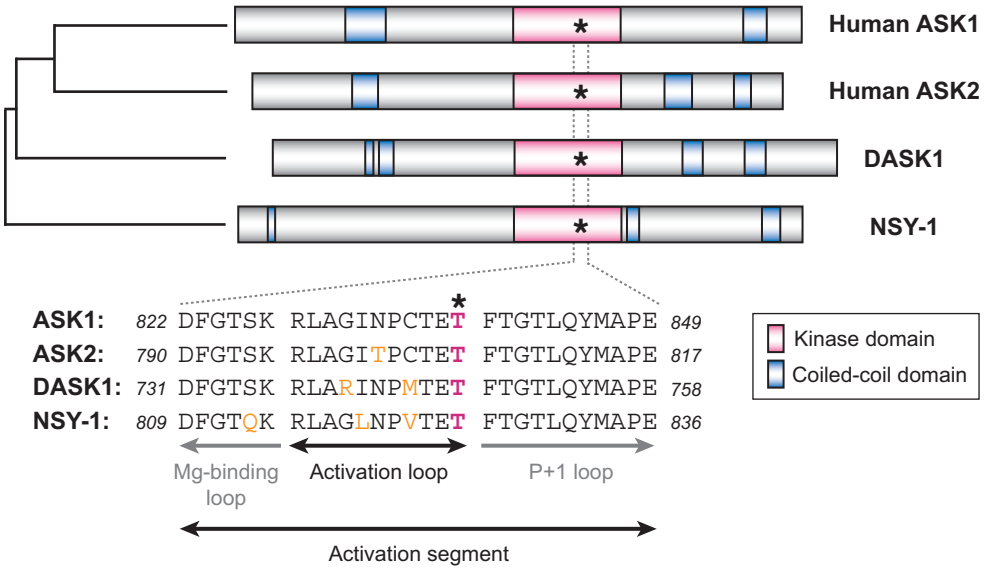

Figure 2

Structural features of ASK family kinases. The ASK family consists of mammalian ASK1 and ASK2 and their single orthologs in *Drosophila* and *C. elegans*, termed DASK1 and NSY-1, respectively. Each molecule possesses a serine/threonine kinase domain in its midportion flanked by long N- and C-terminal regulatory domains. N- and C-terminal coiled-coil domains in ASK1 have been shown to play critical roles in the homophilic interaction between ASK1 molecules, although the roles of coiled-coil domains in other family members have not been extensively examined. Amino acid sequences of the activation segments in the kinase domains are highly conserved among the ASK family members. Asterisks indicate the location of the activating phosphorylation site threonine. Numbers indicate amino acid positions of both ends of the activation segments. Nomenclature for the activation segment follows that of Reference 20.

has indeed been shown that the state of activation of ASK2 is critically controlled by phosphorylation of Thr806 and can be monitored by the phospho-ASK antibody (22). Recently, stress-induced activation of other MAP3Ks, such as MTK1/MEKK4, has also been shown to be regulated by phosphorylation in the activation segment, which appears to be a common mechanism of activation at least in a subset of MAP3Ks (26).

Activation segment: the site of regulatory phosphorylation or interaction with activity modulators within the kinase domains of many kinases

Coiled-coil: a protein folding and assembly motif consisting of α-helices wrapping around each other to form a supercoil

MECHANISM OF ACTIVATION OF ASK1

ASK1 Signalosome

Consistent with the previous finding that homo-oligomerization through the C-terminal coiled-coil (CCC) domain is required for ASK1 activation (25), ASK1 has recently been shown to form a high-molecular-mass (HMM) complex through the CCC domain in the cell (27). When wild-type ASK1 was expressed in cells, ASK1 was found, using gel filtration column chromatography, to form a HMM complex

(>1500 kDa) with relatively high basal activity. On the other hand, an ASK1 mutant that lacked the CCC domain formed a smaller complex (~500 kDa) with no detectable activity. These findings suggest that oligomerization-dependent formation of the HMM complex is required for the kinase activity of ASK1. Under unstimulated conditions, endogenous ASK1 formed a HMM complex similar to that formed by exogenously expressed ASK1. Because this preformed HMM complex includes important regulatory proteins of ASK1 (see below) and appears to serve as a platform for the regulation of basal activity and stress-induced activation of ASK1, we designate this complex the ASK1 signalosome.

Trx: thioredoxin

Reactive oxygen species (ROS): reactive oxygen metabolites including free radicals such as superoxide anion, hydroxyl radical, and singlet oxygen and nonradicals such as hydrogen peroxide

TRAF: TNF receptor-associated factor

MEF: mouse embryonic fibroblast

Roles of Thioredoxin and TRAF Family Proteins in the ASK1 Signalosome

Various ASK1-interacting proteins that have been shown to regulate ASK1 activity are candidates for components of the ASK1 signalosome (**Table 1**). Among them, thioredoxin (Trx) was the first identified ASK1-interacting protein and plays a critical role in the regulation of ASK1 activity (9). Trx inhibits ASK1 kinase activity by direct binding to the N-terminal region of ASK1 (**Figure 3**). Trx has a redox active site, in which two cysteine residues provide the sulfhydryl groups involved in Trx-dependent reducing activity. Only the reduced form [Trx-$(SH)_2$], and not the oxidative form (Trx-S_2) or a mutant of the redox active site of Trx, binds to ASK1. ASK1 activity thus depends on the redox status of Trx (9, 28). In fact, reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), dissociate Trx from ASK1 and thereby activate ASK1 by inducing phosphorylation of a critical threonine residue in the activation loop of ASK1, as described above.

In attempting to determine how Trx exerts its inhibitory effect on ASK1, our group recently found that Trx did not interrupt the homophilic interaction of ASK1 through its CCC domain, which is required for basal activity of ASK1. We found instead that Trx inhibited the homophilic interaction through the N-terminal coiled-coil (NCC) domain of ASK1 in unstimulated cells (28a). Because the NCC domain appears to be required for ROS-induced full activation of ASK1, ASK1 appears to be tightly oligomerized through its NCC domains and thereby to be activated upon ROS-dependent dissociation of Trx from ASK1.

