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Forum Review

Stress-Responsive Protein Kinases in Redox-Regulated Apoptosis Signaling

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ABSTRACT

Both extra- and intracellular stimuli elicit a wide variety of responses, such as cell survival, proliferation, differentiation, and apoptosis, through regulation of cell signaling. Recent studies have revealed that stress-responsive signal transduction pathways are strictly regulated by the intracellular redox state. The redox state of the cell is a consequence of the precise balance between the levels of oxidizing and reducing equivalents, such as reactive oxygen species (ROS) and endogenous antioxidants. The generation of ROS fluctuates in response to alterations of both external and internal environment and, in turn, triggers specific signaling cascades, including mitogen-activated protein kinases, which determine cell survival or cell death. This review focuses on the regulatory mechanisms of stress-responsive protein kinases and their involvement in oxidative stress-induced apoptosis. It also provides recent findings on the molecular mechanisms by which redox signaling cross-talks with stress-responsive protein kinase cascades. *Antioxid. Redox Signal.* 7, 472–481.

INTRODUCTION

A CELL is exposed to a variety of stresses in both the external and internal environment. For aerobic organisms, exposure to reactive oxygen species (ROS) is continuous and unavoidable. ROS, such as superoxide, singlet oxygen, hydroxyl radical, and hydrogen peroxide (H_2O_2), are generated by electron transport through the mitochondrial respiratory chain, NADPH cytochrome P450 reductase in the endoplasmic reticulum (ER), hypoxanthine/xanthine oxidase, NADPH oxidase, lipoxygenase, cyclooxygenase, and γ-ray and ultraviolet (UV) light irradiation. Although the production of ROS is approximately balanced by endogenous antioxidants and reductants, oxidative stress occurs when the production of ROS increases, elimination of ROS or repair of oxidatively damaged macromolecules decreases, or both. Such a serious imbalance between ROS generation and the antioxidant capacity of the cell causes damage to all types of biological molecules, including DNA, RNA, proteins, and lipids, which ultimately leads to cell death and tissue damage.

Recent studies have shown that ROS generated by a variety of stresses and growth factors are not only toxicants to the cell, but also second messengers in signal transduction of cellular activation and cell death, and that the intracellular redox condition plays a crucial role in the regulation of normal physiologic signaling pathways in many cells. The generation of ROS or the fluctuation of the cellular redox state leads to the stimulation of various signaling systems, such as mitogen-activated protein kinases (MAPKs) and other stress-responsive protein kinases. Protein kinases contribute to the 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 or survival. Therefore, the cell's fate is determined by cross talk between the cellular signaling pathways and the cellular redox state through a strict regulation mechanism. This article reviews the redox-regulatory mechanisms of various stress-responsive protein kinases and their involvement in oxidative stress-induced apoptosis.

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MAPKS

MAPK subfamilies

MAPK cascade is evolutionarily well conserved in all eukaryotic cells and typically includes central three-tiered core signaling modules comprising a mitogen-activated protein kinase kinase kinase (MAPKKK), mitogen-activated protein kinase kinase (MAPKK), and MAPK (Fig. 1) (9, 28). All eukaryotic cells possess multiple MAPK pathways. c-Jun N-terminal kinase (JNK), p38 MAPK, and extracellular signal-regulated kinase (ERK) are well characterized subgroups of a large MAPK family. These kinase pathways are structurally similar, but functionally distinct. Whereas the ERK pathway is most commonly linked to the regulation of cell proliferation, the JNK and p38 pathways are primarily activated by various environmental stresses: osmotic shock, UV radiation, heat shock, oxidative stress, protein synthesis inhibitors, stimulation of Fas, and proinflammatory cytokines such as tumor necrosis factor α (TNFα) and interleukin-1 (IL-1) (54). Therefore, JNK and p38 are often grouped together and referred to as stress-activated protein kinases (SAPKs). ERK5/big MAPK 1 (BMK1) was recently identified as the novel class of stress-activated MAPK, which is also activated by oxidative stress and osmotic shock (Fig. 2) (66).

ERK, JNK, and p38 have all been shown to be activated in response to oxidant injury and therefore could potentially contribute to influencing cell survival or cell death. In neuron-like PC12 pheochromocytoma cells, nerve growth factor withdrawal leads to sustained activation of the JNK and p38 MAPK and inhibition of ERK, and both effects are required for the induction of apoptosis (62). Although the idea that ERK and JNK/p38 have opposing functions is still generally accepted, recent studies suggest that activations of SAPKs appear to be important for cell survival and differentiation. As will be discussed below, deletion of apoptosis signal-regulating kinase 1 (ASK1), an upstream MAPKKK of SAPKs, revealed that ASK1−/− cells were resistant to oxidative stress-induced apoptosis, accompanied by the dramatic suppression of the sustained phase of JNK/p38 activations (37, 55). The dynamic balance between the magnitude or duration of ERK and JNK/p38 activations is key to determining whether a cell survives or undergoes apoptosis. In the next sections, we will concretely review regulatory mechanisms of MAPK signal transduction pathways by the intracellular redox state and oxidative stress, and discuss how the redox modulation of MAPK, which is actually implicated in various diseases, controls cell survival and cell death.

