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Forum Review

Physiological Roles of ASK1-Mediated Signal Transduction in Oxidative Stress- and Endoplasmic Reticulum Stress-Induced Apoptosis: Advanced Findings from ASK1 Knockout Mice

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ABSTRACT

Apoptosis, a molecularly regulated form of cell death, is essential for the normal functioning and homeostasis of most multicellular organisms, and can be induced by a range of environmental, physical, and chemical stresses. As the cellular decision to live or to die is made by the coordinated action and balancing of many different pro- and antiapoptotic factors, defects in control of this coordination and balance may contribute to a variety of human diseases, including cancer and autoimmune and neurodegenerative conditions. In recent years, multiple factors associated with the execution of apoptosis, such as caspases and Bcl-2 family members, have been discovered and their complicated signaling and molecular interactions have been demonstrated; however, the precise mechanistic basis for intracellular and/or extracellular stress-induced apoptosis remains to be fully characterized. Protein kinases contribute to regulation of life and death decisions made in response to various stress signals, and the actions of pro- and antiapoptotic factors are often affected by modulation of the phosphorylation status of key elements in the execution of apoptosis. Apoptosis signal-regulating kinase 1 (ASK1) is a member of the mitogen-activated protein (MAP) kinase kinase kinase family, which activates both the MKK4/MKK7–JNK and MKK3/MKK6–p38 MAP kinase pathways and constitutes a pivotal signaling pathway in various types of stress-induced apoptosis. We have recently shown through ASK1 gene ablation in mice that ASK1 plays essential roles in oxidative stress- and endoplasmic reticulum (ER) stress-induced apoptosis. These stresses are closely linked to physiological phenomena in the control of cell fate, and the resultant apoptosis is implicated in the pathophysiology of a broad range of human diseases. This article reviews our new findings on the physiological roles of ASK1-mediated signal transduction in stress responses and the molecular mechanisms by which ASK1 determines cell fate such as survival, differentiation, or apoptosis, with special focus on the regulatory mechanisms of ASK1-mediated apoptosis induced by oxidative stress and ER stress. *Antioxid. Redox Signal.* 4, 415–425.

INTRODUCTION

APOPTOSIS is a highly regulated and organized death process that serves critical functions in the deletion of autoreactive lymphocytes, elimination of virally infected and malignant cells, and development of complex multicellular organisms. Dysregulation of apoptosis results in loss of homeostasis as a consequence of inappropriate cell survival or death and promotes the development of various diseases, including tumorigenesis, atherosclerosis, autoimmune diseases, diabetes mellitus, ischemic tissue damage, and neurodegeneration. The recent astounding pace of research in this area has expanded understanding that this mechanism is regulated largely by pro- and antiapoptotic factors for or against the

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final death event. Although many components implicated in the execution of apoptosis have been determined, the mechanisms regulating stress-induced apoptosis networks through sensing of cell damage remain controversial.

Phosphorylation and dephosphorylation of intracellular effector molecules are the most common and important regulatory mechanisms in signal transduction, and control a variety of cellular events from cell growth to apoptosis. Protein kinases have many substrates implicated in the execution of apoptosis, such as p53 (20, 46), caspases (7), and Bcl-2 family members (4, 13), and the delicate crosstalk between protein kinases and these apoptotic molecules is perfectly suited to regulate life and death decisions made in response to intracellular and/or extracellular signals. Mitogen-activated protein (MAP) kinase cascade is evolutionarily well conserved in all eukaryotic cells and is typically composed of three kinases that establish a sequential activation pathway comprising a MAP kinase kinase kinase (MAPKKK), MAP kinase kinase (MAPKK), and MAP kinase (MAPK) (32). Among these three components, MAPKKKs, as the highest signaling modules, sense the degree of stress-induced cell damage in the upstream induction phase of apoptosis and determine cell fate by regulation of the MAPK cascade; finally, the downstream MAP kinases may phosphorylate various substrates as the effectors and executioners of apoptosis.

c-Jun N-terminal kinase (JNK), p38 MAP kinase, and extracellular signal-regulated kinase (ERK) are well characterized subgroups of a large MAP kinase family. These kinase pathways are structurally similar, but functionally distinct (Fig. 1). Generally, ERK is rapidly activated by a variety of cell growth and differentiation stimuli and plays a central role in mitogenic signaling, whereas JNK and p38 are primarily activated by various environmental stresses, including osmotic shock, UV radiation, heat shock, oxidative stress, protein synthesis inhibitors, chemotherapeutic agents, stimulation of Fas, and proinflammatory cytokines such as tumor necrosis factor-α (TNFα) and interleukin-1. Specific inhibitors of JNK pathways and p38, or dominant-negative mutants of JNK and p38, suppress various types of stress-induced apoptosis (33, 35, 43). In JNK3 knockout mice and JNK1/JNK2 double knockout mice, glutamate-induced hippocampal cell death and UV radiation-induced apoptosis are prevented to remarkable extents (55, 62). It has thus been suggested that JNK and p38 play critical roles in signal transduction of stress-induced apoptosis.

