ANTIOXIDANTS & REDOX SIGNALING
Volume 15, Number 3, 2011

DOI: 10.1089/ars.2010.3392

COMPREHENSIVE INVITED REVIEW

Apoptosis Signaling Kinases: From Stress Response to Health Outcomes

Kohsuke Takeda, Isao Naguro, Hideki Nishitoh, Atsushi Matsuzawa, and Hidenori Ichijo

Abstract

Apoptosis is a highly regulated process essential for the development and homeostasis of multicellular organisms. Whereas caspases, a large family of intracellular cysteine proteases, play central roles in the execution of apoptosis, other proapoptotic and antiapoptotic regulators such as the members of the Bcl-2 family are also critically involved in the regulation of apoptosis. A large body of evidence has revealed that a number of protein kinases are among such regulators and regulate cellular sensitivity to various proapoptotic signals at multiple steps in apoptosis. However, recent progress in the analysis of these apoptosis signaling kinases demonstrates that they generally act as crucial regulators of diverse cellular responses to a wide variety of stressors, beyond their roles in apoptosis regulation. In this review, we have cataloged apoptosis signaling kinases involved in cellular stress responses on the basis of their ability to induce apoptosis and discuss their roles in stress responses with particular emphasis on health outcomes upon their dysregulation. *Antioxid. Redox Signal.* 15, 719–761.

Reviewing Editors: *Kotb Abdelmohsen, Giuseppe Filomeni, Cristina Furdui, Michael Grusch, Derek Hausenloy, Pamela Maher, Hugo Monteiro, Paolo Pinton, Junichi Sadoshima, and Peter Storz*

Laboratory of Cell Signaling, Graduate School of Pharmaceutical Sciences, Strategic Approach to Drug Discovery and Development in Pharmaceutical Sciences, Global Center of Excellence Program and Core Research for Evolutional Science and Technology, Japan Science and Technology Corporation, The University of Tokyo, Tokyo, Japan.

I. Introduction

Apoptosis is an essential process for the maintenance of homeostasis as a defense against stressful changes in internal and external environments as well as for proper development in multicellular organisms. Therefore, apoptosis must be tightly controlled by multiple extracellular and intracellular signaling systems, in which proapoptotic and antiapoptotic factors precisely regulate each other's functions to prevent systemic dysfunction caused by aberrant apoptosis or survival at the cellular level.

Caspases, a large family of intracellular cysteine proteases, are major proapoptotic factors that play central roles in the execution of apoptosis. Caspases are synthesized as inactive zymogens and activated by proteolytic cleavage in response to apoptotic stimuli. In general, initiator caspases, such as caspase-2, -8, -9, and -10, are first activated and, in turn, proteolytically cleave and activate effector caspases, such as caspase-3, -6, and -7, which execute apoptosis by cleaving multiple cellular substrates (208). The Bcl-2 family proteins also critically regulate apoptosis both positively and negatively. This family is divided into two classes: the antiapoptotic Bcl-2 family proteins, such as Bcl-2 itself, Bcl-x_L, and myeloid cell leukemia-1 (Mcl-1), and the proapoptotic Bcl-2 family proteins, such as Bax and Bak. The BH3-only proteins, such as p53-upregulated modulator of apoptosis (Puma), Noxa, Bid, Bim, and Bmf, also are a family of proapoptotic proteins and have the ability to induce apoptosis by binding to and inhibiting the antiapoptotic Bcl-2 proteins. Among these factors, Bax and Bak play a key role; the pro- and antiapoptotic activity of the BH3-only proteins and antiapoptotic Bcl-2 proteins is mediated by positively and negatively regulating Bax/Bak, respectively (410).

A large body of evidence has also revealed that a number of protein kinases are critically involved in the regulation of apoptosis. Many such kinases, designated here as apoptosis signaling kinases, were originally identified due to their ability to induce apoptosis when overexpressed in cultured cells. During the process of characterizing these kinases, it was revealed that the phosphorylation of a variety of intracellular proteins, such as transcriptional factors, cytoskeletal proteins, and adaptor proteins, as well as caspases and the Bcl-2 and BH3-only family proteins, are important events in apoptosis induction. Further analyses and particularly those using genetical approaches with animal models such as gene knockout mice, *Drosophila melanogaster*, and *Caenorhabditis elegans* have revealed that a considerable fraction of apoptosis signaling kinases, at the endogenous level, do not necessarily function in apoptosis induction but rather function in the suppression of apoptosis in a manner dependent on cell types and/or cellular context. Therefore, such kinases appear to regulate cellular sensitivity to various proapoptotic signals at multiple steps in apoptosis as contextual regulators. Further, recent evidence has indicated that apoptosis signaling kinases generally act as crucial regulators of diverse cellular responses to a wide variety of stressors, beyond their roles in apoptosis regulation. In this review, we focus on apoptosis signaling kinases involved in cellular stress responses and their wide range of functions.

II. Components of the Mitogen-Activated Protein Kinase Pathways

A. Stress-activated mitogen-activated protein kinase pathways

The mitogen-activated protein (MAP) kinase cascade is evolutionarily well conserved in all eukaryotic cells and typically includes central three-tiered core signaling modules comprising a MAP kinase kinase kinase (MAP3K), MAP kinase kinase (MAP2K), and MAP kinase (MAPK) (194, 380). All eukaryotic cells possess multiple MAP kinase pathways, and the defined members of the MAPK superfamily include at

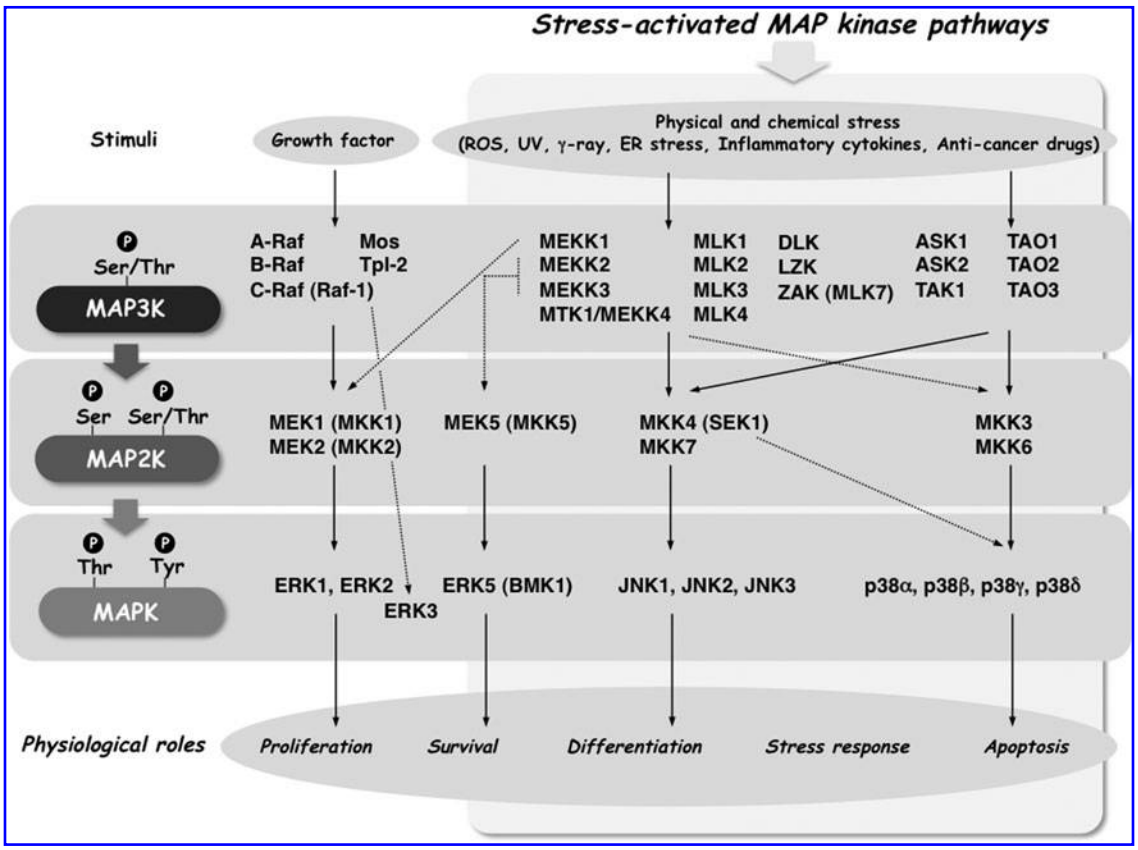

FIG. 1. Overview of the MAP kinase signaling pathways. Each MAP kinase cascade is composed of three classes of kinases that establish a sequential activation pathway comprising a MAP3K, MAP2K, and MAPK. In mammals, three major subgroups of MAPKs, ERKs, JNKs, and p38 MAPKs, have been identified, which are structurally similar to each other, but are functionally distinct. Several other MAPKs, such as ERK5 (BMK1) and ERK3, also exist in these cascades. Whereas ERK is mainly activated by growth factors, JNK and p38 are preferentially activated by various types of environmental stressors, such as UV radiation, oxidative stress, and ER stress, as well as inflammatory cytokines. Therefore, the signaling axes converging to JNKs and p38 MAPKs are called stress-activated MAP kinase pathways. MAPKs phosphorylate downstream targets, such as transcription factors and downstream kinases, and generate diverse and appropriate biological responses. ER, endoplasmic reticulum; ERK, extracellular signal-regulated kinase; JNK, c-Jun N-terminal kinase; MAP, mitogen-activated protein; MAPK, MAP kinase; MAP2K, MAP kinase kinase; MAP3K, MAP kinase kinase kinase; UV, ultraviolet.

least 11 MAPK genes, 7 MAP2K genes, and 21 MAP3K genes in mammals (383) (Fig. 1). 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 from each other. While ERK1 and ERK2 are rapidly activated by a variety of cell growth and differentiation stimuli and play a central role in mitogenic signaling, JNK and p38 are primarily activated by various environmental and chemical stressors such as osmotic shock, ultraviolet (UV) radiation, heat shock, oxidative stress, and protein synthesis inhibitors and proinflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) (341). Therefore, JNK and p38 are often grouped together and referred to as stress-activated protein kinases. ERK5, also known as big MAP kinase 1 (BMK1), was identified as a novel class of stress-activated MAPKs, which is also activated by oxidative stress and osmotic shock (421). MAPKs are activated by dual phosphorylation at tyrosine and threonine residues within the conserved Thr-X-Tyr motif in the activation loop (194). ERK5, as well as ERK1 and ERK2, has the specific sequence Thr-Glu-Tyr in its phosphorylation loop, whereas JNK and p38 have the Thr-Pro-Tyr and Thr-Gly-Tyr motifs, respectively. Atypical MAPK family members, including ERK3, ERK4, ERK7, and NLK, have also been identified, but their functions are still unclear (58).

JNK is a substrate of MKK4/SEK1 and MKK7, and p38 is a substrate of MKK3, MKK4, and MKK6. These MAP2Ks in turn are substrates of the highest signaling modules in the MAPK cascade, MAP3Ks, which include MAP/ERK kinase kinases (MEKKs), mixed-lineage kinases (MLKs), dual-leucine-zipper-bearing kinase (DLK), apoptosis signal-regulating kinases (ASKs), TGF-β activated kinase 1 (TAK1), and thousand and one amino acids (TAOs). MAP3Ks sense the degree of stress-induced cell damage in the upstream phase of the MAPK signaling pathways, so they are important for the determination of cell fate. These signaling axes are stress-activated MAP kinase pathways. While the MEK1/2-ERK1/2 pathway mainly contributes to cell growth and survival, the MKK4/MKK7-JNK and MKK3/MKK6-p38 pathways play a pivotal role in stress-induced apoptosis signaling. In stress-activated MAP kinase pathways, MAP3K

kinases occasionally exist upstream of MAP3Ks. The characteristics of these components will be described in more detail in the later sections. Finally, the stress-activated MAP kinase pathways influence the expression of pro- and antiapoptotic proteins, such as Fas ligand and Bcl-2 family proteins, *via* the phosphorylation of transcription factors, including c-Jun and p53, and regulate the functions of various pro- and antiapoptotic factors, such as caspases, kinases, and Bcl-2 family proteins, by direct phosphorylation as described in detail below.

B. MAP kinases

1. c-Jun N-terminal kinases. JNK has been identified as a family of UV-responsive protein kinases involved in the phosphorylation of c-Jun at Ser63 and Ser73, which is essential for its transcriptional activation (77, 128). There are three genes that encode JNKs in mammals, *JNK1*, *JNK2*, and *JNK3*, each of which has various splicing isoforms. JNKs are preferentially activated in response to various cytotoxic stressors and proinflammatory cytokines and regulate cellular responses to them (194, 380). Although activation of JNKs is mainly regulated by the upstream MAP3Ks and MAP2Ks as described above, the redox-dependent direct regulation of JNKs has also been proposed; nitric oxide negatively regulates JNK activity through S-nitrosylation of a cysteine residue conserved among all three isoforms of JNK (127, 269).

a. JNKs in apoptosis. JNK1 and JNK2 are ubiquitously expressed, whereas JNK3 is mainly found in the brain. $JNK1^{-/-}JNK2^{-/-}$mice have been extensively examined as a useful model for elucidation of the physiological roles of JNK1 and JNK2 in apoptosis. $JNK1^{-/-}JNK2^{-/-}$mice are embryonic lethal due to altered apoptosis during brain development (189). In the brain of these mice, both a decrease and an increase in apoptosis have been observed depending on the brain regions, suggesting that JNKs have the ability to induce and suppress apoptosis. One proposed mechanism by which JNKs differentially regulate pro- and antiapoptosis signals is the duration of JNK activation; the sustained activation of JNKs appears to facilitate apoptosis induction, whereas the acute and transient activation of JNKs appears to protect cells from apoptosis (195, 267). Recently, reactive oxygen species (ROS) have been shown to be critically involved in the regulation of the duration of JNK activation. TNF-α activates the nuclear factor-kappa B (NF-κB) pathway as well as the MAPK pathways. When NF-κB activation is inhibited, JNK is sustainedly activated by TNF-α and contributes to TNF-α-induced apoptosis (267). TNF-induced generation of ROS causes oxidation of the catalytic cysteine, and thus inactivation, of MAPK phosphatases, which negatively regulate JNK by dephosphorylation, allowing sustained activation of JNK (169). These findings demonstrate that phosphatase-dependent regulation of JNK activity is critically involved in ROS-mediated proapoptotic signaling.

$JNK1^{-/-}JNK2^{-/-}$mouse embryonic fibroblasts (MEFs) showed resistance to apoptosis in response to UV radiation, a DNA-alkylating agent methyl methanesulfate, and a translation inhibitor anisomycin (346). These cells were sensitive to Fas ligand-induced apoptosis, suggesting that JNK1 and JNK2 are selectively required for stress-induced apoptosis in MEFs. MEFs derived from $c\text{-}Jun^{AA}$ mice, in which the endogenous *c-jun* gene was replaced by a mutant allele with non-phosphorylatable Ala mutations at Ser63 and Ser73, similarly showed resistance to UV-induced apoptosis (15). These lines of genetic evidence suggest that JNK1 and JNK2 mediate UV-induced apoptosis through phosphorylation of c-Jun. The roles of JNK3 in apoptosis have also been examined using $JNK3^{-/-}$mice (403). The excitotoxic agents, such as kainic acid and glutamic acid, induce apoptosis in hippocampal neurons. $JNK3^{-/-}$hippocampal neurons, but not $JNK1^{-/-}JNK2^{-/-}$neurons, are significantly resistant to glutamic acid-induced apoptosis. Because both $c\text{-}Jun^{AA}$ neurons and $JNK3^{-/-}$neurons are resistant to excitotoxic apoptosis (15), it would appear that the JNK3-c-Jun pathway plays a critical role in excitotoxic-induced apoptosis. Studies using JNK-selective ATP-competitive inhibitors, such as SP600125, and JNK inhibitory peptides have also demonstrated the proapoptotic roles of JNKs (22).

Phosphorylation-mediated activation of various transcription factors has been recognized to mediate a major portion of JNK-induced proapoptotic signals (Fig. 2). For instance, activated JNKs translocate to the nucleus, where they phosphorylate and thus activate c-Jun, inducing proapoptotic genes such as TNF-α, Fas ligand, and the proapoptotic Bcl-2 family member Bak (80). Another well-known factor contributing to JNK-mediated apoptosis is p53, which is the well-characterized tumor suppressor protein that induces cell cycle arrest and apoptosis (387). Phosphorylation of p53 at Ser6 by JNK1 and JNK2 inhibits ubiquitin-mediated degradation of p53, leading to the stabilization of p53 (94). The resulting stabilized p53 induces expression of proapoptotic genes, such as Puma and another apoptotic Bcl-2 family member, Bax, thereby mediating JNK-dependent apoptosis. The stabilization and activation of p73, another member of the p53 family, is also regulated by JNKs in response to

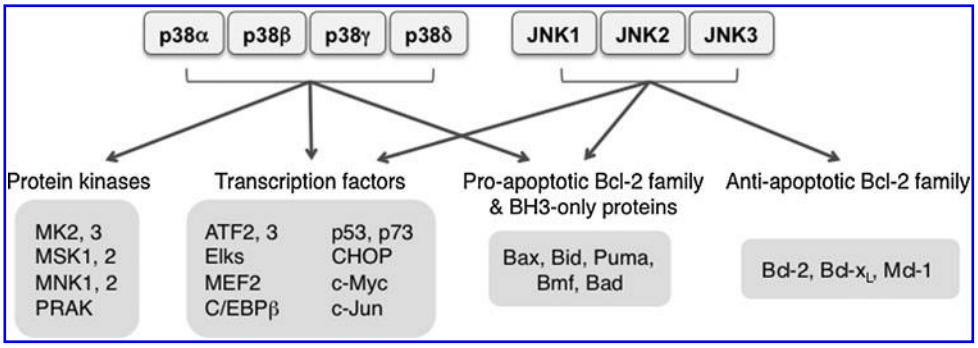

FIG. 2. Substrates of JNKs and p38 MAPKs. Representative groups of substrates of JNKs and p38 MAPKs are shown.

DNA-damaging agents and is required for DNA damage-induced apoptosis through induction of these proapoptotic genes (163).

Several lines of evidence indicate that pro- and antiapoptotic Bcl-2 family proteins contribute to the JNK-mediated apoptosis pathways. The BH3-only protein Bid is cleaved and activated by caspase-8, and the truncated Bid translocates to the mitochondria and triggers cytochrome c release. In response to TNF-α, however, JNK activation induces caspase-8-independent cleavage of Bid at a distinct site to generate its cleaved product jBid, which translocates to the mitochondria and triggers release of another proapoptotic factor, Smac/DIABLO, but not cytochrome c (74). JNKs also phosphorylate and promote the proapoptotic activities of other proapoptotic BH3-only proteins such as Bim, Bmf, and Bad (82, 206, 370). JNK-induced phosphorylation of Bim and Bmf has recently been found to play an important role in apoptosis induced by infection with *Neisseria gonorrhoeae*, the cause agent of the sexually transmitted disease gonorrhea (176). In the case of Bad, JNKs phosphorylate 14-3-3 proteins, which bind to and suppress the proapoptotic activity of Bad, and facilitate dissociation of 14-3-3 from Bad. The liberated Bad appears to translocate to the mitochondria, where Bad exerts its proapoptotic activity (328). During the course of apoptosis induced by treatment of breast cancer cells with the microtubule-damaging agent paclitaxel, JNKs phosphorylated Bcl-2 at Ser70. Mutation of this site inhibited paclitaxel-induced apoptosis, suggesting that JNK suppresses the antiapoptotic activity of Bcl-2 by phosphorylation (400). In addition, JNK phosphorylated another antiapoptotic member of the Bcl-2 family, Mcl-1, at Ser121 and Thr163 in response to oxidative stress, and exogenous expression of a nonphosphorylatable mutant of Mcl-1 enhanced oxidative stress-induced apoptosis (151). It has recently been shown using *JNK1*$^{-/-}$mice and *JNK2*$^{-/-}$mice that phosphorylation of Mcl-1 by JNK1 prevents TNF-α-induced hepatocyte apoptosis through the stabilization of Mcl-1 (186). This may be an important mechanism involved in liver pathology, because TNF-α-induced apoptosis has been implicated in many liver diseases. These findings suggest that JNK-induced phosphorylation of pro- and antiapoptotic Bcl-2 family members is one of the critical events in apoptosis in a variety of cellular contexts.

b. JNKs in proliferation. JNK1 and JNK2 have been shown to differentially regulate proliferation of various cells, such as fibroblasts, erythrocytes, and keratinocytes. *JNK2*$^{-/-}$cells grow slightly faster, whereas *JNK1*$^{-/-}$mice grow slower than their wild-type (WT) counterparts (289). One proposed mechanism is that JNK2 preferentially binds to c-Jun and facilitates its degradation in unstimulated cells. Upon stimulation, JNK1 phosphorylates and stabilizes c-Jun, leading to transcriptional activation (289). Another recent report has shown, using a unique chemical genetics approach, that both JNK1 and JNK2 are positive regulators of c-Jun and contribute to proliferation, and has proposed that the increased c-Jun expression and proliferation observed in *JNK2*$^{-/-}$cells are primarily due to a compensatory increase in JNK1 functions (156). In either case, it is strongly suggested that JNK isoforms have distinct regulatory functions in proliferation. Therefore, elucidation of such isoform-specific functions and their regulation will be needed for a better understanding of the pathophysiological roles of JNKs described in the next section.

c. JNKs in diseases. Recent analyses using genetically modified mice have revealed the roles of JNKs in various pathophysiological conditions (162, 362, 378). Insulin signaling plays an essential role in glucose metabolism and is mediated through tyrosine phosphorylation of the insulin receptor substrate downstream of the insulin receptor in insulin target cells. JNKs phosphorylate a serine residue of insulin receptor substrate-1, which inhibits its association with the insulin receptor and thereby blocks insulin signaling (204). In fact, *JNK1*$^{-/-}$mice were protected against obesity and insulin resistance caused by feeding with a high-fat diet (HFD), suggesting that JNK1 suppresses insulin signaling *in vivo* (130). This is supported by the recent data showing that mice with selective deficiency of JNK1 in adipose tissue suppress HFD-induced insulin resistance in the liver. In addition, these mice are protected against hepatic steatosis (292). However, mice with specific ablation of JNK1 in hepatocytes exhibit hepatic steatosis as well as insulin resistance even under normal feeding conditions (291). These findings clearly indicate that JNK1 functions oppositely in metabolic regulation in adipose tissue and liver. Although JNK1 has been regarded as a potential target for therapeutics for metabolic syndrome, tissue selectivity of JNK1 inhibitors may be a prerequisite for effective therapeutics.

In tumorigenesis, JNKs exert both pro- and antioncogenic functions probably depending on the cell types and/or stages of cancer development (362). In the chemically induced skin tumorigenesis model, papilloma formation was increased and decreased in *JNK1*$^{-/-}$mice and *JNK2*$^{-/-}$mice, respectively, suggesting that JNK isoforms exert distinct functions even in the same tumorigenesis process (38, 314). In contrast to this skin tumorigenesis model, *JNK1*$^{-/-}$mice were resistant to diethylnitrosamine-induced hepatocarcinogenesis, concomitant with decreased expression of cyclin D and vascular endothelial growth factor (VEGF), diminished cell proliferation, and reduced tumor neovascularization (298). With regard to human hepatocarcinogenesis, JNK1 appears to be required for proliferation of human hepatocellular carcinoma (HCC) cells and tumorigenesis after xenotransplantation, suggesting the clinical significance of JNK1 as a therapeutic target for HCCs (144). In line with this, *JNK1*$^{-/-}$mice were also resistant to N-methyl-N-nitrosourea-induced gastric carcinogenesis, probably due mainly to the decreased expression of cyclin D and cyclin-dependent kinase (CDK) as well as decreased cell proliferation (315).

In addition to their roles in the chemically induced tumorigenesis model described above, JNKs also appear to be involved in genetically induced tumorigenesis. *Apc*^{min} mice carry a mutation in the *Apc* tumor suppressor gene and are vulnerable to intestinal tumorigenesis. When *Apc*^{min} mice were crossed with *c-Jun*^{AA} mice, the number of spontaneously generated intestinal tumors was markedly reduced, compared with that in control *Apc*^{min} mice, suggesting that the JNK-c-Jun pathway plays a pro-oncogenic role in the intestines (250). Nevertheless, such a role should be carefully interpreted, because c-Jun appeared not to be essential for intestinal tumorigenesis when mice selectively lacking *c-jun* in the intestinal epithelium were subjected to a similar experiment (123).

Ischemia reperfusion injury is a major clinical problem in several organs, including brain, heart, kidney, lung, and liver. During ischemia reperfusion, cells are exposed to high

oxidative stress environments, which appear to activate JNKs. Liver ischemia reperfusion injury occurs during several conditions, including transplantation, liver tumor resection, and circulatory shock (162). JNKs have been shown to mediate hepatic ischemia reperfusion injury in a rat model using three highly selective JNK inhibitors (CC0209766, CC0223105, and CC-401). Rats treated with either of these inhibitors show the decrease in mortality, which is correlated with the attenuation of necrosis and apoptosis of hepatocytes and sinusoidal endothelial cells (349). Accumulating evidence based on the effectiveness of another highly selective JNK inhibitor, SP600125, demonstrates that JNKs are also involved in ischemia reperfusion injury of other organs, such as brain, kidney, and lung. These findings indicate that JNKs are important general regulators in ischemia reperfusion injury in various organs and serve as potential therapeutic targets (22). Nevertheless, roles of JNKs in myocardial ischemia reperfusion injury seem to be rather complex; *JNK1*$^{-/-}$mice, *JNK2*$^{-/-}$mice, and transgenic mice expressing dominant negative JNK1/2 in the heart showed reduced injury and cellular apoptosis after ischemia reperfusion, but ironically, transgenic mice expressing MKK7, in which cardiac JNK activity was elevated, were also protected from ischemia reperfusion injury (167). This controversial result has been supported by the fact that both the proapoptotic and antiapoptotic roles of JNKs in myocytes have been proposed (372). Thus, the complexity of the roles of JNKs in cellular regulation in heart diseases, as well as in other pathological conditions such as tumorigenesis, may be a barrier to be overcome before clinical application of the JNK inhibitors.

2. p38 MAPKs. In mammals, there are four isoforms of p38 MAPKs, p38α, p38β, p38γ, and p38δ (194, 380) (Fig. 2). p38α and p38β are closely related to each other and have overlapping functions. Although they are ubiquitously expressed among various cell types, p38α and p38β seem to be generally expressed at high and low levels, respectively. p38γ is expressed predominantly in the muscle, and p38δ is expressed mainly in the skin, small intestine, and kidney (61). Most of the published literature on p38 refers to p38α (362). p38α was first identified as a serine/threonine kinase that is rapidly phosphorylated upon treatment of cells with IL-1, lipopolysaccharide (LPS), and heat shock stress (114). Later, it was found that p38 is also activated in response to a wide variety of stress stimuli, such as TNF-α, IL-6, ROS, and UV radiation, and is involved in various biological processes, such as proliferation, differentiation, and apoptosis (61). Substrates of p38 include protein kinases such as MAPKAP kinase-2 (MK2), MK3, p38-regulated/activated kinase (PRAK), mitogen- and stress-activated protein kinase (MSK)-1 and -2, and MAP kinase-interacting kinase (MNK)-1 and -2, as well as transcription factors such as p53, ATF2, Elk1, myocyte enhancer factor 2 (MEF2), cyclic AMP response element binding protein homologous protein (CHOP), and C/EBPβ (61).

a. p38 MAPKs in apoptosis. A number of reports mainly using the inhibitor compounds of p38, such as SB203580, have suggested that p38 MAPKs are involved in apoptosis induction in different cell types. The proapoptotic activity of p38 MAPKs appears to be mediated by transcriptional and posttranscriptional mechanisms that affect the expression and/or activity of death receptors and pro- and antiapoptotic Bcl-2 family proteins (362). Among such mechanisms is the regulation of p53. For instance, p38-mediated phosphorylation of p53 at Ser15 and subsequent activation of p53 has been proposed to be one of the mechanisms of hypoxic stress-induced neuronal cell death (422). In articular chondrocytes, p38-mediated p53 activation was involved in nitric oxide-induced apoptosis (181). Also in neuroblastoma cells and mouse primary cortical neurons, the p38-p53 axis mediated ROS-dependent apoptosis in response to tetrahydrobiopterin, which is an obligatory cofactor for tyrosine hydroxylase and exerts selective toxicity on dopamine-producing cells (31). In addition to Ser15, p38 MAPKs phosphorylate p53 at several other sites, including Ser20, Ser33, Ser37, and Ser46, although it remains unclear whether and how these sites are differentially used downstream of p38 MAPKs (387). In normal human keratinocytes, however, UV radiation induced p38-mediated phosphorylation of p53 at Ser15, which led to the cytoplasmic accumulation of p53 and thereby inhibited UV-induced apoptosis (29). Contribution of the p38-p53 axis to apoptosis may thus depend on the cell types and/or cellular context.

Another setting in which p38 MAPKs play a role in apoptotic processes is the cellular response to endoplasmic reticulum (ER) stress. CHOP is a transcription factor critically involved in ER stress-induced apoptosis (424). The expression level of CHOP is relatively low under nonstressed conditions, but is markedly increased in response to ER stress. Overexpression of CHOP promotes apoptosis in several cell lines, whereas deficiency of CHOP protects cells from ER stress-induced apoptosis (119, 231, 424). Importantly, p38 MAPKs is activated in response to ER stress and in turn phosphorylate and thus activate CHOP (371). Subsequently, CHOP appears to execute apoptosis through downregulation of the antiapoptotic protein Bcl-2 and upregulation of proapoptotic proteins, such as the BH3-only protein Bim and the member of the death receptor family DR5 (233, 277, 397).

b. p38 MAPKs in proliferation and differentiation. Whereas *p38*$\alpha^{-/-}$mice are embryonic lethal due to insufficient vascularization in placental development (2, 4, 246, 336), *p38*$\beta^{-/-}$ mice, *p38*$\gamma^{-/-}$mice, *p38*$\delta^{-/-}$mice, and *p38*$\gamma^{-/-}$*p38*$\delta^{-/-}$mice are all viable and exhibit no apparent abnormalities, at least under unchallenged conditions (14, 290). To analyze the function of p38α in postnatal development, *p38*α conditional knockout (*p38*$\alpha^{f/f}$) mice were generated (88, 251). When *p38*α was specifically deleted in embryos, but not in the placenta, by crossing *p38*$\alpha^{f/f}$ mice to mice with a MORE-cre transgene, the mice died soon after their birth due to lung dysfunction (142). Fetal hematopoietic cells and MEFs from these mice showed enhanced proliferation, suggesting the antiproliferative role of p38α. Consistent with this, *MKK3*$^{-/-}$*MKK6*$^{-/-}$MEFs, which almost completely lacked p38 activity, similarly showed enhanced proliferation, suggesting that the MKK3/6-p38 pathways have the antiproliferative function (25). The antiproliferative roles of p38α have been proposed in other cell types; proliferation of myoblasts, cardiomyocytes, lung cells, and hepatocytes is increased in mice selectively lacking *p38*α in the respective cell types, compared with control cells from *p38*$\alpha^{f/f}$ mice (88, 143, 273, 358).

A large body of evidence has suggested that p38 MAPKs are the major players in skeletal muscle differentiation at

multiple levels. However, the relative contribution of each of the four p38 isoforms is not fully understood. Recently, comprehensive analysis using mice carrying either of the conditional (floxed) *p38α*, *p38β*, *p38γ*, or *p38δ* alleles has revealed that p38α is essential for myoblast differentiation and formation of multinucleated myotubes (273). Consistent with this, p38α-deficient neonatal muscle exhibits delayed maturation *in vivo*. Importantly, this phenotype appears to be associated with a delayed cell cycle withdrawal and continuous proliferation of cells lacking p38α. Whereas myoblasts lacking p38β or p38δ normally differentiate and form myotubes, p38γ-deficient myoblasts exhibit an attenuated fusion capacity, suggesting some contribution of p38γ to myoblast differentiation (61, 273).

One plausible explanation for the antiproliferative roles of p38α is the suppression of the JNK-c-Jun pathway, which we will discuss later (see Crosstalk between the JNK and p38 pathways section). Another important mechanism by which p38α regulates proliferation, particularly in response to inducers of DNA damage, is the control of cell cycle checkpoints (115). MK2, a downstream kinase of p38 MAPKs, phosphorylates Cdc25B and Cdc25C, phosphatases that facilitate G_2/M progression by activation of CDK1 by dephosphorylation of its inhibitory phosphorylation sites. Phosphorylation sites in Cdc25B/C by MK2 are recognized and sequestered by 14-3-3 proteins, leading to cell cycle arrest through inactivation of Cdc25B/C (225). This mechanism appears to play an important role in the control of DNA damage checkpoints, especially in the absence of p53, and may be critically involved in the prevention of carcinogenesis and cancer progression (283).

c. p38 MAPKs in diseases. Recent evidence has strongly suggested that p38 MAPKs are involved in tumor suppression (115, 143, 362). The antiproliferative role of p38α described in the previous section is recognized as a candidate mechanism that may account for p38-dependent tumor suppression. When immortalized *MKK3*$^{-/-}$*MKK6*$^{-/-}$MEFs were injected subcutaneously into athymic nude mice, a dramatic increase in tumorigenesis was observed (25). In a chemically induced liver cancer model, mice with liver-specific deletion of the *p38α* gene exhibited increased tumorigenesis, concomitant with upregulated hepatocyte proliferation (142). Consistent with the finding that p38α suppressed self-renewal of the lung stem and progenitor cell population, K-Ras-induced tumorigenesis was enhanced in p38α-deficient lungs (358).

p38-dependent apoptosis has also been proposed to contribute to tumor suppression. Once activated by ROS-producing carcinogenic stimuli, p38α induces apoptosis and prevents the further accumulation of ROS and their carcinogenic effects, indicating that p38α functions as a sensor of ROS in tumorigenesis (81). p38 MAPKs also regulate oncogene-induced senescence as a barrier to tumor development. PRAK, a kinase directly regulated by p38, induces senescence upon Ras-induced activation of p38, probably through direct phosphorylation of p53 by PRAK (326). Inactivation of PRAK prevents senescence and promotes oncogenic transformation in primary cells, and consistent with this, chemically induced skin tumorigenesis is increased in *PRAK*$^{-/-}$mice, compared with WT mice.