ERK1/2

The best studied MAPK cascade is the ERK1/2 pathway. ERK1/2 is generally activated by growth factors and cytokines, and its activation is related to the stimulation of tyro-

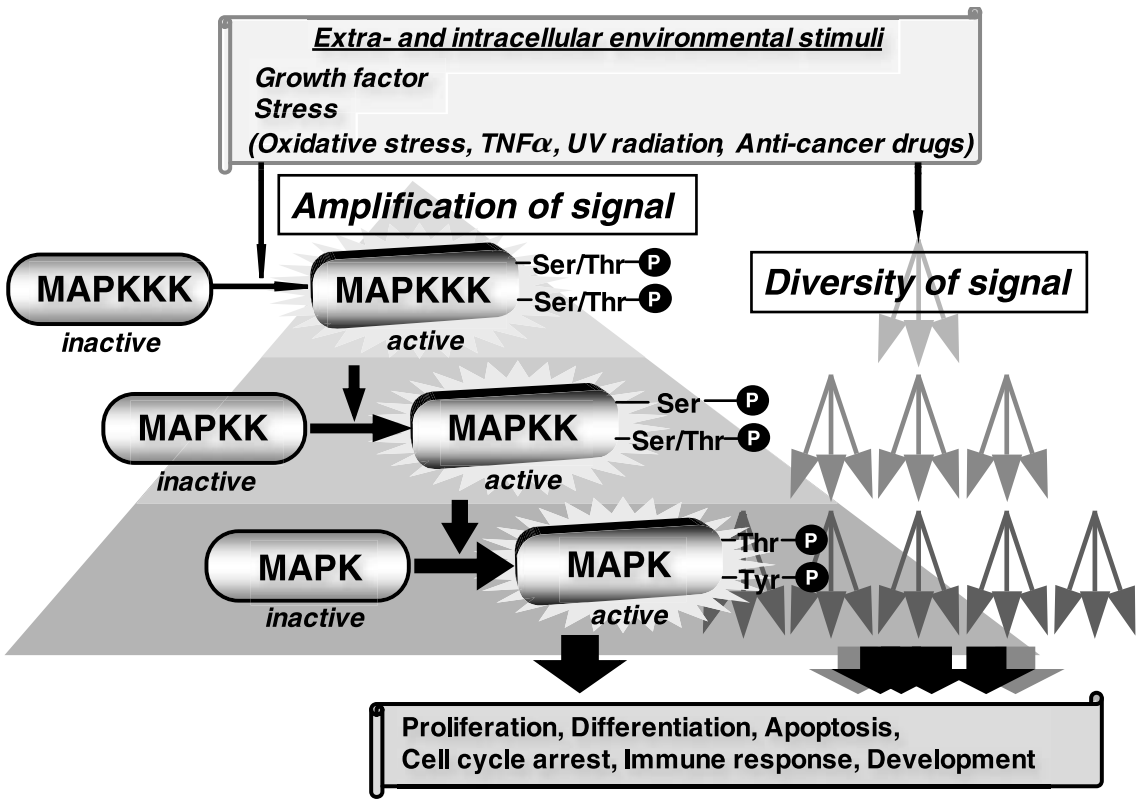

FIG. 1. MAPK cascade. MAPK cascade is typically composed of three kinases that establish a sequential activation pathway comprising a MAPKKK, MAPKK, and MAPK. Extracellular and intracellular stimuli activate MAPK cascade, in which the signals are amplified and transduced step by step to various downstream targets, resulting in diverse and appropriate biological responses.

