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Forum Review

The Role of Apoptosis Signal-Regulating Kinase 1 in Cardiomyocyte Apoptosis

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ABSTRACT

Apoptosis signal-regulating kinase 1 (ASK1), a serine/threonine protein kinase, is a reactive oxygen species–sensitive mitogen-activated protein kinase kinase kinase and activates both p38 and c-Jun N-terminal kinase pathways. Two isoforms of thioredoxin (Trx), cytosolic and mitochondrial Trx (Trx1 and Trx2, respectively), have been identified in mammalian cells. Trx1 was initially identified as an ASK1-binding protein. Trx1 and Trx2 bind directly to the N-terminal regulatory domain of ASK1 and inhibit ASK1-dependent apoptosis. Numerous other proteins interact with ASK1 and regulate its activity. In cardiomyocytes, ASK1 is involved not only in cardiac apoptosis, leading to cardiac remodeling, but also in cardiac hypertrophy as well as nonapoptotic cardiomyocyte death. *Antioxid. Redox Signal.* 8, 1729–1736.

INTRODUCTION

APOPTOSIS IS A GENETICALLY CONTROLLED FORM of cell death that involves specific structural and biochemical changes such as chromatin condensation, nuclear fragmentation, formation of apoptotic bodies, and caspase activation. Reactive oxygen species (ROS) are involved in a wide variety of cellular functions, including cell proliferation, differentiation, and apoptosis. Apoptosis signal–regulating kinase 1 (ASK1), a 160-kDa serine/threonine protein kinase, is an ROS-sensitive mitogen-activated protein (MAP) kinase kinase kinase and activates both p38 and c-Jun N-terminal kinase (JNK) pathways by directly phosphorylating and activating MAP kinase kinase (MKK)4/MKK7 and MKK3/MKK6 (26, 55). ASK1 is ubiquitously expressed in most mammalian cells, and the kinase activity of ASK1 is activated by many stress signals and proinflammatory cytokines, including H_2O_2, tumor necrosis factor (TNF)-α, endoplasmic reticulum stress, and serum withdrawal (16, 26, 38, 44). Overexpression of wild-type ASK1 or the constitutively active mutant of ASK1 induced apoptosis in various cells, whereas oxidative stress and TNF-α–induced apoptosis were suppressed in ASK1 knockout ($ASK1^{-/-}$) cells (44, 51, 59). Furthermore, the expression of a catalytically inactive mutant of ASK1 exhibited a dominant negative effect, inhibiting apoptosis induced by stress signals such as TNF-α and H_2O_2 (16, 26, 44). Conversely, ASK1 was found to induce neurite outgrowth in PC12 cells and keratinocyte differentiation, predominantly by activation of the p38 pathway (45, 48).

Occurrence of cardiomyocyte apoptosis has been reported in a variety of cardiovascular diseases, including myocardial infarction (MI), ischemia–reperfusion, and end-stage heart failure (29). ROS have been shown not only to induce cardiomyocyte apoptosis by upregulating proapoptotic proteins (54), but also to act as intracellular signaling molecules in cardiac hypertrophy *in vitro* (46) and to mediate the hypertrophy induced by several stimuli, such as mechanical stretch (2, 42), endothelin-1 (49), phenylephrine (49), angiotensin II (35), and TNF-α (20, 35). Similar results have been obtained with an *in vivo* study (11).

In cardiomyocytes, ASK1 is reportedly involved not only in cardiac apoptosis, leading to cardiac remodeling (59), but also in cardiac hypertrophy (23, 27). Furthermore, we recently demonstrated that ASK1 is involved in nonapoptotic cardiomyocyte death (58). The purpose of this review article is to clarify the role of ASK1 in the regulation of ASK1-bind-

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ing proteins including thioredoxin (Trx) and in cardiomyocyte apoptosis.

ASK1 ACTIVITY IS REGULATED BY DIRECT TRX BINDING

Human and mouse ASK1 consist of 1,374 and 1,379 amino acids, respectively, and both have a serine/threonine kinase domain in the middle of the molecule (32). The ASK1 protein contains three domains: the N-terminal regulatory domain, the internal kinase domain, and the C-terminal regulatory domain (39, 44).