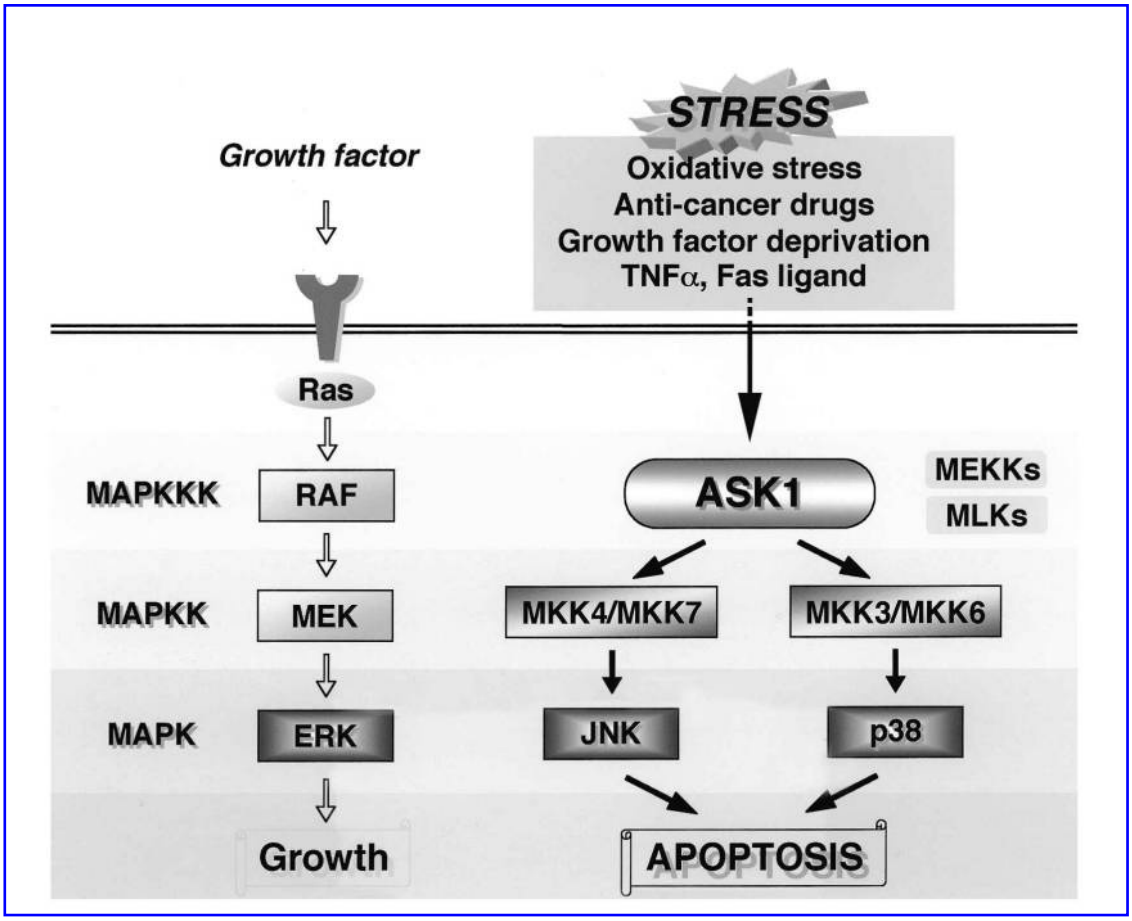

FIG. 1. The mammalian MAP kinase modules and ASK1 as a stress-responsive kinase. MAP kinase cascade is typically composed of three kinases that establish a sequential activation pathway comprising MAPKKK, MAPKK, and MAPK. In mammals, three major subgroups of MAP kinase, ERK, JNK, and p38, have been identified, which are structurally similar, but functionally distinct. MAP kinases regulate and determine cell fate, such as growth, differentiation or apoptosis, in response to environmental changes. ASK1, as a member of the MAPKKK family, is activated in response to a variety of stress, including oxidative stress, anticancer drugs, growth factor deprivation, and death receptor ligands such as TNFα and Fas ligand, and then induces apoptosis through both the MKK4/MKK7–JNK and MKK3/MKK6–p38 signaling cascades.

Apoptosis signal-regulating kinase 1 (ASK1) is a member of the MAPKKK family and activates both the MKK4/MKK7–JNK and MKK3/MKK6–p38 signaling cascades (23, 24). ASK1 is activated in cells treated with death receptor ligands such as TNFα and Fas ligand (8, 44) and subjected to various types of stress such as hydrogen peroxide (H_2O_2), anticancer drugs, and growth factor deprivation (Fig. 1) (10, 28, 49). Overexpression of wild-type ASK1 or the constitutively active mutant induced apoptosis in various types of cells through mitochondria-dependent caspase activation (18, 24, 49, 61). We have also revealed that ASK1 functions as an important molecular sensor of degree of internal and/or external environmental stresses to determine cell fate, such as survival, differentiation, or apoptosis (37, 50, 53). Furthermore, our recent findings have been obtained by analysis of ASK1-deficient mice; we have found that ASK1 plays essential roles in stress-induced apoptosis, especially oxidative stress- and endoplasmic reticulum (ER) stress-induced apoptosis (54; Nishitoh *et al.*, submitted for publication).

In this review, we discuss the physiological roles of ASK1-mediated signal transduction in stress responses and the molecular mechanisms by which ASK1 determines cell fate, on the basis of our new findings obtained by ASK1 gene ablation in mice. We focus here on the regulatory mechanisms of ASK1-mediated apoptosis induced by oxidative stress and ER stress.

OXIDATIVE STRESS-INDUCED APOPTOSIS IS MEDIATED BY ASK1–JNK/P38 PATHWAY

Role of ASK1 as a redox sensor

The reduction–oxidation (redox) state of the cell is a consequence of the precise balance between the levels of oxidizing and reducing equivalents, that is, reactive oxygen species (ROS), such as superoxide anions and H_2O_2, and endogenous thiol buffers present in the cell, such as glutathione and thioredoxin (Trx), which protect cells from oxidative stress-induced cell damage. Under oxidative conditions, increased highly reactive radicals can attack DNA, RNA, proteins, and lipid bilayer, which may compromise various cellular functions and homeostasis. Elevation of ROS in excess of the buffering capacity results in potentially cytotoxic oxidative stress, which leads to apoptosis as a final event (14).