In response to H_2O_2, the ASK1 signalosome forms a higher molecular mass complex following the dissociation of Trx. TNF receptor-associated factor 2 (TRAF2) and TRAF6, members of the TRAF family, which consists of six members, have been identified as components that are specifically recruited to the ASK1 signalosome in ROS-dependent fashion (27, 29). In TRAF6-deficient mouse embryonic fibroblasts (MEFs), the ASK1 signalosome no longer exhibited an H_2O_2-dependent shift toward higher molecular mass, indicating that TRAF6 is required for ROS-induced formation of the activated ASK1 signalosome (27). Consistent with this, H_2O_2-induced activation of ASK1 was strongly suppressed in TRAF2- and TRAF6-deficient MEFs, suggesting that TRAF2 and TRAF6 play important roles in ROS-induced ASK1 activation. These findings suggest that, following ROS-induced dissociation of Trx from the steady-state ASK1 signalosome, reciprocal recruitment of TRAF2 and TRAF6 is

Table 1 ASK1-interacting proteins that have been shown to regulate ASK1 activity

Protein name	Regulation*	Regulatory mechanism	References
14-3-3	–	Interacts through a site involving phosphorylated Ser966 of ASK1	(40–42)
AIP1	+	Induces dissociation of 14-3-3 from ASK1	(94, 95)
Akt	–	Phosphorylates ASK1 at Ser83	(38, 39)
ASK2	+	Phosphorylates ASK1 at Ser838	(22)
Calcineurin	+	Dephosphorylates ASK1 at Ser966	(43)
CaMKII	+	Phosphorylates ASK1	(11)
CDC25A	–	Suppresses oligomerization of ASK1	(96)
CHIP	–	Induces ubiquitination and proteasome-dependent degradation of ASK1	(48)
c-IAP	–	Induces ubiquitination and proteasome-dependent degradation of ASK1	(46)
CIIA	–	Suppresses oligomerization of ASK1	(97)
Daxx	+	Perturbs intramolecular association in ASK1, which may facilitate the oligomerization of ASK1	(98)
FIP200	+	Interacts with ASK1 and TRAF2 and facilitates TNF-induced formation of TRAF2-ASK1 complex	(99)
GSTM1–1	–	Interacts with the N-terminal region of ASK1 and may be involved in heat-shock-induced activation of ASK1	(100, 101)
Grx	–	Interacts with the C-terminal region of ASK1	(102)
HIV-1 Nef	–	Prevents dissociation of Trx from ASK1	(103)
HSP72	–	Suppresses oligomerization of ASK1	(104)
HSP90	–	Facilitates phosphorylation of ASK1 by Akt	(105)
IGF-IR	–	Phosphorylates Tyr residue(s) in the N-terminal region of ASK1	(106)
$p21^{Cip1/WAF1}$	–	Unknown	(107, 108)
PKD	+	Induces dissociation of 14-3-3 from ASK1	(109)
PP5	–	Dephosphorylates ASK1 at Thr838	(34)
QRS	–	Suppresses ASK1 activity by binding in a manner dependent on the cellular glutamine concentration	(110)
Raf-1	–	Interacts with the N-terminal region of ASK1	(111)
Rb	?	Interacts through an LXCXE motif of ASK1 (Leu916-Glu920)	(112)
SKRP1	+	Unknown	(113)
SLK	+	Phosphorylates ASK1	(114)
SOCS1	–	Induces ubiquitination and proteasome-dependent degradation of ASK1	(47)
SUMO-1	–	Physically but not covalently interacts with ASK1 and suppresses the oligomerization of ASK1	(115)
TRAF2	+	Facilitates oligomerization of ASK1	(27, 28, 28a, 29)
TRAF6	+	Facilitates oligomerization of ASK1	(12, 27, 28a, 29)
Trx	–	Interacts with the N-terminal region of ASK1	(9)

*(–) and (+) represent inhibitory and stimulatory effect on ASK1 activity, respectively.

Numbers showing amino acid position are based on human ASK1 sequence. AIP1, ASK1-interacting protein 1; CaMKII, Ca^{2+}/calmodulin-dependent protein kinase type II; CHIP, C-terminus of heat shock protein 70-interacting protein; CIIA, caspase-activated DNase (CAD) inhibitor that interacts with ASK1; FIP200, focal adhesion kinase family interacting protein of 200 kDa; GSTM1-1, glutathione S-transferase Mu 1–1; HSP, heat shock protein; IGF-IR, type 1 insulin-like growth factor receptor; PKD, protein kinase D; PP5, serine/threonine protein phosphatase 5; QRS, glutaminyl-tRNA synthetase; Rb, retinoblastoma protein; SKRP1, stress-activated protein kinase pathway-regulating phosphatase 1; SLK, Ste20-like kinase; SUMO-1, small ubiquitin-related modifier-1; TRAF, TNF receptor-associated factor; Trx, thioredoxin.

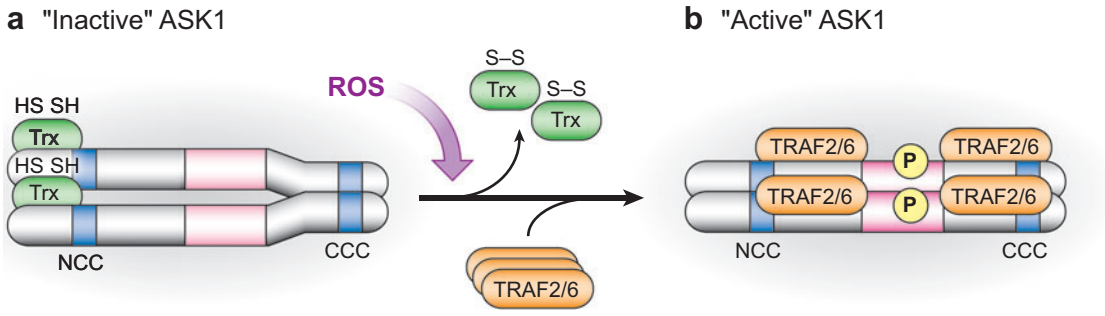

Figure 3

ROS-induced activation of ASK1. The "inactive" ASK1, which is formed by homophilic association of ASK1 through the C-terminal coiled-coil (CCC) domains and the heteromeric complex formation of ASK1 with the reduced form of thioredoxin (Trx), constitutes the core structure of the steady-state ASK1 signalosome. Upon ROS-induced dissociation of Trx from ASK1, TRAF2 and TRAF6 are reciprocally recruited to ASK1 ("active" ASK1) and form the active ASK1 signalosome, in which TRAF2 and TRAF6 appear to facilitate the active configuration of ASK1 at least in part through the homophilic interaction through the N-terminal coiled-coil (NCC) domains.

required at least for effective facilitation of homophilic interaction through the NCC domain, itself a prerequisite for the active configuration of ASK1 signalosome.