Trx is a 12-kDa protein ubiquitously expressed in all living cells and is characterized by the reduction/oxidation (redox) active site sequence Trp-Cys-Gly-Pro-Cys-Lys, which is conserved through evolution (24, 44). The two cysteine residues (Cys32 and Cys35) within the redox active center provide the sulfhydryl groups involved in cytosolic Trx (Trx1)-dependent reducing activity.

Saito *et al.* (44) demonstrated that Trx1 was initially identified from a yeast two-hybrid screening for ASK1-binding proteins. They found that Trx1 bound directly to the N-terminal regulatory domain of ASK1 and inhibited ASK1 kinase activity as well as ASK1-dependent apoptosis. Treatment of cells with *N*-acetyl-L-cysteine also inhibited serum withdrawal–, TNF-α–, and H_2O_2-induced activation of ASK1 as well as apoptosis. The interaction between Trx1 and ASK1 was found to be highly dependent on the redox status of Trx1. Moreover, inhibition of Trx1 by the N-terminus–deleted mutant of ASK1 (ASKΔN) led to constitutive ASK1 kinase activity, which suggests that Trx1 is a physiologic inhibitor of ASK1.

Liu and Min (30) demonstrated that the reduced form of the reactive sulfhydryl groups of Trx1 is necessary for Trx1–ASK1 binding to take place. The oxidized form (intramolecular disulfide between Cys32 and Cys35) or redox-inactive form (the double-mutation at catalytic sites Cys32 and Cys35) of Trx1 did not bind to ASK1. Moreover, inhibition of Trx1 activity by antisense oligonucleotides or by Trx reductase inhibitor resulted in activation of endogenous ASK1 activity. The same investigators demonstrated redox-independent inhibition of ASK1 by Trx1 in cultured bovine aortic endothelial cells. Overexpression of wild-type Trx1 induced ASK1 ubiquitination and degradation, whereas a single mutation of Trx1 at a catalytic site (Cys32 or Cys35) resulted in retention of the binding activity of Trx1 for ASK1 and of its ability to induce ASK1 ubiquitination/degradation. These results suggest that association of Trx1 with ASK1 through a single cysteine is necessary and sufficient for Trx1 to induce ASK1 ubiquitination/degradation, leading to inhibition of ASK1-induced apoptosis (Fig. 1A).

Thr845 is located in the activation loop of the ASK1 kinase domain. Tobiume *et al*. (52) demonstrated that the kinase activity of the alanine-exchange mutant of this residue (T845A) was severely reduced in response to H_2O_2 stimulation and that phosphorylation of Thr845 was essential for ASK1 activation. The mechanism they proposed for the activation of ASK1 after its release from Trx1 starts with the formation by

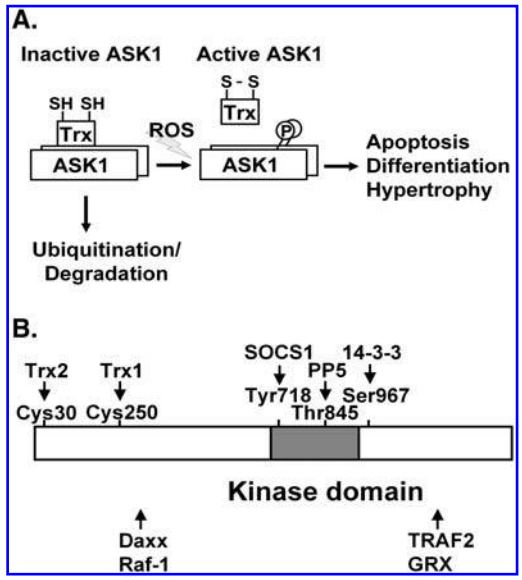

FIG. 1. Model for ASK1 regulation by Trx (A) and schematic illustration of interaction between ASK1 and ASK1 binding proteins (B).