In contrast, *p38δ*$^{-/-}$mice have recently been shown to be less susceptible to chemically induced skin tumorigenesis, the same experimental system as used in the analysis of *PRAK*$^{-/-}$mice (304). This finding suggests that p38 isoforms have different functions both in suppression and promotion of tumorigenesis. Given that inflammation, particularly its chronic form, is regarded as a critical component of tumor promotion, p38 MAPKs may be able to accelerate tumorigenesis through upregulation of inflammation. That p38 MAPKs play critical roles in cytokine production and inflammatory responses has been supported by a number of studies mainly using p38 inhibitor compounds, and is further supported by recent genetic analyses using selective deletion of the *p38α* gene in myeloid or epithelial cells (171, 178).

Roles of p38 MAPKs in other pathological settings have also been investigated; cardiac-specific p38α-deficient mice exhibited abnormal responses to pressure overload, including massive cardiac fibrosis and the appearance of apoptotic cardiomyocytes (251). This finding demonstrates that p38α plays a critical role in the cardiomyocyte survival pathway in response to pressure overload, although both proapoptotic and antiapoptotic regulatory roles of p38 MAPKs in cardiomyocytes have been proposed (372). In fact, targeted inhibition of p38 MAPKs using transgenic mice expressing dominant-negative MKK6 or dominant-negative p38α antagonized cardiac injury and apoptosis after ischemia reperfusion (166). Further, *p38δ*$^{-/-}$mice were protected against HFD-induced insulin resistance and oxidative stress-mediated pancreatic β cell failure, suggesting that p38δ regulates the insulin secretory capacity and survival of β cells (324). Taken together with the roles of p38 MAPKs in a variety of inflammatory diseases, such as rheumatoid arthritis, Crohn's disease, psoriasis, and asthma, as critical regulators of cytokine production (59), these findings imply that p38 MAPKs are profoundly involved in a variety of human diseases. Thus, p38 MAPKs attract serve as potential chemotherapeutic targets, and a number of p38 inhibitors have indeed reached clinical trials (59).

3. Crosstalk between the JNK and p38 pathways. Recently, crosstalk between the JNK and p38 pathways has received much attention as a system that may provide diversity of cellular stress responses. Although JNKs and p38 MAPKs are often activated by the same stimuli through common upstream signaling components and synergize to activate the downstream effectors, these two pathways sometimes act antagonistically to each other. The decreased cell proliferation, increased cellular senescence, and resistance to oncogenic transformation observed in *MKK7*$^{-/-}$ MEFs were all rescued by treatment with the p38 inhibitor, suggesting that the phenotypic changes in the absence of JNK activity are mediated by the p38 pathway and that JNKs suppress p38 functions in WT cells (361). Consistent with this, p38 activity was increased during liver regeneration after partial hepatectomy in mice selectively lacking *c-jun* in the liver and suppressed hepatocyte proliferation through aberrant induction of the CDK inhibitor p21. In fact, conditional deletion of both *p38α* and *c-Jun* in the liver restored hepatocyte proliferation and the normal level of p21 expression (320).

In contrast, aberrant activation of the JNK-c-Jun pathway in the absence of p38α has been proposed to be involved in cell proliferation. Fetal hematopoietic cells, MEFs, and hepatocytes from *p38α*$^{-/-}$mice exhibited increased proliferation resulting from sustained activation of the JNK-c-Jun pathway. Consistent with this, in the diethylnitrosamine-induced

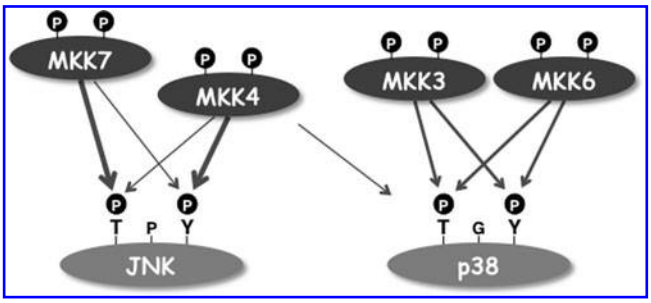

FIG. 3. Regulation of JNKs and p38 MAPKs by MAP2Ks. MKK4 and MKK7 activate JNKs by preferentially phosphorylating Tyr and Thr residues, respectively, of the T-P-Y motif in JNKs, whereas MKK3 and MKK6 activate p38 MAPKs by equally phosphorylating Thr and Tyr residues in the T-G-Y motif in p38 MAPKs. MKK4, but not MKK7, has the ability to phosphorylate and activate p38 MAPKs.

hepatocarcinogenesis model, mice with liver-specific deletion of *p38α* exhibited increased tumor formation, which was restored by conditional co-deletion of *c-jun* in the liver, suggesting that p38α negatively regulates cell proliferation by antagonizing the JNK-c-Jun pathway (142). Further, this regulatory system appears to operate in myogenesis. As described above, p38α-deficient neonatal muscle exhibited delayed maturation, which was associated with continuous proliferation of cells lacking p38α. This proliferation in the absence of p38α also depended on the activation of the JNK-c-Jun pathway (273). Although molecular mechanisms by which crosstalk between the JNK and p38 pathways is regulated are not fully understood, elucidation of such mechanisms is a prerequisite for a full understanding of the physiological roles of the stress-activated MAP kinase pathways.

C. MAP kinase kinases

MAP2Ks are signaling intermediates between MAP3Ks and MAPKs. MAP2Ks are activated upon direct phosphorylation by MAP3Ks and in turn activate MAPKs by dual phosphorylation at threonine and tyrosine residues within the Thr-X-Tyr motif located within the activation loop of the protein kinase subdomain VIII (194, 380). Among the seven MAP2K isoforms in mammals (Fig. 1), we here focus on four MAPKs, MKK4/SEK1, MKK7, MKK3, and MKK6, as regulators of the JNK and p38 pathways.

1. MKK4 and MKK7. MKK4 (also known as SEK1) and MKK7 cooperate to activate JNK *via* dual phosphorylation of the Thr-Pro-Tyr (T-P-Y) motif in the kinase domain of JNK (67). MKK4 and MKK7 have been shown to preferentially phosphorylate at Tyr and Thr residues, respectively, of the T-P-Y motif in JNKs, whereas MKK3 and MKK6 are believed to activate p38 MAPKs by equally phosphorylating Thr and Tyr residues in the T-G-Y motif in p38 MAPKs (198, 255) (Fig. 3). Compared with that in WT cells, JNK activation by proinflammatory cytokines such as TNF-α and IL-1 was strongly inhibited in $MKK7^{-/-}$ cells, whereas substantial JNK activation remained in $MKK4^{-/-}$cells (345). However, JNK activation by environmental stressors, such as UV radiation, heat shock, and osmotic stress, which was almost completely inhibited in embryonic stem cells deficient for both MKK4 and MKK7 ($MKK4^{-/-}MKK7^{-/-}$), was similarly inhibited in both $MKK4^{-/-}$cells and $MKK7^{-/-}$cells (184, 252, 330, 345, 402). These observations suggest that the respective contribution of MKK4 and MKK7 to JNK activation may differ depending at least on the types of cellular stimuli.

Another functional difference between MKK4 and MKK7 is that MKK4 activates not only JNK but also p38 *in vitro*, whereas MKK7 activates only JNK (134, 219, 347, 390, 405). Indeed, the residual p38 activation induced by UV radiation in $MKK3^{-/-}MKK6^{-/-}$MEFs was further attenuated upon MKK4 knockdown, suggesting that MKK4 plays a role in UV-induced p38 activation (25). On the other hand, a contribution of MKK4 to TNF-α-induced p38 activation was not detected in the same experiment, indicating the selective function of MKK4 in the p38 pathway. Therefore, for a more comprehensive understanding of the regulation of the JNK and p38 pathways, it may be important to elucidate the physiological settings in which MKK4 activates p38.

$MKK4^{-/-}$mice and $MKK7^{-/-}$mice have been shown to display severe anemia and die at E11.5-13.5, suggesting that MKK4 and MKK7 have nonredundant functions *in vivo* (98, 252, 254, 299, 373). MKK4 appears to have a crucial role in inducing a survival signal in hepatocytes, since $MKK4^{-/-}$ embryos exhibit severe hemorrhage with increased hepatocyte apoptosis in the liver at E12.5 (254) (Table 1). Deletion of the *MKK4* gene also enhanced TNF-α-induced apoptosis in MEFs, which was accompanied by downregulation of TNF-α-induced proliferative gene products, such as COX-2 and cyclin D1, as well as of antiapoptotic gene products, such as survivin, IAP1, XIAP, Bcl-2, Bcl-x_L, and c-FLIP (309). Further, MKK4 mediated survival signals in $CD4^+CD8^+$ thymocytes and peripheral T cells by protecting the cells from Fas or CD3

Table 1. Cellular Apoptotic Phenotypes of Knockout Mice of Mitogen-Activated Protein Kinase Kinases in the c-Jun N-Terminal Kinase and p38 Pathways

Genotypes	In vivo *phenotypes*		*Cellular apoptotic phenotype*	*References*
$MKK4^{-/-}$	Lethal	↑	Hepatocyte, MEF, Thymocyte, Peripheral T cell	(252–254, 309)
	Lethal	→	Thymocyte, Peripheral T cell	(330)
$MKK7^{-/-}$	Lethal	→	Thymocyte, Mast cell	(299, 360)
$MKK3^{-/-}$	No apparent phenotype	↓	Peripheral T cell	(152, 217, 221, 337)
$MKK6^{-/-}$	No apparent phenotype	↓	Thymocyte	(329, 337)
$MKK3^{-/-}MKK6^{-/-}$	Lethal			(25)

The upward and downward arrows indicate augmented and diminished apoptotic phenotypes, respectively, and the horizontal arrows indicate no apparent changes, compared with the wild-type counterparts.
MEF, mouse embryonic fibroblast.

cross-linking-induced apoptosis (253). However, another group has suggested that MKK4 does not play an essential role in the regulation of apoptosis, since they observed no significant difference in Fas or CD3 cross-linking-induced apoptosis between *MKK4*$^{-/-}$thymocytes and WT thymocytes (330). Unfortunately, no plausible explanation for this discrepancy has been proposed thus far.

While MKK4 has been shown to regulate stress-induced apoptosis, at least in some cellular contexts, the roles of MKK7 in stress-induced apoptosis have not been understood. When *MKK7*$^{-/-}$thymocytes and mast cells were compared with their WT counterparts, UV radiation-, anisomycin-, and osmotic stress-induced cell death was not inhibited, although the JNK activation induced by these stressors was significantly inhibited (299, 360). The MKK7-JNK pathway may thus play no particular role in apoptosis signaling in these cellular contexts. Collectively, MKK4 and MKK7 appear to have distinct functions, especially in the regulation of apoptosis, whereas they cooperatively regulate JNK activity in some cases. Differential usage and/or regulation of MKK4 and MKK7 may account, at least in part, for the divergent functions of the JNK pathway.

2. MKK3 and MKK6. MKK3 and MKK6 activate p38 *via* dual phosphorylation of the Thr-Gly-Tyr (T-G-Y) motif in the kinase domain of p38 (194). Phosphorylation of p38 is mediated, in part, through interaction between the N-terminal region of MKK3 or MKK6 and the docking site on p38 containing the T-G-Y motif (35) (Fig. 3). MKK3 and MKK6 have been proposed to function in the regulation of T cell development, cytokine production, and inflammatory responses (152, 217, 221, 391). Several reports have also demonstrated the involvement of MKK3 and MKK6 in multiple apoptosis-inducing pathways. Overexpression of a constitutively active form of MKK3 potentiated Fas-induced apoptosis (164), and a dominant negative form of MKK3 inhibitednerve growth factor (NGF) withdrawal-induced apoptosis in differentiated PC12 cells (394). Overexpression of a constitutively active form of MKK6 induced apoptosis in NIH3T3 cells (55). Further, MKK6 was activated by overexpression of c-Abl, which is a ubiquitously expressed protein tyrosine kinase activated by DNA damage (see c-Abl section), and a dominant negative form of MKK6, but not dominant negative form of MKK3, inhibited c-Abl- and DNA damage-induced apoptosis (55). These findings demonstrate that MKK3 and MKK6 function as mediators of apoptosis in several cellular contexts. However, the molecular mechanisms by which MKK3 and MKK6 induce proapoptotic signals are still unclear. The specific p38 inhibitor compound SB203580 and a dominant negative form of p38 (p38DN) failed to inhibit NGF withdrawal-, c-Abl-, and MKK6-induced apoptosis (55, 164). Although Fas cross-linking induced activation of MKK6 and the caspase cascade, p38DN had no effect on Fas/MKK6-mediated apoptosis (141). These findings suggest that the MKK3- and MKK6-mediated apoptosis pathways may have some redundancy or branch from the p38 pathway.

The contribution of MKK3 and MKK6 to apoptosis has been analyzed by deletion of their respective genes in mice. *MKK3*$^{-/-}$mice and *MKK6*$^{-/-}$mice are viable and fertile with no apparent developmental abnormalities, including in the thymus and spleen (337, 391) (Table 1). Whereas the number of thymocytes and splenocytes in *MKK3*$^{-/-}$mice and *MKK6*$^{-/-}$mice was comparable with that in WT mice, apoptosis of $CD4^+CD8^+$ double positive thymocytes induced by anti-CD3 antibody treatment (CD3 cross-linking) *in vivo* was inhibited in *MKK6*$^{-/-}$mice, but not in *MKK3*$^{-/-}$mice, compared with WT mice (337). Consistent with this finding, *MKK6*$^{-/-}$thymocytes, but not *MKK3*$^{-/-}$thymocytes, showed a reduction in anti-CD3 antibody-induced p38 activation and apoptosis *in vitro.* Moreover, overexpression of MKK6 in a fetal thymic organ culture induced thymocyte apoptosis and mimicked the negative selection of $CD4^+CD8^+$ thymocytes (323, 329). These findings suggest that MKK6 contributes to the negative selection of $CD4^+CD8^+$ thymocytes through p38 activation. On the other hand, MKK3, but not MKK6, appears to be involved in peripheral T cell apoptosis. Although there was no significant difference in proliferation of T cells among *MKK3*$^{-/-}$mice, *MKK6*$^{-/-}$mice, and WT mice, activated $CD4^+$ T cells derived from *MKK3*$^{-/-}$mice were more resistant to apoptosis induced upon IL-2 withdrawal than those from *MKK6*$^{-/-}$mice or WT mice (337). Collectively, these results suggest that MKK3 and MKK6 are differentially involved in the apoptosis of activated $CD4^+$ peripheral T cells and $CD4^+CD8^+$ thymocytes, respectively.

The role of MKK3 in apoptosis has also been shown using the mouse model of unilateral ureteric obstruction (221). Apoptosis of tubular cells in this model was significantly reduced in *MKK3*$^{-/-}$mice compared with WT mice. Further, primary cultured tubular epithelial cells from *MKK3*$^{-/-}$mice were resistant to oxidative stress-induced apoptosis. These findings suggest that MKK3 contributes to renal cell apoptosis in this pathological setting.

Mice deficient for both MKK3 and MKK6 (*MKK3*$^{-/-}$ *MKK6*$^{-/-}$mice) die during midgestation at embryonic day (E) 11.0-11.5 and appear to be developmentally delayed and exhibit symptoms of severe anoxia, which are consistent with the phenotype of *p38α*$^{-/-}$mice (25). Considering the possibility that phenotypes of *MKK3*$^{-/-}$mice and *MKK6*$^{-/-}$mice are compensated to some extent by MKK6 and MKK3, respectively, *MKK3*$^{-/-}$*MKK6*$^{-/-}$mice will be powerful tools to elucidate the actual contribution of MKK3 and MKK6 to apoptosis induction.

D. MAP kinase kinase kinases

The MAP3Ks are highly divergent in structure and gene number and possess different motifs and domains, which control protein–protein interactions and post-translational modifications, such as ubiquitination, sumoylation, and phosphorylation, and generate specificity for the stimulus-dependent activation of the MAP2K-MAPK pathways. MAP3Ks function as platforms to integrate MAPK activation with physiological responses to different stimuli. The activation of multiple MAP3Ks provides the spatio-temporal control of the MAPK pathways, which produces a wide variety of physiological responses needed for determining cell fate, such as survival and apoptosis, in response to various types of stress.

1. ASK family. ASK1, also called MEKK5 or MAP3K5, is one of the MAP3K family members that activate the JNK and p38 MAPK pathways through MKK4/MKK7 and MKK3/MKK6, respectively (148, 368). ASK2, also called

MEKK6 or MAP3K6, possesses an overall structure similar to that of ASK1 (334, 369), and thus these MAP3Ks compose the ASK family. NSY-1 and *Drosophila* ASK1 (DASK1) are the single orthologs of mammalian ASK family in *C. elegans* and *Drosophila*, respectively, and are considered prototypic molecules of ASK1 and ASK2 (192, 295).

ASK1 is activated in response to various types of stress, such as oxidative stress and ER stress, calcium overload, and inflammatory cytokines such as TNF-α and plays pivotal roles in apoptosis signaling pathways. Overexpression of WT ASK1 or constitutively active ASK1 induces apoptotic cell death (297). ASK1-dependent apoptosis is mainly mediated by mitochondria-dependent caspase activation (124). Expression of a constitutively active mutant of ASK1 induced the release of cytochrome c from the mitochondria and activation of caspase-9 and caspase-3, but not of caspase-8, and ASK1 failed to activate caspase-3 in caspase-9-deficient cells. When activated at the G_2/M phase, the ASK1-JNK pathway inactivated Bcl-2, an antiapoptotic Bcl-2 family member, by phosphorylation (400).

ASK1 activity is upregulated by auto-phosphorylation of a threonine residue within the activation segment in the kinase domain (Thr838 and Thr845 of human and mouse ASK1, respectively) and is downregulated by dephosphorylation of this site by protein phosphatases, such as protein serine/threonine phosphatase 5 (PP5; see the next section) and protein phosphatase 2Cε (244, 296). In addition, Ser83, Ser966, and Ser1033 have been identified as negative phosphorylation sites (95, 102, 213). Among them, Ser83 is particularly drawing much attention, because this site is phosphorylated, and thus inactivated, by the survival-promoting serine/threonine kinase Akt/protein kinase B (177, 411). Akt is activated through phosphoinositide-dependent kinase 1 downstream of phosphatidylinositol 3′ kinase that is activated in response to a variety of growth factors and cytokines, and the activated Akt in turn phosphorylates a wide range of substrate proteins, including several proapoptotic proteins (116, 386). In addition to cell survival, Akt is known to regulate multiple cellular processes such as nutrient metabolism, cell proliferation, cell motility, and angiogenesis. Therefore, Akt may counteract the roles of ASK1 not only in apoptosis induction, but also in other various biological functions.

ASK2 is a recently characterized member of the ASK family and a functional binding partner of ASK1. By the formation of an endogenous heteromeric complex with ASK1, ASK2 is stabilized and exhibits sufficient activity toward the JNK and p38 pathways, accompanied by an increase in apoptosis-inducing caspase-3 activity (334). In the absence of ASK2, H_2O_2-induced JNK activation is clearly diminished, suggesting that the ASK1/ASK2 hetero-complex plays critical roles in regulation of the oxidative stress-induced apoptosis-signaling pathway. Further, chemically induced skin tumorigenesis was promoted in *ASK2*$^{-/-}$mice; in cooperation with ASK1, ASK2 functions as a tumor suppressor by exerting proapoptotic activity in epithelial cells (153). Genetic analyses using *Drosophila* have shown that DASK1 mediates conserved death signaling in fly eyes and cells (192). DASK1 was identified through a dominant modifier screening that explored the mediators of cell death induced by Reaper, a proapoptotic factor during *Drosophila* embryogenesis. The Reaper-induced cell death was clearly suppressed by the expression of a dominant negative mutant of DASK1.

a. ASK1 and oxidative stress. MEFs derived from *ASK1*$^{-/-}$ mice were resistant to oxidative stress- and TNF-α-induced apoptosis (343). Oxidative stress- and TNF-α-induced sustained but not transient activations of JNK and p38 were significantly diminished in *ASK1*$^{-/-}$cells. TNF-α-induced apoptosis also required ROS-dependent activation of the ASK1-JNK and -p38 pathways. Therefore, ROS-mediated sustained activations of the JNK and p38 pathways may be responsible for apoptosis. As a redox sensor, ASK1 may sense the extent of oxidative stress and drive apoptosis signaling only when cells are extensively damaged by excess and prolonged exposure to ROS. Fas, a member of the death receptor family, also induces ASK1 activation through the association between ASK1 and Daxx (36). Although Fas-induced activations of JNK and p38 were reduced in ASK1-deficient thymocytes, Fas-mediated apoptosis was unaffected, suggesting that ASK1 is not required for Fas-induced apoptosis in thymocytes (343). The possible involvement of ASK1 in Fas-induced apoptosis in other cell types and tissues should be examined.

The regulatory mechanisms of ASK1 have been well characterized. In nonstimulated cells, ASK1 forms an inactive complex with thioredoxin (Trx), which is a redox-sensitive protein with inhibitory activity on ASK1 (297). Trx is a redox-regulatory protein that has two redox-sensitive cysteine residues within the active center. ASK1 is dissociated from Trx in an oxidative stress-dependent manner and thereby activated by its homophilic interaction and cross-phosphorylation. ROS produced by various stimuli, such as TNF-α and anticancer drugs, are the most potent activators of the ASK1-mediated apoptosis-signaling pathway (Fig. 4). ASK1 is also activated by endotoxins such as LPS through ROS production, so that mammalian ASK1 as well as its *C. elegans* ortholog NSY-1 play critical roles in innate immune response (179, 232). ROS therefore function not only as inducers of ASK1-mediated apoptosis, but also as enhancers of inflammation mediated by activation of the ASK1-p38 pathway. TNF receptor-associated factor 2 (TRAF2) and TRAF6, originally identified as adaptor proteins between kinases and receptors, activate ASK1 downstream of TNF receptor 1 (TNFR1) and the LPS receptor Toll-like receptor 4 (TLR4), respectively, by enhancing homo-oligomerization and subsequent auto-phosphorylation of ASK1 (232, 258, 261). Moreover, ROS-mediated activation of the ASK1-p38 pathway is required for extracellular ATP-induced apoptosis in macrophages, which is initiated by the ATP-ligated P2X7 purine receptor (260). The ATP-induced apoptosis may be required for termination of inflammatory responses.

ROS generated by angiotensin II (Ang II) plays an important role in pathophysiological cardiac hypertrophy and remodeling. ASK1 was activated upon treatment with Ang II in an ROS-dependent manner in the left ventricle, and Ang II-induced cardiac hypertrophy and remodeling was significantly reduced in *ASK1*$^{-/-}$mice (154). Also in models of myocardial infarction and pressure overload, cardiac hypertrophy and remodeling was inhibited in *ASK1*$^{-/-}$mice (399). In addition, when subjected to myocardial ischemia reperfusion injury, *ASK1*$^{-/-}$mice exhibited decreased infarct size and reduced susceptibility to myocardial cell death (374). These findings strongly suggest that ROS-mediated ASK1 activity is widely involved in the pathogenesis of heart failure.

Alzheimer's disease (AD) is pathologically characterized by cerebral neuritic plaques of amyloid-β (Aβ) peptide and

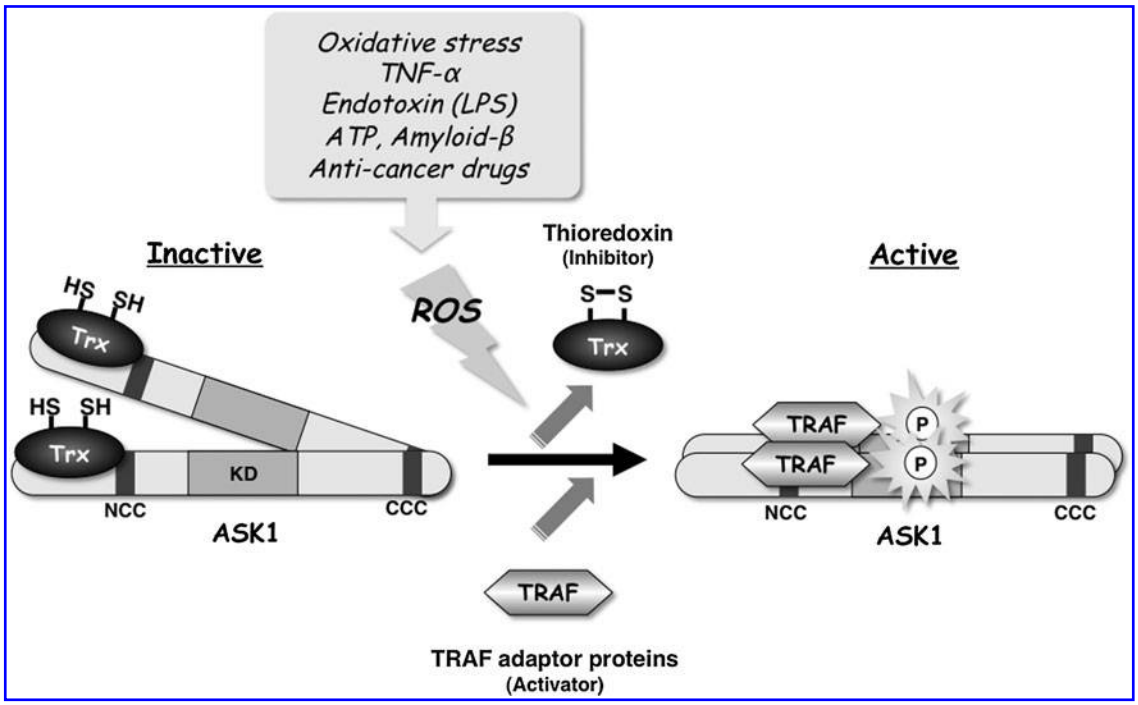

FIG. 4. Regulation of ROS-induced ASK1 activation. ASK1 possesses a kinase domain (KD) in its midportion and two coiled-coil domains, one in the N-terminal and the other in C-terminal, both of which are critical for ASK1 activation through the homophilic interaction. ASK1 is inactivated by interaction with the reduced form of thioredoxin (Trx). Upon ROS-induced dissociation of Trx from ASK1, ASK1 is tightly oligomerized through its N-terminal coiled-coil (NCC) domains in addition to the basal interaction through its C-terminal coiled-coil (CCC) domains. TRAF adaptor proteins, such as TRAF2 and TRAF6, are recruited to ASK1 and facilitate the active configuration of ASK1. ASK1 is fully activated by auto-phosphorylation of a threonine residue in its KD. ROS are produced not only by stress stimuli, such as TNF-α and anticancer drugs, but also by endotoxins, such as lipopolysaccharide (LPS). ROS function as mediators of both apoptosis and immune response. ASK, apoptosis signal-regulating kinase; ROS, reactive oxygen species; TNF-α, tumor necrosis factor-α; TRAF, TNF receptor-associated factor.

neurofibrillary tangles that induce severe neurodegeneration through apoptotic neuronal death. Aβ activates ASK1 through the generation of ROS in neuronal cells, and ASK1-deficient neurons are defective in Aβ-induced JNK activation and cell death, suggesting that ROS-mediated ASK1 activation by Aβ is an important step in the pathogenesis of AD (165).

PP5 is a negative regulator of ASK1 (244). PP5 binds to and dephosphorylates the activated form of ASK1 in response to oxidative stress, inactivating ASK1 in a negative feedback manner. Recently, it has been demonstrated that in response to oxidative stress, activated ASK1 is ubiquitinated and destabilized by proteasomal degradation. USP9X, a deubiquitination enzyme, binds to ASK1 in an oxidative stress-dependent manner and prolongs ASK1 activation through deubiquitination and stabilization of activated and ubiquitinated ASK1. Consistent with this finding, ASK1-mediated apoptosis was enhanced in USP9X-deficient cells (248). HSP72, a heat shock protein, interacts with ASK1 and prevents its dimerization and activation. A mild heat shock induces the ASK1-HSP72 interaction, which protects cells from ROS-induced apoptosis (268).

Reactive nitrogen species (RNS) also regulate ASK1 activity and probably ASK1-mediated apoptosis (127, 325). S-nitrosylation of Trx has been proposed to be a possible mechanism for regulation of ASK1 activity by RNS. Whereas basal S-nitrosylation of Trx Cys69 that lies outside the active site facilitates antiapoptotic functions, S-nitrosylation of possibly the active site cysteine residues (Cys32 and Cys35) exerts proapoptotic activity by facilitating dissociation of ASK1 from Trx (325). On the other hand, ASK1 itself is S-nitrosylated at Cys869, but in this case, ASK1 activity is reduced (270). Thus, it remains to be elucidated whether RNS-induced activation of ASK1 is proapoptotic or antiapoptotic in physiological and pathological settings.

b. ASK1 and ER stress. Accumulation of unfolded and misfolded proteins in the ER induces cellular stress, and persistent exposure to severe ER stress finally leads cells to apoptosis. TRAF2 functions as an adaptor protein of IRE1, which is a transmembrane sensor and a signal transducer protein in ER stress signaling. The TRAF2-ASK1 pathway is required for ER stress-induced apoptosis; ASK1-deficient MEFs were resistant to the ER stress-induced apoptosis, accompanied by suppression of activations of JNK and p38 (257).

Accumulating evidence has suggested that ER stress is critically involved in neurodegenerative disorders, such as AD, Parkinson's disease, and polyglutamine (polyQ) diseases. ER stress caused by polyQ aggregation induced formation of the IRE1-TRAF2-ASK1 complex, which was critical for the ER stress-induced apoptosis (257). Recently, ASK1 has

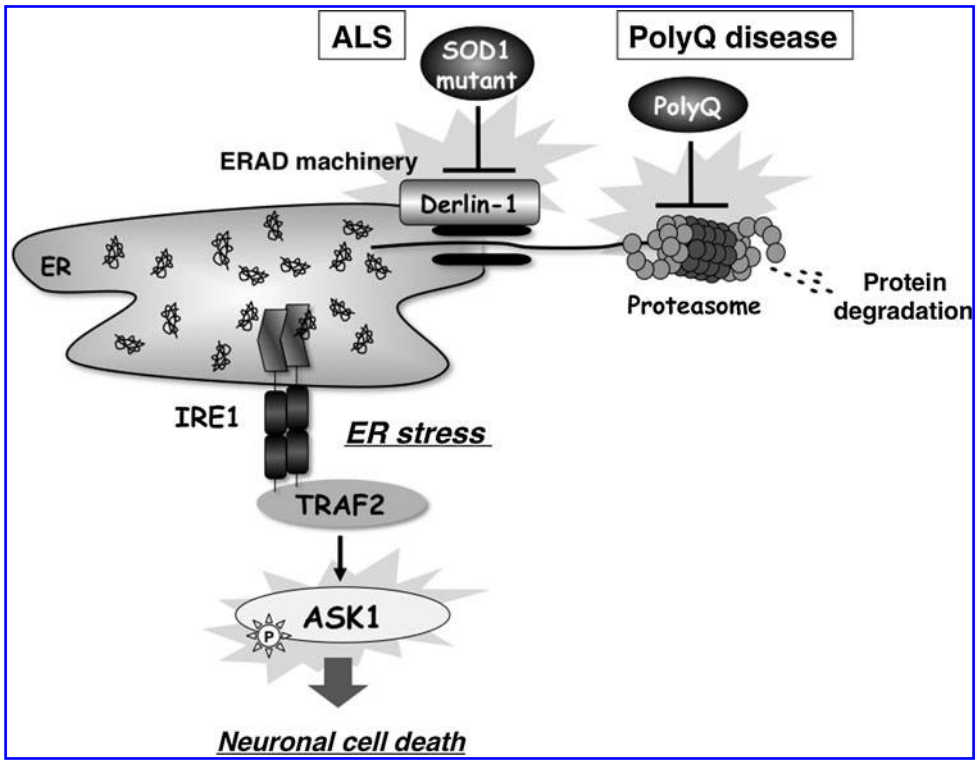

FIG. 5. ER stress-induced ASK1 activation in neurodegenerative disorders. In polyglutamine (polyQ) diseases, aggregated polyQ blocks the ubiquitin-proteasome system, leading to dysfunction of the proteasome-coupled ER-associated degradation (ERAD) machinery. In amyotrophic lateral sclerosis (ALS), a SOD1 mutant specifically interacts with and inhibits Derlin-1, a component of ERAD machinery. In both diseases, unfolded and misfolded proteins are accumulated in the ER through dysfunction of ERAD. The aberrant protein accumulation-induced ER stress activates IRE1 as a sensor molecule, which triggers activation of the TRAF2–ASK1 pathway, resulting in neuronal cell death. SOD1, superoxide dismutase.

been reported to be involved in amyotrophic lateral sclerosis (ALS) (256). Mutation in Cu/Zn-superoxide dismutase is a cause of familial ALS through induction of motor neuron death. The superoxide dismutase mutant specifically interacts with Derlin-1, a component of ER-associated degradation machinery, and induces ER stress through dysfunction of ER-associated degradation. ER stress-induced ASK1 activation appears to be crucial for disease progression of ALS (Fig. 5). Nevertheless, how ASK1 is activated after the interaction with TRAF2 under the ER stress conditions, particularly under these pathological conditions, remains to be elucidated. Since the generation of ROS and the disturbance of Ca^{2+} homeostasis can be seen in cells suffering from ER stress, ROS- and/or Ca^{2+}-induced activation mechanisms of ASK1 may also be involved in pathologies in ER stress-related neurodegenerative disorders (135, 333).