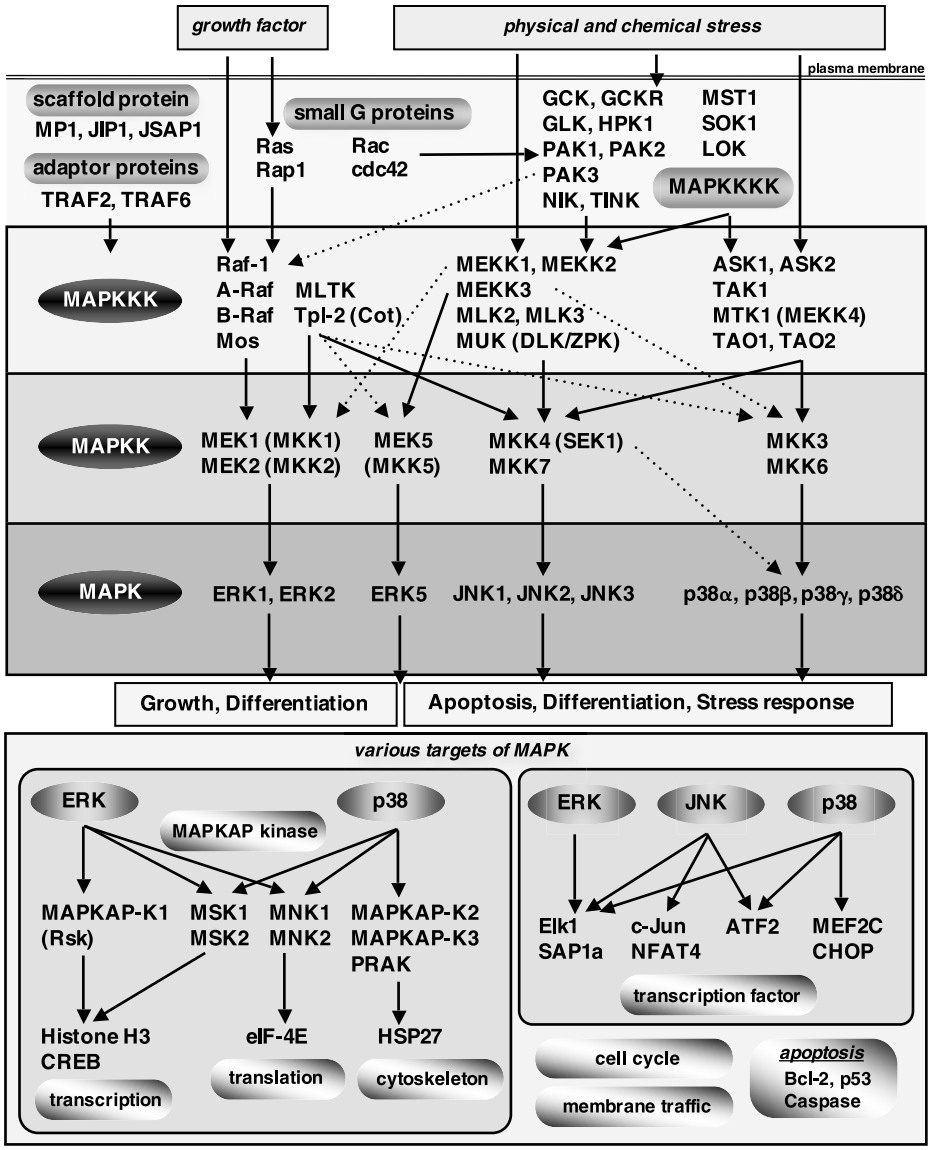

FIG. 2. Overview of MAPK signaling pathway. In mammals, three major subgroups of MAPK, ERK, JNK, and p38, have been identified, which are structurally similar, but functionally distinct. Whereas ERK is activated by cell growth and differentiation stimuli, JNK and p38 are preferentially activated by various types of environmental stress, such as UV radiation, heat shock, and oxidative stress. A novel class of stress-activated MAPK, ERK5/BMK1, is also activated by oxidative stress and osmotic shock. In response to environmental changes, upstream kinases (MAPKKKs and MAPKKKKs), and their various modulators such as small G proteins and adaptor proteins, initiate the activation of the MAPK cascade. MAPKs regulate cellular response or cell fate through phosphorylation of downstream targets, such as transcription or translation factors, cytoskeletal proteins, and regulators of the cell cycle and apoptosis, including p53, caspase, and Bcl-2 family proteins.

sine kinase receptors, which elicits a signaling cascade involving Ras activation, recruitment of Raf-1 MAPKKK to the plasma membrane, and sequential activation/phosphorylation of MEK1/2 and ERK1/2 (28). ERK1 and ERK2 phosphorylate and activate various transcription factors and other protein kinases, thereby influencing a large variety of cellular processes, such as cell survival, differentiation, and cell-cycle regulation. Oxidative stress also leads to substantial activation of ERK1/2, and growth factor receptors play an important role in mediating this effect. A number of growth factor receptors, such as epidermal growth factor (EGF) receptor and platelet-derived growth factor (PDGF) receptor, have been shown to undergo phosphorylation in response to oxidative insults such as H_2O_2 and UV irradiation, and interference of these phosphorylations by using molecular mutagenesis attenuates oxidative stress-induced ERK1/2 activation (26, 46).

Ras, a small G protein, is a target of ROS and reactive nitrogen species, which transduces a signal from tyrosine kinase receptors to the ERK1/2 pathway. It has been shown that nitric oxide (NO) binds to a cysteine residue (Cys118) that is exposed on the surface of the Ras molecule, leading to its activation (29). NO regulates a broad functional spectrum of proteins by *S*-nitrosylation. Evidence has been accumulating that *S*-nitrosylation directly modulates the biological functions

of many intracellular signaling molecules. Furthermore, it has been reported that Ras is not only *S*-nitrosylated but also *S*-thiolated by thiol oxidants such as H_2O_2, *S*-nitrosoglutathione, diamide, and glutathione disulfide at the reactive cysteine residues, and that such modifications of Ras may directly affect the bound GTP/GDP ratio and palmitate lipid turnover (33), resulting in alterations of various biological activities in the Ras-ERK1/2 pathway.

The activation of the growth factor receptor-ERK1/2 pathway by oxidative stress is consistent with the observation that low and adequate concentrations of ROS are mitogenic (7). Although it has been reported that the ERK1/2 pathway contributes to cisplatin-induced apoptosis (60), ERK1/2 generally can function as a survival and antiapoptotic factor following oxidative injury (16).

ERK5/BMK1

ERK5, a putative MEK5 target, was cloned as part of a two-hybrid screening that used MEK5 as bait (66). All MAPKs are activated by a simultaneous dual phosphorylation of Tyr and Thr residues within a conserved Thr-X-Tyr motif in the activation loop. ERK5 is an ~ 90-kDa MAPK and has the specific sequence Thr-Glu-Tyr in its phosphorylation loop like ERK1/2, whereas JNK and p38 have Thr-Pro-Tyr and Thr-Gly-Tyr motifs, respectively. ERK5/BMK1 can be activated by stress stimuli such as oxidative stress, UV irradiation, ischemia, and hyperosmolarity. Several studies have shown that a nonreceptor tyrosine kinase, c-Src, is redox-sensitive and required for activation of ERK5/BMK1 (1, 52). It has been demonstrated that ERK5/BMK1 activation contributes to cell proliferation, although its physiological role remains to be fully characterized.