ASK1 is strongly activated in cells exposed to various oxidants such as H_2O_2 and diamide. Oxidative stress-induced activation of ASK1 leads to apoptosis in various types of cells (24, 49). Trx was identified as a negative regulator of the ASK1–JNK/p38 pathway, through yeast two-hybrid screening for ASK1-binding proteins (49). In resting cells, ASK1 constantly forms an inactive complex with Trx, but upon treatment of cells with TNFα or various oxidants such as H_2O_2 and diamide, ASK1 is dissociated from Trx and activated by subsequent modifications, including oligomerization and auto- and/or cross-phosphorylation at the sites of Thr^{845} within the activation loop of ASK1 (16, 49; Tobiume *et al.*, submitted for publication). Trx is a redox-regulatory protein that has two redox-sensitive cysteine residues within the con-

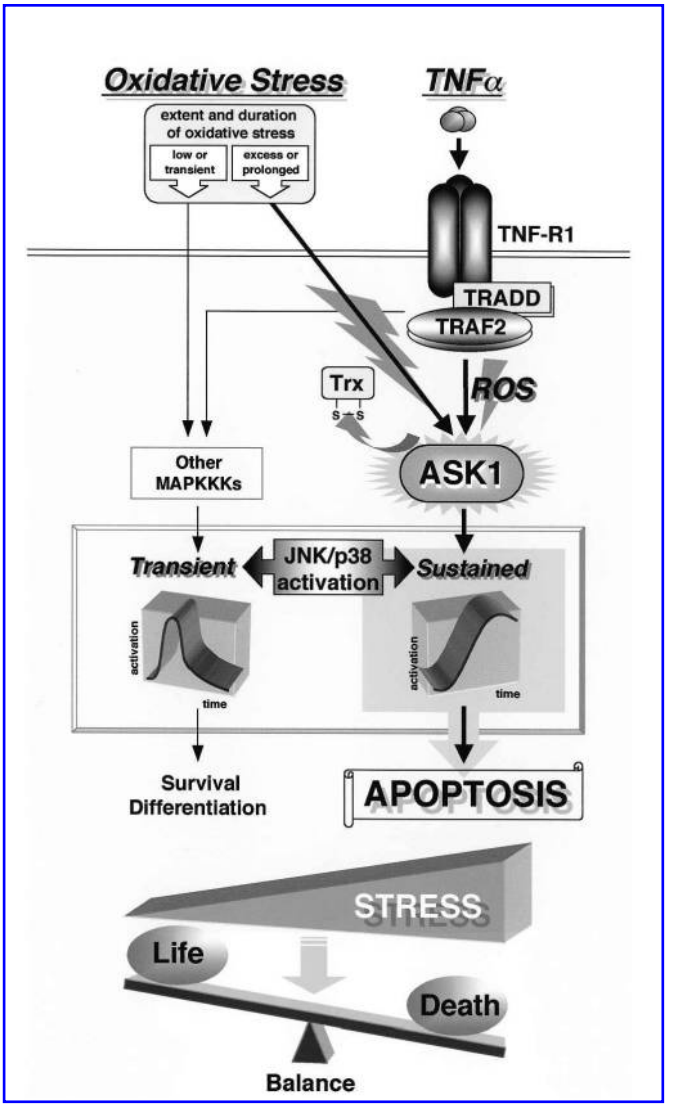

FIG. 2. Sustained activation of JNK/p38 mediated by ASK1 is required for oxidative stress- and TNFα-induced apoptosis. Oxidative stress- and TNFα-induced sustained, but not transient, activations of JNK/p38 are responsible for apoptosis and are mainly mediated by ASK1. Transient activations of JNK/p38 may be mediated by other MAPKKKs and induce cell growth and differentiation but not apoptosis. The extent and duration of exposure to oxidative stress and TNFα are important to determine subsequent cell fate; excess or long exposure leads to apoptosis, whereas low or transient exposure leads to survival/differentiation. ASK1 senses the degree of internal and/or external environmental stresses and drives apoptosis signaling only when cells are damaged lethally by excess and prolonged exposure to oxidative stress and TNFα. In TNFα signaling, TRAF2-mediated activation of ASK1 requires generation of ROS as second messengers. TNFα and oxidative stress induce dissociation of ASK1 from oxidized Trx and sequential activation of ASK1. The ASK1–Trx complex is a redox sensor, which functions as a molecular switch of external and internal redox status for the kinase signaling module. Hence, ASK1 is a determinant of cell fate, "death or life."

served active center. Only a reduced form of Trx is associated with the N-terminal regulatory domain of ASK1 and silences the activity of ASK1; oxidation of Trx results in the dissociation of ASK1 from Trx and thereby switches an inactive form of ASK1 to active kinase. The ASK1–Trx complex is thus thought to be a redox sensor, which functions as a molecular switch of external and internal redox status for the kinase signaling module, in that the reversible systems between thiolate (reduced) and disulfide (oxidized) forms in specific cysteine residues are converted into the similar, but not parallel, reversible systems between phosphorylation and dephosphorylation in specific serine or threonine residues (Figs. 2 and 3). Recently, protein serine/threonine phosphatase 5 (PP5) was identified as another negative regulator of ASK1 (39). PP5 binds to and dephosphorylates the activated form of ASK1 in response to oxidative stress, enabling inactivation of ASK1 by negative feedback. Both negative regulators, Trx and PP5, almost completely eliminate oxidative stress-induced apoptosis in an ASK1-dependent manner, suggesting that ASK1 plays critical roles in signal transduction of oxidative stress-induced apoptosis.

ASK1-mediated JNK/p38 sustained activation is required for oxidative stress-induced apoptosis

To confirm that ASK1 is required for stress-induced apoptosis such as that by H_2O_2 and TNFα, and to assess the pivotal roles played by ASK1–JNK/p38 pathways during apoptosis, we disrupted the ASK1 gene in mice, which were then analyzed *in vivo* and *in vitro*. ASK1-deficient mice were born at the expected Mendelian frequency and were indistinguishable in appearance from wild-type littermates. Histological analysis of ASK1-deficient mice detected no developmental abnormalities. These results suggest that ASK1 may not be necessary for apoptosis associated with fertility and embryogenesis. However, we discovered that ASK1-deficient cells are less sensitive than wild-type cells to death-inducing activities resulting from various types of stresses (54).