2. MEKK family

a. MAP/ERK kinase kinase 1. The MEKK family has four family members, MEKK1, MEKK2, MEKK3, and MTK1. These members are capable of regulating multiple MAP2K-MAPK pathways. MEKK1, also called MAP3K1, was the first member of this family to be identified and characterized, and activates the MEK1/2-ERK1/2, MKK4/MKK7-JNK, and MKK3/MKK6-p38 pathways. It has been shown by analysis of *MEKK1*$^{-/-}$cells that MEKK1 is required for JNK activation induced by microtubule disruption, cold stress, hyperosmolarity, and proinflammatory stimuli, but not for that induced by heat shock, anisomycin, and UV radiation (392, 412). Importantly, loss of MEKK1 expression results in an increase in apoptosis of cells exposed to hyperosmolarity and microtubule disruption (412). These findings demonstrate that MEKK1 plays an important role in protecting cells from apoptosis induced by a certain range of cytotoxic stresses. On the other hand, several studies have revealed that MEKK1 is also implicated in apoptosis (1, 17, 32, 90).

Overexpression of dominant active (DA) MEKK1 in the Jurkat T cell line induces apoptosis in parallel with prolonged JNK activation. DA-MEKK1 also enhances the expression of the Fas ligand on the cell surface of T lymphocytes (90). Overexpression of DA-MEKK1 in neuron-like PC12 cells also promotes apoptosis even in the presence of trophic factors such as NGF, accompanied by activation of JNK and p38. Moreover, MEKK1 contributes to cell death, including anoikis, cell death induced by detachment of cells from the extracellular matrix, and androgen receptor-dependent apoptosis and in which MEKK1 activation is induced by insufficient cell–matrix interactions (1, 32). Expression of death receptors, such as DR4/TRAILR1 and DR5/TRAILR2, is increased by MEKK1 activation (17). These reports have shown that MEKK1 serves as an important mediator of apoptosis in human cancers, such as prostate cancers and malignant lymphomas.

MEKK1 is a direct substrate of caspase-3. MEKK1 is activated by the caspase-3-dependent cleavage during anoikis, DNA damage, and Fas ligation (32, 69, 379) (Fig. 6). MEKK1 is composed of a large N-terminal regulatory domain and a C-terminal kinase domain. The caspase-3-dependent cleavage of MEKK1 releases a constitutively active MEKK1 catalytic domain from a detergent-insoluble cellular compartment into the soluble cytoplasm (69). Exogenous expression of WT MEKK1 or the cleaved catalytic fragment promotes genotoxin-induced apoptosis, whereas a mutant MEKK1 that is resistant to caspase cleavage is impaired in its ability to induce apoptosis (379). Overexpression of the noncleavable MEKK1 mutant also protects cells from anoikis-induced apoptosis, suggesting that caspase-mediated activation of MEKK1 promotes apoptosis (32).

An alternative proposal is that MEKK1 induces apoptosis by promoting the ubiquitination and degradation of c-Jun

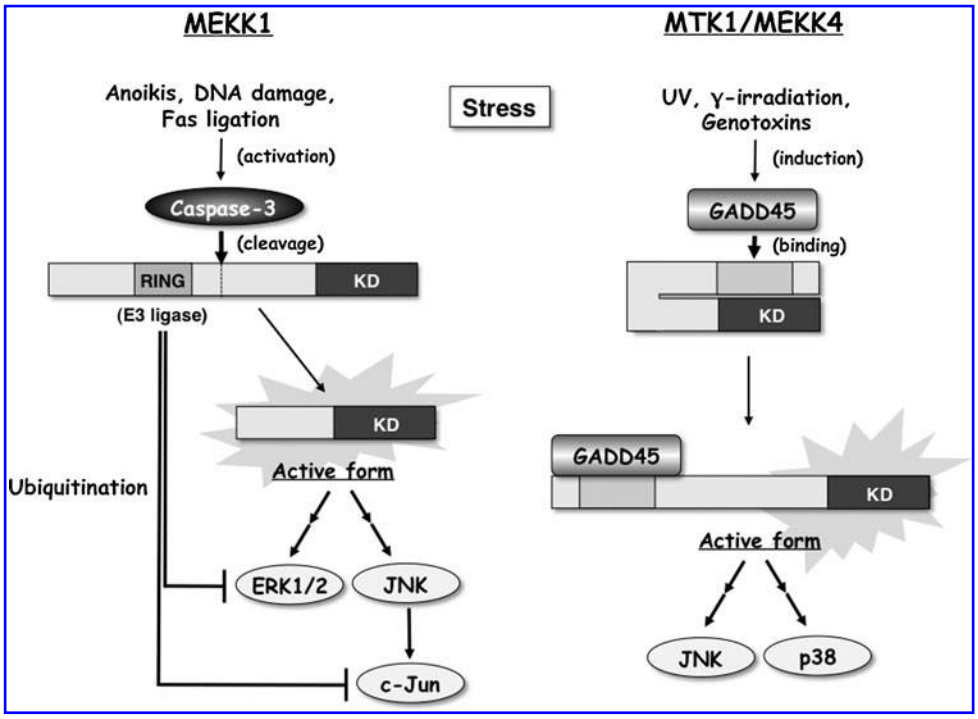

FIG. 6. The activation mechanisms of MEKK1 and MTK1/MEKK4. MEKK1 is activated by the caspase-3-dependent cleavage in response to anoikis, DNA damage, and Fas ligation. The cleaved C-terminal region of MEKK1 including the KD acts as a constitutively active form and activates the ERK and JNK pathways. On the other hand, MEKK1 possesses a RING finger-like structure, which exhibits E3 ubiquitin ligase activity. MEKK1 promotes ubiquitination and degradation of ERK1/2 and c-Jun. MTK1/MEKK4 is activated by interaction with GADD45 proteins, which are induced in response to UV, γ-irradiation, and chemical genotoxins. This interaction releases the KD of MEKK4 from its auto-inhibitory N-terminal domain and activates MTK1 kinase activity, leading to p38 and JNK activation. GADD45, growth arrest and DNA damage inducible 45.

(393). MEKK1 possesses the plant homeo domain (PHD) domain, a RING finger-like structure, which exhibits E3 ubiquitin ligase activity. MEKK1 also associates with ERK1/2 and mediates the polyubiquitination and degradation of ERK1/2 in response to stress stimuli, such as hyperosmotic shock, eventually leading to stress-induced apoptosis (220). Thus, MEKK1 functions as an anti- or proapoptotic kinase in a cell-type- and/or stimuli-dependent manner.

b. MEKK2 and MEKK3. MEKK2 and MEKK3 preferentially activate the MKK4/MKK7-JNK and MKK3/MKK6-p38 pathways, respectively. These MEKKs are structurally similar to each other and can also activate the MKK5-ERK5 pathway (62). In addition, MEKK2 has recently been shown to mediate hyperosmotic stress-ERK1/2 activation (228). Compared with WT thymocytes, *MEKK2*$^{-/-}$thymocytes are more susceptible to anti-CD3-induced apoptosis, but not to apoptosis induced by anti-Fas antibody, UV radiation, and dexamethasone treatment (111). *MEKK3*$^{-/-}$mice are embryonic lethal due to abnormalities in the extraembryonic vasculature of the yolk sac, the embryonic vasculature, and the heart. Primary vessels form, but angiogenesis is not observed. The endocardium fails to adhere to the cardiac myocytes of the ventricle. These defects are partially caused by increased endothelial cell apoptosis (75, 404). MEKK2 and MEKK3 as well as MEKK1 activate the NF-κB pathway, so that MEKK2 and MEKK3 may be important for the survival signaling. TRAF7, a novel TRAF family member, potentiates MEKK3-mediated activation of transcription factors AP1 and CHOP and induces apoptosis (395). However, it is unclear whether MEKK3 is directly implicated in the apoptosis signaling.

c. MTK1/MEKK4. MTK1, also called MEKK4 or MAP3K4, activates the MKK4/MKK7-JNK and MKK3/MKK6-p38 pathways in response to various types of stress, such as osmotic shock, oxidative stress, and UV radiation. *MTK1*$^{-/-}$mice exhibit embryonic lethality due to skeletal defects and exencephaly associated with enhanced apoptosis in the neural tube and a loss of JNK and p38 activation, suggesting that MTK1 exerts antiapoptotic functions *in vivo* (45). Nevertheless, MTK1 also functions as a proapoptotic kinase (335, 340).

Growth arrest and DNA damage inducible 45 (GADD45) α, β, γ proteins bind to the N-terminus of MTK1 (335) (Fig. 6). This interaction releases the kinase domain of MTK1 from its auto-inhibitory N-terminal domain and eventually activates MTK1. GADD45 proteins are induced in response to genotoxic stresses such as UV, γ-irradiation, and chemical genotoxins. Ectopic expression of GADD45 proteins induces p38 and JNK activation and apoptosis, which can be suppressed by a dominant negative mutant of MTK1. The MTK1-JNK/p38 pathways promote apoptosis through induction of GADD45 proteins in response to genotoxic stress, suggesting that the GADD45-MTK1 system provides a positive loop for apoptosis induction through p38 and JNK activation (335). It has also been reported that the BRCA1 tumor suppressor gene induces apoptosis by activation of the MTK1-JNK pathway in breast and ovarian cancer cell lines, which are subjected to various proapoptotic stimuli, including growth factor withdrawal, substratum detachment, ionizing radiation, and treatment with anticancer agents (340).

Recently, the signaling scaffold protein, receptor for activated C-kinase 1 (RACK1), has been identified as another important binding protein of MTK1 (7). RACK1 facilitates

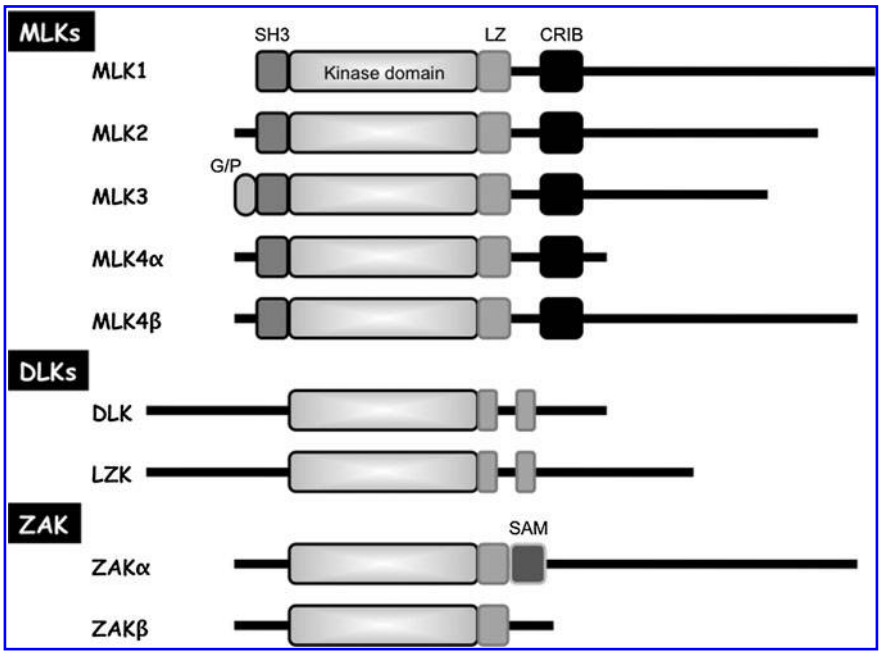

FIG. 7. MLK family kinases. Domain structures of the members of the mammalian MLK family are shown. The MLK family is divided into three subgroups on the basis of domain arrangements and sequence similarity within their catalytic domains. MLK4 and ZAK have two splicing variant forms. CRIB, Cdc42/Rac-interactive binding; G/P, Gly/Pro-rich sequence; LZ, leucine-zipper domain; MLK, mixed-lineage kinase; SAM, sterile-α motif; SH3, Src-homology 3; ZAK, zipper sterile-α-motif kinase.

activation of MTK1 and thus induces apoptosis in response to certain types of stressors, such as UV radiation, H_2O_2, and genotoxic drugs. In response to different types of stressors such as hypoxia, however, RACK1 is sequestered into the so-called stress granules, which are known to accumulate factors of stalled translation initiation complexes, and thereby MTK1 activation and apoptosis are suppressed. These findings suggest that MTK1 contributes to hypoxia-induced resistance to chemotherapy.

3. MLK family. The MLK family is activated in response to various types of stress, such as heat shock, inhibition of protein glycosylation, exposure to inflammatory cytokines, and UV radiation (96). The MLK family, the biggest MAP3K family, consists of seven different members, which are divided into three subgroups on the basis of domain arrangements and sequence similarity within their catalytic domain: the MLKs, which include MLK1, MLK2, MLK3, and MLK4; the DLKs, which include DLK itself and leucine-zipper kinase (LZK); and zipper sterile-α-motif kinase (ZAK) (Fig. 7). The MLKs share conserved domains, including a Src-homology 3 (SH3) domain, kinase domain, leucine-zipper (LZ), and Cdc42/Rac-interactive binding (CRIB) domain. The SH3 domain contributes to kinase autoinhibition, the LZ mediates protein dimerization or oligomerization, and the CRIB domain binds to Cdc42 and Rac, upstream activators of MLKs. The DLKs and ZAK lack the SH3 and CRIB domains, but they contain a kinase domain similar to that in other MLK family members and one or two LZ regions. ZAK has two splicing variants, ZAKα and ZAKβ, the former of which contains a sterile-α motif that mediates homo- or hetero-dimerization. MLK3, DLK, and ZAK activate the MKK4/MKK7-JNK and MKK3/MKK6-p38 pathways, whereas MLK1, MLK2, MLK4, and LZK preferentially activate the MKK4/MKK7-JNK pathway.

a. Mixed-lineage kinases. Among the four MLKs, the functions of MLK1, MLK2, and MLK3 have been widely investigated, whereas that of MLK4 remains unclear. *MLK1*$^{-/-}$mice, *MLK2*$^{-/-}$mice, and mice deficient for both MLK1 and MLK2 (*MLK1*$^{-/-}$*MLK2*$^{-/-}$mice) are viable and fertile, and show no developmental abnormalities (19). *MLK3*$^{-/-}$mice are viable and fertile except for a mild defect in the epidermal tissue of the dorsal midline and a selective reduction in TNF-α-stimulated JNK activation (26).

MLKs play important roles in trophic factor deprivation-induced neuronal cell death. Ectopic expression of MLK2 or MLK3 in PC12 cells induces apoptosis through JNK activation (396). In a study in which apoptosis of superior cervical ganglion (SCG) sympathetic neurons was induced by deprivation of NGF from the culture medium, MLK3 activity was increased (245). Overexpression of MLK3 in SCG neurons activated JNK and induced apoptosis, whereas a kinase inactive MLK3 mutant protein blocked NGF-deprivation-induced apoptosis of SCG neurons. In the rat hippocampal cell line HN33, overexpressed MLK2 or MLK3 induced apoptosis, whereas a kinase inactive mutant of MLK2 or MLK3 suppressed the kainate receptor glutamate receptor 6 (GluR6)-induced apoptosis (302). The induction of apoptosis in HN33 cells appears to require the postsynaptic-density scaffold protein PSD-95, which binds to both GluR6 and MLK2/MLK3 and couples the MLK2/MLK3-JNK pathway to GluR6. Further, the dominant negative forms of MLK1, MLK2, and MLK3 suppressed apoptosis induced by transforming growth factor-β (TGF-β) in hepatoma cells (180). Since this TGF-β-induced apoptosis is dependent on p38 activation, MLKs appear to mediate a proapoptotic signal through the p38 pathway.

It has been proposed that MLK2 is involved in a neurodegenerative disorder, Huntington's disease (HD), which is caused by the expansion of a polyQ stretch near the N-terminus of the responsible gene huntingtin. The length of the polyQ expansion correlates with the severity of HD (214). Under normal conditions, MLK2 interacts with huntingtin and the catalytic activity of MLK2 is inhibited. In HD, polyQ-

expanded huntingtin is aggregated and appears to no longer interact with MLK2. The released MLK2 is activated and promotes JNK-mediated apoptosis.

Many functional analyses of MLKs have been performed by an inhibitor compound of MLKs, CEP-1347, which blocks JNK activation and protects primary cultured motoneurons from trophic factor deprivation- and UV-induced apoptosis (227). CEP-1347 selectively inhibits the activity of MLKs, but not that of other MAP3Ks (226). CEP-1347 is also effective in animal models of HD, AD, and Parkinson's disease, so that MLKs are logical therapeutic targets of neurodegenerative diseases through the prevention of neuronal cell death (5, 23, 24, 54). These studies using CEP-1347 support the notion that the MLKs-JNK pathway plays important pathophysiological roles in apoptosis.

Recently, MLK3 has been shown to be involved in metabolic stress signaling (155). Obesity is one of the risk factors of metabolic syndrome and is associated with free fatty acids (FFA). Intriguingly, FFA induced a high level of JNK activation in WT MEFs, but activated JNK to a lesser extent in *MLK3*$^{-/-}$MEFs, indicating that MLK3 is required for FFA-induced JNK activation. Consistent with this finding, feeding WT mice a HFD, which increases blood FFA, caused MLK3 activation. Further analysis using *MLK3*$^{-/-}$mice demonstrated that MLK3 was required for obesity-induced JNK activation in brown fat. These findings strongly suggest an important role of MLK3 in metabolic stress signaling. On the other hand, MLK3 was not required for obesity-induced JNK activation in white fat, suggesting that other MLKs may be involved in regulation of JNK activity in tissues other than brown fat in metabolic stress signaling (155).

b. DLKs and ZAK. DLK, also called MUK, ZPK, or MAP3K12, was involved in apoptosis induced by neurotrophic factor deprivation in PC12 cells and sympathetic neurons and that ectopic expression of DLK induced apoptosis through JNK activation in PC12 cells (396). The dominant negative form of DLK prevented apoptosis and enhanced long-term survival of dopamine neurons through the blockade of phosphorylation of c-Jun (40). These findings suggest that DLK is an important regulator of apoptosis, particularly in neuronal cells. Although it has not been clarified whether the proapoptotic activity of DLK in neuronal cells is directly involved, DLK plays an essential role in mouse brain development; *DLK*$^{-/-}$mice die perinatally and display abnormal brain development, such as defects in anterior commissure, axon growth, and radial migration of pyramidal neurons (129). Recently, it has been shown, by using *DLK*$^{-/-}$mice and mutant flies lacking the *Drosophila* ortholog of DLK, that DLK promotes the degeneration of severed axons through JNK activation (239).

Although the activation mechanisms of DLK are not fully understood, it has been proposed that activation of DLK is induced by its oligomerization *via* tissue transglutaminase, a family of Ca^{2+}-dependent cross-linking enzymes that form specific cross-linked protein polymers. Since both the expression level and activity of tissue transglutaminase increase in apoptotic cells, tissue transglutaminase-dependent oligomerization, and thus activation, of DLK may be a critical step for apoptosis (126).

The functions of ZAK, also called MLK7, have not yet been clarified, except that ZAK is required for JNK and p38 activation as well as for apoptosis induced by anisomycin or UV radiation (367). Considering that ZAK is the only protein carrying a sterile-α motif domain among the MLK family members, this unique kinase might have a distinct function from other members.

4. Other MAP3Ks

a. TGF-β activated kinase 1. TAK1, also called MAP3K7, was originally identified as a kinase activated by TGF-β (398). TAK1 is activated in response to TGF-β and bone morphogenetic proteins, members of the TGF-β superfamily, and induces activation of the p38 pathway and transcription of genes such as plasminogen activator inhibitor-1, a classical target gene of TGF-β (196). Although the biological outputs of activation of TAK1 by TGF-β have not been well understood, bone morphogenetic protein 2 induces apoptosis through activation of the TAK1-p38 pathway in mouse hybridoma MH60 cells (183). In the neuroepithelium of early *Xenopus* embryos, TAK1 induces apoptosis, which is important for the dorsoventral patterning of early embryos (316). Moreover, ectopic expression of *Drosophila* TAK1 triggers JNK-mediated apoptosis in *Drosophila* embryogenesis and eye development (332). Thus, TAK1-induced apoptosis plays critical roles in embryogenesis and organ development.

TAK1 is also activated in response to proinflammatory stimuli, such as IL-1β, TNF-α, and LPS, and activates the MKK4/MKK7-JNK and MKK3/MKK6-p38 pathways (3). The TAK1 signaling complex consists of several adaptor proteins, including TAK1-associated binding protein 1 (TAB1), TAB2, TAB3, and TRAF6. By this complex, TAK1 also activates the IKKα/IKKβ-IκB-NF-κB survival pathway, so that TAK1 has important roles for the transduction of survival signals. Actually, *TAK1*$^{-/-}$mice are embryonic lethal due to severe vascular defects and substantial delayed growth, and display massive apoptosis of hepatocytes and hematopoietic cells. Moreover, the role of TAK1 in inflammation is also important, because the JNK, p38, and NF-κB pathways are essential for the regulation of cytokine expression. IL-1β-induced IL-6 expression was decreased in *TAK1*$^{-/-}$MEFs, and the cellular responses to TLR ligands, CD40, and B cell receptor cross-linking were considerably impaired in mice with B cell-specific TAK1 deficiency (300). Epidermal-specific TAK1-deficient mice exhibited loss of IL-1β- and TNF-α-induced activation of NF-κB and JNK and a severe inflammatory skin condition by postnatal day 6–8, suggesting that TAK1 plays important roles in epidermal homeostasis such as skin inflammation (266).

b. TAO family. The TAO kinase family consists of three closely related kinases, TAO1 (also known as MARKK/PSK2/KFC-B), TAO2 (also known as PSK1/KFC-C), and TAO3 (also known as JIK/KFC-A), all of which also belong to the Ste20-related kinase family (see below and Fig. 8). These molecules have a common domain organization consisting of a highly conserved kinase domain and a large coiled-coil domain in the N- and C-terminal region, respectively. TAO1 is a founder member of this family and has been characterized as a selective activator of the MKK3-p38 axis (147, 413). Recently, however, TAO1 has also been reported to activate the JNK pathway; TAO1 is activated in response to apoptotic stimuli and in turn induces the caspase-dependent cleavage of

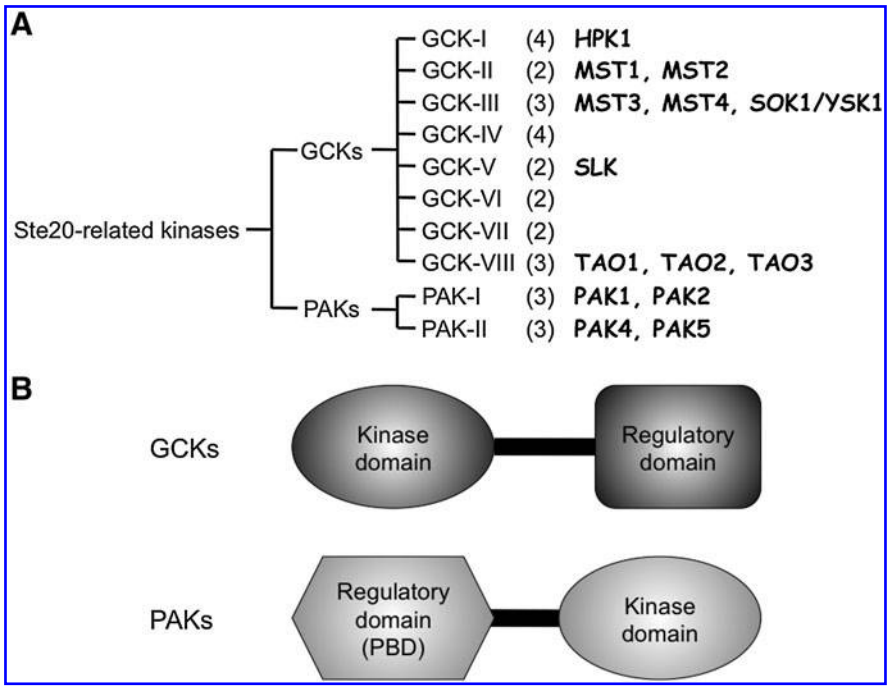

FIG. 8. Mammalian Ste20-related kinases (MSTs). (A) The MSTs consist of 28 Ser/Thr kinases, which are divided into two subfamilies, GCKs and PAKs. All PAKs have the p21-binding domain (PBD) in their regulatory domain through which PAKs interact with Rho-family GTPases. GCKs and PAKs are further divided into 8 and 2 subgroups, respectively, by sequence similarity in their KDs. The numbers of genes encoding kinases in each subgroup are shown in parentheses. The kinases highlighted in the text are shown in bold. **(B)** Simplified domain structures of GCKs and PAKs. GCK, germinal center kinase; PAK, p21-activated kinase.

Rho kinase 1 as well as JNK activation, leading to apoptotic morphological changes that include cell contraction, membrane blebbing, and apoptotic body formation (423). Also, in a physiological context, TAO1 appears to regulate cytoskeletal dynamics through direct phosphorylation and thus activation of microtubule affinity-regulating kinase, which regulates the phosphorylation state of microtubule-associated proteins (342).

TAO2 has the ability to activate both the JNK and p38 pathways, but appears to preferentially activate the p38 pathway, at least under certain physiological circumstances (42, 413). In addition to physicochemical stressors, such as osmotic stress, DNA damage, and microtubule-disrupting agents, carbachol has been found to activate TAO2 through a G protein-coupled receptor (43). Like TAO1, TAO2 also induces apoptotic morphological changes through JNK activation. In response to apoptotic and microtubule-disrupting agents, microtubule-associated TAO2 is activated by the caspase-9-dependent cleavage of TAO2 itself and localizes to the nucleus, leading to induction of apoptotic morphological changes (423).

Unlike TAO1 and TAO2, TAO3 was originally identified as a kinase that inhibited JNK activity (339). Later, however, it was shown that, similar to TAO1 and TAO2, TAO3 had the ability to preferentially activate the p38 pathway (413). TAO3 has also been identified as an antiapoptotic survival factor by two independent unbiased RNAi-based screenings. One was the search for modulators of apoptosis induced by TNF-related apoptosis-inducing ligand (TRAIL) receptor, a widely expressed member of the TNF superfamily. Knockdown of TAO3 enhanced TRAIL-induced apoptosis, indicating that TAO3 promotes survival by opposing TRAIL-induced apoptosis (12). By another screening for signaling intermediates regulating the antiapoptotic survival pathway in unstimulated cells, TAO3 was one of the kinases required for maintaining cell survival (222).

Recent comprehensive analysis of the three members of this family has revealed that each member mediates the activation of p38 in response to various genotoxic stimuli, such as ionizing radiation, UV radiation, and hydroxyurea (281). In this response, TAO kinases appear to be activated by the DNA damage checkpoint kinases ataxia telangiectasia mutated (ATM) and ATM and Rad3-related (ATR) and to control the DNA damage-induced G_2/M checkpoint through p38 and its downstream MK2. This finding indicates that all the TAO family kinases function similarly as key intermediates in the activation of p38 by DNA damage, although they may also have distinct functions, as described above, under other conditions. Taken together with accumulating evidence that p38 is profoundly involved in tumorigenesis (see p38 MAPKs and Crosstalk between the JNK and p38 pathways sections), TAO kinases may function as the upstream regulators that control such roles of p38.

c. MAP3Ks regulating the ERK pathway. Other MAP3Ks, including A-Raf, B-Raf, C-Raf (Raf-1), Mos, and Tpl2, are mainly involved in survival signaling. In response to a variety of mitogens and growth factors, these MAP3Ks activate the MEK1/2-ERK1/2 pathway, which induces cell survival and proliferation. C-Raf and Tpl-2 also activate the NF-κB pathway. Most mice deficient in the Raf family members display increased apoptosis in various embryonic tissues (238, 384). However, these MAP3Ks sometimes contribute to apoptosis signaling pathways. Pharmacological depletion of C-Raf by geldanamycin protects MCF-7 cells from apoptosis induced by the antitumor compound taxol (20). The Raf-MEK1/2-ERK1/2 pathway promotes the induction of apoptosis by various DNA-damaging agents and anticancer drugs. Moreover, the constitutively activated C-Raf mutant promotes apoptosis induced by c-Myc or p53 (30, 173). Considering that the signaling components of the ERK pathway are critically involved in human carcinogenesis, it is crucial to elucidate the

mechanism by which these MAP3Ks differentially contribute to apoptosis and survival signaling pathways.

III. Ste20-Related Kinases

Sterile 20 protein (Ste20p) is a budding yeast Ser/Thr kinase that regulates the MAPK pathway as a MAP3K kinase and that is involved in mating signaling (66, 199). Its homologs exist in mammals, *Drosophila*, *C. elegans*, and other organisms. In mammals, 28 kinases have been identified as the Ste20p homologs by sequence similarity in their kinase domains and are designated as the Ste20-related kinases (72) (Fig. 8A). These kinases are divided into two subfamilies, the germinal center kinases (GCKs) family and the p21-activated kinases (PAKs) family, based on the intramolecular location of the kinase domain; GCKs and PAKs possess the kinase domain in their N- and C-terminus, respectively (Fig. 8B). In this section, we will focus on several members of these families that play critical roles in the regulation of cell proliferation, cytoskeletal remodeling, and apoptosis, all of which are believed to be involved in tumor formation and tumor cell invasiveness (190, 193, 211, 243).

A. Germinal center kinases

GCKs are further divided into eight groups (GCK-I–VIII) by taking account of the structural similarities within and outside the kinase domain (66). Here, we will first focus on the mammalian Ste20-related kinases (MSTs), which are among the most extensively examined GCKs: MST1 and MST2 comprise the GCK-II group, whereas MST3, MST4, and Ste20 oxidant stress response kinase 1 (SOK1)/YSK1 comprise the GCK-III groups. In addition, we will also describe hematopoietic progenitor kinase 1 (HPK1) and Ste20-like kinase (SLK), which are representative members of the GCK-I and GCK-V groups, respectively. The members of the GCK-VIII group, which are the three members of the TAO family, have been characterized as MAP3Ks and were therefore described above in the section on MAP3Ks (II-D-4).

1. Mammalian ste20-related kinases. Among the Ste20-related kinases, MST1 has been the most extensively studied as a component of apoptosis signaling (211). The first indication of the involvement of MST1 in apoptosis signaling came from the observation that MST1 was activated by caspase-mediated proteolytic cleavage during apoptosis in various cell types (108, 168, 200, 284) (Fig. 9). The cleavage site is located between the N-terminal catalytic domain and the C-terminal regulatory domain in MST1. The resulting fragment containing the catalytic domain behaves as a constitutively active kinase, because the regulatory domain includes an autoinhibitory domain that intramolecularly inhibits catalytic activity in the intact kinase (60). Further analysis has revealed that MST1 has two cleavage sites targeted by distinct caspases, probably in an ordered fashion. Importantly, full activation of MST1 during apoptosis appears to require not only cleavage but also phosphorylation of MST1, suggesting that the proapoptotic activity of MST1 is tightly regulated by multiple mechanisms to prevent its aberrant activation (107). Further, overexpression of either full-length MST1 or the N-terminal catalytic domain, but not a kinase-negative mutant of MST1, induces cell shrinkage and apoptotic chromatin condensation through caspase activation, suggesting that

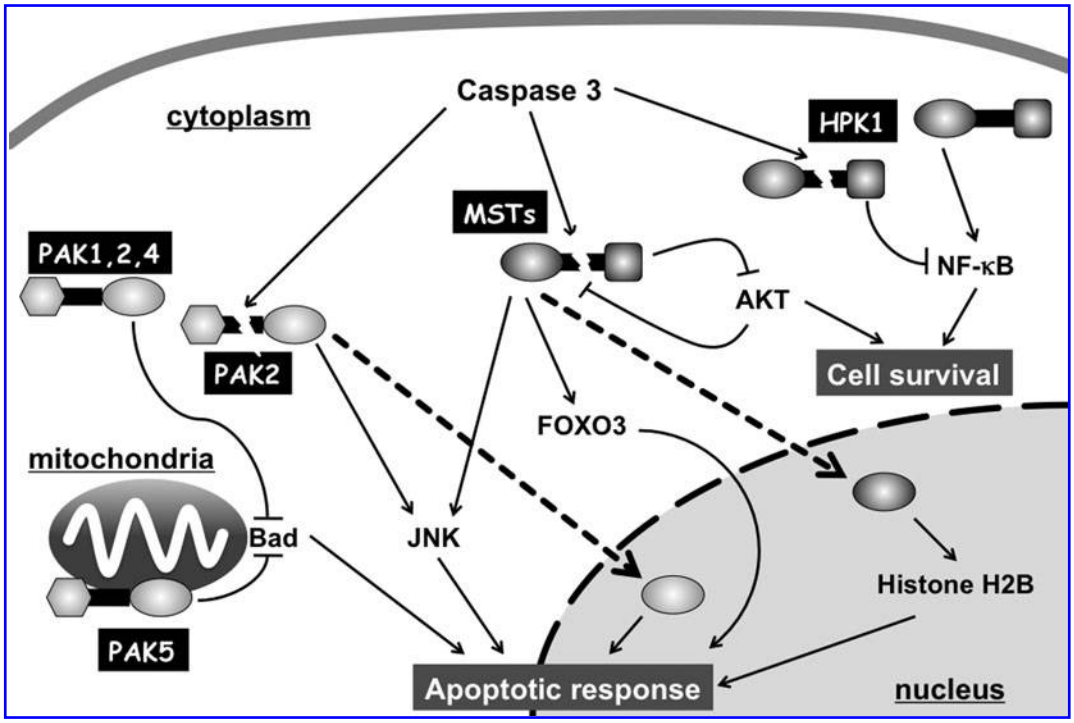

FIG. 9. Signaling pathways regulated by Ste20-related kinases in cell survival and apoptosis. Various Ste20-related kinases are involved in the regulation of pro- and antiapoptotic signaling. MSTs, HPK1, and PAK2 are cleaved by caspase-3, and the cleaved KDs (ellipse) of MSTs and PAK2 are translocated to the nucleus owing to the lack of regulatory domains (square or hexagon). HPK1, hematopoietic progenitor kinase 1.

MST1 is not only a target but also an activator of caspases (108, 201, 351).