JNK and p38 MAPK

Both JNK and p38, which are stress-activated MAPK family members, are regulated by environmental stress and proinflammatory cytokines such as IL-1 and TNFα. JNK is activated by various prooxidants, such as H_2O_2, arsenite, cadmium chloride, and UV-B radiation (16, 21, 59). It has been reported that mice lacking expression of the p66 isoform of the ShcA adaptor protein (p66ShcA) are less susceptible to oxidative stress and have an extended life span (38). Especially, phosphorylation of p66ShcA at serine-36 is critical for the cell death response elicited by light-induced oxidative stress such as UV radiation. JNK was found to be the kinase that phosphorylates p66ShcA at serine-36 (30). The p38 MAPKs are also activated by various types of oxidative stress, such as JNK-activating prooxidants, as well as other cellular stress such as osmotic shock, heat shock, and lipopolysaccharide (13, 16, 17). Singlet oxygen and NO contribute to activate the p38 MAPK pathway, which is required for oxidant-induced apoptosis (11, 67).

Specific inhibitors of JNK and p38 pathways or expression of dominant-negative mutants for JNK and p38 suppressed apoptosis induced by various stresses (54). A cell-penetrating peptide inhibitor of JNK is an extremely potent neuroprotectant *in vivo* against cerebral ischemia and excitotoxicity (4). In the cases of the JNK3 knockout mouse or the JNK1/JNK2 double knockout mouse, glutamate-induced hippocampal cell death or UV radiation-induced apoptosis is remarkably prevented, respectively (56, 64). Thus, many studies have shown that JNK and p38 have a critical role in signal transduction of oxidative stress-induced apoptotic cell death.

Recent data offer some mechanisms linking ROS and SAPK (Fig. 3). Antioxidants and thiol reductants, such as *N*-acetylcysteine and dithiothreitol, and overexpression of antioxidative enzymes, such as manganese superoxide dismutase (MnSOD) and glutathione *S*-transferase π (GSTp), can block or delay apoptosis. GSTp can interact with JNK to suppress its activation. ROS trigger the detachment of JNK from GSTp, and thereby facilitate JNK activation (2). Heat shock proteins (Hsps) are induced upon exposure to oxidative insults and prevent misfolding and aggregation of damaged proteins. Hsp70 can inhibit JNK activity and JNK-mediated apoptosis (44). It has been indicated that serine/threonine and tyrosine phosphatases can be regulated by altering the oxidation state of active-site functional groups, such as an active-site Fe ion in serine/threonine phosphatases or cysteine residues in tyrosine phosphatases. ROS-dependent activations of JNK and p38 may involve downregulations of JNK/p38 phosphatases. JNK- and p38-mediated phosphorylation of p53, which augments the p53 response, may also play a role in their proapoptotic actions (12, 48). However, the mechanisms by which MAPK signaling molecules regulate oxidative stress-induced apoptosis have not yet been clearly understood.

ASK1 is a member of the MAPKKK family, which activates both the JNK and p38 MAPK pathways and constitutes a pivotal signaling pathway in oxidative stress-induced apoptosis (20). In the next section, we will discuss the redox-sensitive ASK1 pathway and the regulatory mechanisms of ASK1-mediated apoptosis.

ASK1 IN REDOX-REGULATED APOPTOSIS SIGNALING

ASK1 is a redox-sensor MAPKKK in oxidative stress-induced apoptosis signaling

ASK1 is structurally a MAPKKK family member that activates both the SEK1(MKK4)/MKK7-JNK and MKK3/MKK6-p38 MAPK signaling cascades (8, 19, 20, 41, 47). ASK1 is activated in cells treated with inflammatory cytokines and various stresses. Overexpression of wild-type ASK1 or the constitutively active mutant induced apoptosis in various cell types. Furthermore, cell death induced by various stresses, such as TNFα and oxidative stress, can be reduced by a dominant-negative mutant form of ASK1, suggesting that ASK1 is a key element in cytokine- and stress-induced apoptosis.

Oxidative stress-induced activation of ASK1 leads to apoptosis. Thioredoxin (Trx) was identified as a negative regulator of the ASK1-JNK/p38 pathway, through yeast two-hybrid screening for ASK1-binding proteins (47). In resting cells, ASK1 constantly forms an inactive complex with Trx, whereas upon treatment of cells with TNFα or H_2O_2 as a ROS donor, ASK1 is dissociated from Trx and thereby fully activated by conformational changes and covalent modifications, such as oligomerization and auto- and/or cross-phosphorylation (14, 20, 47). Trx is a redox-regulatory protein that has two redox-sensitive cysteine residues within the active center. Only a reduced form of Trx is associated with the N-terminal regulatory domain of ASK1 and silences the activity of ASK1, and then the dissociation of ASK1 from oxidized Trx switches an