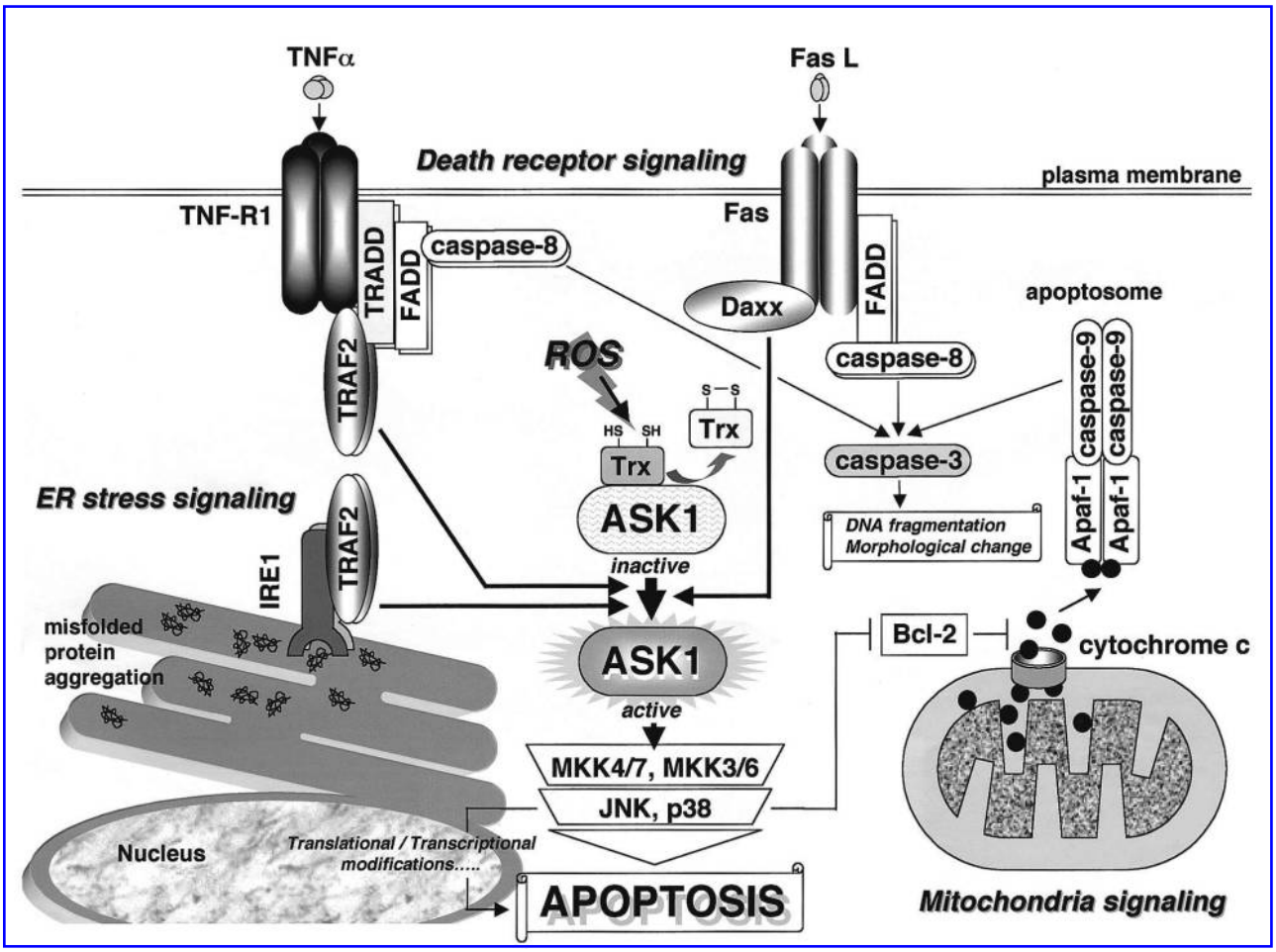

FIG. 3. Overview of ASK1-mediated apoptosis signal transduction. Sensing of cell damage and triggering of apoptosis signal transduction may occur through different cellular organelles, including not only cell-surface receptors and mitochondria, but also ER and the nucleus. Multiple death signals, from death receptors on the cell surface and ER, are integrated into ASK1, and in turn, the signals are transduced to multiple organelles, such as mitochondria and the nucleus, through phosphorylation of a variety of substrates (*e.g.,* Bcl-2 family members, specific transcription and translation factors) for the ASK1–JNK/p38 pathway, eventually leading to apoptosis. Thus, ASK1 can serve as an initial sensor of extracellular and intracellular stresses, such as oxidative stress, TNFα-induced cytotoxicity, and ER stress, to modulate the balance of pro- and antiapoptotic signal transductions and to make appropriate responses, such as survival, differentiation, and apoptosis, for the maintenance of homeostasis.

Mouse embryonic fibroblasts (MEFs) derived from ASK1-deficient mice were significantly resistant to H_2O_2-induced apoptosis. Moreover, it was observed that H_2O_2-induced transient activations of JNK and p38 were indistinguishable between ASK1+/+ and ASK1−/−MEFs, but that its sustained activations of JNK and p38 were dramatically diminished in ASK1-deficient cells. No significant change in activity was apparent for ERK. Similar phenomena were observed in cells stimulated not only by other oxidative reagents, such as diamide and tert-butyl hydroperoxide, but also TNFα as a death receptor ligand. Furthermore, expression of wild-type ASK1 in ASK1−/−MEFs restored H_2O_2- and TNFα-induced JNK/p38 activations at a sustained phase and apoptosis in an ASK1 dose-dependent manner (54). These results indicate that ASK1 plays essential roles in TNFα- and oxidative stress-induced sustained JNK/p38 activations and apoptosis (Fig. 2).

ASK1 is a determinant of cell fate

ROS are implicated in the regulation of diverse cellular functions, including defense against pathogens, signal mediation, cell proliferation, and apoptosis. Activation of a certain MAP kinase can result in distinct phenomena, such as survival, differentiation, and apoptosis. These findings appear paradoxical. Recently, it was demonstrated that the duration of activation of MAP kinases may contribute to determination of cell fate, such as survival, differentiation, and apoptosis (14, 37). Transient and persistent activations of ERK are known to lead to different cell fates, in that early and transient activation of ERK by epidermal growth factor stimulates proliferation of PC12 cells, whereas prolonged and sustained activation by nerve growth factor induces neuronal differentiation in response to nerve growth factor (36, 64). T-cell activation signals are mediated through the costimulator CD28-induced rapid and transient JNK activation, which in turn stimulates cell growth, whereas UV-C or γ-radiation causes delayed and persistent JNK activation, which induces apoptosis. Early/transient and late/sustained activations of JNK and/or p38 induced by the proinflammatory cytokine TNFα or various types of stress have been reported to correlate with survival/differentiation and apoptosis, respectively (9, 17, 41, 48).