The JNK and p38 MAPK pathways are the physiologically relevant downstream effectors of MST1 (108). Overexpression of MST1 activates these pathways. However, expression of a dominant-negative mutant of JNK, but not of p38, inhibits MST1-induced apoptotic morphological changes and caspase activation, suggesting that JNK mediates these apoptotic events induced by MST1 (351). Consistent with this, MST1-induced chromatin condensation during apoptosis is not induced in embryonic stem cells deficient for both MKK4 and MKK7, in which the JNK activation is abolished (352). Given that expression of a dominant-negative mutant of MEKK1 suppressed MST1-induced JNK activation, MEKK1 appears to be one of the MAP3Ks responsible for MST1-induced JNK activation, although the precise mechanism is unknown (107). Recently, MST1 and also MST2 have been shown to mediate activation of the JNK pathway in response to the disruption of the actin cytoskeleton, leading to cell cycle arrest through JNK-dependent stabilization of the CDK inhibitor p21 (76). MSTs thus appear to monitor and sense actin cytoskeletal integrity.

Several lines of evidence indicate that the cleavage of MST1 results not only in its activation but also in the translocation of the activated catalytic domain to the nucleus. The C-terminal regulatory domain of MST1 contains two functional nuclear export signals (NES) by which full-length MST1 is excluded from the nucleus and localized to the cytoplasm. In response to proapoptotic stimuli, the cleaved catalytic domain is liberated from regulation by the NES and thus translocates to the nucleus (201, 350). In fact, forced nuclear translocation of MST1 by mutation of its NES induces chromatin condensation, whereas inhibition of nuclear translocation of MST1 by mutation of its cleavage sites reduces its ability to induce chromatin condensation (350). These findings strongly suggest that nuclear translocation of MST1 is important for the chromatin condensation in apoptosis.

One possible mechanism by which nuclear-translocated MST1 may trigger chromatin condensation is the phosphorylation of histone H2B (H2B). MST1 directly phosphorylates H2B at Ser14, and this action is dependent on the cleavage of MST1 by caspase-3 and correlates well with the apoptotic chromatin condensation (44). Recently, H2B phosphorylation by MST1 has been demonstrated to immobilize RCC1, a guanine nucleotide exchanger factor for Ran, on the chromosomes and reduce nuclear RanGTP levels, leading to suppression of transport of nuclear localization signal-containing proteins, such as the survival factor NF-κB (385). On the other hand, H2B phosphorylation in response to proapoptotic stimuli is also mediated by protein kinase C (PKC)δ, rather than MST1 (137). Thus, H2B phosphorylation may be redundantly regulated by PKCδ or other MST members, such as MST2, depending on the cellular context. Consistent with this idea, caspase-catalyzed cleavage and activation of MST1 correlate with eosinophil apoptosis, but not with neutrophil apoptosis, suggesting the cell type-specific role of MST1 (68).

Another important signaling intermediate that transduces MST1-induced proapoptotic signals is FOXO3. MST1 phosphorylates FOXO3 and disrupts its interaction with 14-3-3 proteins, allowing the translocation of FOXO3 to the nucleus, where it induces a number of proapoptotic genes (139, 205). In this context, MST1 counteracts the survival-promoting kinase Akt, which phosphorylates FOXO3 and promotes its interaction with 14-3-3 proteins, sequestering FOXO3 in the cytosol. Therefore, it has been proposed that FOXO3 is negatively and positively regulated through phosphorylation by Akt and MST1, respectively (139). This signaling appears to be an evolutionarily conserved system; knockdown of *C. elegans* MST1 ortholog CST-1 in nematodes shortens life span and accelerates tissue aging, whereas overexpression of CST-1 promotes life span and delays aging in a manner dependent on the *C. elegans* FOXO ortholog DAF-16 (205). This finding suggests that MST1 plays important roles in the control of longevity beyond those in apoptosis regulation. Intriguingly, MST1 and Akt also directly regulate each other in a negative feedback manner; Akt directly phosphorylates MST1 at Thr387 to prevent its cleavage by caspases, whereas MST1 binds to and inhibits Akt (51, 161).

MST1 not only induces apoptosis, but also negatively regulates hypertrophy of cardiac myocytes (264, 401). A serine/threonine kinase LATS2, a member of the *lats* tumor suppressor family, has recently been found to play a critical role in this regulation (230). The role of LATS2 downstream of MST1 has been proposed from genetic evidence in *Drosophila*. Warts (Wts), the *Drosophila* ortholog of LATS2, is phosphorylated by hippo (Hpo), the *Drosophila* ortholog of mammalian MST1 and MST2, in the ternary complex of Wts, Hpo, and a scaffold protein Salvador (Sav) (121). LATS2, but not LATS1, another member of the *lats* family, mediates the functions of MST1 in cardiac myocytes, such as induction of apoptosis and inhibition of hypertrophy, suggesting the pathophysiological roles of the MST1-LATS2 pathway in heart failure.

The molecular structure, activation mechanism, and proapoptotic function of MST2 are similar to those of MST1. In fact, MST2 also regulates Akt and FOXO3 in a manner similar to MST1 (51, 205). Activation of MST2 during apoptosis is dually regulated by caspase-catalyzed cleavage and phosphorylation (73, 200). Raf-1 is an important regulator of MST2; Raf-1 prevents dimerization and activating phosphorylation of MST2, and the dissociation of Raf-1 from MST2 is proposed to be one mechanism for MST2 activation (263). Concerning the regulation of LATS1 and LATS2, MST2 has been proposed to phosphorylate these substrates more effectively than MST1 (34). Therefore, the tumor suppressive roles through the mammalian counterpart of the *Drosophila* Hpo-Sav-Wts pathway are regarded to be the unique and important function of MST2. In *Drosophila*, Hpo phosphorylates and activates Wts, which in turn negatively regulates the transcription of cell cycle and cell-death regulators, thereby playing an important role in regulating the organ size (301). Recently, Yorkie (Yki), the *Drosophila* ortholog of the mammalian transcriptional coactivator yes-associated protein 1 (YAP1), has been identified as a responsible transcription factor functioning downstream of Wts (140, 301). In analogy to this, YAP1 is a downstream effector of the MST2-LATS1 axis in mammals as well. Although YAP1 is sequestered in the cytoplasm in unstimulated cells, it translocates into the nucleus upon being phosphorylated by LATS, which is activated by *RASSF1A,* a tumor suppressor gene. In the nucleus, YAP1 associates with p73 and exerts tumor suppressive activity through induction of the proapoptotic target genes, such as Puma (229). On the contrary, phosphorylation of YAP1 by

LATS, which is regulated in a manner dependent on cell density, also leads to cytoplasmic translocation and inactivation of YAP1 (418). In the latter case, however, YAP1 appears to rather promote cell growth, suggesting that, in either case, the MST2-LATS axis consistently acts in tumor suppression. Thus, the precise mechanisms by which MST2 exerts its tumor suppressive roles, particularly those in various cellular contexts, remain to be elucidated.

Physiological relevance of MST1 and MST2 in tumor suppression has recently been shown using mice with combined deletion of the *MST1* and *MST2* genes. *MST1*$^{-/-}$mice and *MST2*$^{-/-}$mice are viable and fertile; however, *MST1*$^{-/-}$mice, but not *MST2*$^{-/-}$mice, has a reduced number of mature naive T cells (172, 420). Although *MST1*$^{-/-}$*MST2*$^{-/-}$mice were embryonic lethal, *MST1*$^{+/-}$*MST2*$^{-/-}$mice and *MST1*$^{-/-}$*MST2*$^{+/-}$mice survived but harbored highly aggressive HCCs (265, 419). Importantly, the *MST1* allele and *MST2* allele were found to be lost in HCCs of *MST1*$^{+/-}$*MST2*$^{-/-}$mice and *MST1*$^{-/-}$*MST2*$^{+/-}$mice, respectively. This susceptibility of HCCs was also observed in mice with liver-specific deletion of the *MST2* gene on an *MST1* null background (419). These findings strongly suggest the roles of MST1 and MST2 in tumor suppression *in vivo*.

Similar to MST1 and MST2, MST3 is also cleaved by caspase activity, and thus activated and shuttled to the nucleus in response to several proapoptotic stimuli (138, 203). Although proapoptotic activity of MST3 has been observed, such as in the context of oxidative stress-induced apoptosis (388), the molecular mechanisms by which MST3 induces apoptosis are still unclear. Considering that MST3 does not activate the JNK and p38 pathways (305), MST3-induced apoptosis appears to be regulated by mechanisms different from those for MST1 and MST2. On the other hand, MST3 phosphorylates and inhibits the protein tyrosine phosphatase proline-, glutamine-, serine-, and threonine-rich sequence (PTP-PEST), inhibiting cell migration through regulation of phosphorylation levels of Paxillin, a focal adhesion adaptor protein (218). Since PTP-PEST is directly regulated by caspase-3 and sensitizes cells to TNF-α- and Fas-induced apoptosis, PTP-PEST may also function as the effector of MST3-mediated apoptosis (113).

Similar to MST3, neither MST4 nor SOK1/YSK1 activates the JNK and p38 MAPK pathways, indicating that the GCK-III members share common functional features (65, 276). The roles of MST4 and SOK1 in apoptosis regulation are not fully understood, except that overexpression of MST4 induces apoptosis (66) and the caspase-dependent cleavage and nuclear translocation of SOK1 induce apoptosis after chemical anoxia (262).

2. HPK1 and SLK. HPK1 is predominantly expressed in hematopoietic cells and organs such as the bone marrow, spleen, lymph node, placenta, and thymus. HPK1 activates the JNK pathway through a MAP3K MEKK1 (136), and similar to some of the MSTs, HPK1 is cleaved and activated by proapoptotic stimuli and induces apoptosis, at least in part, through the JNK pathway (41, 307) (Fig. 9). A unique functional feature of HPK1 among Ste20-related kinases is its ability to regulate the NF-κB pathway. Whereas the full-length HPK1 activates the NF-κB pathway, the cleaved C-terminal regulatory region of HPK1 suppresses it by regulating degradation of IκB inhibitory proteins, suggesting that, upon the cleavage of HPK1 during apoptosis, the role of HPK1 is converted from an activator to an inhibitor of NF-κB (10, 307). Recently, it has been reported that HPK1 cleavage by caspases is involved in the elimination of autoreactive T- and B-lymphocytes, the so-called activation-induced cell death, through the C-terminal region-induced inhibition of the NF-κB pathway (27). Moreover, the cleaved HPK1 also mediates myeloid cell survival during monocytic differentiation, through, in this case, the N-terminal catalytic domain-induced sustained activation of the JNK pathway (9).

SLK is a ubiquitously expressed kinase that has the ability to induce apoptosis and activate the JNK pathway (118, 293). Similar to HPK1, SLK is activated through its cleavage by caspase-3 in response to proapoptotic stimuli, and both the resulting N-terminal and C-terminal regions have distinct roles. Whereas the N-terminal region functions as an active kinase after the induction of apoptosis, the C-terminal region regulates actin fiber disassembly (294). SLK is also directly phosphorylated and inhibited by casein kinase II in v-Src-transformed cells, providing indirect evidence that SLK may have tumor suppressive activity and be inactivated upon cellular transformation (33). However, the physiological role of phosphorylation of SLK by casein kinase II in normal cells remains to be elucidated. These unique cleavage-induced functions of HPK1 and SLK, together with those of MSTs, suggest that functions of GCKs regulated by caspase-dependent cleavage are widely involved in various cell fate determinations.

B. p21-activated kinases

PAKs constitute another branch from that of GCKs in MSTs (Fig. 8A) and play roles in various physiological processes, including actin cytoskeletal dynamics and apoptosis (243). PAKs are well known to function as downstream nodes for various oncogenic signaling pathways and recently have received much attention as potential pharmacological targets for cancer therapeutics (190). There are six mammalian PAKs (PAK1-6), which are divided into two subgroups, called PAK-I (PAK1-3) and PAK-II (PAK4-6), based on structural similarities within and outside the kinase domain (66, 72). All PAKs have a highly conserved C-terminal Ser/Thr kinase domain and the ability to bind to the activated forms of Rho-family GTPases, Cdc42 and Rac, through the N-terminal p21-binding domain (PBD) (Fig. 8B). However, only PAK-I subfamily members are indeed activated by the interaction with Cdc42 and Rac, indicating that, in spite of the architectural similarity between the two PAK subfamilies, their mode of regulation and cellular functions are likely different (6, 377).

1. p21-activated kinase-I. Under unstimulated conditions, the PAK-I members form inactive homodimers, in which the active sites of the kinase domain are blocked by an N-terminal regulatory domain. The active GTP-bound forms of Cdc42 and Rac bind to PBD and release the kinase domain from the autoinhibition, thereby allowing autophosphorylation of the kinase domain (275).

PAK1, the best-characterized member of PAKs, is critically involved in tumorigenesis and tumor progression (190). PAK1 expression is widely upregulated in human breast cancers and correlates with breast cancer invasiveness (13). Accumulating evidence also indicates that PAK1 expression and activity are upregulated in several other human cancers,

such as colon cancer, ovarian cancer, neurofibromatosis, and T cell lymphoma (190). The oncogenic activity of PAK1 appears to be mediated through various downstream effectors. One set of substrates of PAK1 is involved in the cytoskeletal regulation. PAK1 regulates actin reorganization through several substrates, including LIM kinase (83), p41-ARC (355), and filamin (354). PAK1-dependent cytoskeletal changes are thought to be required for cell motility and invasion by cancer cells. Another set of substrates is involved in metabolic regulation, alterations of which enable tumor cells to survive under adverse conditions. PAK1 plays a pivotal role in the control of the cellular redox state and metabolic phenotype through phosphorylation-dependent regulation of the $p47^{phox}$ subunit of NADPH oxidase, phosphoglycerate mutase, and phosphoglucomutase (112, 185, 310).

Several lines of evidence also suggest that PAK1 facilitates tumor formation and progression by counteracting the proapoptotic pathways. In response to a cell survival signal, such as IL-3 for lymphoid progenitor cells, PAK1 is activated and in turn directly phosphorylates the proapoptotic Bcl-2 family member Bad, which reduces the binding of Bad to Bcl-2 or Bcl-x_L and thereby promotes cell survival due to the predominant antiapoptotic effects of Bcl-2 and Bcl-x_L (308) (Fig. 9). PAK1 also interacts with and phosphorylate dynein light chain 1 (DLC1), a component of the dynein motor complex. DLC1 exerts antiapoptotic activity by preventing the proapoptotic Bcl-2 family member BimL from binding to Bcl-2 and blocking its survival activity. PAK1 phosphorylates both DLC1 and BimL and further suppresses the inhibitory activity of BimL toward Bcl-2, promoting cell survival (353). Although the precise mechanism is not understood, PAK1 is activated by the critical survival factor Akt through a GTPase-independent mechanism (338). Moreover, PAK1 is a crucial signaling molecule in the induction of NF-κB activation for cell survival in several contexts, further supporting the survival-promoting role of PAK1 (93).

Unlike other PAKs, PAK2 has both proapoptotic and antiapoptotic functions, consistent with the fact that PAK2 is dually regulated by caspase-mediated intramolecular cleavage as well as by binding to GTPases (288). Similar to PAK1, activation of full-length PAK2 by Cdc42 and Rac GTPases promotes cell survival under stress conditions, mainly through the induction of Bad phosphorylation (see above) (158). On the other hand, the cleavage site in PAK2 is located between the N-terminal regulatory domain and the C-terminal catalytic domain, and the proteolytically cleaved catalytic domain acts as a constitutively active kinase and induces JNK activation and apoptosis (202, 288, 359). This functional difference between the activated full-length form and the cleaved form of PAK2 can be explained, at least in part, by their distinct subcellular targeting. Full-length PAK2 is localized in the cytoplasm, whereas cleaved PAK2 translocates to the nucleus because of the loss of NES in the regulatory domain (202). Although the direct substrates of cleaved PAK2 in the nucleus remain to be identified, cleaved PAK2 may phosphorylate key targets involved in the morphological changes during apoptosis. For example, H2B, known as a substrate of MST1 as described above, is one of the candidates because overexpression of cleaved PAK2, but not of full-length PAK2, resulted in chromatin condensation (202). Another plausible explanation for the functional difference between the full-length and cleaved forms of PAK2 is the difference in protein stability; protein degradation of cleaved PAK2 was much faster than that of full-length PAK2 (157). Taken together, these results indicate that caspase activation coverts the antiapoptotic function of PAK2 into the proapoptosis function, and it would be interesting to clarify how this switching system of PAK2 functions under pathophysiological conditions.

2. p21-activated kinase-II. Although all the PAK-II members, PAK4, PAK5, and PAK6, have an N-terminal PBD and a C-terminal kinase domain, the degree of structural similarity of the PAK-II members is much less than that of the PAK-I members (6). In contrast to the PAK-I members, activated Cdc42 had little effect on activation of the PAK-II members, and the members of PAK-I, but not of PAK-II, complemented the function of Ste20p in the yeast mating-response pathway, suggesting the functional difference between the PAK-I and PAK-II members (57). Although less is known about the regulatory mechanisms and functions of PAK-II compared to PAK-I, the PAK-II family members have recently received much attention as regulators of antiapoptotic and proliferative responses in the context of tumorigenesis and tumor progression (89, 243, 377).

While PAK4, similar to PAK1 and PAK2, suppresses apoptosis by regulating Bad phosphorylation (101), PAK4 antagonizes TNF-α-induced apoptosis *via* a mechanism that is independent of Bad phosphorylation. TNF-α exerts both pro- and antiapoptotic activity, and these activities are mediated by distinct signaling complexes: upon TNF-α stimulation, the signaling complex, the so-called complex I, comprised of TNFR1, the TNFR-associated death domain (TRADD), receptor interacting kinase 1 (RIP1) and TRAF2 or TRAF5, is formed and predominantly mediates the survival signal through the NF-κB pathway. After receptor internalization, another complex, the so-called complex II, comprised of TRADD, the Fas-associated death domain (FADD), RIP1, and caspase-8, is formed and mediates the proapoptotic signal (381). PAK4, irrespective of its kinase activity, suppresses TNF-α-induced activation of caspase-8 and caspase-3, probably by inhibiting the recruitment of caspase-8 to complex II (100). On the other hand, PAK4 mediates the survival signal by facilitating recruitment of TRADD to complex I (209). These findings indicate that PAK4 enhances the prosurvival effect of TNF-α signaling by preventing and promoting the pro- and antiapoptotic activity of TNF-α, respectively, and suggest PAK4 has tumor promoting activity. Consistent with this idea, it has recently been reported that expression of PAK4 is upregulated in a variety of human tumors (39, 182, 271).

PAK5 is predominantly expressed in the brain and constitutively localizes to the mitochondria independently of its kinase activity and binding to GTPases (57, 64). This unique localization to the mitochondria, together with the kinase activity, appears to be required for the antiapoptotic activity of PAK5 through phosphorylation of Bad (56, 57). In addition to this direct regulation of Bad by PAK5, PAK5 has recently been shown to activate and target Raf-1 to the mitochondria, where Raf-1 may phosphorylate Bad and thereby promote cell survival (97, 389). Thus, mitochondria-localized PAK5 appears to prevent apoptosis through both direct and indirect regulation of Bad phosphorylation, and these activities may suggest that PAK5 plays a specific role in the regulation of neuronal apoptosis.

IV. Death-Associated Protein Kinase Family

Death-associated protein kinase (DAPK)/DAPK1 is a ubiquitously expressed proapoptotic serine/threonine kinase that was first indentified by an elegant antisense-based genetic screening for molecules that were required for interferon (IFN)-γ-induced cell death (71). DAPK is the founding member of a family of related kinases that includes DAPK2/DAPK-related protein-1, DAPK3/zipper-interacting protein kinase (ZIPK), DAPK-related apoptosis-inducing protein kinase (DRAK)-1/STK17A, and DRAK-2/STK17B, although the molecular size and structure outside the kinase domain differ among these members (16) (Fig. 10). Whereas DAPK, DAPK2, and DAPK3 are closely related to each other, DRAK-1 and −2 are more distantly related to the other three kinases. With regard to the primary structure of the kinase domain, this family is most closely related to myosin light chain kinase, which is one of the Ca^{2+}/calmodulin (CaM)-regulating kinases. In this section, we focus on DAPK, DAPK2, and DAPK3 as the well-characterized members of this family.

DAPK possesses multiple domains in addition to the kinase domain, including a CaM regulatory domain, eight ankyrin repeats, two putative nucleotide-binding domains (P-loops), a cytoskeletal binding domain, and a death domain (DD) (Fig. 10). The CaM regulatory domain suppresses catalytic activity by binding to mask the catalytic center of the kinase domain (52). Upon binding to CaM, the CaM regulatory domain dissociates from the kinase domain, triggering catalytic activity. Another unique activation mechanism of DAPK is regulated by phosphorylation states of Ser308 within the CaM regulatory domain (317). Autophosphorylation of Ser308 reduces the affinity of this CaM regulatory domain to CaM, which strengthens the interaction of this domain with the kinase domain and thus inhibits catalytic activity. Conversely, dephosphorylation of Ser308 increases the affinity of the CaM regulatory domain to CaM and thus promotes catalytic activity even at low CaM levels, suggesting that DAPK is not a kinase whose activation totally depends on the CaM level. Recently, Ser735, Tyr491, and Tyr492 have been shown to be additional phosphorylation sites required for regulation of the catalytic activity of DAPK. ERK phosphorylates Ser735, leading to upregulation of the catalytic activity of DAPK as well as its proapoptotic activity (37). Src tyrosine kinase and leukocyte common antigen-related tyrosine phosphatase respectively phosphorylate and dephosphorylate Tyr491/492, phosphorylation of which inhibits catalytic as well as proapoptotic activity of DAPK (366). ERK-dependent Ser735 phosphorylation and Src-dependent Tyr491/492 phosphorylation of DAPK can commonly occur in response to growth-promoting stimuli such as by EGF but exert opposite effects on catalytic and proapoptotic activity of DAPK. However, it remains elusive whether and how these mechanisms are alternatively regulated.

DAPK2 also possesses the CaM regulatory domain and is regulated in a manner highly homologous to that of DAPK (312). Unlike DAPK and DAPK2, DAPK3 does not possess the CaM regulatory domain, suggesting that its catalytic activity is regulated independently from CaM levels. Instead, the catalytic activity of DAPK3 appears to be regulated by phosphorylation of Thr180, Thr225, and Thr265, among which Thr180 is located in the activation loop in the kinase domain that is generally an important structure for the regulation of kinase activity (109).

A large body of evidence has revealed that DAPK has the ability to induce cell death in a manner dependent on its kinase activity. DAPK is involved in cell death induced by TNF-α, Fas, IFN-γ, TGF-β, ceramide, and various mitochondrial toxins (53, 160, 272, 311). UNC5H2, a DD-containing receptor for a diffusible laminin-related protein netrin-1, interacts with and activates DAPK by reducing the autophosphorylation of Ser308, whereas netrin-1 suppresses UNC5H2-dependent activation of DAPK (215). Moreover, upon oncogenic transformation by overexpression of oncogenes such as c-myc and E2F, DAPK is upregulated and in turn induces p19ARF/p53-dependent apoptosis (282). Importantly, some of these lines of evidence have been established using $DAPK^{-/-}$cells, in which stimulation-induced death is attenuated (215, 272, 282). Consistent with the findings from these *in vitro* studies, retinal ganglion cells in $DAPK^{-/-}$mice injected with glutamate are

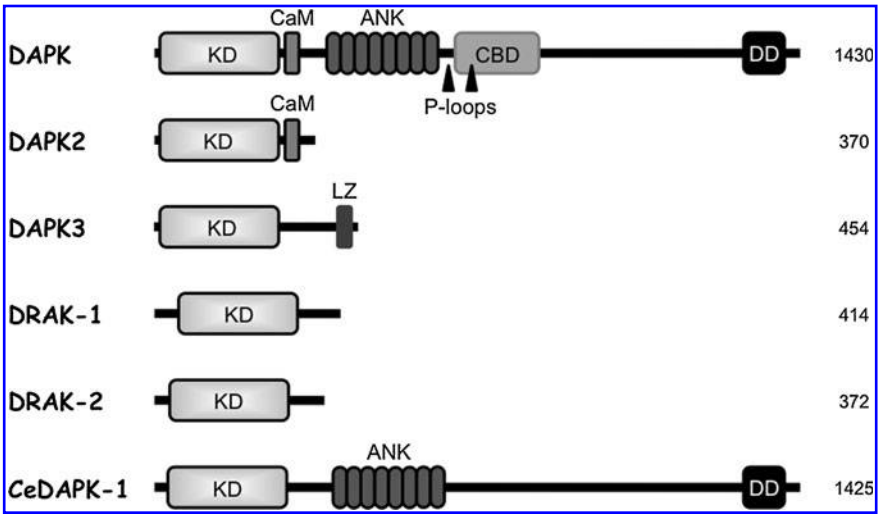

FIG. 10. DAPK family kinases. Members of the mammalian DAPK family, DAPK (DAPK1), DAPK2 (DRP-1), DAPK3 (ZIPK), DRAK-1/STK17A, and DRAK-2/STK17B, and the *Caenorhabditis elegans* ortholog of DAPK (CeDAPK-1) are shown. Although their kinase structures are similar to each other, molecular size and structure outside the KD differ among the members. Whether CeDAPK-1 possesses CaM regulatory domains and cytoskeletal binding domains has not been specified. The black triangles indicate P-loops. The numbers indicate amino acid length. ANK, ankyrin repeats; CaM, Ca^{2+}/calmodulin regulatory domain; CBD, cytoskeletal binding domain; DAPK, death-associated protein kinase; DD, death domain; DRAK, DAPK-related apoptosis-inducing protein kinase; DRP-1, DAPK-related protein-1.

more resistant than those in identically treated WT mice (306). DAPK also plays a major role in cellular responses to oxidative stress. DAPK physically interacts with and activates protein kinase D (PKD; see PKDs section) in response to oxidative stress, and activated PKD in turn induces activation of the JNK pathway. Further, DAPK is required for oxidative stress-induced necrotic cell death as well as JNK activation (85). DAPK2 and DAPK3 also show death-inducing activity in various cellular settings (149, 150, 174). Although DAPK3 localizes to the nucleus (174, 187), the death-inducing activity of DAPK3 appears to depend on its cytoplasmic distribution, which is regulated by DAPK-induced phosphorylation of multiple sites within the extracatalytic C-terminal domain of DAPK3 (313).

While these three members of the DAPK family induce caspase-dependent apoptotic cell death (53, 150, 160, 174, 187, 282), they also induce autophagic cell death, an alternate type of death, called Type II cell death, which does not depend on caspase activation (149, 313). Although autophagy is a cellular process for degradation of proteins and organelles and primarily plays a protective role for cells, it can also be involved in cell death (242). The mechanisms by which DAPKs induce autophagy are not fully understood, but DAPK-dependent phosphorylation of beclin-1, an essential component for autophagy, and phosphorylation of MAP1B, which interacts with another essential autophagy protein Atg8 (LC3), appear to be crucial events (120, 414). Recently, it has been shown that not only caspase-3-dependent apoptosis, but also DAPK-dependent autophagic cell death plays an important role in response to ER stress (106). *DAPK*$^{-/-}$mice are protected from kidney damage caused by injection of the ER stress inducer tunicamycin. Consistent with this, tunicamycin-induced cell death is attenuated in *DAPK*$^{-/-}$MEFs, in which the caspase activation and autophagy induction that precede cell death are also attenuated. The roles of DAPK in autophagy are further supported by the finding that DAPK-1, the *C. elegans* ortholog of DAPK, regulates the extent of starvation-induced autophagy in *C. elegans* (170).

One of the human diseases in which DAPKs play a key role is cancer. In addition to promoting tumor suppression *via* its proapoptotic activity, DAPK also contributes to tumor suppression by blocking the integrin-mediated polarity pathway and thereby helping to inhibit cell motility (191). The frequent reduction in DAPK expression through hypermethylation of the *DAPK* promoter, which correlates well with the recurrence and/or metastasis incidence of several cancers, is another evidence suggesting the tumor suppressive role of DAPK (237). The requirement of DAPKs for cytokine-induced cell death also suggests the possible roles of DAPKs in inflammatory disorders. DAPK has also recently been shown to play a novel role independent of its role in cell death; a signaling cascade composed of DAPK and DAPK3 participated in the translational control of inflammatory gene expression by modulating the IFN-γ-activated inhibitor of translation complex. Finally, in support of the idea that DAPKs are important for immune responses, DAPK-1 has been shown to negatively regulate the innate immune responses to epidermal damage in *C. elegans* (344). These findings strongly suggest that DAPKs regulate a wide variety of cellular events beyond induction of cell death, probably *via* mechanisms that are dependent on their kinase activity and/or cellular context.

V. RIP Family

In addition to apoptosis, necrosis is another important form of programmed cell death (103). Although necrosis has long been considered to be uncontrolled and accidentally evoked, recent evidence indicates that it is tightly regulated through specific intracellular signaling pathways. In this regard, the term programmed necrosis or necroptosis has been used. The RIP1/RIP/RIPK1 and RIP3 are emerging key regulators of necrosis (356). These kinases, together with RIP2 (also known as CARDIAK/RICK) and RIP4 (also known as DIK/PKK), comprise a family of related serine/threonine kinases (Fig. 11). They share significant homology in their N-terminal kinase domains, but their C-termini differ from each other. RIP1, RIP2, and RIP4 possess a DD, a caspase recruitment domain and ankyrin repeats, respectively, in their C-termini. Additionally, the RIP homotypic interaction motif, which is required for the interaction between RIP1 and RIP3, exists in the intermediate region between the kinase domain and DD of RIP1 and in the C-terminal region of RIP3 (327). Intramolecular cleavage by caspases has been shown to inactivate RIP1, RIP3, and RIP4 (91, 210, 236). In this section, we will not focus on RIP2 and RIP4 because little has been reported about their active involvement in cell death induction; nevertheless, they have been characterized as critical regulators of the NF-κB pathway (122, 236).

RIP1, through its DD domain, binds to death receptors, such as TNFR1, Fas, TRAIL1, and TRAIL2, as well as DD-containing adaptor proteins that are required for caspase-8 activation and apoptosis, such as TRADD and FADD (356). Involvement of RIP1 in the regulation of cell survival and death has been suggested by the finding that *RIP1*$^{-/-}$ cultured cells are highly sensitive to TNF-induced cell death, which is accounted for by a failure to activate the survival signaling through NF-κB (175). The ubiquitination state of RIP1 has been proposed to determine whether it functions as a signaling intermediate for cell survival or death. Upon activation of TNFR1, RIP1 is recruited to a membrane-associated complex, the so-called complex I, composed of TNFR1,

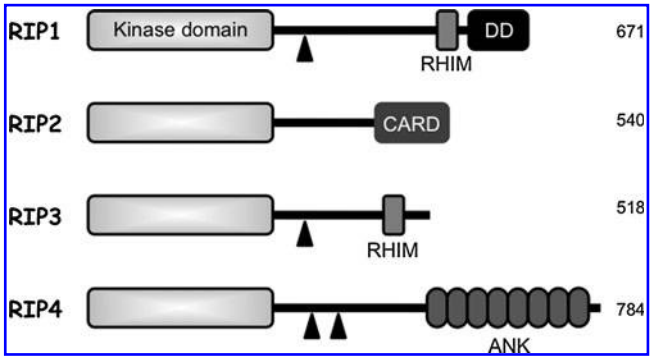

FIG. 11. RIP family kinases. RIP1 (RIP/RIPK1), RIP2 (CARDIAK/RICK), RIP3, and RIP4 (DIK/PKK) comprise a family of related serine/threonine kinases. They share significant homology in their N-terminal KDs, but their C-termini differ from each other. The black triangles indicate intramolecular cleavage sites by caspases. The numbers indicate amino acid length. CARD, caspase recruitment domain; RHIM, RIP homotypic interaction motif; RIP, receptor interacting kinase.

TRADD, TRAF2, or TRAF5, and the inhibitor of apoptosis proteins cIAP1 and cIAP2, where cIAP1 and cIAP2 form polyubiquitin chains linked through lysine 63 (K63) of ubiquitin on RIP1 (224, 357) (Fig. 12). The resulting polyubiquitinated form of RIP1 mediates prosurvival signals through activation of NF-κB and JNK and p38 MAPKs.

On the other hand, when K63-polyubiquitination of RIP1 is removed by the deubiquitinating enzymes CYLD and A20, which are upregulated by NF-κB in a negative feedback manner, RIP1 loses its ability to activate NF-κB and is included in the secondary cytosolic complex, the so-called complex II, formed after receptor internalization (381). In one type of the complex II that is formed in a manner dependent on TRADD, caspase-8 is activated and induces apoptosis, concomitant with cleavage of RIP1. Another type of complex II is formed independently of TRADD in the presence of Smac mimetics that facilitate proteasomal degradation of cIAPs and thus promotes the accumulation of unubiquitinated RIP1. In the latter complex, caspase-8 is activated in a RIP1 kinase-dependent manner and induces apoptosis (364).

When apoptosis is suppressed by the experimental treatment of cells with pan-caspase inhibitors or by pathophysiological conditions such as viral infection, necrosis is alternatively induced, and RIP1 and RIP3 play crucial roles in this induction (46, 70, 125, 131, 415). In this setting, RIP1 and RIP3 form a so-called necrosome complex containing FADD and caspase-8 together with or without TRADD. Direct or indirect (through an unknown kinase) phosphorylation between RIP1 and RIP3 appears to consolidate this complex and activate necrotic signaling (46, 125). In the search for necrosis-inducing factors that function downstream of RIP3, three metabolic enzymes—glycogen phosphorylase (PYGL), glutamate-ammonia ligase (GLUL), and glutamate dehydrogenase 1 (GLUD1)—have been identified as proteins that interact with RIP3 (415). PYGL catalyzes the rate-limiting step in the degradation of glycogen by releasing glucose-1-phosphate, thereby having a key role in using reserved glycogen as an energy source. GLUL and GLUD1 are essential for the use of amino acid glutamate or glutamine as substrates for ATP production in oxidative phosphorylation. RIP3 phosphorylates and activates these enzymes and thereby increases energy metabolism, facilitating TNF-induced necrosis, at least in part, through ROS production (415). Therefore, RIP3 appears to mediate TNF-induced necrosis through increasing energy metabolism-associated ROS production, in spite of increased overall ATP synthesis, which is generally regarded to support cell survival.