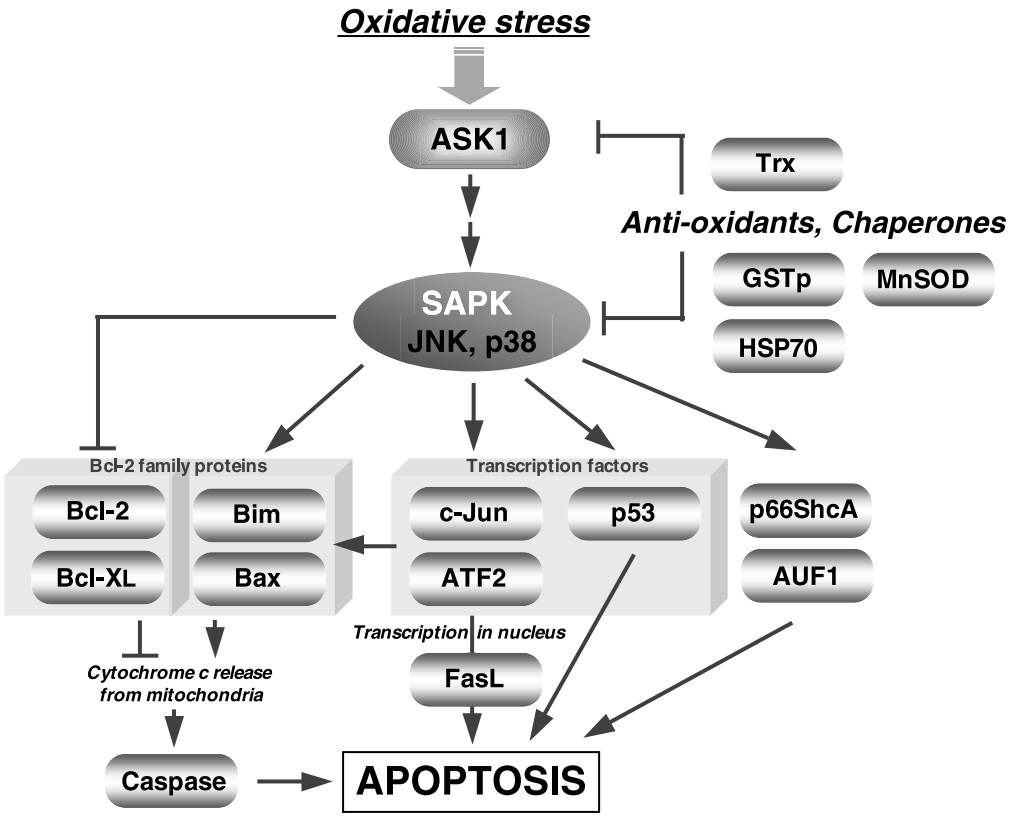

FIG. 3. The possible involvement of SAPKs in the execution of oxidative stress-induced apoptosis. Oxidative stress-induced activation of SAPKs including JNK and p38, is mediated by the stimulation of upstream kinases, such as ASK1. A variety of antioxidative enzymes and molecular chaperones can inhibit the activation of these kinases. Oxidative stress-induced activation of SAPK is involved in the execution of apoptosis through direct or indirect regulation of various downstream targets. It has been reported that SAPKs may influence the expression of pro- and antiapoptotic proteins, such as FasL (Fas ligand) and Bcl-2 family proteins, via the phosphorylation of transcription factors. The functions of various pro- and antiapoptotic factors, such as Bcl-2 family proteins, p66ShcA (p66 isoform of ShcA adaptor protein), and AUF1 (AU-rich element mRNA binding protein), can be modified directly by SAPK-mediated phosphorylation. SAPK-mediated phosphorylation and expression of Bcl-2 family proteins enhance cytochrome *c* release from the mitochondria and caspase activation. Finally, these effects lead to apoptosis.

inactive form of ASK1 to active kinase; the ASK1-Trx complex is thought to be a redox sensor, which functions as a molecular switch of external and internal redox status to the kinase signaling module (Fig. 4).

Recently, protein serine/threonine phosphatase 5 (PP5) was identified as another negative regulator of ASK1 (40). PP5 binds to and dephosphorylates the activated form of ASK1 in response to oxidative stress, enabling inactivation of ASK1 by negative feedback. Trx and PP5 can block oxidative stress-induced apoptosis, suggesting that ASK1 plays critical roles in signal transduction of oxidative stress-induced apoptosis.

ASK1-deficient mice

To confirm that ASK1 is required for oxidative stress-induced apoptosis, we disrupted the ASK1 gene in mice, which were then analyzed *in vivo* and *in vitro* (37, 55). Mouse embryonic fibroblasts (MEFs) derived from ASK1-deficient mice were significantly resistant to oxidative stress-induced apoptosis. Whereas oxidative stress-induced transient activations of JNK and p38 were indistinguishable between ASK1+/+ and ASK1−/− MEFs, sustained activations of JNK and p38 were dramatically diminished in ASK1-deficient cells. No significant change in activity was apparent for ERK1/2. Similar results were observed in cells treated not only with oxidative reagents, such as H_2O_2, diamide, and *tert*-butyl hydroperoxide, but also with TNFα. We found that TNFα-induced apoptosis also requires activation of the ASK1-JNK/p38 pathway mediated by ROS as a second messenger.