ASK2: An Emerging Component of the ASK1 Signalosome

ASK2, a recently characterized member of the ASK family, is identical to MAPKKK6, which has previously been reported to be a MAP3K that activates JNK only weakly but does not activate ERK or p38 at all (30). It has recently been demonstrated that this low activity of ASK2 is attributable to its intrinsic instability (22). Because ASK2 is extremely unstable in ASK1-deficient cells, ASK1 appeared to be required for the stability of ASK2. Indeed, endogenous ASK1 and ASK2 formed a heteromeric complex in unstimulated cells, and, surprisingly, ASK2 in this complex exhibited sufficient basal activity toward the JNK and p38 pathways as a MAP3K. Moreover, ASK2 in a heteromeric complex with ASK1 was activated in response to H_2O_2, and ASK2 was shown to be required for H_2O_2-induced activation of JNK by knockdown experiments. ASK2 thus appears to be a novel component of the ASK1 signalosome, and the ASK1 signalosome may be a suitable platform for ASK2 to exert its activity in response to oxidative stress.

Intriguingly, the kinase activity of ASK1 was not required to support the protein stability and kinase activity of ASK2 by ASK1 (22). On the other hand, ASK2 was found to activate ASK1 by direct phosphorylation of ASK1 Thr838, suggesting that ASK1 and ASK2 facilitate each other's activity by distinct mechanisms in the ASK1 signalosome (**Figure 4**). Whereas the stability and activity of ASK2 depend on ASK1, ASK1 exhibits sufficient stability and activity by itself. Two types of ASK1 signalosomes may thus exist: one with a core structure of the hetero-oligomer comprised of ASK1 and ASK2, and the other with a core structure of the homo-oligomer of

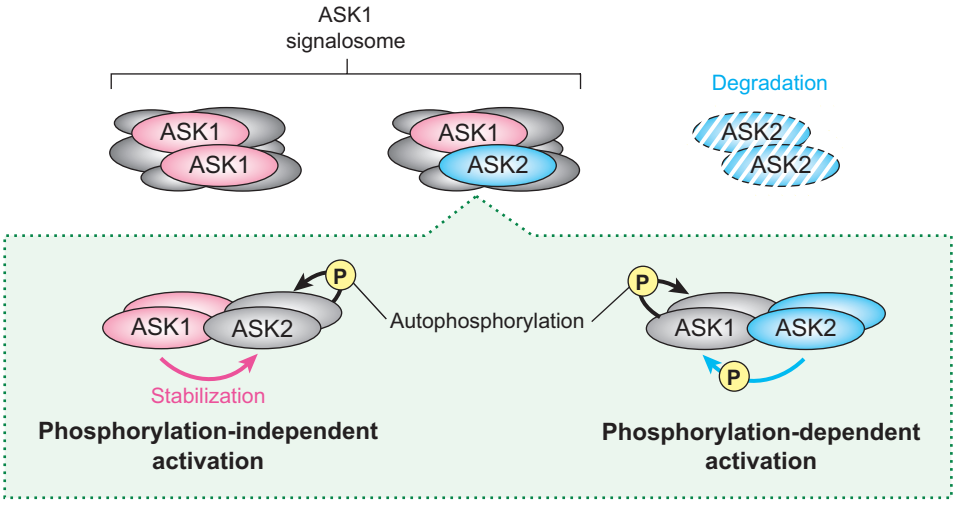

Figure 4

Heteromeric complex of ASK1 and ASK2 in the ASK1 signalosome. Because the stability of ASK2 depends on ASK1, but not vice versa, two types of ASK1 signalosomes may exist: one has a core structure of the homo-oligomer of ASK1 (*upper left*), whereas the other has that of a hetero-oligomer comprised of ASK1 and ASK2 (*upper middle*). In the ASK1-ASK2 complex-based signalosome, ASK1, independent of its kinase activity, supports the stability and active configuration of ASK2, and leads to autophosphorylation of ASK2 (*lower left*). On the other hand, ASK2 induces activation of ASK1 by direct phosphorylation, accelerating the autophosphorylation of ASK1 (*lower right*).

ASK1. Alternative usages of these types of signalosomes may modulate the capacity and sensitivity of cells to various types of stress and contribute to a variety of cellular stress response.

ASK1 in Receptor-Mediated Signaling through ROS Generation

Several lines of evidence have indicated that ROS is generated in response to growth factors and cytokines through their cognate receptors and functions as a signaling intermediate for appropriate cellular response. ASK1 appears to be involved in certain types of receptor-mediated signaling in an ROS-dependent fashion.

TNF is a well-characterized proinflammatory cytokine that regulates inflammation, proliferation, and apoptosis. TNF has been shown to activate the ASK1-JNK and -p38 pathways. The requirement of ASK1 for TNF signaling has been demonstrated using ASK1-deficient MEFs, in which TNF-induced JNK and p38 activation and apoptosis are reduced (15). Interestingly, TNF-induced activation of ASK1 is dependent on the ROS-dependent changes in the ASK1 signalosome, i.e., dissociation of Trx followed by the association of TRAF2 (9, 27, 28). This finding is consistent with a previous one that ROS is intracellularly generated upon TNF treatment (31, 32). ASK1 thus appears to be specifically involved in ROS-dependent elements of TNF signaling.

LPS is one of the pathogen-associated molecular patterns (PAMPs) that initiates a wide range of signal transduction. Among members of the Toll-like receptor (TLR) family, which recognize PAMPs and initiate signal transduction for a variety of cellular responses, TLR4 exists in a heteromeric complex with MD2 and CD14 that specifically recognizes LPS. Upon ligation of LPS, TLR4 recruits TRAF6 by intracellular complex formation, including other adaptor proteins and triggers signaling through the intracellular kinase cascades (33). Recently, ROS and TRAF6 have been found to play critical roles in LPS-induced activation of ASK1 (12). In a macrophage cell line, LPS-induced activation of the ASK1–p38 pathway was suppressed by treatment with antioxidants such as N-acetyl-L-cysteine and propyl gallate. Furthermore, LPS-dependent interaction between TRAF6 and ASK1 was also disrupted by treatment with these antioxidants. Together with the finding that ROS was generated in cells treated with LPS, these results suggest that ROS generated upon TLR4 activation triggers the dissociation of Trx from ASK1 and facilitates active configuration of the ASK1 signalosome by recruiting TRAF6. The biological significance of LPS-induced activation of the ASK1-p38 pathway is discussed below.