Based on the above-described critical roles of RIP1 and RIP3 in necrosis induction, their pathophysiological roles have been further investigated using *RIP3*$^{-/-}$ mice (46, 125, 415). Cerulein-induced pancreatitis is a well-established model of necrosis-associated diseases. Whereas many WT mice administered with cerulein exhibited multiple areas of pancreas acinar cell loss and necrosis, all *RIP3*$^{-/-}$littermates exhibited a much milder phenotype, suggesting that RIP3-dependent necrotic signaling exerts an adverse effect, at least on this disease model (125, 415). As another pathophysiological model, the vaccinia virus (VV), which encodes the viral caspase inhibitor B13R/Spi2, has been used (46). When T cells from *RIP3*$^{-/-}$mice were infected with VV, they exhibited

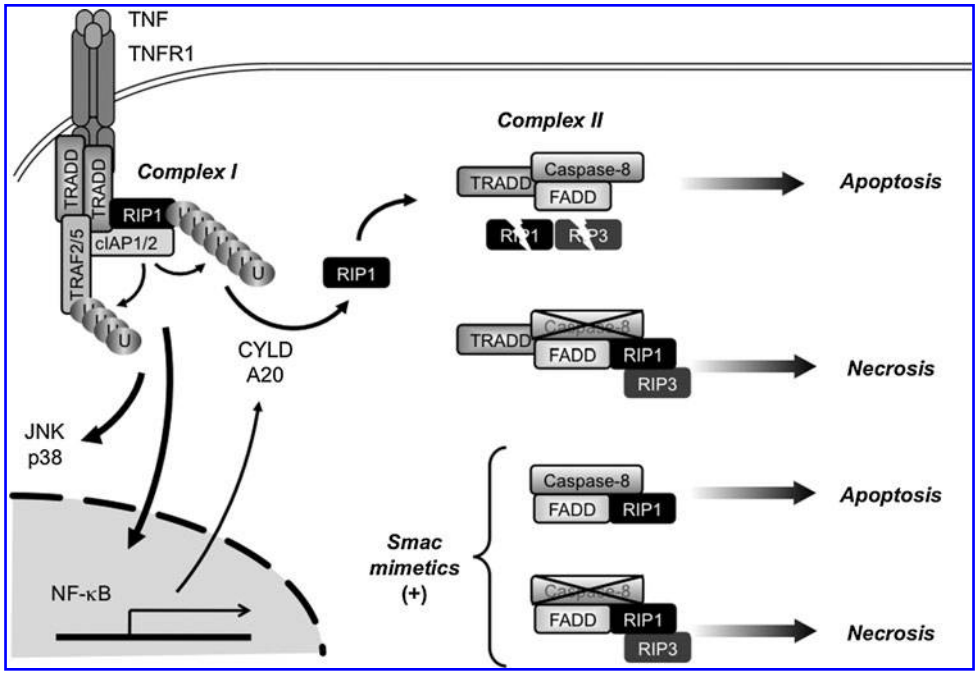

FIG. 12. Regulation of apoptosis and necrosis by RIP1 and RIP3. Upon activation of TNFR1, RIP1 is recruited to the membrane-associated complex I, in which cIAP1 and cIAP2 form polyubiquitin chains linked through K63 of ubiquitin (indicated as U) on RIP1. The resulting polyubiquitinated form of RIP1 induces activation of NF-κB, JNKs, and p38 MAPKs. When the polyubiquitin chain of RIP1 is removed by the deubiquitinating enzymes CYLD and A20, which are upregulated by NF-κB in a negative feedback manner, RIP1 loses its ability to activate NF-κB and is included in the secondary cytosolic complex II, formed in a TRADD-dependent manner. In this TRADD-dependent complex II, caspase-8 is activated and induces apoptosis, concomitant with cleavage of RIP1 and RIP3. When caspase-8 is inactivated, on the other hand, RIP1 and RIP3 stabilize and form a so-called necrosome complex containing FADD and probably TRADD, activating necrotic signaling. In the presence of Smac mimetics, another type of complex II is formed independently of TRADD, and caspase-8 is activated in a RIP1 kinase-dependent manner and induces apoptosis. In addition, when caspase-8 is inactivated in the presence of Smac mimetics, RIP1 and RIP3 play a key role in necrosis induction. FADD, Fas-associated death domain; NF-κB, nuclear factor-kappa B; TNFR1, TNF receptor 1; TRADD, TNFR-associated death domain.

reduced activation-induced cell death, indicating that RIP3 indeed functions as a necrosis inducer in this infection model. When WT mice were infected with VV, *RIP3*$^{-/-}$mice were much more vulnerable to VV infection than WT mice. Importantly, in *RIP3*$^{-/-}$mice, necrotic injury and virus-induced inflammation were impaired, whereas virus titers were dramatically increased, compared with those in WT mice. These findings strongly suggest that RIP3 and probably RIP1 critically regulate innate immune responses by promoting necrotic cell death, which triggers the release of intracellular danger-associated molecular patterns. Taken together with the fact that necrotic cell death occurs in a broad range of other pathogenic conditions, such as ischemia reperfusion damage during cardiac infarction, stroke, traumatic brain injury, and organ transplantation, the development of inhibitors of the RIP family kinases, as exemplified by the necrostatin RIP1 inhibitors, will be of importance for preventing and treating various human diseases (70, 356).

VI. Other Miscellaneous But Important Kinases

A. Homeodomain-interacting protein kinase 2

The serine/threonine kinase homeodomain-interacting protein kinase 2 (HIPK2) is a member of the HIPK family, which is comprised of four closely related kinases, and predominantly localizes in a nuclear compartment called nuclear bodies (NBs) (319). Although HIPK2 was originally identified as a transcriptional regulator and corepressor for homeodomain transcription factors, accumulating evidence has suggested that it is a crucial regulator of apoptosis, particularly in response to a variety of DNA damage-inducing stimuli.

In response to severe, nonrepairable DNA damage, HIPK2 colocalizes and interacts with the tumor suppressor p53 in promyelocytic leukemia NBs, where HIPK2 phosphorylates p53 at Ser46 (Fig. 13). Phosphorylation of this residue facilitates acetylation of p53 at Lys382, which is mediated by the CREB-binding protein that is also colocalized in promyelocytic leukemia-NBs, and thus promotes apoptosis through the expression of p53 target genes such as *Puma*, *Bax*, *Noxa*, and *p53AIP1* (63, 132). HIPK2 also exerts its proapoptotic activity in the absence of p53. HIPK2 phosphorylates Ser422 of the C-terminal binding protein (CtBP), a transcriptional corepressor that exerts antiapoptotic activity through the repression of several proapoptotic genes, and induces the proteasomal degradation of CtBP, subsequently promoting apoptosis even in p53-deficient cells (417). In addition, HIPK2 induces activation of JNK, and JNK in turn phosphorylates CtBP at Ser422, suggesting that HIPK2 regulates CtBP by direct phosphorylation and indirect phosphorylation through JNK (133, 365). This HIPK2-JNK axis operates in TGF-β-induced apoptosis in p53-deficient hepatoma cells (133).

In addition to the role of p53 as a downstream effector of HIPK2, p53 also functions as a regulator of HIPK2. In response to severe DNA damage, HIPK2 was cleaved at Asp916 and Asp977, which eliminated the autoinhibitory domain existing in the most C-terminal region of the molecule and thus generated a constitutively active kinase (Fig. 13). Intriguingly, caspase-6, which is a target gene of p53, was a responsible protease for the DNA damage-dependent cleavage of HIPK2 (110). These findings suggest that HIPK2 and p53 activate each other in a feed-forward manner in response to severe DNA damage. In response to relatively mild, sublethal DNA damage, on the other hand, p53 negatively regulates HIPK2 in a proteasome-dependent manner (Fig. 14). In this regard, two distinct mechanisms are proposed: one is dependent on murine double minute 2 and its human ortholog HDM2 and the other is dependent on seven in absentia homolog-1 (Siah-1), both of which are p53-inducible E3 ubiquitin ligases (286, 382). After cells are exposed to sublethal DNA damage, downregulation of HIPK2 may be a prerequisite for recovery of cells from DNA damage.

In addition to the cleavage-dependent activation, DNA-dependent stabilization of HIPK2 is another mechanism of a net upregulation of HIPK2 activity. It has recently been shown that the DNA damage checkpoint kinases ATM and ATR, which are well known to coordinate cellular responses to DNA damage, phosphorylate Siah-1 at Ser19. This phosphorylation disrupts the HIPK2–Siah-1 complex, resulting in stabilization and accumulation of HIPK2 (382) (Fig. 14). Another E3 ligase, WSB-1 (WD-repeat and suppressor of cytokine signaling (SOCS) box-containing-1), also associates with HIPK2 and induces degradation of HIPK2 through the ubiquitin-proteasome system. Upon DNA damage, the HIPK2–WSB-1 complex is disrupted and the resulting liberated HIPK2 stabilizes and exerts its proapoptotic activity (47).

When subjected to chemically induced skin tumorigenesis, *HIPK2*$^{-/-}$mice showed a significant increase in tumor number, compared with that in WT mice (376). Whereas tumors in all WT mice were benign papillomas, many tumors in *HIPK2*$^{-/-}$mice showed features of carcinoma *in situ* or invasive squamous cell carcinoma. This tumor-suppressive ac-

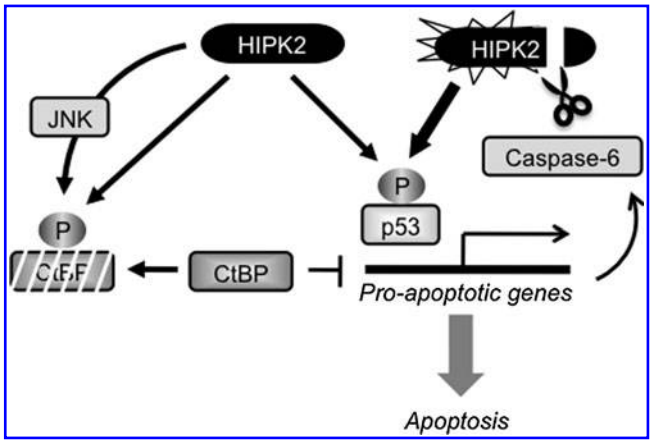

FIG. 13. Roles of HIPK2 in DNA damage-induced apoptosis. In response to severe, nonrepairable DNA damage, HIPK2 phosphorylates p53 at Ser46 and thus promotes apoptosis through the expression of proapoptotic genes. Caspase-6 is one such gene and cleaves HIPK2 at Asp916 and Asp977, which generates a constitutively active kinase by eliminating the autoinhibitory domain existing in the most C-terminal region of the molecule. HIPK2 and p53 thus appear to activate each other in a feed-forward manner in response to severe DNA damage. Even in the absence of p53, HIPK2 induces apoptosis by inducing degradation of CtBP, a transcription factor that represses several proapoptotic genes, through direct phosphorylation by HIPK2 and JNK, the latter of which is activated downstream of HIPK2. CtBP, C-terminal binding protein; HIPK2, homeodomain-interacting protein kinase 2.

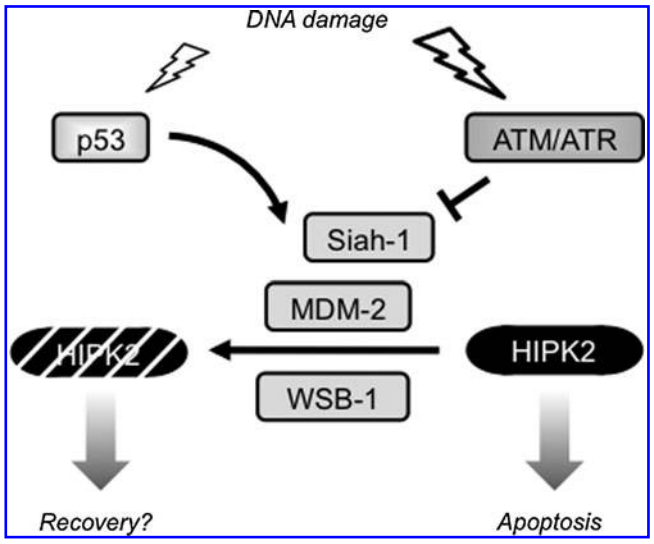

FIG. 14. Regulation of HIPK2 degradation. In response to relatively mild, sublethal DNA damage, p53 negatively regulates HIPK2 in a manner dependent on MDM2 or Siah-1, both of which are p53-inducible E3 ubiquitin ligases. Another E3 ligase, WSB-1, also induces degradation of HIPK2. Although not depicted in this scheme, the HIPK2–WSB-1 complex is disrupted upon DNA damage, and the resulting liberated HIPK2 stabilizes and exerts its proapoptotic activity. ATM and ATR phosphorylate Siah-1 at Ser19, which results in stabilization and accumulation of HIPK2. WSB-1, WD-repeat and suppressor of cytokine signaling (SOCS) box-containing-1; ATM, ataxia telangiectasia mutated; ATR, ATM and Rad3-related; MDM2, murine double minute 2; Siah-1, seven in absentia homolog-1.

tivity of HIPK2 appears to be accounted for not by its proapoptotic activity, but rather by suppression of cell proliferation through downregulation of cyclin D1 (376). Although there has not been enough evidence indicating the tumor-suppressive role of HIPK2 in humans, oncogene-dependent regulation of intracellular localization of HIPK2 has recently been proposed to be critically involved at least in some forms of cancers. The high-mobility group A1, a proto-oncogene frequently overexpressed in multiple human cancers, has the ability to promote translocation and retention of HIPK2 in the cytoplasm and thus inhibit the apoptotic function of p53. In fact, a strong correlation among overexpression of high-mobility group A1, cytoplasmic localization of HIPK2 and low spontaneous apoptosis was observed in WT p53-expressing human breast cancer samples (274). In analogy to this, it has been suggested that the leukemogenic chimeric protein core-binding factor-β-smooth muscle myosin heavy chain, which is generated by the chromosome inversion inv(16)(p13;q22) present in $\sim$12% of cases of human acute myeloid leukemia, sequesters HIPK2 in the cytosol and thereby compromises regular hematopoiesis, eventually leading to leukemogenesis (375). These findings strongly suggest that HIPK2 plays a tumor-suppressive role. Nevertheless, frequent *HIPK2* amplification and increased HIPK2 expression in pilocytic astrocytomas, the most common brain tumors in the pediatric and adolescent population, have also been reported (78). Consistent with this, overexpression of HIPK2 promotes cell growth in U87 glioma cells (78). Therefore, the precise roles of HIPK2, particularly in growth control during tumorigenesis, remain elusive and will require further investigation.

Finally, with respect to the control of apoptosis, HIPK2 is not necessarily a general apoptosis inducer. *HIPK2*$^{-/-}$mice exhibited a selective loss of midbrain dopamine neurons due to increased apoptosis without obvious defects in their neurogenesis *per se*. This finding indicates that HIPK2 functions as an antiapoptotic factor at least in neuronal development (416). Considering all these results together, two types of comparative analysis will be required for the comprehensive understanding of multifunctional HIPK2: one is focusing on its functions in apoptosis induction *versus* suppression and the other is focusing its functions in growth promotion *versus* suppression. In this regard, further analysis of various disease models using *HIPK2*$^{-/-}$mice may shed light on the important functions of HIPK2, especially those relevant to human pathophysiology.

B. Protein kinase Cδ

The PKC family is composed of a series of serine/threonine kinases that regulate diverse cellular functions (259). PKC are classified into three groups: conventional PKCs (α, β, and γ), novel PKCs (δ, ε, η, and θ), and atypical PKCs (ζ and λ/ι). Conventional PKCs are activated by diacylglycerol (DAG) and Ca^{2+}, whereas novel PKCs are activated by DAG but not by Ca^{2+}. Atypical PKCs are insensitive to both DAG and Ca^{2+}.

While many members of this family are known to critically regulate cell proliferation and survival, PKCδ functions as a positive regulator of apoptosis in many cell types (285). PKCδ is activated by various cytotoxic stressors, such as ionizing radiation, UV radiation, anticancer drugs, and ROS as well as growth factors and cytokines. PKCδ is normally activated by DAG generated from receptor-mediated hydrolysis of inositol phospholipids. In addition to or alternatively to this conventional activation mechanism, phosphorylation of PKCδ on tyrosine residues by various tyrosine kinases, such as the Src family kinases and c-Abl, is regarded as a crucial step, particularly for the induction of apoptosis in response to various cytotoxic stressors. Phosphorylations of multiple tyrosine residues of PKCδ have been reported so far, but the phosphorylations of different residues may be regulated in a manner dependent on cell types and/or types of stimulus. For instance, when COS-7 cells expressing exogenous PKCδ were exposed to H_2O_2, Tyr311, Tyr332, and Tyr512 were phosphorylated, among which Tyr311 was required for an increase in the catalytic activity (188). In glioma cells, phosphorylation of Tyr64 and Tyr187 appears to be required for apoptosis in response to etoposide, a topoisomerase II inhibitor (21).

Phosphorylation of tyrosine residues also appears to play a major role in the nuclear translocation of PKCδ. In fact, PKCδ possesses an nuclear localization signal and translocates from the cytoplasm to the nucleus in response to various proapoptotic stimuli, and this translocation is required for the proapoptotic activity of PKCδ (79). In the nucleus, PKCδ is cleaved between N-terminal regulatory and C-terminal catalytic domains by caspase-3, generating a constitutively active catalytic fragment (86, 99). Consistent with this nuclear regulation of PKCδ, many substrates of PKCδ exist in the nucleus, such as DNA-dependent protein kinase, the nuclear structural protein lamin B and the checkpoint protein Rad9, all of which

are especially important in genotoxic stress-induced apoptosis (406). Phosphorylation of p53 at Ser46 by PKCδ has also been proposed to be a critical regulation of genotoxic stress-induced apoptosis (408). Intriguingly, PKCδ has recently been shown to transactivate p53 expression at the transcriptional level by interacting with the death-promoting transcription factor Btf (Bcl-2-associated transcription factor) to co-occupy the p53 core promoter element (212).

Proapoptotic roles of PKCδ have been clearly demonstrated using *PKCδ*$^{-/-}$mice. *PKCδ*$^{-/-}$mice develop normally and are fertile, but smooth muscle cells from *PKCδ*$^{-/-}$mice are more resistant to various stimuli, such as UV, H_2O_2, and cytokines, than those from WT mice (207). Consistent with this finding, vein bypass graft experiments showed that vein segments from *PKCδ*$^{-/-}$mice isografted to carotid arteries of either WT or *PKCδ*$^{-/-}$recipient mice led to a more severe arteriosclerosis than was seen with vein segments from WT mice. When mice were subjected to γ-irradiation, apoptosis in the parotid glands was significantly suppressed in *PKCδ*$^{-/-}$mice, compared with WT mice. Primary parotid cells from *PKCδ*$^{-/-}$ mice were also defective in mitochondria-dependent apoptosis (145). These results demonstrate the pathophysiological significance of the proapoptotic function of PKCδ in cellular stress responses. In addition to such a role in stress response, recent studies using *PKCδ*$^{-/-}$mice have also demonstrated a critical role of this kinase in the maintenance of immune integrity (234, 235, 241). The spleens and lymph nodes of *PKCδ*$^{-/-}$mice are enlarged due to an increase in the number of B cells. Deficiency in PKCδ prevents B-cell tolerance and allows maturation and terminal differentiation of self-reactive B cells, leading to production of auto-reactive antibodies and immune-complex-type glomerulonephritis as well as lymphocyte infiltration in many organs. These findings strongly suggest that PKCδ is critically involved in human autoimmune diseases.

PKCδ also plays a key role in the injury associated with reperfusion of ischemic cardiac tissue (50). Activation of PKCδ during reperfusion appears to result in its translocation to the mitochondria, where it negatively regulates mitochondrial function and increases generation of mitochondrial ROS, inducing apoptosis through release of cytochrome c into the cytosol. Inhibition of PKCδ by a PKCδ-specific peptide inhibitor during reperfusion after ischemia improves myocardial cell survival by blocking the accumulation of Bad, inactivation of Akt, release of cytochrome c, and the following apoptotic events such as poly(ADP-ribose) polymerase cleavage and DNA fragmentation (247). Intriguingly, PKCε, another novel PKC isoform that is highly homologous to PKCδ, plays an opposing role in myocardial ischemia reperfusion injury; activation of PKCε before ischemia prevents ischemia-reperfusion-induced cell death by protecting mitochondrial function and decreasing apoptosis, suggesting the role of PKCε in ischemic preconditioning, a state produced by short bouts of sublethal ischemia reperfusion that protect tissue from subsequent lethal ischemia (8, 50).

These counteracting roles of PKCδ and PKCε are also involved in ischemic brain injury (49). Whereas cerebral infarcts are similarly induced after permanent middle cerebral artery occlusion in *PKCδ*$^{+/+}$ and *PKCδ*$^{-/-}$mice, *PKCδ*$^{-/-}$mice exhibit a striking reduction in infarct size compared with *PKCδ*$^{+/+}$mice after transient middle cerebral artery occlusion and reperfusion (48). This reduction in infarction in *PKCδ*$^{-/-}$ mice appears to be attributable in part to neutrophil PKCδ. In response to cerebral ischemia reperfusion, neutrophil PKCδ activates resting neutrophils to adhere to cerebral blood vessels and migrate into ischemic brain tissue. Neutrophil PKCδ is thought to regulate the release of superoxide anion through NADPH oxidase activation and promote neutrophil degranulation (48, 49). Brain PKCδ also plays a role in reperfusion injury; intravascular administration of a PKCδ-specific peptide inhibitor during reperfusion, but not before ischemia, decreased infarct size concomitant with reduced apoptotic signaling in the brain (28). Thus, PKCδ in neutrophils and neurons mediates reperfusion injury after cerebral ischemia. On the other hand, contribution of PKCε to protection against ischemia reperfusion injury through neuronal ischemic preconditioning has been demonstrated in various culture systems such as hippocampal slices and primary neuronal cultures using PKCε-specific activators and inhibitors (49). Taken these findings into account, PKCδ and PKCε would be promising therapeutic targets for ischemia-reperfusion-related complications.

C. Protein kinase Ds

The PKD family belongs to the CaM-dependent protein kinase group of serine/threonine kinases and consists of three isoforms, PKD1 (formerly known as PKCμ), PKD2, and PKD3 (formerly known as PKCν. These three isoforms share a similar domain structure; each consists of a C-terminal catalytic domain and an N-terminal regulatory domain, the latter of which further contains two zinc finger-like cysteine-rich domains and a pleckstrin homology domain (11, 287). The regulatory domain inhibits the activity of the catalytic domain in an autoinhibitory manner, and deletion or mutation of this domain leads to constitutive activation of the kinase. DAG, phorbol esters, and G protein-coupled receptor agonists bind to the cysteine-rich domains with high affinity and facilitate the release of PKD from its autoinhibition and the translocation of PKD from the cytosol to cellular membrane. Subsequently, PKD is activated through direct phosphorylation of two conserved serine residues within the activation loop of the catalytic domain by DAG-responsive PKC isoforms (287).

As an alternative activation mechanism of PKD, the proteolytic cleavage between the regulatory domain and the catalytic domain has been proposed (87). PKD is cleaved by caspase-3 in response to genotoxic agents, and the released catalytic domain gains its kinase activity and sensitizes cells to DNA damage-induced apoptosis. PKD also promotes anoikis by modulating the activity of Bcl-2 inhibitor transcription (Bit1), a mitochondrial protein that is released into the cytosol and induces caspase-independent apoptosis upon loss of cell attachment (18, 159). PKD directly phosphorylates serine residues in Bit1 and facilitates the release of Bit1 from the mitochondria to the cytosol. Since cell attachment to fibronectin was found to inhibit PKD activity, PKD appears to be activated in response to cell detachment by an unknown mechanism and function as a proapoptotic signaling intermediate (18).

In contrast to these proapoptotic roles, PKD also plays roles in promoting cell survival and inhibiting apoptosis. The accumulation of mitochondrial ROS leads to translocation of PKD from the cytosol to the mitochondria, where tyrosine kinases Src and Abl phosphorylate a tyrosine residue within

the pleckstrin homology domain of PKD and induce conformational changes that release autoinhibition of PKD (322). This tyrosine phosphorylation further facilitates phosphorylation of the activation loop of PKD by PKCδ, mediating full activation of PKD. Subsequently, PKD induces expression of the *SOD2* gene, which controls cellular detoxication from superoxide, and the antiapoptotic gene encoding A20 through activation of the NF-κB transcription factor. Tyrosine phosphorylated PKD, but not conventionally activated PKD such as through DAG generation, induces NF-κB, suggesting that tyrosine phosphorylation is an important mechanism by which PKD exerts its specific function as a sensor of mitochondrial ROS (321). JNK and c-Jun have also been proposed to targets of PKD when PKD exerts its antiapoptotic function (146). PKD associates with JNK and probably with c-Jun in a manner dependent on the kinase activity of PKD and appears to impair the ability of JNK to phosphorylate and thus activate c-Jun by directly phosphorylating alternative sites in the c-Jun N-terminus.

In addition to the roles in cell death/survival control, PKD has been implicated in cell migration, invasion, and motility, all of which are critical processes in cancer progression and metastasis (197). For instance, PKD phosphorylates slingshot 1 like, a phosphatase that dephosphorylates and reactivates the actin-remodeling protein cofilin at the leading edge of migrating cells. This phosphorylation blocks the localization of slingshot 1 like to the actin cytoskeleton and subsequently inhibits cell motility through sustained cofilin inactivation (84). PKD has also been implicated in angiogenesis, which plays a major role in tumorigenesis by providing growing tumors with oxygen and nutrients (197). PKD is activated by VEGF and required for VEGF-induced endothelial cell proliferation, migration, and *in vivo* angiogenesis (278). Consistent with these experimental findings, dysregulation of expression, activity, and intracellular distribution of all PKD isoforms in a variety of human tumor samples has been demonstrated (197).

PKD also plays a major role in cellular responses to oxidative stress. PKD has been focused on as a regulator of the cardiovascular system because cardiac PKD is activated in response to hypertension, pressure overload, and chronic neurohormonal signaling (11). Recently, mice with cardiac-specific deletion of the *PRKD1* gene that encodes PKD1 have been found to be viable and show diminished hypertrophy, fibrosis, and fetal gene activation as well as improved cardiac function in response to pressure overload or chronic adrenergic and Ang II signaling (92). These findings demonstrate that PKD1 activity plays a key role in mediating stress-dependent remodeling and reprogramming of gene expression in the adult heart. In this regulation, phosphorylation of class II histone deacetylases (HDACs) such as HDAC5 by PKD has been proposed to be crucial. Class II HDACs function as negative regulators of pathological cardiac remodeling through association with the MEF2 transcription factor, an activator of heart diseases; however, phosphorylation of class II HDACs by PKD results in their dissociation from MEF2, allowing the activation of MEF2 target genes (11).

According to the recent progress in the elucidation of the pathophysiological roles of PKD as described in this section, various types of chemical inhibitors have been developed (197). To further validate PKD as a chemotherapeutic target, however, it would be important to investigate to what extent the PKD isoforms are different in their functions, because no isoform-specific inhibitors have been reported so far.

D. c-Abl

c-Abl is a tyrosine kinase closely related to the Src family kinases and essential for mouse development, as demonstrated by the embryonic and neonatal lethality of *c-abl*$^{-/-}$ mice (348). Whereas the overall structure of the N-terminal half of c-Abl, which includes an Src-homology 2 domain, an SH3 domain, and the kinase domain, is homologous to that of the Src family kinases, the C-terminal half of c-Abl has a unique structure composed of binding elements for SH3 domains, nuclear localization and export signals, a bipartite DNA-binding domain, and an actin-binding domain (Fig. 15). The basal catalytic activity of c-Abl is tightly regulated by autoinhibitory mechanisms through tyrosyl phosphorylation and N-terminal myristoylation within the molecule (117, 249).

c-Abl is well known to have oncogenic potential. When the gene encoding c-Abl is aberrantly fused to the breakpoint cluster region (*BCR*) gene, the autoinhibitory mechanisms of c-Abl are disrupted and BCR-Abl acts as a constitutive kinase, resulting in the disease chronic myelogenous leukemia (303). On the other hand, accumulating evidence has suggested that c-Abl also functions as an inducer of cell death (363). c-Abl is preferentially activated by DNA damage inducers, such as ionizing radiation, cisplatin, mitomycin C, and topoisomerase inhibitors, all of which are known to induce apoptosis, and the requirement of c-Abl for DNA damage-induced apoptosis has been demonstrated by studies using cells from *c-abl*$^{-/-}$mice (104).

With regard to the control of oncogenic *versus* proapoptotic activity of c-Abl, its intracellular distribution appears to be a critical determinant. It has been proposed that nuclear c-Abl is associated with induction of DNA damage-induced apoptosis, whereas cytoplasmic c-Abl contributes to cell proliferation and survival (406). Recently, the mechanisms by which cytoplasmic c-Abl is targeted to the nucleus in response to DNA damage have been revealed. c-Abl is sequestered in the cytoplasm by binding to 14-3-3 proteins through phosphorylation of c-Abl at Thr735. In response to DNA damage, JNK is

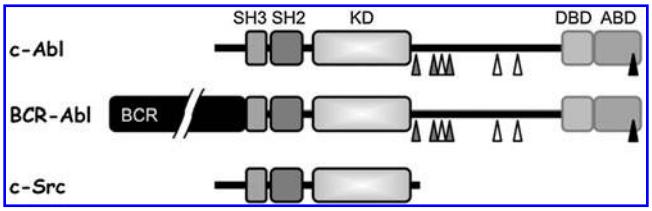

FIG. 15. Domain structure of c-Abl. The overall structure of the N-terminal half of c-Abl, which includes an SH2 domain, an SH3 domain, and the KD, is homologous to that of c-Src. The C-terminal half of c-Abl has a unique structure composed of binding elements for SH3 domains (Pro-X-X-Pro sequence; indicated by the gray triangles), nuclear localization and export signals (indicated by the white and black triangles, respectively), a bipartite DNA-binding domain (DBD), and an actin-binding domain (ABD). BCR-Abl is a fusion protein, in which the BCR protein is fused upstream of the SH3 domain of c-Abl. BCR, breakpoint cluster region; SH2, Src-homology 2.

activated and induces phosphorylation of 14-3-3, which dissociates 14-3-3 from c-Abl. The liberated c-Abl subsequently is translocated into the nucleus, where it exerts its proapoptotic activity (409). The MUC1 transmembrane glycoprotein is another important molecule that tethers c-Abl in the cytoplasm. MUC1 is an oncoprotein overexpressed in many human cancers and is known to suppress apoptosis induced by genotoxic agents (318). MUC1 associates with and thus inhibits nuclear translocation of c-Abl through direct binding of the Src-homology 2 domain of c-Abl to MUC1 Tyr60, which is phosphorylated by c-Abl (280). Binding of MUC1 to c-Abl attenuates phosphorylation of c-Abl at Thr735 and the interaction between c-Abl and 14-3-3, implying that MUC1 not only tethers c-Abl in the cytoplasm but also disrupts DNA damage-induced, 14-3-3-dependent regulation of c-Abl localization. This mechanism may contribute to the resistance of cancer cells, particularly those overexpressing MUC1, to anticancer agents.

Recent precise analysis of *c-abl*$^{-/-}$mice has revealed the critical role of c-Abl in cardiac growth and development. *c-abl*$^{-/-}$mice with a C57BL/6J background exhibit severe perinatal lethality, probably as a result of cardiac hyperplasia that is caused not by decreased apoptosis, but rather by abnormally increased cardiomyocyte proliferation during the later stages of embryogenesis (279). The proapoptotic activity of c-Abl may thus be tightly confined to the situation where cells need to correctly respond to DNA damage.

E. DYRK2

The dual-specificity tyrosine-regulated kinases, DYRK1A, DYRK1B, DYRK2, DYRK3, and DYRK4, constitute a unique family of protein kinases that autophosphorylate an essential tyrosine residue within their activation loop and phosphorylate their substrates only at serine and threonine residues (407). Among the family members, DYRK1A has been paid the most attention because it appears to be involved in some of the neurological defects of Down syndrome patients, and because the human *DYRK1A* gene is consistently located on chromosome 21 in the Down syndrome critical region.

Although there has been little evidence of involvement of DYRKs in apoptosis regulation, DYRK2 has recently been found to be critically involved in the induction of DNA damage-dependent apoptosis (331). Whereas DYRK2 exists predominantly in the cytoplasm in unstimulated cells, it translocates into the nucleus in response to DNA damage. In the nucleus, DYRK2 phosphorylates p53 at Ser46, which induces expression of proapoptotic genes, such as p53AIP1, and thereby induces apoptosis. ATM appears to function as an upstream regulator of DYRK2. Since ATM appears not to be required for DNA damage-induced nuclear translocation of DYRK2, ATM might regulate the catalytic activity of DYRK2 in response to DNA damage. These findings suggest that DYRK2 functions as a suppressor of DNA damage-induced tumorigenesis.

However, DYRK2 is also suggested to be a potential oncogene, since gene amplification and overexpression of DYRK2 have been reported in gastrointestinal and lung cancers (105, 240). Recently, a possible underlying mechanism of the oncogenic aspect of DYRK2 has been proposed. In *C. elegans*, MBK-2, the *C. elegans* ortholog of mammalian DYRK2, phosphorylated and regulated MEI-1, the *C. elegans* ortholog of mammalian katanin p60 that is a microtubule AAA-ATPase required for severing microtubules at the mitotic spindles (216). Consistent with this, DYRK2 served as a scaffold for an E3 ligase complex, designated as the EDVP complex, containing an E3 ligase EDD (also known as UBR5), DNA damage binding protein 1, and a WD40 domain-containing protein VPRBP (also known as DCAF1) (223). In this complex, DYRK2 phosphorylated katanin p60 and thereby facilitated its degradation through the ubiquitin-proteasome system. Importantly, siRNA-mediated downregulation of DYRK2, which upregulates katanin, resulted in abnormal accumulation of cells with a *4N* DNA content. In addition, DYRK2-mediated phosphorylation of katanin was required for proper cell cycle progression. These findings suggest that DYRK2 positively controls cell proliferation and that aberrant mitosis and altered cell cycle progression through overexpression and/or hyper-activation of DYRK2 may play a key role in oncogenic transformation.