The extent and/or duration of activation of MAPKs may contribute to determination of cell fate, such as survival, differentiation, and apoptosis (36, 37). It has been reported that early/transient and late/sustained activations of JNK and/or p38 induced by oxidative stress and TNFα correlate with diverse cellular processes, such as survival/differentiation or apoptosis (10, 15, 45). For example, delayed and persistent JNK activation caused by UV-C or γ radiation is important to induce apoptosis (10). 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. Transient activations of JNK/p38 (as well as ERK1/2 activation) may be mediated by other MAPKKKs, such as MEKKs and MLKs. As a redox sensor, ASK1 may sense the degree of oxidative stress and drive apoptosis signaling only when cells are extensively damaged by excess and prolonged exposure to oxidative stress. ASK1 thus appears to be a deter-

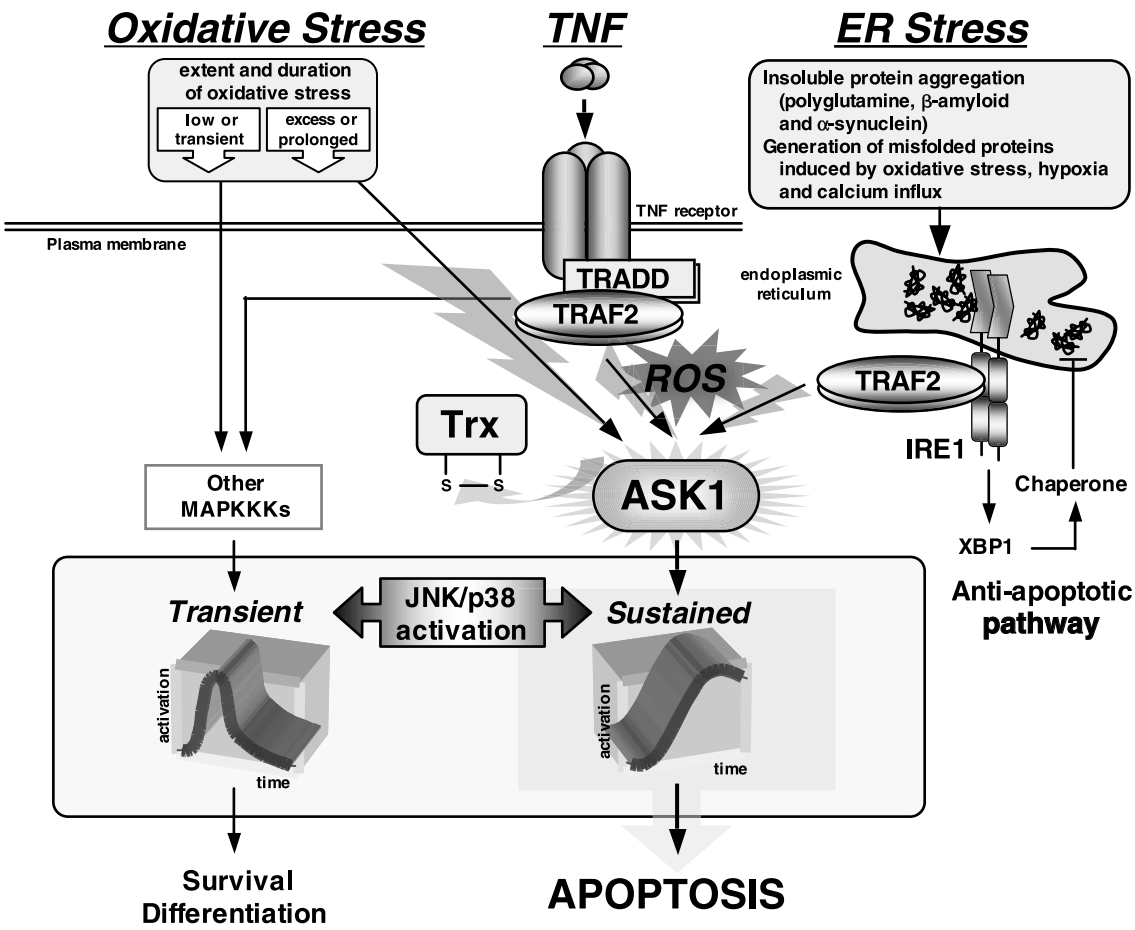

FIG. 4. ASK1 is a redox sensor and regulates apoptosis signaling pathway. ASK1 is a member of the MAPKKK family, which activates both JNK and p38 MAPK pathways and constitutes a pivotal signaling pathway in oxidative stress-induced apoptosis. In response to oxidative stress and TNFα, ASK1 is dissociated from Trx, a negative regulator of the ASK1-JNK/p38 pathway, and is activated. The ASK1-Trx complex is a redox sensor. TNFα- and oxidative stress-induced sustained activations of JNK and p38 may be responsible for apoptosis. The extent and/or duration of activation of MAPKs probably contribute to determination of the cell's fate, such as survival, differentiation, and apoptosis. Furthermore, the TRAF2-ASK1 module mediates not only TNFα-induced but also ER stress-induced apoptosis, which is implicated in various diseases, such as neurodegeneration and atherosclerosis. Stress-responsive signals diverge in both survival and apoptosis pathways, and the balance between pro- and anti-apoptotic pathways determines the cell's fate.

minant of cell fate, such as survival, differentiation, or apoptosis, in redox signaling (Fig. 4).