TLR: Toll-like receptor

Negative Regulation of ASK1 by PP5

In ASK1-deficient MEFs, the sustained, but not the transient, phase of JNK and p38 activation concomitant with cell death in response to H_2O_2 or TNF is considerably impaired, suggesting that duration of ROS-induced activation of ASK1 is a critical factor in decisions regarding the cell fate (15). Determination of the mechanism of negative feedback regulation of ASK1 is thus of considerable importance.

Protein phosphatase 5 (PP5), a member of the serine/threonine protein phosphatase family, plays a central role in the negative regulation of ASK1 (34). PP5 specifically binds to the activated form of ASK1 in response to ROS and accomplishes dephosphorylation of Thr838, and thus inactivation of ASK1 in a negative feedback mechanism (**Figure 5**). It has recently been shown that the phosphatase activity of PP5 is inhibited by amino acid deprivation or treatment with rapamycin in a mammalian target of rapamycin (mTOR)-dependent fashion (35), as well as by natural polyamines such as spermidine and spermine (36). It has also been shown that expression of PP5 is transcriptionally induced in hypoxic conditions (37). The findings that these novel types of regulation of activity and expression of PP5 appear to profoundly affect ASK1 activity suggest that PP5 may be a critical determinant of the cellular context-dependent duration of ASK1 activation.

Regulation of ASK1 by Posttranslational Modification

As indicated by the requirement of phosphorylation of Thr838 for ASK1 activation, posttranslational modifications appear to critically modulate the function of ASK1 (**Figure 5**). In addition to the phosphorylation of Thr838, the existence of an activating phosphorylation site(s) has been proposed. Calcium/calmodulin-dependent protein kinase type II (CaMKII) is a conserved activator of ASK1 in, at least, *C. elegans*

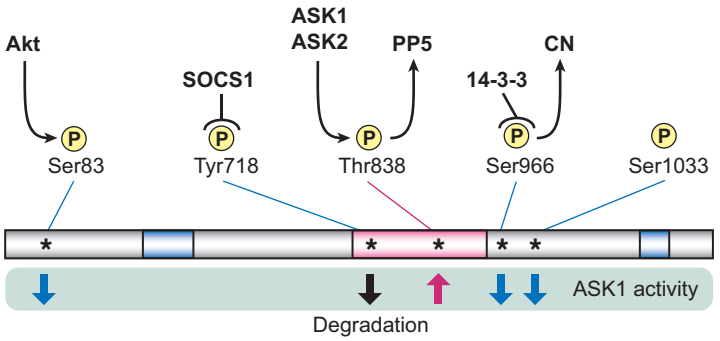

Figure 5

Regulation of ASK1 by phosphorylation. Thr838 is an activating phosphorylation site that is phosphorylated by ASK1 and ASK2 and dephosphorylated by PP5. Ser83 and Ser1033 are inactivating phosphorylation sites, the former of which is phosphorylated by Akt, although mechanisms of the inhibition of ASK1 remain to be determined. Phosphorylation of Ser966 induces association of ASK1 with the 14-3-3 family proteins, which suppresses ASK1 activity by an unknown mechanism. Recently, calcium/calmodulin-activated protein phosphatase calcineurin (CN) has been shown to directly dephosphorylate this site. SOCS1 binds to ASK1 by recognizing phosphorylation of Tyr718 and induces ASK1 degradation in endothelial cells.

and mammals (11, 23). CaMKII activates ASK1, probably by direct phosphorylation of an unidentified residue(s) other than Thr838.

Ser83, Ser966, and Ser1033 have been identified as negative phosphorylation sites. Serine/threonine kinases of the Akt (protein kinase B) family promote cellular survival through inactivation of a number of proapoptotic proteins by direct phosphorylation. Akt has been demonstrated to phosphorylate Ser83 of ASK1 and suppress kinase activity of ASK1 (38, 39). Although the kinases responsible for it remain to be identified, phosphorylation of Ser966 induces association of ASK1 with the 14-3-3 family proteins, which suppresses ASK1 activity by an unknown mechanism (40–42). Recently, calcium/calmodulin-activated protein phosphatase calcineurin (protein phosphatase 2B) has been shown to directly dephosphorylate ASK1 at Ser966 and thus induce dissociation of 14-3-3 from ASK1 (43). The mechanisms of phosphorylation/dephosphorylation and by which phosphorylation of Ser1033 suppresses ASK1 remain to be determined (44).

Another reported type of posttranslational modification of ASK1 is ubiquitination, which triggers proteasome-mediated degradation. TNF binds to two distinct receptors, TNF receptor 1 (TNFR1; expressed ubiquitously) and TNF receptor 2 (TNFR2; expressed predominantly in cells of the immune system) (45). Recently, TNFR2, but not TNFR1, signaling has been shown to induce ubiquitination and subsequent degradation of ASK1 in TNFR2-expressing cells, such as B lymphocytes (46). Cellular inhibitor of apoptosis protein 1 (c-IAP) has been identified as an ubiquitin protein ligase (E3) for ASK1. This system appears to be responsible for termination of TNF-induced MAPK activation by a negative feedback mechanism. However, analyses using endothelial cells have revealed that a member of the suppressor of cytokine signaling (SOCS) family, SOCS1, constitutively binds to ASK1

and induces degradation of ASK1 through the ubiquitin-proteasome system in unstimulated conditions. Upon TNF treatment, ASK1 dissociates from SOCS1 and stabilizes, leading to enhancement of TNF-induced MAPK activation (47). The C terminus of heat shock protein 70–interacting protein (CHIP) has also been identified as an ubiquitin ligase of ASK1, although the physiological settings in which CHIP stimulates ASK1 degradation remain to be determined (48). At present, control of ASK1 stability by ubiquitination appears to strongly depend on cell types. Further investigation is needed to determine whether ubiquitination is a general regulatory modification for ASK1. In determining alternative consequences of ubiquitination, it will be important to examine whether it affects the molecular characteristics of ASK1, e.g., its activity and substrate specificity as a MAP3K.