VII. Concluding Remarks

Here, we have focused on the representative apoptosis signaling kinases and discussed their roles in stress response as well as apoptosis signaling. By overviewing this substantial collection, one can clearly recognize that such kinases play multiple important roles in coordinating various intracellular signaling pathways, such as proapoptotic, antiapoptotic, proliferative, and differentiation signaling pathways, to enable cells to respond properly to various levels of changes in internal and external environments. The reversibility of protein phosphorylation may be a plausible explanation for why kinases accomplish such fine-tuning of cellular responses. In this regard, further investigation of apoptosis signaling phosphatases will be an important task to fully understand the functions and regulatory mechanisms of apoptosis signaling kinases. In this review, we have also focused on health outcomes upon dysregulation of some of the kinases, which have been revealed mainly by genetic approaches using genetically modified mice. The cumulative evidence described here suggests that most of the apoptosis signaling kinases merit attention as potential therapeutic targets for human diseases. Thus, further investigation into the pathophysiological roles of the kinases using genetically modified mice will surely lead to cures or treatments for various obstinate diseases.

Acknowledgments

We thank all the members of Cell Signaling Laboratory for their critical comments. This work was supported by grants-in aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan, the Program for Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation, and the Uehara Memorial Foundation.

References

1. Abreu-Martin MT, Chari A, Palladino AA, Craft NA, and Sawyers CL. Mitogen-activated protein kinase kinase kinase 1 activates androgen receptor-dependent transcription

and apoptosis in prostate cancer. *Mol Cell Biol* 19: 5143–5154, 1999.
2. Adams RH, Porras A, Alonso G, Jones M, Vintersten K, Panelli S, Valladares A, Perez L, Klein R, and Nebreda AR. Essential role of p38α MAP kinase in placental but not embryonic cardiovascular development. *Mol Cell* 6: 109–116, 2000.
3. Adhikari A, Xu M, and Chen ZJ. Ubiquitin-mediated activation of TAK1 and IKK. *Oncogene* 26: 3214–3226, 2007.
4. Allen M, Svensson L, Roach M, Hambor J, McNeish J, and Gabel CA. Deficiency of the stress kinase p38α results in embryonic lethality: characterization of the kinase dependence of stress responses of enzyme-deficient embryonic stem cells. *J Exp Med* 191: 859–870, 2000.
5. Apostol BL, Simmons DA, Zuccato C, Illes K, Pallos J, Casale M, Conforti P, Ramos C, Roarke M, Kathuria S, Cattaneo E, Marsh JL, and Thompson LM. CEP-1347 reduces mutant huntingtin-associated neurotoxicity and restores BDNF levels in R6/2 mice. *Mol Cell Neurosci* 39: 8–20, 2008.
6. Arias-Romero LE and Chernoff J. A tale of two Paks. *Biol Cell* 100: 97–108, 2008.
7. Arimoto K, Fukuda H, Imajoh-Ohmi S, Saito H, and Takekawa M. Formation of stress granules inhibits apoptosis by suppressing stress-responsive MAPK pathways. *Nat Cell Biol* 10: 1324–1332, 2008.
8. Armstrong SC. Protein kinase activation and myocardial ischemia/reperfusion injury. *Cardiovasc Res* 61: 427–436, 2004.
9. Arnold R, Frey CR, Muller W, Brenner D, Krammer PH, and Kiefer F. Sustained JNK signaling by proteolytically processed HPK1 mediates IL-3 independent survival during monocytic differentiation. *Cell Death Differ* 14: 568–575, 2007.
10. Arnold R, Liou J, Drexler HC, Weiss A, and Kiefer F. Caspase-mediated cleavage of hematopoietic progenitor kinase 1 (HPK1) converts an activator of NFκB into an inhibitor of NFκB. *J Biol Chem* 276: 14675–14684, 2001.
11. Avkiran M, Rowland AJ, Cuello F, and Haworth RS. Protein kinase D in the cardiovascular system: emerging roles in health and disease. *Circ Res* 102: 157–163, 2008.
12. Aza-Blanc P, Cooper CL, Wagner K, Batalov S, Deveraux QL, and Cooke MP. Identification of modulators of TRAIL-induced apoptosis via RNAi-based phenotypic screening. *Mol Cell* 12: 627–637, 2003.
13. Balasenthil S, Sahin AA, Barnes CJ, Wang RA, Pestell RG, Vadlamudi RK, and Kumar R. p21-activated kinase-1 signaling mediates cyclin D1 expression in mammary epithelial and cancer cells. *J Biol Chem* 279: 1422–1428, 2004.
14. Beardmore VA, Hinton HJ, Eftychi C, Apostolaki M, Armaka M, Darragh J, McIlrath J, Carr JM, Armit LJ, Clacher C, Malone L, Kollias G, and Arthur JS. Generation and characterization of p38β (MAPK11) gene-targeted mice. *Mol Cell Biol* 25: 10454–10464, 2005.
15. Behrens A, Sibilia M, and Wagner EF. Amino-terminal phosphorylation of c-Jun regulates stress-induced apoptosis and cellular proliferation. *Nat Genet* 21: 326–329, 1999.
16. Bialik S and Kimchi A. The death-associated protein kinases: structure, function, and beyond. *Annu Rev Biochem* 75: 189–210, 2006.
17. Bild AH, Mendoza FJ, Gibson EM, Huang M, Villanueva J, Garrington TP, Jove R, Johnson GL, and Gibson SB. MEKK1-induced apoptosis requires TRAIL death receptor activation and is inhibited by AKT/PKB through inhibition of MEKK1 cleavage. *Oncogene* 21: 6649–6656, 2002.
18. Biliran H, Jan Y, Chen R, Pasquale EB, and Ruoslahti E. Protein kinase D is a positive regulator of Bit1 apoptotic function. *J Biol Chem* 283: 28029–28037, 2008.
19. Bisson N, Tremblay M, Robinson F, Kaplan DR, Trusko SP, and Moss T. Mice lacking both mixed-lineage kinase genes Mlk1 and Mlk2 retain a wild type phenotype. *Cell Cycle* 7: 909–916, 2008.
20. Blagosklonny MV, Schulte T, Nguyen P, Trepel J, and Neckers LM. Taxol-induced apoptosis and phosphorylation of Bcl-2 protein involves c-Raf-1 and represents a novel c-Raf-1 signal transduction pathway. *Cancer Res* 56: 1851–1854, 1996.
21. Blass M, Kronfeld I, Kazimirsky G, Blumberg PM, and Brodie C. Tyrosine phosphorylation of protein kinase Cδ is essential for its apoptotic effect in response to etoposide. *Mol Cell Biol* 22: 182–195, 2002.
22. Bogoyevitch MA and Arthur PG. Inhibitors of c-Jun N-terminal kinases: JuNK no more? *Biochim Biophys Acta* 1784: 76–93, 2008.
23. Boll JB, Geist MA, Kaminski Schierle GS, Petersen K, Leist M, and Vaudano E. Improvement of embryonic dopaminergic neurone survival in culture and after grafting into the striatum of hemiparkinsonian rats by CEP-1347. *J Neurochem* 88: 698–707, 2004.
24. Bozyczko-Coyne D, O'Kane TM, Wu ZL, Dobrzanski P, Murthy S, Vaught JL, and Scott RW. CEP-1347/KT-7515, an inhibitor of SAPK/JNK pathway activation, promotes survival and blocks multiple events associated with Aβ-induced cortical neuron apoptosis. *J Neurochem* 77: 849–863, 2001.
25. Brancho D, Tanaka N, Jaeschke A, Ventura JJ, Kelkar N, Tanaka Y, Kyuuma M, Takeshita T, Flavell RA, and Davis RJ. Mechanism of p38 MAP kinase activation *in vivo*. *Genes Dev* 17: 1969–1978, 2003.
26. Brancho D, Ventura JJ, Jaeschke A, Doran B, Flavell RA, and Davis RJ. Role of MLK3 in the regulation of mitogen-activated protein kinase signaling cascades. *Mol Cell Biol* 25: 3670–3681, 2005.
27. Brenner D, Golks A, Becker M, Muller W, Frey CR, Novak R, Melamed D, Kiefer F, Krammer PH, and Arnold R. Caspase-cleaved HPK1 induces CD95L-independent activation-induced cell death in T and B lymphocytes. *Blood* 110: 3968–3977, 2007.
28. Bright R, Raval AP, Dembner JM, Perez-Pinzon MA, Steinberg GK, Yenari MA, and Mochly-Rosen D. Protein kinase Cδ mediates cerebral reperfusion injury *in vivo*. *J Neurosci* 24: 6880–6888, 2004.
29. Bulavin DV, Saito S, Hollander MC, Sakaguchi K, Anderson CW, Appella E, and Fornace AJ, Jr. Phosphorylation of human p53 by p38 kinase coordinates N-terminal phosphorylation and apoptosis in response to UV radiation. *EMBO J* 18: 6845–6854, 1999.
30. Cagnol S and Chambard JC. ERK and cell death: mechanisms of ERK-induced cell death—apoptosis, autophagy and senescence. *FEBS J* 277: 2–21, 2010.
31. Cardaci S, Filomeni G, Rotilio G, and Ciriolo MR. p38(MAPK)/p53 signalling axis mediates neuronal apoptosis in response to tetrahydrobiopterin-induced oxidative stress and glucose uptake inhibition: implication for neurodegeneration. *Biochem J* 430: 439–451, 2010.
32. Cardone MH, Salvesen GS, Widmann C, Johnson G, and Frisch SM. The regulation of anoikis: MEKK-1 activation requires cleavage by caspases. *Cell* 90: 315–323, 1997.

33. Chaar Z, O'Reilly P, Gelman I, and Sabourin LA. v-Src-dependent down-regulation of the Ste20-like kinase SLK by casein kinase II. *J Biol Chem* 281: 28193–28199, 2006.
34. Chan EH, Nousiainen M, Chalamalasetty RB, Schafer A, Nigg EA, and Sillje HH. The Ste20-like kinase Mst2 activates the human large tumor suppressor kinase Lats1. *Oncogene* 24: 2076–2086, 2005.
35. Chang CI, Xu BE, Akella R, Cobb MH, and Goldsmith EJ. Crystal structures of MAP kinase p38 complexed to the docking sites on its nuclear substrate MEF2A and activator MKK3b. *Mol Cell* 9: 1241–1249, 2002.
36. Chang HY, Nishitoh H, Yang X, Ichijo H, and Baltimore D. Activation of apoptosis signal-regulating kinase 1 (ASK1) by the adapter protein Daxx. *Science* 281: 1860–1863, 1998.
37. Chen CH, Wang WJ, Kuo JC, Tsai HC, Lin JR, Chang ZF, and Chen RH. Bidirectional signals transduced by DAPK-ERK interaction promote the apoptotic effect of DAPK. *EMBO J* 24: 294–304, 2005.
38. Chen N, Nomura M, She QB, Ma WY, Bode AM, Wang L, Flavell RA, and Dong Z. Suppression of skin tumorigenesis in c-Jun NH(2)-terminal kinase-2-deficient mice. *Cancer Res* 61: 3908–3912, 2001.
39. Chen S, Auletta T, Dovirak O, Hutter C, Kuntz K, El-Ftesi S, Kendall J, Han H, Von Hoff DD, Ashfaq R, Maitra A, Iacobuzio-Donahue CA, Hruban RH, and Lucito R. Copy number alterations in pancreatic cancer identify recurrent PAK4 amplification. *Cancer Biol Ther* 7: 1793–1802, 2008.
40. Chen X, Rzhetskaya M, Kareva T, Bland R, During MJ, Tank AW, Kholodilov N, and Burke RE. Antiapoptotic and trophic effects of dominant-negative forms of dual leucine zipper kinase in dopamine neurons of the substantia nigra *in vivo*. *J Neurosci* 28: 672–680, 2008.
41. Chen YR, Meyer CF, Ahmed B, Yao Z, and Tan TH. Caspase-mediated cleavage and functional changes of hematopoietic progenitor kinase 1 (HPK1). *Oncogene* 18: 7370–7377, 1999.
42. Chen Z and Cobb MH. Regulation of stress-responsive mitogen-activated protein (MAP) kinase pathways by TAO2. *J Biol Chem* 276: 16070–16075, 2001.
43. Chen Z, Raman M, Chen L, Lee SF, Gilman AG, and Cobb MH. TAO (thousand-and-one amino acid) protein kinases mediate signaling from carbachol to p38 mitogen-activated protein kinase and ternary complex factors. *J Biol Chem* 278: 22278–22283, 2003.
44. Cheung WL, Ajiro K, Samejima K, Kloc M, Cheung P, Mizzen CA, Beeser A, Etkin LD, Chernoff J, Earnshaw WC, and Allis CD. Apoptotic phosphorylation of histone H2B is mediated by mammalian sterile twenty kinase. *Cell* 113: 507–517, 2003.
45. Chi H, Sarkisian MR, Rakic P, and Flavell RA. Loss of mitogen-activated protein kinase kinase kinase 4 (MEKK4) results in enhanced apoptosis and defective neural tube development. *Proc Natl Acad Sci USA* 102: 3846–3851, 2005.
46. Cho YS, Challa S, Moquin D, Genga R, Ray TD, Guildford M, and Chan FK. Phosphorylation-driven assembly of the RIP1-RIP3 complex regulates programmed necrosis and virus-induced inflammation. *Cell* 137: 1112–1123, 2009.
47. Choi DW, Seo YM, Kim EA, Sung KS, Ahn JW, Park SJ, Lee SR, and Choi CY. Ubiquitination and degradation of homeodomain-interacting protein kinase 2 by WD40 repeat/SOCS box protein WSB-1. *J Biol Chem* 283: 4682–4689, 2008.
48. Chou WH, Choi DS, Zhang H, Mu D, McMahon T, Kharazia VN, Lowell CA, Ferriero DM, and Messing RO. Neutrophil protein kinase Cδ as a mediator of stroke-reperfusion injury. *J Clin Invest* 114: 49–56, 2004.
49. Chou WH and Messing RO. Protein kinase C isozymes in stroke. *Trends Cardiovasc Med* 15: 47–51, 2005.
50. Churchill EN and Mochly-Rosen D. The roles of PKCδ and ε isoenzymes in the regulation of myocardial ischaemia/reperfusion injury. *Biochem Soc Trans* 35: 1040–1042, 2007.
51. Cinar B, Fang PK, Lutchman M, Di Vizio D, Adam RM, Pavlova N, Rubin MA, Yelick PC, and Freeman MR. The pro-apoptotic kinase Mst1 and its caspase cleavage products are direct inhibitors of Akt1. *EMBO J* 26: 4523–4534, 2007.
52. Cohen O, Feinstein E, and Kimchi A. DAP-kinase is a Ca2+/calmodulin-dependent, cytoskeletal-associated protein kinase, with cell death-inducing functions that depend on its catalytic activity. *EMBO J* 16: 998–1008, 1997.
53. Cohen O, Inbal B, Kissil JL, Raveh T, Berissi H, Spivak-Kroizaman T, Feinstein E, and Kimchi A. DAP-kinase participates in TNF-α- and Fas-induced apoptosis and its function requires the death domain. *J Cell Biol* 146: 141–148, 1999.
54. Conforti P, Ramos C, Apostol BL, Simmons DA, Nguyen HP, Riess O, Thompson LM, Zuccato C, and Cattaneo E. Blood level of brain-derived neurotrophic factor mRNA is progressively reduced in rodent models of Huntington's disease: restoration by the neuroprotective compound CEP-1347. *Mol Cell Neurosci* 39: 1–7, 2008.
55. Cong F and Goff SP. c-Abl-induced apoptosis, but not cell cycle arrest, requires mitogen-activated protein kinase kinase 6 activation. *Proc Natl Acad Sci USA* 96: 13819–13824, 1999.
56. Cotteret S and Chernoff J. Nucleocytoplasmic shuttling of Pak5 regulates its antiapoptotic properties. *Mol Cell Biol* 26: 3215–3230, 2006.
57. Cotteret S, Jaffer ZM, Beeser A, and Chernoff J. p21-Activated kinase 5 (Pak5) localizes to mitochondria and inhibits apoptosis by phosphorylating BAD. *Mol Cell Biol* 23: 5526–5539, 2003.
58. Coulombe P and Meloche S. Atypical mitogen-activated protein kinases: structure, regulation and functions. *Biochim Biophys Acta* 1773: 1376–1387, 2007.
59. Coulthard LR, White DE, Jones DL, McDermott MF, and Burchill SA. p38(MAPK): stress responses from molecular mechanisms to therapeutics. *Trends Mol Med* 15: 369–379, 2009.
60. Creasy CL, Ambrose DM, and Chernoff J. The Ste20-like protein kinase, Mst1, dimerizes and contains an inhibitory domain. *J Biol Chem* 271: 21049–21053, 1996.
61. Cuenda A and Rousseau S. p38 MAP-kinases pathway regulation, function and role in human diseases. *Biochim Biophys Acta* 1773: 1358–1375, 2007.
62. Cuevas BD, Abell AN, and Johnson GL. Role of mitogen-activated protein kinase kinase kinases in signal integration. *Oncogene* 26: 3159–3171, 2007.
63. D'Orazi G, Cecchinelli B, Bruno T, Manni I, Higashimoto Y, Saito S, Gostissa M, Coen S, Marchetti A, Del Sal G, Piaggio G, Fanciulli M, Appella E, and Soddu S. Homeodomain-interacting protein kinase-2 phosphorylates p53 at Ser 46 and mediates apoptosis. *Nat Cell Biol* 4: 11–19, 2002.
64. Dan C, Nath N, Liberto M, and Minden A. PAK5, a new brain-specific kinase, promotes neurite outgrowth in N1E-115 cells. *Mol Cell Biol* 22: 567–577, 2002.
65. Dan I, Ong SE, Watanabe NM, Blagoev B, Nielsen MM, Kajikawa E, Kristiansen TZ, Mann M, and Pandey A.

Cloning of MASK, a novel member of the mammalian germinal center kinase III subfamily, with apoptosis-inducing properties. *J Biol Chem* 277: 5929–5939, 2002.
66. Dan I, Watanabe NM, and Kusumi A. The Ste20 group kinases as regulators of MAP kinase cascades. *Trends Cell Biol* 11: 220–230, 2001.
67. Davis RJ. Signal transduction by the JNK group of MAP kinases. *Cell* 103: 239–252, 2000.
68. De Souza PM, Kankaanranta H, Michael A, Barnes PJ, Giembycz MA, and Lindsay MA. Caspase-catalyzed cleavage and activation of Mst1 correlates with eosinophil but not neutrophil apoptosis. *Blood* 99: 3432–3438, 2002.
69. Deak JC, Cross JV, Lewis M, Qian Y, Parrott LA, Distelhorst CW, and Templeton DJ. Fas-induced proteolytic activation and intracellular redistribution of the stress-signaling kinase MEKK1. *Proc Natl Acad Sci USA* 95: 5595–5600, 1998.
70. Degterev A, Hitomi J, Germscheid M, Ch'en IL, Korkina O, Teng X, Abbott D, Cuny GD, Yuan C, Wagner G, Hedrick SM, Gerber SA, Lugovskoy A, and Yuan J. Identification of RIP1 kinase as a specific cellular target of necrostatins. *Nat Chem Biol* 4: 313–321, 2008.
71. Deiss LP, Feinstein E, Berissi H, Cohen O, and Kimchi A. Identification of a novel serine/threonine kinase and a novel 15-kD protein as potential mediators of the γ interferon-induced cell death. *Genes Dev* 9: 15–30, 1995.
72. Delpire E. The mammalian family of sterile 20p-like protein kinases. *Pflugers Arch* 458: 953–967, 2009.
73. Deng Y, Pang A, and Wang JH. Regulation of mammalian STE20-like kinase 2 (MST2) by protein phosphorylation/dephosphorylation and proteolysis. *J Biol Chem* 278: 11760–11767, 2003.
74. Deng Y, Ren X, Yang L, Lin Y, and Wu X. A JNK-dependent pathway is required for TNFα-induced apoptosis. *Cell* 115: 61–70, 2003.
75. Deng Y, Yang J, McCarty M, and Su B. MEKK3 is required for endothelium function but is not essential for tumor growth and angiogenesis. *Am J Physiol Cell Physiol* 293: C1404– C1411, 2007.
76. Densham RM, O'Neill E, Munro J, Konig I, Anderson K, Kolch W, and Olson MF. MST kinases monitor actin cytoskeletal integrity and signal via c-Jun N-terminal kinase stress-activated kinase to regulate p21Waf1/Cip1 stability. *Mol Cell Biol* 29: 6380–6390, 2009.
77. Derijard B, Hibi M, Wu IH, Barrett T, Su B, Deng T, Karin M, and Davis RJ. JNK1: a protein kinase stimulated by UV light and Ha-Ras that binds and phosphorylates the c-Jun activation domain. *Cell* 76: 1025–1037, 1994.
78. Deshmukh H, Yeh TH, Yu J, Sharma MK, Perry A, Leonard JR, Watson MA, Gutmann DH, and Nagarajan R. High-resolution, dual-platform aCGH analysis reveals frequent HIPK2 amplification and increased expression in pilocytic astrocytomas. *Oncogene* 27: 4745–4751, 2008.
79. DeVries TA, Neville MC, and Reyland ME. Nuclear import of PKCδ is required for apoptosis: identification of a novel nuclear import sequence. *EMBO J* 21: 6050–6060, 2002.
80. Dhanasekaran DN and Reddy EP. JNK signaling in apoptosis. *Oncogene* 27: 6245–6251, 2008.
81. Dolado I, Swat A, Ajenjo N, De Vita G, Cuadrado A, and Nebreda AR. p38α MAP kinase as a sensor of reactive oxygen species in tumorigenesis. *Cancer Cell* 11: 191–205, 2007.
82. Donovan N, Becker EB, Konishi Y, and Bonni A. JNK phosphorylation and activation of BAD couples the stress-activated signaling pathway to the cell death machinery. *J Biol Chem* 277: 40944–40949, 2002.
83. Edwards DC, Sanders LC, Bokoch GM, and Gill GN. Activation of LIM-kinase by Pak1 couples Rac/Cdc42 GTPase signalling to actin cytoskeletal dynamics. *Nat Cell Biol* 1: 253–259, 1999.
84. Eiseler T, Doppler H, Yan IK, Kitatani K, Mizuno K, and Storz P. Protein kinase D1 regulates cofilin-mediated F-actin reorganization and cell motility through slingshot. *Nat Cell Biol* 11: 545–556, 2009.
85. Eisenberg-Lerner A and Kimchi A. DAP kinase regulates JNK signaling by binding and activating protein kinase D under oxidative stress. *Cell Death Differ* 14: 1908–1915, 2007.
86. Emoto Y, Manome Y, Meinhardt G, Kisaki H, Kharbanda S, Robertson M, Ghayur T, Wong WW, Kamen R, Weichselbaum R, *et al.* Proteolytic activation of protein kinase Cδ by an ICE-like protease in apoptotic cells. *EMBO J* 14: 6148–6156, 1995.
87. Endo K, Oki E, Biedermann V, Kojima H, Yoshida K, Johannes FJ, Kufe D, and Datta R. Proteolytic cleavage and activation of protein kinase C [micro] by caspase-3 in the apoptotic response of cells to 1-β-D-arabinofuranosylcytosine and other genotoxic agents. *J Biol Chem* 275: 18476–18481, 2000.
88. Engel FB, Schebesta M, Duong MT, Lu G, Ren S, Madwed JB, Jiang H, Wang Y, and Keating MT. p38 MAP kinase inhibition enables proliferation of adult mammalian cardiomyocytes. *Genes Dev* 19: 1175–1187, 2005.
89. Eswaran J, Soundararajan M, and Knapp S. Targeting group II PAKs in cancer and metastasis. *Cancer Metastasis Rev* 28: 209–217, 2009.
90. Faris M, Latinis KM, Kempiak SJ, Koretzky GA, and Nel A. Stress-induced Fas ligand expression in T cells is mediated through a MEK kinase 1-regulated response element in the Fas ligand promoter. *Mol Cell Biol* 18: 5414–5424, 1998.
91. Feng S, Yang Y, Mei Y, Ma L, Zhu DE, Hoti N, Castanares M, and Wu M. Cleavage of RIP3 inactivates its caspase-independent apoptosis pathway by removal of kinase domain. *Cell Signal* 19: 2056–2067, 2007.
92. Fielitz J, Kim MS, Shelton JM, Qi X, Hill JA, Richardson JA, Bassel-Duby R, and Olson EN. Requirement of protein kinase D1 for pathological cardiac remodeling. *Proc Natl Acad Sci USA* 105: 3059–3063, 2008.
93. Frost JA, Swantek JL, Stippec S, Yin MJ, Gaynor R, and Cobb MH. Stimulation of NFκB activity by multiple signaling pathways requires PAK1. *J Biol Chem* 275: 19693–19699, 2000.
94. Fuchs SY, Adler V, Buschmann T, Yin Z, Wu X, Jones SN, and Ronai Z. JNK targets p53 ubiquitination and degradation in nonstressed cells. *Genes Dev* 12: 2658–2663, 1998.
95. Fujii K, Goldman EH, Park HR, Zhang L, Chen J, and Fu H. Negative control of apoptosis signal-regulating kinase 1 through phosphorylation of Ser-1034. *Oncogene* 23: 5099–5104, 2004.
96. Gallo KA and Johnson GL. Mixed-lineage kinase control of JNK and p38 MAPK pathways. *Nat Rev Mol Cell Biol* 3: 663–672, 2002.
97. Galmiche A and Fueller J. RAF kinases and mitochondria. *Biochim Biophys Acta* 1773: 1256–1262, 2007.
98. Ganiatsas S, Kwee L, Fujiwara Y, Perkins A, Ikeda T, Labow MA, and Zon LI. SEK1 deficiency reveals mitogen-activated protein kinase cascade crossregulation and leads

to abnormal hepatogenesis. *Proc Natl Acad Sci USA* 95: 6881–6886, 1998.
99. Ghayur T, Hugunin M, Talanian RV, Ratnofsky S, Quinlan C, Emoto Y, Pandey P, Datta R, Huang Y, Kharbanda S, Allen H, Kamen R, Wong W, and Kufe D. Proteolytic activation of protein kinase Cδ by an ICE/CED 3-like protease induces characteristics of apoptosis. *J Exp Med* 184: 2399–2404, 1996.
100. Gnesutta N and Minden A. Death receptor-induced activation of initiator caspase 8 is antagonized by serine/threonine kinase PAK4. *Mol Cell Biol* 23: 7838–7848, 2003.
101. Gnesutta N, Qu J, and Minden A. The serine/threonine kinase PAK4 prevents caspase activation and protects cells from apoptosis. *J Biol Chem* 276: 14414–14419, 2001.
102. Goldman EH, Chen L, and Fu H. Activation of apoptosis signal-regulating kinase 1 by reactive oxygen species through dephosphorylation at serine 967 and 14–3-3 dissociation. *J Biol Chem* 279: 10442–10449, 2004.
103. Golstein P and Kroemer G. Cell death by necrosis: towards a molecular definition. *Trends Biochem Sci* 32: 37–43, 2007.
104. Gong JG, Costanzo A, Yang HQ, Melino G, Kaelin WG, Jr., Levrero M, and Wang JY. The tyrosine kinase c-Abl regulates p73 in apoptotic response to cisplatin-induced DNA damage. *Nature* 399: 806–809, 1999.
105. Gorringe KL, Boussioutas A, and Bowtell DD. Novel regions of chromosomal amplification at 6p21, 5p13, and 12q14 in gastric cancer identified by array comparative genomic hybridization. *Genes Chromosomes Cancer* 42: 247–259, 2005.
106. Gozuacik D, Bialik S, Raveh T, Mitou G, Shohat G, Sabanay H, Mizushima N, Yoshimori T, and Kimchi A. DAP-kinase is a mediator of endoplasmic reticulum stress-induced caspase activation and autophagic cell death. *Cell Death Differ* 15: 1875–1886, 2008.
107. Graves JD, Draves KE, Gotoh Y, Krebs EG, and Clark EA. Both phosphorylation and caspase-mediated cleavage contribute to regulation of the Ste20-like protein kinase Mst1 during CD95/Fas-induced apoptosis. *J Biol Chem* 276: 14909–14915, 2001.
108. Graves JD, Gotoh Y, Draves KE, Ambrose D, Han DK, Wright M, Chernoff J, Clark EA, and Krebs EG. Caspase-mediated activation and induction of apoptosis by the mammalian Ste20-like kinase Mst1. *EMBO J* 17: 2224–2234, 1998.
109. Graves PR, Winkfield KM, and Haystead TA. Regulation of zipper-interacting protein kinase activity *in vitro* and *in vivo* by multisite phosphorylation. *J Biol Chem* 280: 9363–9374, 2005.
110. Gresko E, Roscic A, Ritterhoff S, Vichalkovski A, del Sal G, and Schmitz ML. Autoregulatory control of the p53 response by caspase-mediated processing of HIPK2. *EMBO J* 25: 1883–1894, 2006.
111. Guo Z, Clydesdale G, Cheng J, Kim K, Gan L, McConkey DJ, Ullrich SE, Zhuang Y, and Su B. Disruption of MEKK2 in mice reveals an unexpected role for MEKK2 in modulating T-cell receptor signal transduction. *Mol Cell Biol* 22: 5761–5768, 2002.
112. Gururaj A, Barnes CJ, Vadlamudi RK, and Kumar R. Regulation of phosphoglucomutase 1 phosphorylation and activity by a signaling kinase. *Oncogene* 23: 8118–8127, 2004.
113. Halle M, Liu YC, Hardy S, Theberge JF, Blanchetot C, Bourdeau A, Meng TC, and Tremblay ML. Caspase-3 regulates catalytic activity and scaffolding functions of the protein tyrosine phosphatase PEST, a novel modulator of the apoptotic response. *Mol Cell Biol* 27: 1172–1190, 2007.
114. Han J, Lee JD, Bibbs L, and Ulevitch RJ. A MAP kinase targeted by endotoxin and hyperosmolarity in mammalian cells. *Science* 265: 808–811, 1994.
115. Han J and Sun P. The pathways to tumor suppression via route p38. *Trends Biochem Sci* 32: 364–371, 2007.
116. Hanada M, Feng J, and Hemmings BA. Structure, regulation and function of PKB/AKT—a major therapeutic target. *Biochim Biophys Acta* 1697: 3–16, 2004.
117. Hantschel O, Nagar B, Guettler S, Kretzschmar J, Dorey K, Kuriyan J, and Superti-Furga G. A myristoyl/phosphotyrosine switch regulates c-Abl. *Cell* 112: 845–857, 2003.
118. Hao W, Takano T, Guillemette J, Papillon J, Ren G, and Cybulsky AV. Induction of apoptosis by the Ste20-like kinase SLK, a germinal center kinase that activates apoptosis signal-regulating kinase and p38. *J Biol Chem* 281: 3075–3084, 2006.
119. Harding HP, Zhang Y, Zeng H, Novoa I, Lu PD, Calfon M, Sadri N, Yun C, Popko B, Paules R, Stojdl DF, Bell JC, Hettmann T, Leiden JM, and Ron D. An integrated stress response regulates amino acid metabolism and resistance to oxidative stress. *Mol Cell* 11: 619–633, 2003.
120. Harrison B, Kraus M, Burch L, Stevens C, Craig A, Gordon-Weeks P, and Hupp TR. DAPK-1 binding to a linear peptide motif in MAP1B stimulates autophagy and membrane blebbing. *J Biol Chem* 283: 9999–10014, 2008.
121. Harvey K and Tapon N. The Salvador-Warts-Hippo pathway—an emerging tumour-suppressor network. *Nat Rev Cancer* 7: 182–191, 2007.
122. Hasegawa M, Fujimoto Y, Lucas PC, Nakano H, Fukase K, Nunez G, and Inohara N. A critical role of RICK/RIP2 polyubiquitination in Nod-induced NF-κB activation. *EMBO J* 27: 373–383, 2008.
123. Hasselblatt P, Gresh L, Kudo H, Guinea-Viniegra J, and Wagner EF. The role of the transcription factor AP-1 in colitis-associated and β-catenin-dependent intestinal tumorigenesis in mice. *Oncogene* 27: 6102–6109, 2008.
124. Hatai T, Matsuzawa A, Inoshita S, Mochida Y, Kuroda T, Sakamaki K, Kuida K, Yonehara S, Ichijo H, and Takeda K. Execution of apoptosis signal-regulating kinase 1 (ASK1)-induced apoptosis by the mitochondria-dependent caspase activation. *J Biol Chem* 275: 26576–26581, 2000.
125. He S, Wang L, Miao L, Wang T, Du F, Zhao L, and Wang X. Receptor interacting protein kinase-3 determines cellular necrotic response to TNF-α. *Cell* 137: 1100–1111, 2009.
126. Hebert SS, Daviau A, Grondin G, Latreille M, Aubin RA, and Blouin R. The mixed lineage kinase DLK is oligomerized by tissue transglutaminase during apoptosis. *J Biol Chem* 275: 32482–32490, 2000.
127. Hess DT, Matsumoto A, Kim SO, Marshall HE, and Stamler JS. Protein S-nitrosylation: purview and parameters. *Nat Rev Mol Cell Biol* 6: 150–166, 2005.
128. Hibi M, Lin A, Smeal T, Minden A, and Karin M. Identification of an oncoprotein- and UV-responsive protein kinase that binds and potentiates the c-Jun activation domain. *Genes Dev* 7: 2135–2148, 1993.
129. Hirai S, Cui de F, Miyata T, Ogawa M, Kiyonari H, Suda Y, Aizawa S, Banba Y, and Ohno S. The c-Jun N-terminal kinase activator dual leucine zipper kinase regulates axon growth and neuronal migration in the developing cerebral cortex. *J Neurosci* 26: 11992–12002, 2006.
130. Hirosumi J, Tuncman G, Chang L, Gorgun CZ, Uysal KT, Maeda K, Karin M, and Hotamisligil GS. A central role for