ASK1 of a constitutive homo-oligomer in nonstressed cells through direct association at its C-terminus. On H_2O_2 stimulation, oxidized Trx1 is dissociated from ASK1, and ASK1 creates a new interface in the preexisting oligomeric complex. At the same time, the upstream Thr845 kinase phosphorylates at least one Thr845 of ASK1, leading to a robust autophosphorylation of Thr845 in the homo-oligomer in a transmolecular manner (Fig. 1A).

Because the mitochondria are the major physiologic source of ROS generated during respiration, the production of ROS in mitochondria is strictly regulated by mitochondrial antioxidant systems. In addition to Trx1, mitochondrial Trx (Trx2) has been recently identified in mammalian cells. Trx1 and Trx2 are both encoded by distinct nuclear genes, whereas Trx2 contains a mitochondrial targeting signal peptide. Trx2 is ubiquitously expressed in most mammalian cells (7, 10), and the two cysteine residues within its redox active center are Cys90 and Cys93 (7). Tanaka *et al*. (50) used a Trx2-deficient chicken B-cell line to determine that Trx2 regulates the mitochondrial apoptosis signaling pathway and suggested that it may play a crucial role in the scavenging ROS in mitochondria.

Zhang *et al.* (64) demonstrated that ASK1 is localized in both cytoplasm and mitochondria and binds to Trx1 and Trx2 in the resting state. The proapoptotic stimuli TNF and oxidative stress were found to cause dissociation of Trx1/Trx2 from ASK1, thus leading to enhanced mitochondria-dependent apoptosis. Cys250 is critical for binding of Trx1 in the N-terminal domain of ASK1, and Cys30, for that of Trx2. Mutation of ASK1 at Cys250 was shown to enhance ASK1-induced JNK activation and apoptosis, whereas mutation of ASK1 at Cys30 specifically or specific knockdown of Trx2 increased ASK1-induced apoptosis without affecting JNK activation. These findings suggest that ASK1 in mitochondria may mediate a JNK-independent apoptotic pathway.

NUMEROUS PROTEINS INTERACT WITH ASK1 AND REGULATE ASK1 ACTIVITY

The most common ASK1 regulation is through protein–protein interactions, and numerous proteins have been shown to bind ASK1 to exert their regulatory function (Fig. 1B). The binding of TNF receptor–associated factor (TRAF) 2 (39) or death domain–associated protein (Daxx) (5) enhances ASK1 function, whereas the kinase and proapoptotic activities of ASK1 are inhibited by many other associated proteins, including Trx (30, 44, 64), glutaredoxin (GRX) (47), Cdc25A (66), Hsp 72 (41), ASK1-interacting protein 1 (65), SOCS1 (18), and 14–3-3 proteins (15, 63).

ASK1 binds TRAF2 within the conserved C-terminal regulatory domain, and on TNF stimulation, ASK1 is recruited to TRAF2 and activates the JNK but not the nuclear factor-κB (NF-κB) pathway (39).

Daxx was originally cloned as a Fas-interacting protein and modulator of Fas-induced cell death. On Fas activation, Daxx binds to the N-terminal domain of ASK1 and subsequently activates JNK, which may sensitize cells to apoptosis (5).

14-3-3 belongs to a family of phosphoserine/phosphothreonine-binding molecules. Through phosphorylation-dependent protein–protein interactions, 14-3-3 plays an important role in the regulation of many cellular processes including cell proliferation and survival signaling. When ASK1 is inhibited by Trx, the 14-3-3 proteins bind to ASK1 through phosphorylation of Ser967 by an unidentified kinase. 14-3-3 also was found to inhibit ASK1 activity and ASK1-induced apoptosis. On dissociation from Trx, homodimerized and Thr845-phosphorylated ASK1 was subjected to dephosphorylation at Ser967, which induced dissociation of 14-3-3 and mediated the recruitment of the TRAF-2 protein (15, 63).