The TRAF2-ASK1 pathway mediates ER stress

TNF receptor-associated factor 2 (TRAF2) is an adaptor protein that connects TNF receptor with downstream signaling molecules. Overexpression of TRAF2 fosters the production of ROS in transfected cells, and the interaction between TRAF2 and ASK1 is redox-sensitive and can be prevented by free-radical scavengers. Although the mechanism by which oxygen radicals are generated downstream of TRAF2 is unknown, TNFα-induced apoptosis is also mediated in part by ROS-triggered activation of the ASK1-JNK/p38 pathway.

ASK1−/− MEFs were resistant to the ER stress-induced apoptosis, accompanied by the drastic suppression of the activations of JNK and p38 (42). Accumulation of unfolded and misfolded proteins in the ER induces cellular stress and triggers the expression of a number of molecular chaperones, such as Bip/GRP78 and GRP94, which assist protein folding and promote cell survival. However, the excess amount of persistent exposure to ER stress eventually leads to apoptosis (39, 57). It is reported that the mutation or deletion of presenilin-1 involved in Alzheimer's disease profoundly influences the ER stress signaling pathway and enhances neuronal apoptosis in patients (23, 43, 49). IRE1, a transmembrane sensor protein in ER stress signaling, activates the JNK pathway through recruiting of TRAF2 (58). Recently, we found that ER stress caused by polyglutamine aggregation induces the formation of the IRE1-TRAF2-ASK1 complex, and that the ASK1-JNK pathway mediates the ER stress-induced apoptosis. ASK1 appears to play an important role in the progress of neurodegenerative disorders such as polyglutamine disease (42). It is unclear whether ER stress-induced activation of the IRE1-TRAF2-ASK1-JNK signaling pathway is also mediated by ROS generation (Fig. 4).

PHOSPHOINOSITIDE 3-KINASE (PI3K) AND AKT PATHWAY

PI3K and Akt in redox-regulated apoptosis signaling

Akt, also known as protein kinase B, is a serine/threonine kinase that plays a key role in multiple cellular processes, such as cell proliferation, angiogenesis, glucose metabolism, and apoptosis. Akt is activated in a variety of stresses and growth factors via a PI3K pathway, in which PI3K-mediated generation of 3′-phosphorylated phosphoinositides leads to the recruitment of Akt to the cell membrane where it undergoes phosphorylation by kinases such as PDK1 (5).

Several studies have reported that a variety of environmental stresses lead to the down-regulation of the PI3K-Akt pathway, suggesting that such down-regulation is important in the apoptotic process. Activation of Akt in response to oxidative stress appears to be mediated through growth factor receptors. It has been reported that H_2O_2- and peroxynitrite-induced activations of the PI3K-Akt pathway rely on the EGF receptor and PDGF receptor, respectively (25, 61). Moreover, evidence suggesting that oxidative stress-induced activation of the PI3K-Akt pathway was required for cell survival was obtained by using the PI3K inhibitor wortmannin and overexpression of Akt (61). PTEN (phosphatase and tensin homologue), a phosphatidylinositol 3,4,5-triphosphate 3-phosphatase, regulates many cellular processes through direct antagonism of PI3K signaling. Recently, it was revealed that oxidative stress activates PI3K-dependent signaling via inactivation of PTEN (31).

Mechanisms for antiapoptotic effects of Akt

The PI3K-Akt pathway transduces its survival signals through the phosphorylation-dependent regulation of both pro- and antiapoptotic factors, such as BAD, caspase 9, forkhead transcription factor, GSK3, and IKKα. ASK1 is also a target of Akt. It was reported that Akt-mediated phosphorylation of ASK1 inhibited its ability to activate JNK/p38, its downstream target ATF2, and Bax, and protected cells against H_2O_2- and cisplatin-induced apoptosis (24, 65). These studies not only offer a mechanism for the antiapoptotic effects of Akt during oxidant injury, but also suggest the existence of cross talk between pro- and antiapoptotic signaling pathways, such as the PI3K-Akt and ASK1-JNK pathways (Fig. 5).

OTHER STRESS-RESPONSIVE KINASES

Protein kinase Cδ (PKCδ)

PKC represents a family of phospholipid-dependent serine/threonine kinases involved in various signaling pathways that regulate cell growth, death, and stress responses. Although other family members contribute to suppression of apoptosis, PKCδ, a member of the novel PKC subfamily, is especially associated with apoptosis induction (6). Activation of PKCδ is induced by a variety of oxidative stress, such as H_2O_2, cisplatin, and γ-ray and UV radiation. H_2O_2-induced activation of PKCδ results in targeting it to the mitochondria and promotes apoptosis signaling (32). During apoptosis, PKCδ is phosphorylated by c-Abl tyro-