Transcriptional Control of the *ASK1* Gene

Previous studies have demonstrated that expression of ASK1 in rats is induced in the palatal epithelium after mucoperiosteal injury and in the spinal cord injured by extradural static weight-compression (49, 50). These findings raise the possibility that expression of ASK1 is regulated not only posttranslationally through the ubiquitin-proteasome system, as described above, but also at the transcriptional level. This possibility has been strongly supported by identification, using oligonucleotide microarrays, of the *ASK1* gene as a target of the E2F family of transcription factors, which plays critical roles in cell cycle control (51). mRNA expression of ASK1 was indeed found to be regulated in a cell cycle–dependent fashion at least in human neuroblastoma cells. Moreover, E2F1, E2F2, E2F3, and E2F4, representative members of the E2F family, directly bound to the *ASK1* promoter, and mutation of the E2F-binding site in the promoter abolished responsiveness to E2Fs (52). Several lines of evidence have also suggested that E2Fs play major roles in the regulation of apoptosis. Interestingly, E2F1-induced expression of ASK1 has been suggested to be involved in E2F1-induced apoptosis in a cell type– or cellular context–dependent fashion (53–55). Although there is currently no evidence that E2F-mediated transcriptional control of ASK1 is involved in the injury-induced expression of ASK1 in vivo, regulation of ASK1 expression at the transcriptional level is an important mechanism requiring extensive study for comprehensive understanding of mediation of stress responses through ASK1.

ASK1 IN STRESS RESPONSE

ASK1 in Stress-Induced Cell Death

Excessively generated ROS are generally counteracted by ubiquitously expressed antioxidant proteins, such as glutathione, glutaredoxin, and Trx. Once the generation of ROS exceeds the capacity of such antioxidant proteins or occurs in inappropriate cellular contexts, the intracellular reduction/oxidation (redox) balance is disturbed and cells are subject to oxidative stress. One of the consequences of strong activation of intracellular signaling by severe oxidative stress is the induction of cell death. In ASK1-deficient cells, H_2O_2-induced cell death was significantly impaired, providing

direct evidence that ASK1 is required for oxidative stress-induced cell death (15). Various cytotoxic stressors such as methylglyoxal, a reactive endogenous metabolite that is produced through the process of degradation of triose-phosphates (56), certain metal ions (57) and anticancer drugs (58), and UV irradiation (59) have been found to trigger cell death at least in part though ROS-mediated ASK1 activation.

Recently, ASK1 has also been found to be required for ER stress-mediated cell death (10). ER stress, which is caused by the accumulation of unfolded and/or misfolded proteins in the ER lumen, is one type of intracellular stressor (60). ER stress triggers the unfolded protein response (UPR), which includes the general attenuation of protein synthesis and the transcriptional activation of the genes encoding ER-resident chaperones and molecules involved in the ER-associated degradation (ERAD). Whereas the UPR in coordinated fashion reduces ER stress by restoration of the protein-folding capacity of the ER, severe and/or prolonged ER stress eventually leads to cell death.

Among several ER-resident transmembrane proteins that serve as apical signaling molecules of ER stress, IRE1, a type 1 transmembrane protein that has a serine/threonine kinase domain in the cytoplasmic region, appears to play a role in ER stress-mediated cell death through MAPK activation. TRAF2 and the ASK1-JNK pathway play pivotal roles when IRE1 triggers cytoplasmic signaling for ER stress-induced cell death (10, 61). TRAF2 binds to IRE1 and recruits ASK1 in an ER stress-dependent fashion, with resulting activation of ASK1 in the IRE1-TRAF2-ASK1 complex. ASK1 thus activates the JNK pathway, although ER stress-induced activation of the p38 pathway through ASK1 has not yet been extensively examined. This mechanism of activation is supported by the findings that JNK activation induced by the treatment with thapsigargin, which triggered ER stress by depleting lumenal calcium stores, or the overexpression of IRE1 was abolished in ASK1-deficient MEFs (10). ER stress-induced cell death is also strongly impaired in MEFs and primary neurons derived from ASK1-deficient mice, suggesting that the TRAF2-ASK1-JNK pathway plays central roles in ER stress-induced cell death. ASK1 may thus be critically involved in decisions regarding cell fate in response to various stressors.

The existence of death-inducing activity of ASK1 has been strongly supported by genetic analyses using *Drosophila*. DASK1 was, together with a *Drosophila* ortholog of TRAF (DTRAF1), identified through a dominant modifier screen exploring the mediators of cell death induced by Reaper, a proapoptotic factor during *Drosophila* embryogenesis (24). Overexpression of DTRAF1 activated DASK1 and DJNK and induced cell death, and mutation of DTRAF1 or expression of a dominant negative mutant of either DASK1 or *Drosophila* JNK (DJNK) suppressed Reaper-induced cell death. These findings strongly suggest that the TRAF-ASK1-JNK pathway mediates conserved death signaling in mammalian and *Drosophila* cells.

Is Cell Death the Only Consequence of Stress-Induced ASK1 Activation?

Apoptotic elimination of cells that have suffered severe stress-induced injury is an effective means of maintaining homeostasis in multicellular organisms. In response

to weak or transient stress, however, cells modulate cellular status, e.g., by activating intrinsic survival signals and by stabilizing or enhancing differentiated phenotypes to adapt to or resist such stress. The requirement of ASK1 for the production of inflammatory factors upon LPS challenge in macrophagic cells (12) (details of which are provided in the next section) provides a hint that is useful for evaluation of the function of ASK1 other than induction of cell death.

ASK1ΔN, a constitutively active mutant of ASK1, is useful for exploring such functions of ASK1. The rat pheochromocytoma cell line PC12, which differentiates with sympathetic neuron-like characteristics, including neurite outgrowth upon treatment with nerve growth factor (NGF), was shown to extend neurites upon expression of ASK1ΔN even in the absence of NGF (16). ASK1ΔN-expressing PC12 cells were also found to survive in serum-starved conditions, suggesting that ASK1 may mediate signals for differentiation and survival. Consistent with these findings, expression of ASK1ΔN in keratinocytes induces an enhanced differentiated phenotype, as represented by morphological changes concomitant with upregulation of differentiation marker proteins (17). Given that the epidermis is a primary barrier against the external environment and is maintained by precise regulation of keratinocyte proliferation, differentiation, and apoptosis in self-renewing fashion, ASK1 may participate in stress responses in keratinocytes by regulating their state of differentiation (62).