GRX, a ubiquitously expressed 12-kDa cytosolic protein rather similar to Trx, was found to act as a cytoprotective antioxidant and to inhibit ASK1. Like Trx, GRX catalyzes the reduction of protein disulfide bonds using a Cys-Pro-Tyr-Cys active site (Cys22 and Cys25). Song *et al.* (47) demonstrated through deletion mutant analysis that the C-terminal regulatory domain of ASK1 binds GRX and that glucose deprivation induces dissociation of GRX from ASK1. They also found that overexpression of GRX inhibits activation of ASK1-MKK4-JNK1 signaling during glucose deprivation, indicating that GRX is a negative regulator of ASK1.

Morita *et al.* (34) demonstrated that protein serine/threonine phosphatase 5 (PP5) directly dephosphorylates Thr845 of ASK1 and inactivates its kinase activity both *in vitro* and *in vivo*. As the interaction between PP5 and ASK1 is induced by H_2O_2 stimulation, it seems to operate as a negative-feedback system of ASK1 activation.

He *et al.* (18) established that SOCS1, via its SH2 domain, binds to the phosphotyrosine residue (Tyr718) on ASK1 and that SOCS1 functions as a negative regulator of TNF-induced inflammation in endothelial cells by inducing ASK1 degradation.

ASK1 IS INVOLVED IN CARDIOMYOCYTE HYPERTROPHY

A growing number of intracellular signaling pathways have been characterized as important transducers of the hypertrophic response, including specific G protein isoforms, low-molecular-weight GTPases (Ras, RhoA, and Rac), MAPK cascades, protein kinase C, calcineurin, gp130-signal transducer and activator of transcription, insulin-like growth factor I receptor pathway, and many others. Each of these signaling pathways has been implicated as a hypertrophic transducer, which collectively suggests an emerging paradigm whereby multiple pathways operate in concert to orchestrate a hypertrophic response (33). We demonstrated a novel ASK1–NF-κB pathway regulating cardiomyocyte hypertrophy in this paragraph. We suggest that this pathway also cooperates with other pathways.

ROS have been shown to act as intracellular signaling molecules during stress response in a variety of cell types, leading to apoptosis, proliferation, and transformation (1). In an *in vitro* study, ROS also were found to be involved in cardiomyocytes in relation to cardiac hypertrophy, which is an important adaptive process (46), and to mediate hypertrophy induced by several stimuli, such as mechanical stretch (2, 42), endothelin-1 (49), phenylephrine (49), angiotensin II (35), and TNF-α (20, 35). Similar results have been obtained with an *in vivo* study (11).

Results of our *in vitro* studies (20, 23) indicate that ROS, the ROS-sensitive transcriptional factor NF-κB, and ASK1 are involved in cardiac hypertrophy induced by G protein–coupled receptor (GPCR) agonists, such as angiotensin II, and cytokines, such as TNF-α. The GPCR agonists and TNF-αa were seen to generate ROS and activate NF-κB in rat neonatal cardiomyocytes. Pretreatment with *N*-acetyl-L-cysteine eliminated the GPCR agonists or TNF-α–induced ASK1 activation, NF-κB activation, and hypertrophic responses. Inhibition of NF-κB activation resulted in elimination of the agonist-induced cardiomyocyte hypertrophy, suggesting that NF-kB is required for the hypertrophic growth of cardiomyocytes. Moreover, GPCR agonists activated ASK1, and overexpression of a dominant negative form of ASK1, ASK(KM), inhibited GPCR agonist–induced cardiomyocyte hypertrophy. Finally, the constitutively active form of ASK1, ASKΔN, activated NF-κB, leading to cardiomyocyte hypertrophy. These indicated that NF-κB and ASK1 are involved in cardiomyocytes hypertrophy. Because *N*-acetyl-L-cysteine inhibited GPCR agonist–induced ASK1 activation, the Trx-ASK1 system could be one of the signaling mechanisms for ROS-mediated cardiac hypertrophy. ASK1 activates the MKK7-JNK and MKK3/6-p38 pathways (26). Upstream activators for p38 (36, 56) and JNK (57) elicit characteristic hypertrophic responses in cardiomyocytes. NF-κB is known to be activated by p38 in cardiomyocytes (61). Overexpression of MEK kinase 1, which functions as the MAPKKK in the JNK signaling pathway, leads to activation of NF-κB (21). These data suggested that ASK1 is involved in GPCR agonist–induced NF-κB activation via p38 and/or JNK. However, ASK1 may have another pathway that activates NF-κB, mediated through ASK1 binding proteins.