Analysis of ASK1-deficient mice suggests that TNFα- and oxidative stress-induced sustained, but not transient, activations of JNK/p38 may be responsible for apoptosis, and that the ASK1–JNK/p38 pathway mainly mediates apoptosis, among various TNFα- and oxidative stress-activated kinases. Transient activations of JNK/p38 (as well as ERK activation) may be mediated by other MAPKKKs, such as MEKKs or MLKs, and induce cell growth and differentiation, but not apoptosis. The extent and duration of exposure to oxidative stress and TNFα are important in determining subsequent cell fate; excess or long exposure leads to apoptosis, whereas low or transient exposure leads to survival/differentiation. For example, in immune cells, large-scale production of superoxide by neutrophils or of nitric oxide by macrophages provides a necessary host defense and causes apoptosis, whereas these same ROS, which are appropriately produced by nonphagocytic cells, also function as signaling molecules, leading to cell growth and cytokine release. The mechanisms by which organisms regulate generation of endogenous ROS have been partially characterized. Large- *versus* small-scale production of ROS is regulated by homologous but distinct systems, that is, inducible *versus* constitutive enzyme systems, such as nitric oxide synthases for nitric oxide and NADPH oxidase systems for superoxide (15). However, the molecular systems sensing the extent and duration of stress are not fully understood. As a redox sensor, ASK1 may sense the degree of internal and/or external environmental stresses and drive apoptosis signaling only when cells are damaged lethally by excess and prolonged exposure to oxidative stress. ASK1 is a determinant of cell fate, such as survival, differentiation, or apoptosis, in ROS-mediated redox signaling (Fig. 2).

TNFα-INDUCED APOPTOSIS REQUIRES ROS-MEDIATED ACTIVATION OF ASK1–JNK/P38 PATHWAY

Death receptor signaling

As an increasing number of factors involved in apoptosis have been identified, concepts of different, but interacting apoptosis signaling pathways have been delineated. The signaling pathways of apoptosis can be divided into two components, including either mitochondrial signals or death receptor signals (Fig. 3) (19). In the mitochondrial pathway, cytochrome *c* is released as a trigger of cell death from the intermembrane space of mitochondria. Binding of cytochrome *c* and ATP/dATP to the caspase-9-cofactor protein Apaf-1 results in activation of Apaf-1, formation of the "apoptosome" (a multimeric complex of apoptotic factors), and triggering of the caspase-9–caspase-3 cascade, finally leading to activation or degradation of various effector molecules, such as DNases and cytoskeletal proteins. The mitochondrial pathways play central roles in many forms of apoptosis, which are often regulated by pro- and antiapoptotic Bcl-2 family proteins. On the other hand, the death receptor pathway is initiated by interactions of cell-surface death receptors, such as TNF receptor 1 (TNF-R1) and Fas, with their cognate ligands, followed by recruiting and activation of caspase-8 through adaptor molecules such as TRADD (TNF-R1-associated death domain protein) and FADD (Fas-associated death domain-containing protein). Once activated, caspase-8 activates downstream caspases such as caspase-3. As Bid, a proapoptotic Bcl-2 family protein, translocates to mitochondria through caspase-8-mediated cleavage and enhances cytochrome *c* release, this molecule functions as a mediator linking death receptor signals to mitochondria-dependent death signals.

Within the death receptor family, TNF-R1 appears to play unique roles; activation of TNF-R1 can mediate opposing cellular functions, such as cell growth and apoptosis, in the same cell, which are regulated in particular by various adaptor proteins interacting with the "death domain," an intracellular domain that is conserved among the death receptor family members (2). Upon TNFα binding to TNF-R1, trimerization of TNF-R1 occurs and results in aggregation of death domains, allowing recruitment of TRADD. TRADD mediates recruit-

ment of an adaptor or scaffold molecule, TRAF2 (TNF receptor-associated factor 2). TRADD also recruits the FADD–caspase-8 complex, leading to acute execution of apoptosis. Hence, the TNF-R1 signaling complex is mainly formed by interaction of TRADD, TRAF2, and FADD, allowing multiple and subdivided signals to regulate both pro- and antiapoptotic activities (12, 22, 47). Among these molecules, TRAF2, in particular, represents an integration point for pro- *versus* anti-apoptotic signals, in that TNF-R1-mediated recruitment of TRAF2 leads to bifurcate activations of the JNK/p38 *versus* NF-κB (nuclear factor for κ chain gene in B cells) pathways, respectively.

ASK1 is required for TNFα-induced apoptosis

Interestingly, TRAF2 is a strong activator of ASK1. TNFα, as well as various oxidative reagents, also activates ASK1. The C-terminal domain of ASK1 directly binds TRAF family proteins, including TRAF1, TRAF2, TRAF3, TRAF5, and TRAF6, by overexpression, and can be activated by TRAF2, TRAF5, and TRAF6 (44). A dominant-negative mutant of TRAF2 blocks TNFα-induced ASK1 activation, as well as JNK/p38 activation. A kinase-inactive mutant of ASK1 strongly inhibits TNFα- and TRAF2-induced JNK/p38 activations. Furthermore, ASK1 associates with endogenous TRAF2 in a TNFα-dependent manner (21, 44). Thus, ASK1 is a direct downstream target of TRAF2, and recruitment of ASK1 to the active TRAF2 components is a specific mechanism by which TNFα activates both JNK and p38 pathways.

As described above, MEFs derived from ASK1-deficient mice were significantly resistant to TNFα-induced apoptosis accompanied by remarkable reduction of sustained activations of JNK and p38, as well as H_2O_2-induced apoptosis (54). These results indicate that ASK1 is required for TNFα-induced apoptosis mediated by sustained JNK/p38 activation, and that TNFα-induced ASK1–TRAF2 interaction leads to apoptosis through JNK/p38 activation. Furthermore, it is important that TRAF2-mediated activation of ASK1 was found to be required for ROS generation, by comparison between TNFα- and Fas-induced apoptosis in ASK1-deficient mice, as indicated below.