JNK in obesity and insulin resistance. *Nature* 420: 333–336, 2002.
131. Hitomi J, Christofferson DE, Ng A, Yao J, Degterev A, Xavier RJ, and Yuan J. Identification of a molecular signaling network that regulates a cellular necrotic cell death pathway. *Cell* 135: 1311–1323, 2008.
132. Hofmann TG, Moller A, Sirma H, Zentgraf H, Taya Y, Droge W, Will H, and Schmitz ML. Regulation of p53 activity by its interaction with homeodomain-interacting protein kinase-2. *Nat Cell Biol* 4: 1–10, 2002.
133. Hofmann TG, Stollberg N, Schmitz ML, and Will H. HIPK2 regulates transforming growth factor-β-induced c-Jun NH(2)-terminal kinase activation and apoptosis in human hepatoma cells. *Cancer Res* 63: 8271–8277, 2003.
134. Holland PM, Suzanne M, Campbell JS, Noselli S, and Cooper JA. MKK7 is a stress-activated mitogen-activated protein kinase kinase functionally related to hemipterous. *J Biol Chem* 272: 24994–24998, 1997.
135. Homma K, Katagiri K, Nishitoh H, and Ichijo H. Targeting ASK1 in ER stress-related neurodegenerative diseases. *Expert Opin Ther Targets* 13: 653–664, 2009.
136. Hu MC, Qiu WR, Wang X, Meyer CF, and Tan TH. Human HPK1, a novel human hematopoietic progenitor kinase that activates the JNK/SAPK kinase cascade. *Genes Dev* 10: 2251–2264, 1996.
137. Hu Y, Liu Z, Yang SJ, and Ye K. Acinus-provoked protein kinase Cδ isoform activation is essential for apoptotic chromatin condensation. *Cell Death Differ* 14: 2035–2046, 2007.
138. Huang CY, Wu YM, Hsu CY, Lee WS, Lai MD, Lu TJ, Huang CL, Leu TH, Shih HM, Fang HI, Robinson DR, Kung HJ, and Yuan CJ. Caspase activation of mammalian sterile 20-like kinase 3 (Mst3). Nuclear translocation and induction of apoptosis. *J Biol Chem* 277: 34367–34374, 2002.
139. Huang H and Tindall DJ. Dynamic FoxO transcription factors. *J Cell Sci* 120: 2479–2487, 2007.
140. Huang J, Wu S, Barrera J, Matthews K, and Pan D. The Hippo signaling pathway coordinately regulates cell proliferation and apoptosis by inactivating Yorkie, the Drosophila Homolog of YAP. *Cell* 122: 421–434, 2005.
141. Huang S, Jiang Y, Li Z, Nishida E, Mathias P, Lin S, Ulevitch RJ, Nemerow GR, and Han J. Apoptosis signaling pathway in T cells is composed of ICE/Ced-3 family proteases and MAP kinase kinase 6b. *Immunity* 6: 739–749, 1997.
142. Hui L, Bakiri L, Mairhorfer A, Schweifer N, Haslinger C, Kenner L, Komnenovic V, Scheuch H, Beug H, and Wagner EF. p38α suppresses normal and cancer cell proliferation by antagonizing the JNK-c-Jun pathway. *Nat Genet* 39: 741–749, 2007.
143. Hui L, Bakiri L, Stepniak E, and Wagner EF. p38α: a suppressor of cell proliferation and tumorigenesis. *Cell Cycle* 6: 2429–2433, 2007.
144. Hui L, Zatloukal K, Scheuch H, Stepniak E, and Wagner EF. Proliferation of human HCC cells and chemically induced mouse liver cancers requires JNK1-dependent p21 downregulation. *J Clin Invest* 118: 3943–3953, 2008.
145. Humphries MJ, Limesand KH, Schneider JC, Nakayama KI, Anderson SM, and Reyland ME. Suppression of apoptosis in the protein kinase Cδ null mouse *in vivo*. *J Biol Chem* 281: 9728–9737, 2006.
146. Hurd C, Waldron RT, and Rozengurt E. Protein kinase D complexes with C-Jun N-terminal kinase via activation loop phosphorylation and phosphorylates the C-Jun N-terminus. *Oncogene* 21: 2154–2160, 2002.
147. Hutchison M, Berman KS, and Cobb MH. Isolation of TAO1, a protein kinase that activates MEKs in stress-activated protein kinase cascades. *J Biol Chem* 273: 28625–28632, 1998.
148. Ichijo H, Nishida E, Irie K, ten Dijke P, Saitoh M, Moriguchi T, Takagi M, Matsumoto K, Miyazono K, and Gotoh Y. Induction of apoptosis by ASK1, a mammalian MAPKKK that activates SAPK/JNK and p38 signaling pathways. *Science* 275: 90–94, 1997.
149. Inbal B, Bialik S, Sabanay I, Shani G, and Kimchi A. DAP kinase and DRP-1 mediate membrane blebbing and the formation of autophagic vesicles during programmed cell death. *J Cell Biol* 157: 455–468, 2002.
150. Inbal B, Shani G, Cohen O, Kissil JL, and Kimchi A. Death-associated protein kinase-related protein 1, a novel serine/threonine kinase involved in apoptosis. *Mol Cell Biol* 20: 1044–1054, 2000.
151. Inoshita S, Takeda K, Hatai T, Terada Y, Sano M, Hata J, Umezawa A, and Ichijo H. Phosphorylation and inactivation of myeloid cell leukemia 1 by JNK in response to oxidative stress. *J Biol Chem* 277: 43730–43734, 2002.
152. Inoue T, Boyle DL, Corr M, Hammaker D, Davis RJ, Flavell RA, and Firestein GS. Mitogen-activated protein kinase kinase 3 is a pivotal pathway regulating p38 activation in inflammatory arthritis. *Proc Natl Acad Sci USA* 103: 5484–5489, 2006.
153. Iriyama T, Takeda K, Nakamura H, Morimoto Y, Kuroiwa T, Mizukami J, Umeda T, Noguchi T, Naguro I, Nishitoh H, Saegusa K, Tobiume K, Homma T, Shimada Y, Tsuda H, Aiko S, Imoto I, Inazawa J, Chida K, Kamei Y, Kozuma S, Taketani Y, Matsuzawa A, and Ichijo H. ASK1 and ASK2 differentially regulate the counteracting roles of apoptosis and inflammation in tumorigenesis. *EMBO J* 28: 843–853, 2009.
154. Izumiya Y, Kim S, Izumi Y, Yoshida K, Yoshiyama M, Matsuzawa A, Ichijo H, and Iwao H. Apoptosis signal-regulating kinase 1 plays a pivotal role in angiotensin II-induced cardiac hypertrophy and remodeling. *Circ Res* 93: 874–883, 2003.
155. Jaeschke A and Davis RJ. Metabolic stress signaling mediated by mixed-lineage kinases. *Mol Cell* 27: 498–508, 2007.
156. Jaeschke A, Karasarides M, Ventura JJ, Ehrhardt A, Zhang C, Flavell RA, Shokat KM, and Davis RJ. JNK2 is a positive regulator of the cJun transcription factor. *Mol Cell* 23: 899–911, 2006.
157. Jakobi R, McCarthy CC, Koeppel MA, and Stringer DK. Caspase-activated PAK-2 is regulated by subcellular targeting and proteasomal degradation. *J Biol Chem* 278: 38675–38685, 2003.
158. Jakobi R, Moertl E, and Koeppel MA. p21-activated protein kinase γ-PAK suppresses programmed cell death of BALB3T3 fibroblasts. *J Biol Chem* 276: 16624–16634, 2001.
159. Jan Y, Matter M, Pai JT, Chen YL, Pilch J, Komatsu M, Ong E, Fukuda M, and Ruoslahti E. A mitochondrial protein, Bit1, mediates apoptosis regulated by integrins and Groucho/TLE corepressors. *Cell* 116: 751–762, 2004.
160. Jang CW, Chen CH, Chen CC, Chen JY, Su YH, and Chen RH. TGF-β induces apoptosis through Smad-mediated expression of DAP-kinase. *Nat Cell Biol* 4: 51–58, 2002.
161. Jang SW, Yang SJ, Srinivasan S, and Ye K. Akt phosphorylates MstI and prevents its proteolytic activation, blocking

FOXO3 phosphorylation and nuclear translocation. *J Biol Chem* 282: 30836–30844, 2007.
162. Johnson GL and Nakamura K. The c-jun kinase/stress-activated pathway: regulation, function and role in human disease. *Biochim Biophys Acta* 1773: 1341–1348, 2007.
163. Jones EV, Dickman MJ, and Whitmarsh AJ. Regulation of p73-mediated apoptosis by c-Jun N-terminal kinase. *Biochem J* 405: 617–623, 2007.
164. Juo P, Kuo CJ, Reynolds SE, Konz RF, Raingeaud J, Davis RJ, Biemann HP, and Blenis J. Fas activation of the p38 mitogen-activated protein kinase signalling pathway requires ICE/CED-3 family proteases. *Mol Cell Biol* 17: 24–35, 1997.
165. Kadowaki H, Nishitoh H, Urano F, Sadamitsu C, Matsuzawa A, Takeda K, Masutani H, Yodoi J, Urano Y, Nagano T, and Ichijo H. Amyloid β induces neuronal cell death through ROS-mediated ASK1 activation. *Cell Death Differ* 12: 19–24, 2005.
166. Kaiser RA, Bueno OF, Lips DJ, Doevendans PA, Jones F, Kimball TF, and Molkentin JD. Targeted inhibition of p38 mitogen-activated protein kinase antagonizes cardiac injury and cell death following ischemia-reperfusion *in vivo*. *J Biol Chem* 279: 15524–15530, 2004.
167. Kaiser RA, Liang Q, Bueno O, Huang Y, Lackey T, Klevitsky R, Hewett TE, and Molkentin JD. Genetic inhibition or activation of JNK1/2 protects the myocardium from ischemia-reperfusion-induced cell death *in vivo*. *J Biol Chem* 280: 32602–32608, 2005.
168. Kakeya H, Onose R, and Osada H. Caspase-mediated activation of a 36-kDa myelin basic protein kinase during anticancer drug-induced apoptosis. *Cancer Res* 58: 4888–4894, 1998.
169. Kamata H, Honda S, Maeda S, Chang L, Hirata H, and Karin M. Reactive oxygen species promote TNFα-induced death and sustained JNK activation by inhibiting MAP kinase phosphatases. *Cell* 120: 649–661, 2005.
170. Kang C, You YJ, and Avery L. Dual roles of autophagy in the survival of *Caenorhabditis elegans* during starvation. *Genes Dev* 21: 2161–2171, 2007.
171. Kang YJ, Chen J, Otsuka M, Mols J, Ren S, Wang Y, and Han J. Macrophage deletion of p38α partially impairs lipopolysaccharide-induced cellular activation. *J Immunol* 180: 5075–5082, 2008.
172. Katagiri K, Katakai T, Ebisuno Y, Ueda Y, Okada T, and Kinashi T. Mst1 controls lymphocyte trafficking and interstitial motility within lymph nodes. *EMBO J* 28: 1319–1331, 2009.
173. Kauffmann-Zeh A, Rodriguez-Viciana P, Ulrich E, Gilbert C, Coffer P, Downward J, and Evan G. Suppression of c-Myc-induced apoptosis by Ras signalling through PI(3)K and PKB. *Nature* 385: 544–548, 1997.
174. Kawai T, Matsumoto M, Takeda K, Sanjo H, and Akira S. ZIP kinase, a novel serine/threonine kinase which mediates apoptosis. *Mol Cell Biol* 18: 1642–1651, 1998.
175. Kelliher MA, Grimm S, Ishida Y, Kuo F, Stanger BZ, and Leder P. The death domain kinase RIP mediates the TNF-induced NF-κB signal. *Immunity* 8: 297–303, 1998.
176. Kepp O, Gottschalk K, Churin Y, Rajalingam K, Brinkmann V, Machuy N, Kroemer G, and Rudel T. Bim and Bmf synergize to induce apoptosis in Neisseria gonorrhoeae infection. *PLoS Pathog* 5: e1000348, 2009.
177. Kim AH, Khursigara G, Sun X, Franke TF, and Chao MV. Akt phosphorylates and negatively regulates apoptosis signal-regulating kinase 1. *Mol Cell Biol* 21: 893–901, 2001.
178. Kim C, Sano Y, Todorova K, Carlson BA, Arpa L, Celada A, Lawrence T, Otsu K, Brissette JL, Arthur JS, and Park JM. The kinase p38α serves cell type-specific inflammatory functions in skin injury and coordinates pro- and anti-inflammatory gene expression. *Nat Immunol* 9: 1019–1027, 2008.
179. Kim DH, Feinbaum R, Alloing G, Emerson FE, Garsin DA, Inoue H, Tanaka-Hino M, Hisamoto N, Matsumoto K, Tan MW, and Ausubel FM. A conserved p38 MAP kinase pathway in *Caenorhabditis elegans* innate immunity. *Science* 297: 623–626, 2002.
180. Kim KY, Kim BC, Xu Z, and Kim SJ. Mixed lineage kinase 3 (MLK3)-activated p38 MAP kinase mediates transforming growth factor-β-induced apoptosis in hepatoma cells. *J Biol Chem* 279: 29478–29484, 2004.
181. Kim SJ, Ju JW, Oh CD, Yoon YM, Song WK, Kim JH, Yoo YJ, Bang OS, Kang SS, and Chun JS. ERK-1/2 and p38 kinase oppositely regulate nitric oxide-induced apoptosis of chondrocytes in association with p53, caspase-3, and differentiation status. *J Biol Chem* 277: 1332–1339, 2002.
182. Kimmelman AC, Hezel AF, Aguirre AJ, Zheng H, Paik JH, Ying H, Chu GC, Zhang JX, Sahin E, Yeo G, Ponugoti A, Nabioullin R, Deroo S, Yang S, Wang X, McGrath JP, Protopopova M, Ivanova E, Zhang J, Feng B, Tsao MS, Redston M, Protopopov A, Xiao Y, Futreal PA, Hahn WC, Klimstra DS, Chin L, and DePinho RA. Genomic alterations link Rho family of GTPases to the highly invasive phenotype of pancreas cancer. *Proc Natl Acad Sci USA* 105: 19372–19377, 2008.
183. Kimura N, Matsuo R, Shibuya H, Nakashima K, and Taga T. BMP2-induced apoptosis is mediated by activation of the TAK1-p38 kinase pathway that is negatively regulated by Smad6. *J Biol Chem* 275: 17647–17652, 2000.
184. Kishimoto H, Nakagawa K, Watanabe T, Kitagawa D, Momose H, Seo J, Nishitai G, Shimizu N, Ohata S, Tanemura S, Asaka S, Goto T, Fukushi H, Yoshida H, Suzuki A, Sasaki T, Wada T, Penninger JM, Nishina H, and Katada T. Different properties of SEK1 and MKK7 in dual phosphorylation of stress-induced activated protein kinase SAPK/JNK in embryonic stem cells. *J Biol Chem* 278: 16595–16601, 2003.
185. Knaus UG, Morris S, Dong HJ, Chernoff J, and Bokoch GM. Regulation of human leukocyte p21-activated kinases through G protein—coupled receptors. *Science* 269: 221–223, 1995.
186. Kodama Y, Taura K, Miura K, Schnabl B, Osawa Y, and Brenner DA. Antiapoptotic effect of c-Jun N-terminal Kinase-1 through Mcl-1 stabilization in TNF-induced hepatocyte apoptosis. *Gastroenterology* 136: 1423–1434, 2009.
187. Kogel D, Bierbaum H, Preuss U, and Scheidtmann KH. C-terminal truncation of Dlk/ZIP kinase leads to abrogation of nuclear transport and high apoptotic activity. *Oncogene* 18: 7212–7218, 1999.
188. Konishi H, Yamauchi E, Taniguchi H, Yamamoto T, Matsuzaki H, Takemura Y, Ohmae K, Kikkawa U, and Nishizuka Y. Phosphorylation sites of protein kinase Cδ in H2O2-treated cells and its activation by tyrosine kinase *in vitro*. *Proc Natl Acad Sci USA* 98: 6587–6592, 2001.
189. Kuan CY, Yang DD, Samanta Roy DR, Davis RJ, Rakic P, and Flavell RA. The Jnk1 and Jnk2 protein kinases are required for regional specific apoptosis during early brain development. *Neuron* 22: 667–676, 1999.

190. Kumar R, Gururaj AE, and Barnes CJ. p21-activated kinases in cancer. *Nat Rev Cancer* 6: 459–471, 2006.
191. Kuo JC, Wang WJ, Yao CC, Wu PR, and Chen RH. The tumor suppressor DAPK inhibits cell motility by blocking the integrin-mediated polarity pathway. *J Cell Biol* 172: 619–631, 2006.
192. Kuranaga E, Kanuka H, Igaki T, Sawamoto K, Ichijo H, Okano H, and Miura M. Reaper-mediated inhibition of DIAP1-induced DTRAF1 degradation results in activation of JNK in Drosophila. *Nat Cell Biol* 4: 705–710, 2002.
193. Kurokawa M and Kornbluth S. Caspases and kinases in a death grip. *Cell* 138: 838–854, 2009.
194. Kyriakis JM and Avruch J. Mammalian mitogen-activated protein kinase signal transduction pathways activated by stress and inflammation. *Physiol Rev* 81: 807–869, 2001.
195. Lamb JA, Ventura JJ, Hess P, Flavell RA, and Davis RJ. JunD mediates survival signaling by the JNK signal transduction pathway. *Mol Cell* 11: 1479–1489, 2003.
196. Landstrom M. The TAK1-TRAF6 signalling pathway. *Int J Biochem Cell Biol* 42: 585–589, 2010.
197. LaValle CR, George KM, Sharlow ER, Lazo JS, Wipf P, and Wang QJ. Protein kinase D as a potential new target for cancer therapy. *Biochim Biophys Acta* 1806: 183–192, 2010.
198. Lawler S, Fleming Y, Goedert M, and Cohen P. Synergistic activation of SAPK1/JNK1 by two MAP kinase kinases *in vitro*. *Curr Biol* 8: 1387–1390, 1998.
199. Leberer E, Dignard D, Harcus D, Thomas DY, and Whiteway M. The protein kinase homologue Ste20p is required to link the yeast pheromone response G-protein $\beta\gamma$ subunits to downstream signalling components. *EMBO J* 11: 4815–4824, 1992.
200. Lee KK, Murakawa M, Nishida E, Tsubuki S, Kawashima S, Sakamaki K, and Yonehara S. Proteolytic activation of MST/Krs, STE20-related protein kinase, by caspase during apoptosis. *Oncogene* 16: 3029–3037, 1998.
201. Lee KK, Ohyama T, Yajima N, Tsubuki S, and Yonehara S. MST, a physiological caspase substrate, highly sensitizes apoptosis both upstream and downstream of caspase activation. *J Biol Chem* 276: 19276–19285, 2001.
202. Lee N, MacDonald H, Reinhard C, Halenbeck R, Roulston A, Shi T, and Williams LT. Activation of hPAK65 by caspase cleavage induces some of the morphological and biochemical changes of apoptosis. *Proc Natl Acad Sci USA* 94: 13642–13647, 1997.
203. Lee WS, Hsu CY, Wang PL, Huang CY, Chang CH, and Yuan CJ. Identification and characterization of the nuclear import and export signals of the mammalian Ste20-like protein kinase 3. *FEBS Lett* 572: 41–45, 2004.
204. Lee YH, Giraud J, Davis RJ, and White MF. c-Jun N-terminal kinase (JNK) mediates feedback inhibition of the insulin signaling cascade. *J Biol Chem* 278: 2896–2902, 2003.
205. Lehtinen MK, Yuan Z, Boag PR, Yang Y, Villen J, Becker EB, DiBacco S, de la Iglesia N, Gygi S, Blackwell TK, and Bonni A. A conserved MST-FOXO signaling pathway mediates oxidative-stress responses and extends life span. *Cell* 125: 987–1001, 2006.
206. Lei K and Davis RJ. JNK phosphorylation of Bim-related members of the Bcl2 family induces Bax-dependent apoptosis. *Proc Natl Acad Sci USA* 100: 2432–2437, 2003.
207. Leitges M, Mayr M, Braun U, Mayr U, Li C, Pfister G, Ghaffari-Tabrizi N, Baier G, Hu Y, and Xu Q. Exacerbated vein graft arteriosclerosis in protein kinase Cδ-null mice. *J Clin Invest* 108: 1505–1512, 2001.
208. Li J and Yuan J. Caspases in apoptosis and beyond. *Oncogene* 27: 6194–6206, 2008.
209. Li X and Minden A. PAK4 functions in tumor necrosis factor (TNF) α-induced survival pathways by facilitating TRADD binding to the TNF receptor. *J Biol Chem* 280: 41192–41200, 2005.
210. Lin Y, Devin A, Rodriguez Y, and Liu ZG. Cleavage of the death domain kinase RIP by caspase-8 prompts TNF-induced apoptosis. *Genes Dev* 13: 2514–2526, 1999.
211. Ling P, Lu TJ, Yuan CJ, and Lai MD. Biosignaling of mammalian Ste20-related kinases. *Cell Signal* 20: 1237–1247, 2008.
212. Liu H, Lu ZG, Miki Y, and Yoshida K. Protein kinase Cδ induces transcription of the TP53 tumor suppressor gene by controlling death-promoting factor Btf in the apoptotic response to DNA damage. *Mol Cell Biol* 27: 8480–8491, 2007.
213. Liu Q, Wilkins BJ, Lee YJ, Ichijo H, and Molkentin JD. Direct interaction and reciprocal regulation between ASK1 and calcineurin-NFAT control cardiomyocyte death and growth. *Mol Cell Biol* 26: 3785–3797, 2006.
214. Liu YF, Dorow D, and Marshall J. Activation of MLK2-mediated signaling cascades by polyglutamine-expanded huntingtin. *J Biol Chem* 275: 19035–19040, 2000.
215. Llambi F, Lourenco FC, Gozuacik D, Guix C, Pays L, Del Rio G, Kimchi A, and Mehlen P. The dependence receptor UNC5H2 mediates apoptosis through DAP-kinase. *EMBO J* 24: 1192–1201, 2005.
216. Lu C and Mains PE. The *C. elegans* anaphase promoting complex and MBK-2/DYRK kinase act redundantly with CUL-3/MEL-26 ubiquitin ligase to degrade MEI-1 microtubule-severing activity after meiosis. *Dev Biol* 302: 438–447, 2007.
217. Lu HT, Yang DD, Wysk M, Gatti E, Mellman I, Davis RJ, and Flavell RA. Defective IL-12 production in mitogen-activated protein (MAP) kinase kinase 3 (Mkk3)-deficient mice. *EMBO J* 18: 1845–1857, 1999.
218. Lu TJ, Lai WY, Huang CY, Hsieh WJ, Yu JS, Hsieh YJ, Chang WT, Leu TH, Chang WC, Chuang WJ, Tang MJ, Chen TY, Lu TL, and Lai MD. Inhibition of cell migration by autophosphorylated mammalian sterile 20-like kinase 3 (MST3) involves paxillin and protein-tyrosine phosphatase-PEST. *J Biol Chem* 281: 38405–38417, 2006.
219. Lu X, Nemoto S, and Lin A. Identification of c-Jun NH2-terminal protein kinase (JNK)-activating kinase 2 as an activator of JNK but not p38. *J Biol Chem* 272: 24751–24754, 1997.
220. Lu Z, Xu S, Joazeiro C, Cobb MH, and Hunter T. The PHD domain of MEKK1 acts as an E3 ubiquitin ligase and mediates ubiquitination and degradation of ERK1/2. *Mol Cell* 9: 945–956, 2002.
221. Ma FY, Tesch GH, Flavell RA, Davis RJ, and Nikolic-Paterson DJ. MKK3-p38 signaling promotes apoptosis and the early inflammatory response in the obstructed mouse kidney. *Am J Physiol Renal Physiol* 293: F1556– F1563, 2007.
222. MacKeigan JP, Murphy LO, and Blenis J. Sensitized RNAi screen of human kinases and phosphatases identifies new regulators of apoptosis and chemoresistance. *Nat Cell Biol* 7: 591–600, 2005.
223. Maddika S and Chen J. Protein kinase DYRK2 is a scaffold that facilitates assembly of an E3 ligase. *Nat Cell Biol* 11: 409–419, 2009.
224. Mahoney DJ, Cheung HH, Mrad RL, Plenchette S, Simard C, Enwere E, Arora V, Mak TW, Lacasse EC, Waring J, and

Korneluk RG. Both cIAP1 and cIAP2 regulate TNFα-mediated NF-κB activation. *Proc Natl Acad Sci USA* 105: 11778–11783, 2008.
225. Manke IA, Nguyen A, Lim D, Stewart MQ, Elia AE, and Yaffe MB. MAPKAP kinase-2 is a cell cycle checkpoint kinase that regulates the G2/M transition and S phase progression in response to UV irradiation. *Mol Cell* 17: 37–48, 2005.
226. Maroney AC, Finn JP, Connors TJ, Durkin JT, Angeles T, Gessner G, Xu Z, Meyer SL, Savage MJ, Greene LA, Scott RW, and Vaught JL. Cep-1347 (KT7515), a semisynthetic inhibitor of the mixed lineage kinase family. *J Biol Chem* 276: 25302–25308, 2001.
227. Maroney AC, Glicksman MA, Basma AN, Walton KM, Knight E, Jr., Murphy CA, Bartlett BA, Finn JP, Angeles T, Matsuda Y, Neff NT, and Dionne CA. Motoneuron apoptosis is blocked by CEP-1347 (KT 7515), a novel inhibitor of the JNK signaling pathway. *J Neurosci* 18: 104–111, 1998.
228. Maruyama T, Kadowaki H, Okamoto N, Nagai A, Naguro I, Matsuzawa A, Shibuya H, Tanaka K, Murata S, Takeda K, Nishitoh H, and Ichijo H. CHIP-dependent termination of MEKK2 regulates temporal ERK activation required for proper hyperosmotic response. *EMBO J* 29: 2501–2514, 2010.
229. Matallanas D, Romano D, Yee K, Meissl K, Kucerova L, Piazzolla D, Baccarini M, Vass JK, Kolch W, and O'Neill E. RASSF1A elicits apoptosis through an MST2 pathway directing proapoptotic transcription by the p73 tumor suppressor protein. *Mol Cell* 27: 962–975, 2007.
230. Matsui Y, Nakano N, Shao D, Gao S, Luo W, Hong C, Zhai P, Holle E, Yu X, Yabuta N, Tao W, Wagner T, Nojima H, and Sadoshima J. Lats2 is a negative regulator of myocyte size in the heart. *Circ Res* 103: 1309–1318, 2008.
231. Matsumoto M, Minami M, Takeda K, Sakao Y, and Akira S. Ectopic expression of CHOP (GADD153) induces apoptosis in M1 myeloblastic leukemia cells. *FEBS Lett* 395: 143–147, 1996.
232. Matsuzawa A, Saegusa K, Noguchi T, Sadamitsu C, Nishitoh H, Nagai S, Koyasu S, Matsumoto K, Takeda K, and Ichijo H. ROS-dependent activation of the TRAF6-ASK1-p38 pathway is selectively required for TLR4-mediated innate immunity. *Nat Immunol* 6: 587–592, 2005.
233. McCullough KD, Martindale JL, Klotz LO, Aw TY, and Holbrook NJ. Gadd153 sensitizes cells to endoplasmic reticulum stress by down-regulating Bcl2 and perturbing the cellular redox state. *Mol Cell Biol* 21: 1249–1259, 2001.
234. Mecklenbrauker I, Kalled SL, Leitges M, Mackay F, and Tarakhovsky A. Regulation of B-cell survival by BAFF-dependent PKCδ-mediated nuclear signalling. *Nature* 431: 456–461, 2004.
235. Mecklenbrauker I, Saijo K, Zheng NY, Leitges M, and Tarakhovsky A. Protein kinase Cδ controls self-antigen-induced B-cell tolerance. *Nature* 416: 860–865, 2002.
236. Meylan E, Martinon F, Thome M, Gschwendt M, and Tschopp J. RIP4 (DIK/PKK), a novel member of the RIP kinase family, activates NF-κB and is processed during apoptosis. *EMBO Rep* 3: 1201–1208, 2002.
237. Michie AM, McCaig AM, Nakagawa R, and Vukovic M. Death-associated protein kinase (DAPK) and signal transduction: regulation in cancer. *FEBS J* 277: 74–80, 2010.
238. Mikula M, Schreiber M, Husak Z, Kucerova L, Ruth J, Wieser R, Zatloukal K, Beug H, Wagner EF, and Baccarini M. Embryonic lethality and fetal liver apoptosis in mice lacking the c-raf-1 gene. *EMBO J* 20: 1952–1962, 2001.
239. Miller BR, Press C, Daniels RW, Sasaki Y, Milbrandt J, and DiAntonio A. A dual leucine kinase-dependent axon self-destruction program promotes Wallerian degeneration. *Nat Neurosci* 12: 387–389, 2009.
240. Miller CT, Aggarwal S, Lin TK, Dagenais SL, Contreras JI, Orringer MB, Glover TW, Beer DG, and Lin L. Amplification and overexpression of the dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 2 (DYRK2) gene in esophageal and lung adenocarcinomas. *Cancer Res* 63: 4136–4143, 2003.
241. Miyamoto A, Nakayama K, Imaki H, Hirose S, Jiang Y, Abe M, Tsukiyama T, Nagahama H, Ohno S, Hatakeyama S, and Nakayama KI. Increased proliferation of B cells and auto-immunity in mice lacking protein kinase Cδ. *Nature* 416: 865–869, 2002.
242. Mizushima N, Levine B, Cuervo AM, and Klionsky DJ. Autophagy fights disease through cellular self-digestion. *Nature* 451: 1069–1075, 2008.
243. Molli PR, Li DQ, Murray BW, Rayala SK, and Kumar R. PAK signaling in oncogenesis. *Oncogene* 28: 2545–2555, 2009.
244. Morita K, Saitoh M, Tobiume K, Matsuura H, Enomoto S, Nishitoh H, and Ichijo H. Negative feedback regulation of ASK1 by protein phosphatase 5 (PP5) in response to oxidative stress. *EMBO J* 20: 6028–6036, 2001.
245. Mota M, Reeder M, Chernoff J, and Bazenet CE. Evidence for a role of mixed lineage kinases in neuronal apoptosis. *J Neurosci* 21: 4949–4957, 2001.
246. Mudgett JS, Ding J, Guh-Siesel L, Chartrain NA, Yang L, Gopal S, and Shen MM. Essential role for p38α mitogen-activated protein kinase in placental angiogenesis. *Proc Natl Acad Sci USA* 97: 10454–10459, 2000.
247. Murriel CL, Churchill E, Inagaki K, Szweda LI, and Mochly-Rosen D. Protein kinase Cδ activation induces apoptosis in response to cardiac ischemia and reperfusion damage: a mechanism involving BAD and the mitochondria. *J Biol Chem* 279: 47985–47991, 2004.
248. Nagai H, Noguchi T, Homma K, Katagiri K, Takeda K, Matsuzawa A, and Ichijo H. Ubiquitin-like sequence in ASK1 plays critical roles in the recognition and stabilization by USP9× and oxidative stress-induced cell death. *Mol Cell* 36: 805–818, 2009.
249. Nagar B, Hantschel O, Young MA, Scheffzek K, Veach D, Bornmann W, Clarkson B, Superti-Furga G, and Kuriyan J. Structural basis for the autoinhibition of c-Abl tyrosine kinase. *Cell* 112: 859–871, 2003.
250. Nateri AS, Spencer-Dene B, and Behrens A. Interaction of phosphorylated c-Jun with TCF4 regulates intestinal cancer development. *Nature* 437: 281–285, 2005.
251. Nishida K, Yamaguchi O, Hirotani S, Hikoso S, Higuchi Y, Watanabe T, Takeda T, Osuka S, Morita T, Kondoh G, Uno Y, Kashiwase K, Taniike M, Nakai A, Matsumura Y, Miyazaki J, Sudo T, Hongo K, Kusakari Y, Kurihara S, Chien KR, Takeda J, Hori M, and Otsu K. p38α mitogen-activated protein kinase plays a critical role in cardiomyocyte survival but not in cardiac hypertrophic growth in response to pressure overload. *Mol Cell Biol* 24: 10611–10620, 2004.
252. Nishina H, Fischer KD, Radvanyi L, Shahinian A, Hakem R, Rubie EA, Bernstein A, Mak TW, Woodgett JR, and Penninger JM. Stress-signalling kinase Sek1 protects thymocytes from apoptosis mediated by CD95 and CD3. *Nature* 385: 350–353, 1997.
253. Nishina H, Radvanyi L, Raju K, Sasaki T, Kozieradzki I, and Penninger JM. Impaired TCR-mediated apoptosis and