To clarify the detailed hypertrophic pathway mediated through ASK1–NF-κB, we also examined several studies. The small guanine nucleotide–binding protein Rac has been implicated in these hypertrophic responses by mediating both the morphologic and transcriptional changes. Clerk *et al*. (8) demonstrated that GPCR agonists induce rapid Rac1 activation in cardiomyocytes. We used an adenovirus expressing a constitutively active mutant of Rac1, a dominant negative mutant of Rac1, a degradation-resistant form of IκBα, or ASK(KM) to demonstrate that Rac1 induces cardiomyocyte hypertrophy mediated through ROS, ASK1, and NF-κB (19).

The Ca^{2+}-sensitive tyrosine kinase Pyk2 is an important downstream target of Ca^{2+} in the hypertrophic signaling pathways. We have further established that Pyk2 is involved in GPCR agonist–induced cardiomyocyte hypertrophy mediated through Rac1 activation and ROS generation (22).

Ca^{2+}/calmodulin-dependent protein kinase (CaMK) is also an important downstream target of Ca^{2+} in the hypertrophic signaling pathways. We used an adenovirus expressing CaMKIIδ3 or ASK(KM) or KN93, an inhibitor of CaMKII, to show that CaMKIIδ3 induces cardiomyocyte hypertrophy mediated through the ASK1–NF-κB signal-transduction pathway (28). The hypertrophic pathways mediated through ASK1–NF-κB are summarized in Fig. 2.

However, the *in vivo* role of ASK1 in cardiac hypertrophy remains controversial. An *in vivo* murine model of pressure overload by thoracic transverse aortic constriction (TAC) leads to hyperfunctional hypertrophy after 1 week, without any signs of heart failure (43). We demonstrated that pressure overload–induced oxidative stress in hearts and the resultant cardiac hypertrophy are attenuated by treatment with the antioxidant mercaptopropionyl glycine (11). In addition, we provided evidence using an *in vivo* murine model that the free radical scavenger 3-methyl-1-phenyl-2-pyrazolin-5-one (edaravone), which has already been used in Japan as an antioxidant in the treatment of patients with ischemic brain damage, can significantly attenuate pressure overload–induced cardiac hypertrophy (53). This attenuation is mediated through the antioxidative function of edaravone and subsequent inhibition of the ASK1 signaling pathway. These findings clearly indicate that ROS are involved in cardiac hypertrophy by TAC. Izumiya *et al*. (27) showed *in vivo* that hypertrophic responses are attenuated in $ASK1^{-/-}$ mice infused with angiotensin II, indicating that ASK1 is involved in GPCR agonist–induced cardiac hypertrophy.

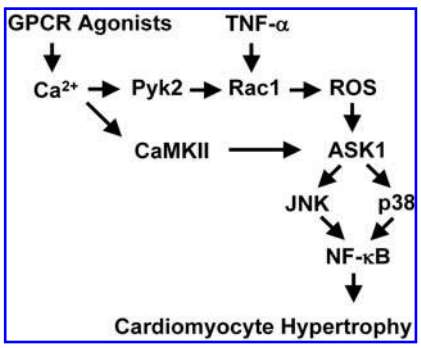

FIG. 2. Hypertrophic pathway mediated through ASK1–NF-κB.