Differential roles of ASK1 in TNFα- or Fas-mediated apoptosis: importance of TRAF2-mediated ROS generation

Fas, a well characterized member of the death receptor family, can enhance apoptosis in certain types of cells. Activation of Fas by Fas-ligand recruits FADD, permitting acute execution of apoptosis by caspase-8 activation (40). ASK1 activation by Fas appears to occur through activation of a second pathway that involves another adaptor protein for Fas, called Daxx (63). Daxx binds to the N-terminal noncatalytic domain of ASK1 in a ligand-dependent manner and thereby activates JNK, which may sensitize cells to caspase-induced apoptosis (18). However, the inhibitory effects of dominant-negative forms of Daxx and ASK1 on Fas-induced apoptosis are much weaker than those of caspase inhibitors. We observed that Fas-induced JNK and p38 activations were strongly suppressed in thymocytes from ASK1-deficient mice, but that the sensitivities to Fas-induced apoptosis of ASK1+/+ and ASK1−/− thymocytes were indistinguishable (54), suggesting that the Daxx–ASK1–JNK/p38 axis is not required for Fas-induced apoptosis, at least in thymocytes.

Fas- and TNF-R1-mediated signalings play different roles in regulating cell fate. In general, Fas signaling is essential for acute execution of apoptosis to terminate sustained immune response or to reduce autoreactive lymphocytes, whereas TNFα contributes not only to apoptosis of malignant and virally infected cells, but also to cell growth and cytokine production. Interestingly, the caspase-8 activity induced by TNFα was significantly less than that induced by Fas, although the magnitudes of apoptosis induced by TNFα and Fas were indistinguishable (54). An alternate pathway may not be required for Fas-induced cell death with sufficient activation of the FADD–caspase-8 pathway; however, weaker activation of the FADD–caspase-8 pathway by TNFα may be compensated for by simultaneous activation of ASK1–JNK/p38 death signals. Thus, a clear difference exists between TNFα- and Fas-induced apoptosis pathways in dependency on ASK1–JNK/p38 and caspase-8 pathways. Although the physiological roles of ASK1-mediated JNK/p38 activation induced by Fas are presently unknown, it is clear that Fas and TNFα differentially utilize the ASK1–JNK/p38 pathway for regulation of apoptosis. Such branching and reintegration of receptor signals could generate discrete biological responses to a variety of outside challenges, "death or life."

Fas-induced apoptosis can occur in low oxygen conditions and does not appear to require the generation of ROS, whereas TNFα-induced cell death can be inhibited by antioxidants, such as *N*-acetyl-L-cysteine (Nac), as previously reported (26, 51). In fact, recent studies have demonstrated that overexpression of TRAF2 fosters the production of ROS in transfected cells, and that the interaction between TRAF2 and ASK1 is redox-sensitive and can be prevented by free-radical scavengers. Overexpression of Trx in excess of coexpressed TRAF2 almost completely inhibits the TRAF2–ASK1 interaction in a process that is reversed by ROS, whereas overexpression of wild-type TRAF2, but not a dominant-negative form of TRAF2, removes coexpressed Trx from the ASK–Trx complex (34). Importantly, the antioxidant Nac inhibited TNFα-induced apoptosis in ASK1+/+ MEFs to the similar extent of apoptosis observed in TNFα-treated ASK1−/− MEFs. Moreover, Nac effectively inhibited only the sustained phases of TNFα-induced JNK/p38 activations in ASK1+/+ MEFs, which followed the same time course as those in ASK1−/− MEFs. TNFα-induced apoptosis and sustained JNK/p38 activations in ASK1−/− MEFs were no more sensitive to Nac (54). Generation of ROS appears to be required for TRAF2-mediated activation of ASK1 and sequential apoptosis through the TNFα death receptor signal pathway (Fig. 2). These findings suggest that ROS are involved in signal transduction of apoptosis as second messengers, rather than as directly cytotoxic molecules. ASK1-mediated sustained JNK/p38 activation may be the common pathway for various types of stress-induced apoptosis, as well as oxidative stress and TNFα, following generation of ROS as physiological messengers.

ASK1 IS ESSENTIAL FOR ER STRESS- AND ABNORMAL PROTEIN AGGREGATION-INDUCED APOPTOSIS THROUGH THE CONSERVED TRAF2–ASK1–JNK/P38 SIGNALING MODULE

ER as a novel determinant of cell fate

Many molecules that control cell death have been identified; consequently, it has been revealed that two main pathways, death receptor signals and mitochondrial signals, are required for execution of apoptosis, as described above. However, recent increasing evidence implies that the sensing of cell damage and triggering of apoptosis signal transduction may occur through different cellular organelles, including ER and the nucleus, as well as cell-surface receptors and mitochondria (5, 6).

The ER is a key component that coordinates the synthesis, folding, export, and degradation of nascent proteins. Protein synthesis and mature folding are carried out by various steps and posttranslational modifications, including binding to heat-shock proteins, glycosylation, and thiol-disulfide formations in the ER. Each of these events must constantly be under the control of cells, to ensure that proteins are accurately expressed, correctly folded, and targeted to the correct compartment. When these processes are out of balance, unfolded and/or misfolded proteins accumulate not only in the ER lumen, but also in the nucleus and cytoplasm, which potentially damage cell function and homeostasis. This accumulation of unfolded and/or misfolded proteins, so-called ER stress, triggers the expression of a number of molecular chaperones, such as Bip/GRP78, GRP94, and protein disulfide isomerase, which assist correct protein refolding and promote cell survival. However, excess extent and/or long duration of ER stress eventually leads to apoptosis (30, 38, 52). In ER stress, whether cells die or survive appears to depend on the net balance between these two programmed responses, one of which protects the stressed cell, and the other sacrifices it. ER stress is provoked by diverse pathophysiological conditions or a large number of physical and chemical insults, including disruption of calcium homeostasis, inhibition of protein glycosylation, reduction of disulfide bonds, hypoxia, ischemia, heat shock, and oxidative stress. Thus, the ER serves as a primary sensor of extracellular and/or intracellular environmental stresses and can determine cell fate by triggering apoptosis or survival signal transduction.