Erythropoiesis also requires a correct balance among cell proliferation, differentiation, and apoptosis. It has recently been demonstrated that erythroid differentiation is mediated by the death receptor Fas and activation of its downstream signals through the ASK1-JNK/p38 pathways and the caspase cascade (18). Expression of ASK1 protein (the state of activation of which has unfortunately not been examined) increased during in vitro differentiation of primary fetal liver-derived erythroblasts, and JNK and p38 were activated especially in the terminal differentiation stage. On the other hand, in homozygous *lpr* mutant erythroblasts, which exhibited a clear lack of differentiation and no cell-surface expression of Fas, ASK1 induction and JNK/p38 activation were severely impaired, suggesting that ASK1 may regulate erythroid differentiation. Together with the previous finding that erythroid differentiation was stimulated through transient activation of JNK and p38 in response to certain environmental stressors (63), these findings suggest that ASK1 may function as a stress-regulated modulator of fate in cells of the erythroid lineage.

Cellular senescence, which was originally described as the irreversible arrest of cell growth after prolonged proliferation under nonphysiological conditions, has been considered a type of cellular response to various stressors, such as oxidative stress, DNA damage, telomere dysfunction, and some anticancer drugs (64). In the case of tumor formation, induction of senescence is generally required to suppress tumor progression by maintaining the tumor cells in a premalignant state. Consistent with accumulating evidence indicating the critical involvement of p38 in the senescence-executing signaling in response to diverse stimuli (65, 66), ASK1 has recently been shown to induce senescence in endothelial cells (67). Expression of ASK1ΔN induced endothelial cell senescence, and ASK1 appeared to be required for the induction of senescence in endothelial cells cultured with high glucose, which mimics the hyperglycemia commonly present in diabetic patients (the pathophysiological role of ASK1

in this setting is discussed below). This finding provides further evidence that ASK1 mediates diverse types of biological signaling involved in decisions regarding cell fate in the presence of various stressors.

ASK1 IN IMMUNE RESPONSES

NSY-1 in *C. elegans* Immune Response

The innate immune system represents a first line of host defense against a wide range of pathogens. Because this system is evolutionarily conserved from invertebrates to vertebrates, genetic models, such as *C. elegans* and *Drosophila*, can be used as efficient systems for study of it. By genetic screening for *C. elegans* mutants with enhanced susceptibility to pathogen (Esp), *esp-2* and *esp-8* were identified as genes required for pathogen resistance (19). *esp-2* and *esp-8* mutants were highly vulnerable to pathogenic bacteria such as gram-negative *Pseudomonas aeruginosa* and gram-positive *Enterococcus facecalis* and *Staphylococcus aureus*, suggesting the critical involvement of these genes in response to a broad spectrum of bacterial infections (19, 68). *esp-2* and *esp-8* were found to encode SEK-1, a MAP2K homologous to mammalian MKK3/MKK6 and MKK4, and NSY-1, respectively. In *esp-2* and *esp-8* mutants, infection-induced activation of PMK-1 (a *C. elegans* ortholog of mammalian p38) was abolished. Moreover, RNA interference-based knockdown of PMK-1 revealed that PMK-1 was also required for resistance to pathogenic bacteria. These findings strongly suggest that the NSY-1-SEK1-PMK-1 axis plays pivotal roles in the innate immune response in *C. elegans*.

ASK1 in Innate Immune Signaling

Involvement of the ASK1-p38 axis in mammalian innate immunity has also been proposed (12). In splenocytes and bone marrow–derived dendritic cells (BMDCs) derived from ASK1-deficient mice, LPS-induced activation of p38 was attenuated. However, neither JNK activation nor the signaling pathway leading to the activation of transcription factor nuclear factor-κB (NF-κB) was affected in LPS-treated ASK1-deficient cells. LPS-induced production of inflammatory cytokines, such as TNF, IL-6, and IL-1β, was also attenuated in ASK1-deficient splenocytes and BMDCs, indicating that activation of the ASK1-p38 pathway is required for inflammation in response to LPS. Consistent with these in vitro findings, ASK1-deficient mice were resistant to LPS-induced septic shock. The levels of two principal factors responsible for septic shock, TNF and nitric oxide, were reduced in the serum of ASK1-deficient mice. These findings strongly suggest that the signaling through ASK1 in cellular responses not only to general stressors but also to microbial infection is highly conserved among species (69). The common involvement of ASK1 in both responses to stress and the innate immune response also suggests that ASK1 may integrate the stress signaling that conveys information about internal and external cellular conditions and the innate immune signaling that alerts cells to the presence of pathogenic microorganisms.

As described above, ASK1 is activated downstream of TLR4 and TRAF6 in a manner strongly dependent on ROS generation (12). The selective requirement of

ASK1 for TLR4 signaling was demonstrated by the finding that activation of ASK1 and p38 in response to peptidoglycan (a chief pathogen-associated molecular pattern for gram-positive bacteria and a ligand for TLR2), poly(I;C) (a synthetic double-stranded RNA and a ligand for TLR3), and unmethylated CpG DNA (a ligand for TLR9) was not diminished in ASK1-deficient cells. Although the mechanisms underlying ROS generation downstream of TLR4 are not fully understood, the possibility of involvement of NOX4, a member of the NOX family of NADPH oxidases that transfer electrons from NADPH to O_2 to generate superoxide anions (70), has been suggested. It has recently been shown that NOX4 interacts with TLR4 in response to LPS and is required for LPS-induced ROS generation and NF-κB activation (71). Moreover, LPS-dependent activation of interferon (IFN) regulatory factor (IRF) 3, a ubiquitously expressed transcription factor that induces a distinct subset of IFN-stimulated regulatory element-containing genes, has also been found to depend on NOX4-induced ROS generation followed by activation of the ASK1-p38 cascades (72). These findings are further evidence of the importance of ROS as a signaling intermediate for immune response through ASK1 activation.