Conversely, we found no differences between hypertrophic responses induced by TAC in $ASK1^{-/-}$ and wild-type mice (59). One week after TAC, heart weight and heart-to–body weight ratio had increased to a similar degree in wild-type and $ASK1^{-/-}$, and changes in the myocyte cross-sectional area of $ASK1^{-/-}$ did not differ from those in wild-type mice. Furthermore, the myocytes isolated from $ASK1^{-/-}$ showed no significant difference in the mean cell area compared with those from wild-type, 1 week after either operation. This suggests either that the endogenous ASK1 does not play a role in the regulation of cardiomyocyte hypertrophy in this model or that the function of ASK1 in the hypertrophy process is compensated for by that of other hypertrophic signaling molecules.

ASK1 PLAYS A PIVOTAL ROLE IN STRESS-INDUCED APOPTOSIS LEADING TO CARDIAC REMODELING

Previous studies have demonstrated that overexpression of wild-type ASK1 or of ASKΔN, the constitutively active mutant of ASK1, induced apoptosis in noncardiomyocytes (44). In addition, we established that ASK1 plays a role in cardiomyocyte apoptosis. Infection of isolated rat neonatal cardiomyocytes with an adenovirus expressing ASKΔN at a multiplicity of infection of 10 plaque-forming units per cell resulted in cardiomyocyte hypertrophy without any signs of apoptosis (23), whereas higher expression levels of activated ASK1 did induce apoptosis, and neonatal $ASK1^{-/-}$ cardiomyocytes were found to be resistant to H_2O_2-induced apoptosis (59).

In the same study using $ASK1^{-/-}$ mice, we also demonstrated that ASK1 plays an important role in stress-induced apoptosis and that stress-induced apoptosis mediated through ASK1 is essential for left ventricular remodeling (59). Left ventricular structural and functional remodeling were determined 4 weeks after left coronary artery (LCA) ligation or TAC. Compared with wild-type mice, $ASK1^{-/-}$ mice showed significantly smaller increases in left ventricular end-diastolic and end-systolic ventricular dimensions and smaller reductions in fractional shortening in both experimental models. The number of terminal deoxynucleotidyl transferase biotin-dUDP nick end-labeling (TUNEL)-positive myocytes after MI or TAC was reduced in $ASK1^{-/-}$ mice compared with that in wild-type mice. An *in vitro* kinase assay showed increased ASK1 activity in wild-type mouse heart after either MI or TAC. The JNK phosphorylation level had significantly increased in wild-type hearts 2 days and 1 week after either LCA ligation or TAC, whereas the increase in phosphorylation levels of JNK was significantly attenuated in $ASK1^{-/-}$ hearts. p38 phosphorylation, conversely, had increased to a similar degree in both wild-type and $ASK1^{-/-}$ hearts after either LCA ligation or TAC. In cardiomyocytes, JNK plays a proapoptotic role in several studies including the cycloheximide-treatment study (4). In contrast, JNK plays a protective

role in ischemia–reperfusion (12) or nitric oxide–induced study (3). p38 also plays both protective and promoting roles in the regulation of cell death in cardiomyocytes (9, 25, 31, 40, 56, 62). Various aspects of stress, such as time, place, quality, and quantity, may determine the precise function of JNK or p38. However, LCA ligation or TAC operated–study in ASK1$^{-/-}$ indicates that the ASK1–JNK signaling pathway, but not the ASK1–p38 signaling pathway, plays a pivotal role in stress-induced apoptosis leading to cardiac remodeling.

The result of another of our studies, using cardiac muscle–specific p38α knockout (p38αCKO) mice, lends support to the notion that cardiomyocyte apoptosis by TAC is mediated through the ASK1–JNK signaling pathway (37). In response to pressure overload to the left ventricle by TAC, p38αCKO mice developed cardiac dysfunction and heart dilatation. Mitochondrion-mediated apoptosis was detected in TAC-operated p38αCKO mice, indicating that p38α plays a critical role in the cardiomyocyte-survival pathway in response to pressure overload. The ASK1-dependent apoptotic signaling pathway resulting from pressure overload is illustrated in Fig. 3.