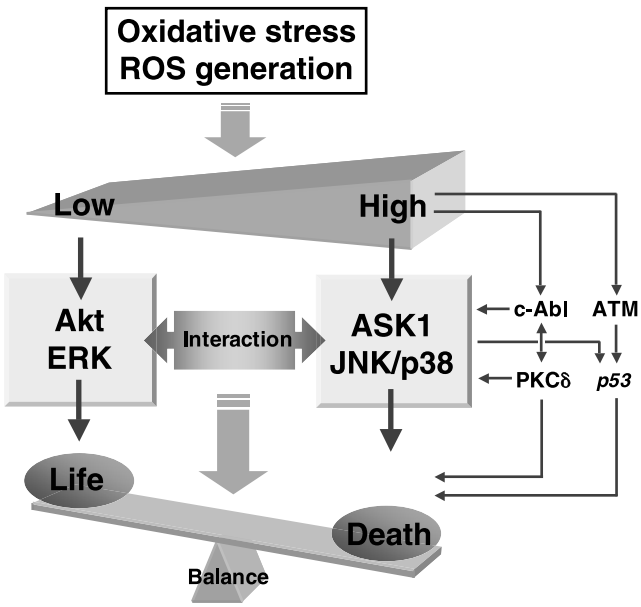

FIG. 5. The cell's fate is determined by cross talk between stress-responsive kinase pathways that are regulated by the severity of oxidative damage. ROS elicit a wide spectrum of responses. These responses depend on the magnitude of the dose of ROS generation and/or the duration of exposure. Typically, low doses of ROS are mitogenic and promote cell growth accompanied by activations of antiapoptotic kinases, including Akt and ERK1/2, whereas severe oxidative stress activates proapoptotic kinases, such as ASK1, JNK, and p38, and ultimately causes cell death. The cell's fate is determined by cross talk between pro- and antiapoptotic signaling pathways, such as Akt versus ASK1, and ERK1/2 versus JNK/p38, which play a significant role in altering susceptibility to apoptosis. More complicated interactions between these pathways and other stress-responsive kinases also exist. Such complicated and antagonistic machineries composed of many pro- and antiapoptosis stress-responsive kinases are necessary for a variety of cellular outcomes.

sine kinase (51). The downstream targets of PKCδ are DNA-dependent protein kinase, lamin B, and p73, which may function in apoptosis induction. It has been reported that PKCs, such as PKCα and PKCδ, promote apoptosis through activation of p38 MAPK and inhibition of the Akt survival pathway (53).

Ataxia-telangiectasia mutated kinase (ATM) and p53

The tumor suppressor protein p53 is a universal sensor of genotoxic stress and, as a transcription factor, plays a critical role in regulating expression of genes involved in mediating growth arrest and apoptosis. ROS are involved in the p53 signaling. Optimal induction and activation of p53, after various types of oxidative stress such as ionizing radiation (IR) and H_2O_2, require the activation of ATM, a member of the PI3K-like kinase family (34). The H_2O_2-induced phosphorylation of p53 on multiple serine residues was blocked in ATM−/− cells (63). Serine-20 phosphorylation of p53 is important for p53 stabilization by dissociation from its inhibitor Mdm2. Chk2, a kinase capable of phosphorylating serine-20 of p53, is directly phosphorylated by ATM after exposure to IR (18, 35). Although the mechanisms for stress-induced ATM activation remain unclear, it was reported that DNA damage activates ATM through intermolecular autophosphorylation and dimer dissociation (3). ATM may act as a sensor of ROS and oxidative stress.

c-Abl tyrosine kinase

c-Abl, a nonreceptor tyrosine kinase, is activated by oxidative stress and ER stress, leading to the induction of apoptosis (22, 50). In the case of H_2O_2, the interaction between PKCδ and c-Abl is important for the activation of c-Abl, targeting it to the mitochondria where it takes part in initiating apoptosis (27, 51). c-Abl is also targeted to the mitochondria by ER stress. Cells deficient in c-Abl show attenuated cytochrome *c* release and reduced apoptosis in response to H_2O_2 (50). c-Abl may enhance oxidative stress-induced apoptosis through modulation or interference of other signaling molecules, such as suppression of Akt or enhancing of JNK and p38 with activations of upstream kinases MEKK1 and MKK6 (34). Such complicated cross talk between stress-responsive kinases is required for a variety of cellular outcomes.

CONCLUSIONS

Recently, it has been noted that ROS generation and redox imbalance are closely linked to a wide range of sporadic and inherited diseases, including inflammation, cancer, ischemic tissue damage, and neurodegenerative disorders. Small shifts in the redox state may influence cellular downstream responses, which vary with the cell type, the extent of the damage, and the duration of the stress. Whether cells are committed to death or life probably depends on the particular circumstances or disease condition. Stress-responsive kinases can serve as an initial sensor of generation of ROS or fluctuation of the cellular redox, to modulate the balance of pro- and antiapoptotic signal transductions for maintenance of tissue homeostasis. ROS and stress-responsive kinases affect each other and together play an important role in determining the cell's responsiveness to apoptotic signals. Further studies of stress signaling and stress-responsive kinases may facilitate the development of improved therapeutic strategies for apoptosis-dysregulated diseases.

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ABBREVIATIONS

ASK1, apoptosis signal-regulating kinase 1; ATM, ataxia-telangiectasia mutated kinase; BMK1, big mitogen-activated protein kinase 1; EGF, epidermal growth factor; ER, endoplasmic reticulum; ERK, extracellular signal-regulated kinase; GSTp, glutathione *S*-transferase π; H_2O_2, hydrogen peroxide; Hsp, heat shock protein; IL-1, interleukin-1; IR, ionizing radiation; 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; NO, nitric oxide; PDGF, platelet-derived growth factor; PI3K, phosphoinositide 3-kinase; PKC, protein kinase C; PP5, protein serine/threonine phosphatase 5; PTEN, phosphatase and tensin homologue; ROS, reactive oxygen species; SAPK, stress-activated protein kinase; TNFα, tumor necrosis factor-α; TRAF2, TNF receptor-associated factor 2; Trx, thioredoxin; UV, ultraviolet.

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