TRAF2–ASK1–JNK/p38 signaling module is essential for ER stress-induced apoptosis

ER stress signals have recently attracted considerable attention as a novel pathway responsible for pathological intracellular stress, because mutations or deletions of presenilin-1, which are linked to Alzheimer's disease, influence the ER stress signaling pathway and facilitate neuronal apoptosis in patients (29, 42, 45). ER stress transducers, such as IRE1, PERK, and ATF6, have been identified and characterized, and an ER-transmembrane sensor protein IRE1 is one of the downstream targets of presenilin-1 and activates the JNK pathway by recruiting TRAF2 (57). In response to ER stress, a serine/threonine protein kinase IRE1 is activated by oligomerization-dependent autophosphorylation, which induces apoptosis, as well as up-regulation of chaperone genes (25, 59). Based on the finding that ASK1–TRAF2 interaction leads to apoptosis through JNK activation in TNFα signaling, we investigated the role of ASK1 in ER stress signaling, using ASK1-deficient mice.

We found that ASK1−/− MEFs were resistant to ER stress-induced apoptosis accompanied by almost complete loss of activations of JNK and p38 (Nishitoh *et al.*, submitted for publication). In fact, ASK1 was activated in response to ER stress. Similar results were observed in cells exposed to distinct ER stresses, such as disturbance of calcium homeostasis, glycosylation, and disulfide formation in the ER, which were chemically induced by thapsigargin, tunicamycin, and dithiothreitol, respectively. Furthermore, it was found on immunoprecipitation analysis that an IRE1–TRAF2–ASK1 complex was formed in an ER stress-dependent manner and then caused JNK activation. These results indicate that ASK1 plays essential roles in ER stress-induced apoptosis, and that the formation of the IRE1–TRAF2–ASK1 complex is critical for the ER stress-induced apoptotic pathway through JNK activation. Therefore, the TRAF2–ASK1–JNK signaling pathway is a common module for apoptosis induced not only by death receptor signals, but also by ER stress signals, in that it can integrate internal or external stress-initiated divergent signals into a common pathway of apoptosis (Fig. 4). ASK1 is required for the novel apoptotic signal pathway triggered by another stress-sensing organelle, the ER, in addition to well known cell-surface receptors and mitochondria.

ASK1 is required for polyglutamine-induced neuronal death

Accumulation of unfolded proteins appears to play a critical role in pathogenesis of a wide variety of systemic diseases, including amyloidosis, hypercholesterolemia, diabetes mellitus, and neurodegenerative disorders (1, 31). Abnormal protein aggregation can cause chronic activation of ER stress, finally leading to apoptosis. Recently, it has been demonstrated that, in various types of neurodegenerative diseases, the pathology and eventual death of specific neuronal populations occur due to common mechanisms; intracellular aggregation of insoluble proteins is a characteristic feature of neurodegenerativediseases, such as β-amyloid or tau in Alzheimer's disease, α-synuclein in Parkinson's disease, superoxide dismutase in amyotrophic lateral sclerosis, prions in prion disease, and polyglutamine aggregation in polyglutamine diseases (*e.g.,* Huntington's disease or Machado–Joseph disease) (27, 52).

Interestingly, ASK1 activation was observed by overexpression of abnormal length polyglutamine derived from C-terminal fragments of the Machado–Joseph disease proteins in neuronal cells, which was correlated with induction of ER stress. Moreover, ASK1−/− primary neurons were clearly more resistant to polyglutamine aggregation-induced apoptosis than ASK1+/+ neurons, accompanied by almost complete loss of JNK activation (Nishitoh *et al.*, submitted for publication). These results indicate that ASK1 is required for polyglutamine-induced neuronal death, and that ASK1 may func-