We further demonstrated that ASK1 plays an important role in regulating left ventricular remodeling by promoting apoptosis in cardiac muscle–specific Raf-1 knockout (RafCKO) mice, which exhibited left ventricular systolic dysfunction and heart dilatation without cardiac hypertrophy or lethality (60). The RafCKO mice also showed a significant increase in the number of apoptotic cardiomyocytes. The expression level and activation of MEK1/2 or extracellular signal–regulated kinase of these mice were not different from those of control littermates, but the kinase activity of ASK1, JNK, or p38 increased significantly. Raf-1 has been shown to bind to the N-terminal domain of ASK1 (6) (Fig. 1B). The ablation of ASK1 in Raf CKO/ASK1$^{-/-}$ double-knockout mice rescued the heart dysfunction and dilatation as well as cardiac fibrosis observed in RafCKO mice, indicating that activation of the ASK1 signaling pathway seems to have an important function in apoptosis in RafCKO mice.

He *et al*. (17) provided evidence of another role of ASK1, its involvement in the regulation of cardiac contractile function by means of cardiac troponin T phosphorylation and its possible participation in cytokine/ROS-induced pathogenesis of cardiomyopathy and heart failure.

These reports indicate that ASK1 plays a pivotal role in the stress-induced apoptosis leading to cardiac remodeling.

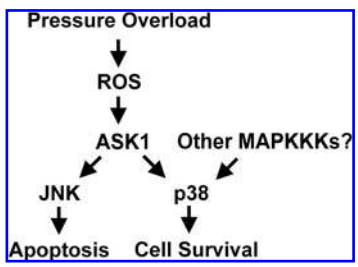

FIG. 3. ASK1-dependent apoptotic signaling pathway by pressure overload.

ASK1 IS INVOLVED NOT ONLY IN APOPTOSIS BUT ALSO IN NONAPOPTOTIC CARDIOMYOCYTE DEATH

Both necrosis and apoptosis are reportedly involved in myocardial injury after ischemia–reperfusion (13, 14), but the relative contribution of necrosis and apoptosis to ischemia–reperfusion injury remains controversial. Because ASK1 plays such an important role in stress-induced apoptosis, we studied ASK1$^{-/-}$ mice to examine the role of ASK1 in ischemia–reperfusion injury (58). In the wild-type heart, ischemia–reperfusion resulted in necrotic injury, whereas infarct size was drastically reduced in the ASK1$^{-/-}$ heart. The necrotic injury was not accompanied by any evidence of apoptosis, such as an increase in TUNEL-positive cells, DNA fragmentation, or the activation of caspase-3. ASK1$^{-/-}$ cardiomyocytes proved to be more resistant to H_2O_2- or Ca^{2+}-induced apoptotic and nonapoptotic cell death than were wild-type cardiomyocytes. These findings suggest that ASK1 is involved in necrosis as well as apoptosis and that ASK1-dependent necrosis is likely to contribute to myocardial cell death in the ischemia–reperfused heart.

CONCLUSIONS

In this review article, we focused on the functional regulation of ASK1 in heart. We conclude that ASK1 is a key molecule in hypertrophy, apoptosis, and necrosis.

ABBREVIATIONS

ASK1, apoptosis signal-regulating kinase 1; ASK1$^{-/-}$, ASK1 knockout; ASKΔN, N-terminus-deleted mutant of ASK1; ASK(KM), dominant negative mutant of ASK1; CaMK, Ca^{2+}/calmodulin-dependent protein kinase; Daxx, death domain–associated protein; GPCR, G protein–coupled receptor; GRX, glutaredoxin; JNK, c-Jun N-terminal kinase; LCA, left coronary artery; MAP, mitogen-activated protein; MI, myocardial infarction; MKK, MAP kinase kinase; NF-κB, nuclear factor-κB; p38αCKO, cardiac- muscle specific p38α knockout; PP5, protein serine/threonine phosphatase 5; RafCKO, cardiac muscle–specific Raf-1 knockout; ROS, reactive oxygen species; TAC, thoracic transverse aortic constriction; TNF, tumor necrosis factor; TRAF, TNF receptor–associated factor; Trx, thioredoxin; TUNEL, terminal deoxynucleotidyl transferase biotin-dUDP nick end-labeling.

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