Unexpected Role of ASK1 in Injury-Induced Hair Growth

A study demonstrating increase in ASK1 expression upon epithelial injury in rats raised the possibility that ASK1 plays roles in epithelial wound healing (49). However, in a recent study, no clear differences in the wound healing process after skin injury were found between wild-type and ASK1-deficient mice (73). Instead, and unexpectedly, dramatic retardation of wound-induced hair regrowth in the skin was observed in ASK1-deficient mice, whereas hair follicle development in embryos and plucking-induced hair regrowth, another experimental model of induced hair growth, appeared normal (73). Consistent with oligonucleotide microarray findings showing that the expression of macrophage-specific markers, as well as that of macrophage-specific chemotactic and activating factors, was reduced in the wounded skin of ASK1-deficient mice, diminished recruitment of macrophages to wounds was immunohistochemically demonstrated in ASK1-deficient mice. Interestingly, intracutaneous transplantation of bone marrow-derived macrophages (BMDMs) stimulated hair growth in wild-type but not ASK1-deficient mice. On the other hand, intracutaneous transplantation of cytokine-activated BMDMs stimulated hair growth in both wild-type and ASK1-deficient mice. These findings strongly suggest that ASK1 is required for wounding-induced infiltration and activation of macrophages, suggesting broad involvement of ASK1 in systemic immune responses in pathological conditions.

ASK1 IN HUMAN DISEASES

ASK1-deficient mice exhibit no developmental abnormalities and appear healthy under standard laboratory conditions. Once ASK1-deficient mice are subjected to certain pathological insults, however, they exhibit pathological phenotypes that may be less or more severe than those of identically treated wild-type mice, as summarized in **Table 2**. These models of disease in mice provide valuable tools for dissecting the

Table 2 Phenotypes of ASK1-deficient mice in disease-associated models

Challenge	Phenotype	Reference
Ang II infusion	Attenuated cardiac hypertrophy and remodeling	(75)
MI caused by LCA ligation Pressure overload by TAC	Attenuated cardiac hypertrophy and remodeling	(76)
Ischemia-reperfusion (LCA ligation followed by reperfusion)	Reduced size of myocardial infarction	(78)
Crossed with Raf CKO mice	Reduced heart dysfunction/dilation and cardiac fibrosis/apoptosis that are observed in Raf CKO mice	(116)
Cuff-induced vascular injury	Attenuated neointimal formation	(79)
Unilateral hindlimb ischemia	Less developed collateral vessels and angiogenesis Reduced infiltration of macrophages and T lymphocytes Reduced expression of VEGF and MCP-1	(117)
STZ infusion (a diabetes model)	Reduced induction of senescent endothelial cells in aorta Reduced elevation of plasma PAI-1 levels	(67)
Ischemic retinal injury	Increased number of surviving retinal neurons	(118)

Ang, angiotensin; MI, Myocardial infarction; LCA, left coronary artery; TAC, transverse thoracic aorta constriction; Raf CKO, cardiac muscle-specific Raf-1 knockout; VEGF, vascular endothelial growth factor; MCP-1, monocyte chemoattractant protein-1; STZ, streptozotocin; PAI-1, plasminogen activator inhibitor-1.

pathophysiological roles of ASK1. In this section, we focus on representative diseases in which ASK1 appears to be involved.

Cardiovascular Diseases

Cardiac hypertrophy, which can occur in response to a variety of stimuli to the heart, is thought to be an initial compensatory response to preserve cardiac function. However, sustained hypertrophic stimulation becomes a risk factor for cardiac morbidity and mortality. There is increasing evidence that ROS generated by angiotensin II (Ang II) through its cognate G protein–coupled receptor plays an important role in pathophysiological cardiac hypertrophy and remodeling (74). It has recently been reported that ASK1 is a key molecule in Ang II–induced cardiac hypertrophy and remodeling in vivo (75). In the left ventricle (LV), ASK1 was activated in a ROS-dependent fashion upon treatment with Ang II. In ASK1-deficient mice, Ang II–induced activation of JNK and p38 was markedly reduced compared with that in wild-type mice. Moreover, Ang II–induced cardiac hypertrophy and remodeling were significantly inhibited in ASK1-deficient mice. In addition, in models of myocardial infarction and pressure overload, cardiac hypertrophy and remodeling were inhibited in ASK1-deficient mice (76). These findings suggest that the ASK1-JNK/p38 pathways are critically involved in cardiac diseases.

Although reperfusion of coronary flow is required to salvage the ischemic myocardium, paradoxically, reperfusion of an ischemic region results in organ dysfunction and cell death. One of the major mediators of reperfusion injury is ROS generated by injured myocytes and endothelial cells in the ischemic zone as well as neutrophils

that enter the ischemic zone (77). A recent study has suggested that ASK1 is involved in myocardial cell death in ischemic-reperfused heart (78). When subjected to myocardial ischemia-reperfusion injury, ASK1-deficient mice exhibited decreased infarct size and reduced susceptibility to myocardial cell death. Consistent with this, ASK1-deficient cardiomyocytes were more resistant to H_2O_2-induced cell death than wild-type cells in vitro. Together with the involvement of ASK1 in cardiac hypertrophy and remodeling, these findings suggest that ASK1 may be widely involved in the pathogenesis of heart failure.

Vascular intimal hyperplasia occurs primarily as a part of the pathogenesis of coronary artery disease or secondary to therapeutic intervention in relieving vascular occlusion. Neointimal formation after cuff placement around the femoral artery in ASK1-deficient mice was found to be significantly attenuated compared with that in wild-type mice (79). Cultured vascular smooth muscle cells from ASK1-deficient mice were found to be defective in both proliferation and migration. These findings indicate that ASK1 plays a key role in vascular intimal hyperplasia.

Polyglutamine (polyQ) disease: an inherited neurodegenerative disorder that is caused by expanded polyglutamine within a pathogenic protein

Neurodegenerative Diseases

Several lines of evidence suggest that neuronal cell death plays critical roles in the pathogenesis of neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), polyglutamine (polyQ) diseases, amyotrophic lateral sclerosis (ALS), and prion diseases. ASK1 has recently been proposed to be involved in the pathogenesis of neurodegenerative diseases as a proapoptotic signaling intermediate (80).