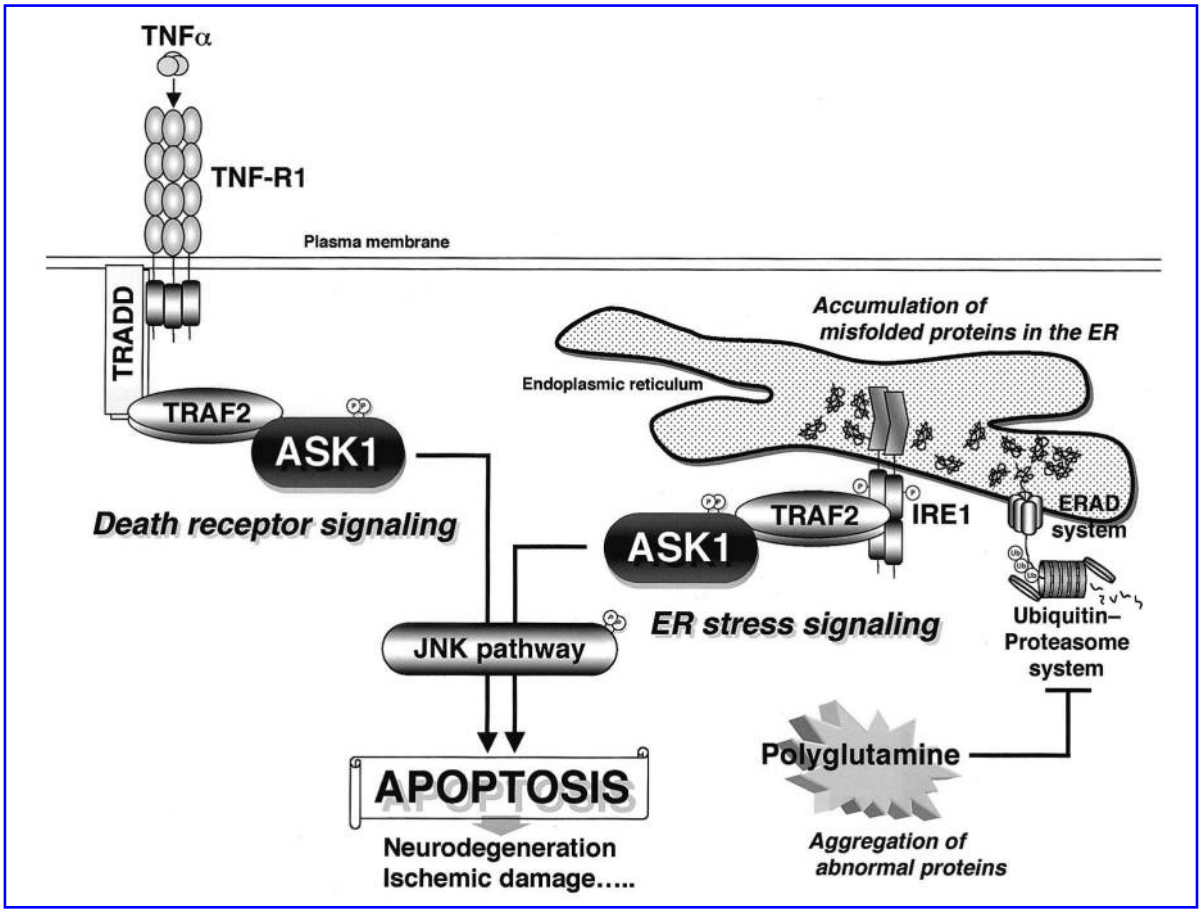

FIG. 4. TRAF2–ASK1–JNK signaling pathway as a common module for TNFα- and ER stress-induced apoptosis. ASK1 plays essential roles in not only TNFα- but also ER stress-induced apoptosis. TRAF2–ASK1–JNK signaling pathway can integrate internal or external stress-initiated divergent signals into a common pathway to apoptosis. ASK1 is also required for polyglutamine-induced neuronal death. As shown in this figure, we speculate that blockage of ubiquitin-proteasome systems by polyglutamine aggregation leads to dysfunction of the proteasome-coupled ERAD system, followed by the accumulation of misfolded proteins that are generated at the normal protein turnover in the ER, and that this protein accumulation-induced ER stress activates IRE1 as a sensor molecule, by which this signal is relayed to the TRAF2–ASK1–JNK pathway, finally resulting in apoptosis. ASK1 may contribute to the pathogenesis of various abnormal protein aggregation-induced disorders, such as neurodegeneration and ischemic damage.

tion as a common signal transducer of apoptosis induced by abnormal protein aggregation and contribute to the pathogenesis of various neurodegenerative diseases (Fig. 4).

Is ASK1 a common therapeutic target for conformational diseases?

Protein degradation is the most important process in homeostasis in the ER. Impairment of the protein degradation process strongly induces ER stress (11, 31, 52). Although autophagy, lysosomal digestion, and the proteasome system are well known mechanisms by which cells eliminate potentially toxic protein aggregates, degradation of integral membrane proteins and secretory proteins, a process known as ERAD (ER-associated degradation), is mediated by cytoplasmic proteasomes, and requires retrotranslocation or dislocation of polypeptide chains across the Sec61 translocon in the ER membrane (56, 60). Recent studies revealed that polyglutamine aggregates are colocalized with various molecular chaperons and proteasome components and impair the function of the ubiquitin–proteasome systems (3, 58). We found that polyglutamine aggregation triggered ER stress through proteasomal dysfunction, and that ASK1 −/− primary neurons were defective in proteasome inhibitor-induced JNK activation and apoptosis, indicating that ASK1 is also required for proteasomal dysfunction-induced cell death, which is the same as ER stress-induced cell death triggered by polyglutamine aggregation. Our hypothesis is that blockage of ubiquitin–proteasome systems by polyglutamine aggregation leads to dysfunction of the proteasome-coupled ERAD system, followed by the accumulation of misfolded proteins that are generated at the normal protein turnover in the ER, and that this protein accumulation-induced ER stress activates IRE1 as a sensor molecule, by which this signal is relayed to the TRAF2–ASK1–JNK pathway, finally resulting in apoptosis (Fig. 4).

With aging, the cell's capacity to handle misfolded proteins becomes insufficient to prevent their accumulation and toxic consequences. At present, there are no mechanism-based

therapies available for "conformational diseases," such as neurodegenerative disorders and various systemic diseases caused by abnormal protein accumulation, even though the prevalence of these diseases has increased as human life expectancy has risen. Understanding the common cell-biological mechanisms by which abnormal protein aggregation leads to conformational diseases will be essential for the development of effective therapies. ASK1 appears to function as a common signal transducer of apoptosis induced by conformational diseases such as polyglutamine diseases and Alzheimer's disease, actually in those patients' brain. ASK1 may thus be a therapeutic target for conformational diseases.

CONCLUSION

Recently, it has been noted that both ROS generation and insoluble abnormal protein aggregation are closely linked to a wide range of sporadic and inherited diseases, including ischemic tissue damage and neurodegenerative disorders. The decision as to whether cells are committed to death or to life probably depends on the extent and duration of intracellular and/or extracellular stresses, such as ROS-induced oxidative stress, TNFα-induced cytotoxicity, and abnormal protein aggregation-induced ER stress, which are eventually integrated into ASK1, to switch on the stress-responsive JNK/p38 kinase signaling pathways for the final death event. Thus, ASK1 can serve as an initial sensor of these internal and external environmental conditions, to modulate the appropriate balance of pro- and antiapoptotic signal transductions for maintenance of tissue homeostasis (Fig. 3).

Many unresolved issues remain concerning the precise mechanisms of the pathogenesis of apoptosis-dysregulated diseases. Novel apoptosis-based therapeutic strategies require a more detailed understanding of how cells survive and die, but one candidate is clearly within our grasp.

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ABBREVIATIONS

ASK1, apoptosis signal-regulating kinase 1; ER, endoplasmic reticulum; ERAD, ER-associated degradation; ERK, extracellular signal-regulated kinase; FADD, Fas-associated death domain-containing protein; H_2O_2, hydrogen peroxide; JNK, c-Jun N-terminal kinase; MAP, mitogen-activated protein; MAPK, MAP kinase; MAPKK, MAP kinase kinase; MAPKKK, MAP kinase kinase kinase; MEF, mouse embryonic fibroblast; Nac, *N*-acetyl-L-cysteine; PP5, phosphatase 5; redox, reduction–oxidation; ROS, reactive oxygen species; TNFα, tumor necrosis factor-α; TNF-R1, TNF receptor 1; TRADD, TNF-R1-associated death domain protein; TRAF2, TNF receptor-associated factor 2; Trx, thioredoxin.

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