It is widely accepted that the intra- or extracellular aggregation of structurally abnormal proteins is a feature common to these diseases. Recent evidence indicates that perturbation of the UPR and subsequent ER stress-mediated cell death might be the link between such protein aggregation and the pathogenesis of neurodegenerative diseases. It has been demonstrated that ASK1 is required for expanded polyQ-induced neuronal cell death through a mechanism involving ER stress evoked by proteasomal dysfunction (10). Expression of expanded polyQ activated IRE1 and induced the transcription of UPR target genes, such as CHOP and BiP, in PC12 cells and primary neurons, indicating that ER stress is induced by polyQ aggregation. In addition, expanded polyQ fragments impaired proteasome activity. Treatment of cells with proteasome inhibitors also induced UPR, suggesting that expanded polyQ induces ER stress in part through proteasomal dysfunction. PolyQ-induced ER stress also activated the IRE1-TRAF2-ASK1-JNK pathway. In ASK1-deficient primary neurons, the cell death induced by treatment with proteasome inhibitors or expression of expanded polyQ was attenuated. The ER stress-induced cell death pathway through ASK1 thus appears to be triggered by pathogenic polyQ through proteasomal dysfunction and to play an important role in the pathogenesis of polyQ diseases.

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. Parkin has been found to be responsible for autosomal recessive juvenile Parkinsonism (AR-JP) and to possess E3 ubiquitin ligase activity,

which is defective in AR-JP-linked mutants (81). A study using *Drosophila* genetics suggested the existence of functional interactions between Parkin and JNK signaling (82). *Drosophila parkin* loss-of-function mutants exhibited shrinkage of dopaminergic neurons with increased DJNK activity. Results of epistatic analysis between *parkin* and the JNK pathway suggested that Parkin might negatively regulate DJNK and the upstream activators of the DJNK pathway, such as DASK1 and Hep (*Drosophila* MKK7), in E3 activity-dependent fashion. These findings suggest that loss of Parkin function activates the DASK1-JNK pathway and promotes degeneration of dopaminergic neurons. Importantly, this negative regulation of the JNK pathway by Parkin is also conserved in mammalian cells (82), suggesting that the ASK1-JNK pathway may be involved in the pathogenesis of PD.

Amyloid-β (Aβ): 39–43–amino acid peptides cleaved by β- and γ-secretases from the amyloid precursor protein (APP)

AD, a neurodegenerative disorder with progressive loss of memory and cognitive impairment, is pathologically characterized by cerebral neuritic plaques of amyloid-β (Aβ) peptide and neurofibrillary tangles. It has been reported that Aβ impairs mitochondrial redox activity and increases the generation of ROS, leading to apoptotic neuronal death in an antioxidant-sensitive fashion, whereas Aβ has also been suggested to exert neurotoxic effects through the induction of ER stress (83–85). It has recently been shown that Aβ activates ASK1 mainly through the generation of ROS and not through ER stress in cultured neuronal cells, and that ASK1-deficient neurons are defective in Aβ-induced JNK activation and cell death (86). These findings suggest that ROS-mediated ASK1 activation by Aβ is an important step in the pathogenesis of AD.

Diabetes

Impairment of tight regulation of insulin signaling results in the development of diabetes. Tyrosine phosphorylation of the insulin receptor substrate (IRS) proteins, including IRS-1 and IRS-2, through the insulin receptor, which functions as a protein tyrosine kinase, is an early key event in the insulin signaling (87). However, serine/threonine phosphorylation of IRS proteins serves as a negative control mechanism that inhibits the activity of IRS proteins (88). JNK-induced serine phosphorylation of IRF-1 has recently been shown to play a critical role in the impairment of activity of insulin in response to TNF produced by adipose tissue; JNK-induced phosphorylation of IRS-1 uncoupled IRS-1 from the insulin receptor (89). Furthermore, deficiency of JNK1, an isoform of JNK, in mice resulted in improved insulin sensitivity and enhanced insulin receptor signaling capacity (90). More recently, ASK1 was suggested to be involved in TNF-induced serine phosphorylation of IRF-1 through JNK, and TNF-induced ROS generation was found to be critical for IRS-1 phosphorylation through activation of ASK1 and JNK (91). Because insulin resistance is evoked by diverse factors such as free fatty acids and cellular stress, as well as TNF, ASK1 might play a role in the increase in insulin resistance induced by a wide variety of oxidative stressors.

Vascular aging is accelerated in diabetic patients. Accumulating evidence indicates that diabetes promotes endothelial cell senescence in part through the hyperglycemia-induced signaling pathways, including ASK1, as described above (67, 92). In mice

challenged with streptozotocin (STZ), a well-established rodent model of diabetes (93), induction of senescent endothelial cells in the aorta and elevation of a clinical marker of impaired fibrinolysis, plasma plasminogen activator inhibitor-1 (PAI-1) levels, a risk factor for cardiovascular diseases, were observed. In STZ-challenged ASK1-deficient mice, these changes were significantly attenuated, indicating the critical involvement of ASK1 in hyperglycemia-induced endothelial cell senescence and PAI-1 expression (67). ASK1 may thus be a therapeutic target for prevention of diabetic vascular complications.

SUMMARY POINTS

1. ASK1 and closely related kinases comprise the ASK family, each member of which serves as a MAP3K of the JNK and p38 MAP kinase pathways and plays pivotal roles in response to cellular stress and the innate immune response.
2. The basal activity and stimulation-dependent activation of ASK1 are highly regulated in a high-molecular-weight mass protein complex designated the ASK1 signalosome.
3. ROS-dependent activation of ASK1 is accomplished by the dissociation of thioredoxin from ASK1 followed by inclusion of TRAF2 and TRAF6 in the ASK1 signalosome.
4. ASK1 participates in receptor-mediated signaling, such as that by TNF and LPS, in a ROS-dependent fashion.
5. To appropriately respond to a wide variety of cellular stress, ASK1 mediates diverse biological signals leading to cell death, differentiation, survival, and senescence.
6. ASK1 is an evolutionarily conserved signaling intermediate for innate immunity. In mammals, ASK1 is activated downstream of TLR4 and TRAF6 in a ROS-dependent fashion and is required for LPS-induced production of inflammatory factors.
7. ASK1 appears to play roles in the pathogenesis of human diseases that are closely related to dysfunction of cellular responses to oxidative and ER stressors.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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Contents

Indexes

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