Bcl-XL expression in T cells lacking the stress kinase activator SEK1/MKK4. *J Immunol* 161: 3416–3420, 1998.
254. Nishina H, Vaz C, Billia P, Nghiem M, Sasaki T, De la Pompa JL, Furlonger K, Paige C, Hui C, Fischer KD, Kishimoto H, Iwatsubo T, Katada T, Woodgett JR, and Penninger JM. Defective liver formation and liver cell apoptosis in mice lacking the stress signaling kinase SEK1/MKK4. *Development* 126: 505–516, 1999.
255. Nishina H, Wada T, and Katada T. Physiological roles of SAPK/JNK signaling pathway. *J Biochem* 136: 123–126, 2004.
256. Nishitoh H, Kadowaki H, Nagai A, Maruyama T, Yokota T, Fukutomi H, Noguchi T, Matsuzawa A, Takeda K, and Ichijo H. ALS-linked mutant SOD1 induces ER stress- and ASK1-dependent motor neuron death by targeting Derlin-1. *Genes Dev* 22: 1451–1464, 2008.
257. Nishitoh H, Matsuzawa A, Tobiume K, Saegusa K, Takeda K, Inoue K, Hori S, Kakizuka A, and Ichijo H. ASK1 is essential for endoplasmic reticulum stress-induced neuronal cell death triggered by expanded polyglutamine repeats. *Genes Dev* 16: 1345–1355, 2002.
258. Nishitoh H, Saitoh M, Mochida Y, Takeda K, Nakano H, Rothe M, Miyazono K, and Ichijo H. ASK1 is essential for JNK/SAPK activation by TRAF2. *Mol Cell* 2: 389–395, 1998.
259. Nishizuka Y. Protein kinase C and lipid signaling for sustained cellular responses. *FASEB J* 9: 484–496, 1995.
260. Noguchi T, Ishii K, Fukutomi H, Naguro I, Matsuzawa A, Takeda K, and Ichijo H. Requirement of reactive oxygen species-dependent activation of ASK1-p38 MAPK pathway for extracellular ATP-induced apoptosis in macrophage. *J Biol Chem* 283: 7657–7665, 2008.
261. Noguchi T, Takeda K, Matsuzawa A, Saegusa K, Nakano H, Gohda J, Inoue J, and Ichijo H. Recruitment of tumor necrosis factor receptor-associated factor family proteins to apoptosis signal-regulating kinase 1 signalosome is essential for oxidative stress-induced cell death. *J Biol Chem* 280: 37033–37040, 2005.
262. Nogueira E, Fidalgo M, Molnar A, Kyriakis J, Force T, Zalvide J, and Pombo CM. SOK1 translocates from the Golgi to the nucleus upon chemical anoxia and induces apoptotic cell death. *J Biol Chem* 283: 16248–16258, 2008.
263. O'Neill E, Rushworth L, Baccarini M, and Kolch W. Role of the kinase MST2 in suppression of apoptosis by the proto-oncogene product Raf-1. *Science* 306: 2267–2270, 2004.
264. Odashima M, Usui S, Takagi H, Hong C, Liu J, Yokota M, and Sadoshima J. Inhibition of endogenous Mst1 prevents apoptosis and cardiac dysfunction without affecting cardiac hypertrophy after myocardial infarction. *Circ Res* 100: 1344–1352, 2007.
265. Oh S, Lee D, Kim T, Kim TS, Oh HJ, Hwang CY, Kong YY, Kwon KS, and Lim DS. Crucial role for Mst1 and Mst2 kinases in early embryonic development of the mouse. *Mol Cell Biol* 29: 6309–6320, 2009.
266. Omori E, Matsumoto K, Sanjo H, Sato S, Akira S, Smart RC, and Ninomiya-Tsuji J. TAK1 is a master regulator of epidermal homeostasis involving skin inflammation and apoptosis. *J Biol Chem* 281: 19610–19617, 2006.
267. Papa S, Bubici C, Zazzeroni F, Pham CG, Kuntzen C, Knabb JR, Dean K, and Franzoso G. The NF-κB-mediated control of the JNK cascade in the antagonism of programmed cell death in health and disease. *Cell Death Differ* 13: 712–729, 2006.
268. Park HS, Cho SG, Kim CK, Hwang HS, Noh KT, Kim MS, Huh SH, Kim MJ, Ryoo K, Kim EK, Kang WJ, Lee JS, Seo JS, Ko YG, Kim S, and Choi EJ. Heat shock protein hsp72 is a negative regulator of apoptosis signal-regulating kinase 1. *Mol Cell Biol* 22: 7721–7730, 2002.
269. Park HS, Huh SH, Kim MS, Lee SH, and Choi EJ. Nitric oxide negatively regulates c-Jun N-terminal kinase/stress-activated protein kinase by means of S-nitrosylation. *Proc Natl Acad Sci USA* 97: 14382–14387, 2000.
270. Park HS, Yu JW, Cho JH, Kim MS, Huh SH, Ryoo K, and Choi EJ. Inhibition of apoptosis signal-regulating kinase 1 by nitric oxide through a thiol redox mechanism. *J Biol Chem* 279: 7584–7590, 2004.
271. Parsons DW, Wang TL, Samuels Y, Bardelli A, Cummins JM, DeLong L, Silliman N, Ptak J, Szabo S, Willson JK, Markowitz S, Kinzler KW, Vogelstein B, Lengauer C, and Velculescu VE. Colorectal cancer: mutations in a signalling pathway. *Nature* 436: 792, 2005.
272. Pelled D, Raveh T, Riebeling C, Fridkin M, Berissi H, Futerman AH, and Kimchi A. Death-associated protein (DAP) kinase plays a central role in ceramide-induced apoptosis in cultured hippocampal neurons. *J Biol Chem* 277: 1957–1961, 2002.
273. Perdiguero E, Ruiz-Bonilla V, Gresh L, Hui L, Ballestar E, Sousa-Victor P, Baeza-Raja B, Jardi M, Bosch-Comas A, Esteller M, Caelles C, Serrano AL, Wagner EF, and Munoz-Canoves P. Genetic analysis of p38 MAP kinases in myogenesis: fundamental role of p38α in abrogating myoblast proliferation. *EMBO J* 26: 1245–1256, 2007.
274. Pierantoni GM, Rinaldo C, Mottolese M, Di Benedetto A, Esposito F, Soddu S, and Fusco A. High-mobility group A1 inhibits p53 by cytoplasmic relocalization of its proapoptotic activator HIPK2. *J Clin Invest* 117: 693–702, 2007.
275. Pirruccello M, Sondermann H, Pelton JG, Pellicena P, Hoelz A, Chernoff J, Wemmer DE, and Kuriyan J. A dimeric kinase assembly underlying autophosphorylation in the p21 activated kinases. *J Mol Biol* 361: 312–326, 2006.
276. Pombo CM, Bonventre JV, Molnar A, Kyriakis J, and Force T. Activation of a human Ste20-like kinase by oxidant stress defines a novel stress response pathway. *EMBO J* 15: 4537–4546, 1996.
277. Puthalakath H, O'Reilly LA, Gunn P, Lee L, Kelly PN, Huntington ND, Hughes PD, Michalak EM, McKimm-Breschkin J, Motoyama N, Gotoh T, Akira S, Bouillet P, and Strasser A. ER stress triggers apoptosis by activating BH3-only protein Bim. *Cell* 129: 1337–1349, 2007.
278. Qin L, Zeng H, and Zhao D. Requirement of protein kinase D tyrosine phosphorylation for VEGF-A165-induced angiogenesis through its interaction and regulation of phospholipase Cγ phosphorylation. *J Biol Chem* 281: 32550–32558, 2006.
279. Qiu Z, Cang Y, and Goff SP. c-Abl tyrosine kinase regulates cardiac growth and development. *Proc Natl Acad Sci USA* 107: 1136–1141, 2010.
280. Raina D, Ahmad R, Kumar S, Ren J, Yoshida K, Kharbanda S, and Kufe D. MUC1 oncoprotein blocks nuclear targeting of c-Abl in the apoptotic response to DNA damage. *EMBO J* 25: 3774–3783, 2006.
281. Raman M, Earnest S, Zhang K, Zhao Y, and Cobb MH. TAO kinases mediate activation of p38 in response to DNA damage. *EMBO J* 26: 2005–2014, 2007.
282. Raveh T, Droguett G, Horwitz MS, DePinho RA, and Kimchi A. DAP kinase activates a p19ARF/p53-mediated apoptotic checkpoint to suppress oncogenic transformation. *Nat Cell Biol* 3: 1–7, 2001.

283. Reinhardt HC, Aslanian AS, Lees JA, and Yaffe MB. p53-deficient cells rely on ATM- and ATR-mediated checkpoint signaling through the p38MAPK/MK2 pathway for survival after DNA damage. *Cancer Cell* 11: 175–189, 2007.
284. Reszka AA, Halasy-Nagy JM, Masarachia PJ, and Rodan GA. Bisphosphonates act directly on the osteoclast to induce caspase cleavage of mst1 kinase during apoptosis. A link between inhibition of the mevalonate pathway and regulation of an apoptosis-promoting kinase. *J Biol Chem* 274: 34967–34973, 1999.
285. Reyland ME. Protein kinase Cδ and apoptosis. *Biochem Soc Trans* 35: 1001–1004, 2007.
286. Rinaldo C, Prodosmo A, Mancini F, Iacovelli S, Sacchi A, Moretti F, and Soddu S. MDM2-regulated degradation of HIPK2 prevents p53Ser46 phosphorylation and DNA damage-induced apoptosis. *Mol Cell* 25: 739–750, 2007.
287. Rozengurt E, Rey O, and Waldron RT. Protein kinase D signaling. *J Biol Chem* 280: 13205–13208, 2005.
288. Rudel T and Bokoch GM. Membrane and morphological changes in apoptotic cells regulated by caspase-mediated activation of PAK2. *Science* 276: 1571–1574, 1997.
289. Sabapathy K, Hochedlinger K, Nam SY, Bauer A, Karin M, and Wagner EF. Distinct roles for JNK1 and JNK2 in regulating JNK activity and c-Jun-dependent cell proliferation. *Mol Cell* 15: 713–725, 2004.
290. Sabio G, Arthur JS, Kuma Y, Peggie M, Carr J, Murray-Tait V, Centeno F, Goedert M, Morrice NA, and Cuenda A. p38γ regulates the localisation of SAP97 in the cytoskeleton by modulating its interaction with GKAP. *EMBO J* 24: 1134–1145, 2005.
291. Sabio G, Cavanagh-Kyros J, Ko HJ, Jung DY, Gray S, Jun JY, Barrett T, Mora A, Kim JK, and Davis RJ. Prevention of steatosis by hepatic JNK1. *Cell Metab* 10: 491–498, 2009.
292. Sabio G, Das M, Mora A, Zhang Z, Jun JY, Ko HJ, Barrett T, Kim JK, and Davis RJ. A stress signaling pathway in adipose tissue regulates hepatic insulin resistance. *Science* 322: 1539–1543, 2008.
293. Sabourin LA and Rudnicki MA. Induction of apoptosis by SLK, a Ste20-related kinase. *Oncogene* 18: 7566–7575, 1999.
294. Sabourin LA, Tamai K, Seale P, Wagner J, and Rudnicki MA. Caspase 3 cleavage of the Ste20-related kinase SLK releases and activates an apoptosis-inducing kinase domain and an actin-disassembling region. *Mol Cell Biol* 20: 684–696, 2000.
295. Sagasti A, Hisamoto N, Hyodo J, Tanaka-Hino M, Matsumoto K, and Bargmann CI. The CaMKII UNC-43 activates the MAPKKK NSY-1 to execute a lateral signaling decision required for asymmetric olfactory neuron fates. *Cell* 105: 221–232, 2001.
296. Saito J, Toriumi S, Awano K, Ichijo H, Sasaki K, Kobayashi T, and Tamura S. Regulation of apoptosis signal-regulating kinase 1 by protein phosphatase 2Cε. *Biochem J* 405: 591–596, 2007.
297. Saitoh M, Nishitoh H, Fujii M, Takeda K, Tobiume K, Sawada Y, Kawabata M, Miyazono K, and Ichijo H. Mammalian thioredoxin is a direct inhibitor of apoptosis signal-regulating kinase (ASK) 1. *EMBO J* 17: 2596–2606, 1998.
298. Sakurai T, Maeda S, Chang L, and Karin M. Loss of hepatic NF-κB activity enhances chemical hepatocarcinogenesis through sustained c-Jun N-terminal kinase 1 activation. *Proc Natl Acad Sci USA* 103: 10544–10551, 2006.
299. Sasaki T, Wada T, Kishimoto H, Irie-Sasaki J, Matsumoto G, Goto T, Yao Z, Wakeham A, Mak TW, Suzuki A, Cho SK, Zuniga-Pflucker JC, Oliveira-dos-Santos AJ, Katada T, Nishina H, and Penninger JM. The stress kinase mitogen-activated protein kinase kinase (MKK)7 is a negative regulator of antigen receptor and growth factor receptor-induced proliferation in hematopoietic cells. *J Exp Med* 194: 757–768, 2001.
300. Sato S, Sanjo H, Takeda K, Ninomiya-Tsuji J, Yamamoto M, Kawai T, Matsumoto K, Takeuchi O, and Akira S. Essential function for the kinase TAK1 in innate and adaptive immune responses. *Nat Immunol* 6: 1087–1095, 2005.
301. Saucedo LJ and Edgar BA. Filling out the Hippo pathway. *Nat Rev Mol Cell Biol* 8: 613–621, 2007.
302. Savinainen A, Garcia EP, Dorow D, Marshall J, and Liu YF. Kainate receptor activation induces mixed lineage kinase-mediated cellular signaling cascades via post-synaptic density protein 95. *J Biol Chem* 276: 11382–11386, 2001.
303. Sawyers CL. Chronic myeloid leukemia. *N Engl J Med* 340: 1330–1340, 1999.
304. Schindler EM, Hindes A, Gribben EL, Burns CJ, Yin Y, Lin MH, Owen RJ, Longmore GD, Kissling GE, Arthur JS, and Efimova T. p38δ Mitogen-activated protein kinase is essential for skin tumor development in mice. *Cancer Res* 69: 4648–4655, 2009.
305. Schinkmann K and Blenis J. Cloning and characterization of a human STE20-like protein kinase with unusual cofactor requirements. *J Biol Chem* 272: 28695–28703, 1997.
306. Schori H, Yoles E, Wheeler LA, Raveh T, Kimchi A, and Schwartz M. Immune-related mechanisms participating in resistance and susceptibility to glutamate toxicity. *Eur J Neurosci* 16: 557–564, 2002.
307. Schulze-Luehrmann J, Santner-Nanan B, Jha MK, Schimpl A, Avots A, and Serfling E. Hematopoietic progenitor kinase 1 supports apoptosis of T lymphocytes. *Blood* 100: 954–960, 2002.
308. Schurmann A, Mooney AF, Sanders LC, Sells MA, Wang HG, Reed JC, and Bokoch GM. p21-activated kinase 1 phosphorylates the death agonist bad and protects cells from apoptosis. *Mol Cell Biol* 20: 453–461, 2000.
309. Sethi G, Ahn KS, Xia D, Kurie JM, and Aggarwal BB. Targeted deletion of MKK4 gene potentiates TNF-induced apoptosis through the down-regulation of NF-κB activation and NF-κB-regulated antiapoptotic gene products. *J Immunol* 179: 1926–1933, 2007.
310. Shalom-Barak T and Knaus UG. A p21-activated kinase-controlled metabolic switch up-regulates phagocyte NADPH oxidase. *J Biol Chem* 277: 40659–40665, 2002.
311. Shang T, Joseph J, Hillard CJ, and Kalyanaraman B. Death-associated protein kinase as a sensor of mitochondrial membrane potential: role of lysosome in mitochondrial toxin-induced cell death. *J Biol Chem* 280: 34644–34653, 2005.
312. Shani G, Henis-Korenblit S, Jona G, Gileadi O, Eisenstein M, Ziv T, Admon A, and Kimchi A. Autophosphorylation restrains the apoptotic activity of DRP-1 kinase by controlling dimerization and calmodulin binding. *EMBO J* 20: 1099–1113, 2001.
313. Shani G, Marash L, Gozuacik D, Bialik S, Teitelbaum L, Shohat G, and Kimchi A. Death-associated protein kinase phosphorylates ZIP kinase, forming a unique kinase hierarchy to activate its cell death functions. *Mol Cell Biol* 24: 8611–8626, 2004.
314. She QB, Chen N, Bode AM, Flavell RA, and Dong Z. Deficiency of c-Jun-NH(2)-terminal kinase-1 in mice enhances skin tumor development by 12-O-tetradecanoylphorbol-13-acetate. *Cancer Res* 62: 1343–1348, 2002.

315. Shibata W, Maeda S, Hikiba Y, Yanai A, Sakamoto K, Nakagawa H, Ogura K, Karin M, and Omata M. c-Jun NH2-terminal kinase 1 is a critical regulator for the development of gastric cancer in mice. *Cancer Res* 68: 5031–5039, 2008.
316. Shibuya H, Iwata H, Masuyama N, Gotoh Y, Yamaguchi K, Irie K, Matsumoto K, Nishida E, and Ueno N. Role of TAK1 and TAB1 in BMP signaling in early Xenopus development. *EMBO J* 17: 1019–1028, 1998.
317. Shohat G, Spivak-Kroizman T, Cohen O, Bialik S, Shani G, Berrisi H, Eisenstein M, and Kimchi A. The pro-apoptotic function of death-associated protein kinase is controlled by a unique inhibitory autophosphorylation-based mechanism. *J Biol Chem* 276: 47460–47467, 2001.
318. Singh PK and Hollingsworth MA. Cell surface-associated mucins in signal transduction. *Trends Cell Biol* 16: 467–476, 2006.
319. Sombroek D and Hofmann TG. How cells switch HIPK2 on and off. *Cell Death Differ* 16: 187–194, 2009.
320. Stepniak E, Ricci R, Eferl R, Sumara G, Sumara I, Rath M, Hui L, and Wagner EF. c-Jun/AP-1 controls liver regeneration by repressing p53/p21 and p38 MAPK activity. *Genes Dev* 20: 2306–2314, 2006.
321. Storz P. Mitochondrial ROS—radical detoxification, mediated by protein kinase D. *Trends Cell Biol* 17: 13–18, 2007.
322. Storz P, Doppler H, and Toker A. Protein kinase D mediates mitochondrion-to-nucleus signaling and detoxification from mitochondrial reactive oxygen species. *Mol Cell Biol* 25: 8520–8530, 2005.
323. Sugawara T, Moriguchi T, Nishida E, and Takahama Y. Differential roles of ERK and p38 MAP kinase pathways in positive and negative selection of T lymphocytes. *Immunity* 9: 565–574, 1998.
324. Sumara G, Formentini I, Collins S, Sumara I, Windak R, Bodenmiller B, Ramracheya R, Caille D, Jiang H, Platt KA, Meda P, Aebersold R, Rorsman P, and Ricci R. Regulation of PKD by the MAPK p38δ in insulin secretion and glucose homeostasis. *Cell* 136: 235–248, 2009.
325. Sumbayev VV and Yasinska IM. Regulation of MAP kinase-dependent apoptotic pathway: implication of reactive oxygen and nitrogen species. *Arch Biochem Biophys* 436: 406–412, 2005.
326. Sun P, Yoshizuka N, New L, Moser BA, Li Y, Liao R, Xie C, Chen J, Deng Q, Yamout M, Dong MQ, Frangou CG, Yates JR, 3rd, Wright PE, and Han J. PRAK is essential for ras-induced senescence and tumor suppression. *Cell* 128: 295–308, 2007.
327. Sun X, Yin J, Starovasnik MA, Fairbrother WJ, and Dixit VM. Identification of a novel homotypic interaction motif required for the phosphorylation of receptor-interacting protein (RIP) by RIP3. *J Biol Chem* 277: 9505–9511, 2002.
328. Sunayama J, Tsuruta F, Masuyama N, and Gotoh Y. JNK antagonizes Akt-mediated survival signals by phosphorylating 14-3-3. *J Cell Biol* 170: 295–304, 2005.
329. Suzuki H, Wu J, Hossain K, Ohhata T, Du J, Akhand AA, Hayakawa A, Kimura H, Hagiwara M, and Nakashima I. Involvement of MKK6 in TCR$\alpha\beta$(int)CD69lo: a target population for apoptotic cell death in thymocytes. *FASEB J* 17: 1538–1540, 2003.
330. Swat W, Fujikawa K, Ganiatsas S, Yang D, Xavier RJ, Harris NL, Davidson L, Ferrini R, Davis RJ, Labow MA, Flavell RA, Zon LI, and Alt FW. SEK1/MKK4 is required for maintenance of a normal peripheral lymphoid compartment but not for lymphocyte development. *Immunity* 8: 625–634, 1998.
331. Taira N, Nihira K, Yamaguchi T, Miki Y, and Yoshida K. DYRK2 is targeted to the nucleus and controls p53 via Ser46 phosphorylation in the apoptotic response to DNA damage. *Mol Cell* 25: 725–738, 2007.
332. Takatsu Y, Nakamura M, Stapleton M, Danos MC, Matsumoto K, O'Connor MB, Shibuya H, and Ueno N. TAK1 participates in c-Jun N-terminal kinase signaling during Drosophila development. *Mol Cell Biol* 20: 3015–3026, 2000.
333. Takeda K, Matsuzawa A, Nishitoh H, Tobiume K, Kishida S, Ninomiya-Tsuji J, Matsumoto K, and Ichijo H. Involvement of ASK1 in Ca2+-induced p38 MAP kinase activation. *EMBO Rep* 5: 161–166, 2004.
334. Takeda K, Shimozono R, Noguchi T, Umeda T, Morimoto Y, Naguro I, Tobiume K, Saitoh M, Matsuzawa A, and Ichijo H. Apoptosis signal-regulating kinase (ASK) 2 functions as a mitogen-activated protein kinase kinase kinase in a heteromeric complex with ASK1. *J Biol Chem* 282: 7522–7531, 2007.
335. Takekawa M and Saito H. A family of stress-inducible GADD45-like proteins mediate activation of the stress-responsive MTK1/MEKK4 MAPKKK. *Cell* 95: 521–530, 1998.
336. Tamura K, Sudo T, Senftleben U, Dadak AM, Johnson R, and Karin M. Requirement for p38α in erythropoietin expression: a role for stress kinases in erythropoiesis. *Cell* 102: 221–231, 2000.
337. Tanaka N, Kamanaka M, Enslen H, Dong C, Wysk M, Davis RJ, and Flavell RA. Differential involvement of p38 mitogen-activated protein kinase kinases MKK3 and MKK6 in T-cell apoptosis. *EMBO Rep* 3: 785–791, 2002.
338. Tang Y, Zhou H, Chen A, Pittman RN, and Field J. The Akt proto-oncogene links Ras to Pak and cell survival signals. *J Biol Chem* 275: 9106–9109, 2000.
339. Tassi E, Biesova Z, Di Fiore PP, Gutkind JS, and Wong WT. Human JIK, a novel member of the STE20 kinase family that inhibits JNK and is negatively regulated by epidermal growth factor. *J Biol Chem* 274: 33287–33295, 1999.
340. Thangaraju M, Kaufmann SH, and Couch FJ. BRCA1 facilitates stress-induced apoptosis in breast and ovarian cancer cell lines. *J Biol Chem* 275: 33487–33496, 2000.
341. Tibbles LA and Woodgett JR. The stress-activated protein kinase pathways. *Cell Mol Life Sci* 55: 1230–1254, 1999.
342. Timm T, Li XY, Biernat J, Jiao J, Mandelkow E, Vandekerckhove J, and Mandelkow EM. MARKK, a Ste20-like kinase, activates the polarity-inducing kinase MARK/PAR-1. *EMBO J* 22: 5090–5101, 2003.
343. Tobiume K, Matsuzawa A, Takahashi T, Nishitoh H, Morita K, Takeda K, Minowa O, Miyazono K, Noda T, and Ichijo H. ASK1 is required for sustained activations of JNK/p38 MAP kinases and apoptosis. *EMBO Rep* 2: 222–228, 2001.
344. Tong A, Lynn G, Ngo V, Wong D, Moseley SL, Ewbank JJ, Goncharov A, Wu YC, Pujol N, and Chisholm AD. Negative regulation of *Caenorhabditis elegans* epidermal damage responses by death-associated protein kinase. *Proc Natl Acad Sci USA* 106: 1457–1461, 2009.
345. Tournier C, Dong C, Turner TK, Jones SN, Flavell RA, and Davis RJ. MKK7 is an essential component of the JNK signal transduction pathway activated by proinflammatory cytokines. *Genes Dev* 15: 1419–1426, 2001.
346. Tournier C, Hess P, Yang DD, Xu J, Turner TK, Nimnual A, Bar-Sagi D, Jones SN, Flavell RA, and Davis RJ. Requirement

of JNK for stress-induced activation of the cytochrome c-mediated death pathway. *Science* 288: 870–874, 2000.
347. Tournier C, Whitmarsh AJ, Cavanagh J, Barrett T, and Davis RJ. Mitogen-activated protein kinase kinase 7 is an activator of the c-Jun NH2-terminal kinase. *Proc Natl Acad Sci USA* 94: 7337–7342, 1997.
348. Tybulewicz VL, Crawford CE, Jackson PK, Bronson RT, and Mulligan RC. Neonatal lethality and lymphopenia in mice with a homozygous disruption of the c-abl proto-oncogene. *Cell* 65: 1153–1163, 1991.
349. Uehara T, Bennett B, Sakata ST, Satoh Y, Bilter GK, Westwick JK, and Brenner DA. JNK mediates hepatic ischemia reperfusion injury. *J Hepatol* 42: 850–859, 2005.
350. Ura S, Masuyama N, Graves JD, and Gotoh Y. Caspase cleavage of MST1 promotes nuclear translocation and chromatin condensation. *Proc Natl Acad Sci USA* 98: 10148–10153, 2001.
351. Ura S, Masuyama N, Graves JD, and Gotoh Y. MST1-JNK promotes apoptosis via caspase-dependent and independent pathways. *Genes Cells* 6: 519–530, 2001.
352. Ura S, Nishina H, Gotoh Y, and Katada T. Activation of the c-Jun N-terminal kinase pathway by MST1 is essential and sufficient for the induction of chromatin condensation during apoptosis. *Mol Cell Biol* 27: 5514–5522, 2007.
353. Vadlamudi RK, Bagheri-Yarmand R, Yang Z, Balasenthil S, Nguyen D, Sahin AA, den Hollander P, and Kumar R. Dynein light chain 1, a p21-activated kinase 1-interacting substrate, promotes cancerous phenotypes. *Cancer Cell* 5: 575–585, 2004.
354. Vadlamudi RK, Li F, Adam L, Nguyen D, Ohta Y, Stossel TP, and Kumar R. Filamin is essential in actin cytoskeletal assembly mediated by p21-activated kinase 1. *Nat Cell Biol* 4: 681–690, 2002.
355. Vadlamudi RK, Li F, Barnes CJ, Bagheri-Yarmand R, and Kumar R. p41-Arc subunit of human Arp2/3 complex is a p21-activated kinase-1-interacting substrate. *EMBO Rep* 5: 154–160, 2004.
356. Vandenabeele P, Declercq W, Van Herreweghe F, and Vanden Berghe T. The role of the kinases RIP1 and RIP3 in TNF-induced necrosis. *Sci Signal* 3: re4, 2010.
357. Varfolomeev E, Goncharov T, Fedorova AV, Dynek JN, Zobel K, Deshayes K, Fairbrother WJ, and Vucic D. c-IAP1 and c-IAP2 are critical mediators of tumor necrosis factor α (TNFα)-induced NF-κB activation. *J Biol Chem* 283: 24295–24299, 2008.
358. Ventura JJ, Tenbaum S, Perdiguero E, Huth M, Guerra C, Barbacid M, Pasparakis M, and Nebreda AR. p38α MAP kinase is essential in lung stem and progenitor cell proliferation and differentiation. *Nat Genet* 39: 750–758, 2007.
359. Vilas GL, Corvi MM, Plummer GJ, Seime AM, Lambkin GR, and Berthiaume LG. Posttranslational myristoylation of caspase-activated p21-activated protein kinase 2 (PAK2) potentiates late apoptotic events. *Proc Natl Acad Sci USA* 103: 6542–6547, 2006.
360. Wada T, Joza N, Cheng HY, Sasaki T, Kozieradzki I, Bachmaier K, Katada T, Schreiber M, Wagner EF, Nishina H, and Penninger JM. MKK7 couples stress signalling to G2/M cell-cycle progression and cellular senescence. *Nat Cell Biol* 6: 215–226, 2004.
361. Wada T, Stepniak E, Hui L, Leibbrandt A, Katada T, Nishina H, Wagner EF, and Penninger JM. Antagonistic control of cell fates by JNK and p38-MAPK signaling. *Cell Death Differ* 15: 89–93, 2008.
362. Wagner EF and Nebreda AR. Signal integration by JNK and p38 MAPK pathways in cancer development. *Nat Rev Cancer* 9: 537–549, 2009.
363. Wang JY. Regulation of cell death by the Abl tyrosine kinase. *Oncogene* 19: 5643–5650, 2000.
364. Wang L, Du F, and Wang X. TNF-α induces two distinct caspase-8 activation pathways. *Cell* 133: 693–703, 2008.
365. Wang SY, Iordanov M, and Zhang Q. c-Jun NH2-terminal kinase promotes apoptosis by down-regulating the transcriptional co-repressor CtBP. *J Biol Chem* 281: 34810–34815, 2006.
366. Wang WJ, Kuo JC, Ku W, Lee YR, Lin FC, Chang YL, Lin YM, Chen CH, Huang YP, Chiang MJ, Yeh SW, Wu PR, Shen CH, Wu CT, and Chen RH. The tumor suppressor DAPK is reciprocally regulated by tyrosine kinase Src and phosphatase LAR. *Mol Cell* 27: 701–716, 2007.
367. Wang X, Mader MM, Toth JE, Yu X, Jin N, Campbell RM, Smallwood JK, Christe ME, Chatterjee A, Goodson T, Jr., Vlahos CJ, Matter WF, and Bloem LJ. Complete inhibition of anisomycin and UV radiation but not cytokine induced JNK and p38 activation by an aryl-substituted dihydropyrrolopyrazole quinoline and mixed lineage kinase 7 small interfering RNA. *J Biol Chem* 280: 19298–19305, 2005.
368. Wang XS, Diener K, Jannuzzi D, Trollinger D, Tan TH, Lichenstein H, Zukowski M, and Yao Z. Molecular cloning and characterization of a novel protein kinase with a catalytic domain homologous to mitogen-activated protein kinase kinase kinase. *J Biol Chem* 271: 31607–31611, 1996.
369. Wang XS, Diener K, Tan TH, and Yao Z. MAPKKK6, a novel mitogen-activated protein kinase kinase kinase, that associates with MAPKKK5. *Biochem Biophys Res Commun* 253: 33–37, 1998.
370. Wang XT, Pei DS, Xu J, Guan QH, Sun YF, Liu XM, and Zhang GY. Opposing effects of Bad phosphorylation at two distinct sites by Akt1 and JNK1/2 on ischemic brain injury. *Cell Signal* 19: 1844–1856, 2007.
371. Wang XZ and Ron D. Stress-induced phosphorylation and activation of the transcription factor CHOP (GADD153) by p38 MAP Kinase. *Science* 272: 1347–1349, 1996.
372. Wang Y. Mitogen-activated protein kinases in heart development and diseases. *Circulation* 116: 1413–1423, 2007.
373. Watanabe T, Nakagawa K, Ohata S, Kitagawa D, Nishitai G, Seo J, Tanemura S, Shimizu N, Kishimoto H, Wada T, Aoki J, Arai H, Iwatsubo T, Mochita M, Satake M, Ito Y, Matsuyama T, Mak TW, Penninger JM, Nishina H, and Katada T. SEK1/MKK4-mediated SAPK/JNK signaling participates in embryonic hepatoblast proliferation via a pathway different from NF-κB-induced anti-apoptosis. *Dev Biol* 250: 332–347, 2002.
374. Watanabe T, Otsu K, Takeda T, Yamaguchi O, Hikoso S, Kashiwase K, Higuchi Y, Taniike M, Nakai A, Matsumura Y, Nishida K, Ichijo H, and Hori M. Apoptosis signal-regulating kinase 1 is involved not only in apoptosis but also in non-apoptotic cardiomyocyte death. *Biochem Biophys Res Commun* 333: 562–567, 2005.
375. Wee HJ, Voon DC, Bae SC, and Ito Y. PEBP2-β/CBF-β-dependent phosphorylation of RUNX1 and p300 by HIPK2: implications for leukemogenesis. *Blood* 112: 3777–3787, 2008.
376. Wei G, Ku S, Ma GK, Saito S, Tang AA, Zhang J, Mao JH, Appella E, Balmain A, and Huang EJ. HIPK2 represses β-catenin-mediated transcription, epidermal stem cell expansion, and skin tumorigenesis. *Proc Natl Acad Sci USA* 104: 13040–13045, 2007.

377. Wells CM and Jones GE. The emerging importance of group II PAKs. *Biochem J* 425: 465–473, 2010.
378. Weston CR and Davis RJ. The JNK signal transduction pathway. *Curr Opin Cell Biol* 19: 142–149, 2007.
379. Widmann C, Gerwins P, Johnson NL, Jarpe MB, and Johnson GL. MEK kinase 1, a substrate for DEVD-directed caspases, is involved in genotoxin-induced apoptosis. *Mol Cell Biol* 18: 2416–2429, 1998.
380. Widmann C, Gibson S, Jarpe MB, and Johnson GL. Mitogen-activated protein kinase: conservation of a three-kinase module from yeast to human. *Physiol Rev* 79: 143–180, 1999.
381. Wilson NS, Dixit V, and Ashkenazi A. Death receptor signal transducers: nodes of coordination in immune signaling networks. *Nat Immunol* 10: 348–355, 2009.
382. Winter M, Sombroek D, Dauth I, Moehlenbrink J, Scheuermann K, Crone J, and Hofmann TG. Control of HIPK2 stability by ubiquitin ligase Siah-1 and checkpoint kinases ATM and ATR. *Nat Cell Biol* 10: 812–824, 2008.
383. Winter-Vann AM and Johnson GL. Integrated activation of MAP3Ks balances cell fate in response to stress. *J Cell Biochem* 102: 848–858, 2007.
384. Wojnowski L, Zimmer AM, Beck TW, Hahn H, Bernal R, Rapp UR, and Zimmer A. Endothelial apoptosis in Braf-deficient mice. *Nat Genet* 16: 293–297, 1997.
385. Wong CH, Chan H, Ho CY, Lai SK, Chan KS, Koh CG, and Li HY. Apoptotic histone modification inhibits nuclear transport by regulating RCC1. *Nat Cell Biol* 11: 36–45, 2009.
386. Woodgett JR. Recent advances in the protein kinase B signaling pathway. *Curr Opin Cell Biol* 17: 150–157, 2005.
387. Wu GS. The functional interactions between the p53 and MAPK signaling pathways. *Cancer Biol Ther* 3: 156–161, 2004.
388. Wu HY, Lin CY, Lin TY, Chen TC, and Yuan CJ. Mammalian Ste20-like protein kinase 3 mediates trophoblast apoptosis in spontaneous delivery. *Apoptosis* 13: 283–294, 2008.
389. Wu X, Carr HS, Dan I, Ruvolo PP, and Frost JA. p21 activated kinase 5 activates Raf-1 and targets it to mitochondria. *J Cell Biochem* 105: 167–175, 2008.
390. Wu Z, Wu J, Jacinto E, and Karin M. Molecular cloning and characterization of human JNKK2, a novel Jun NH2-terminal kinase-specific kinase. *Mol Cell Biol* 17: 7407–7416, 1997.
391. Wysk M, Yang DD, Lu HT, Flavell RA, and Davis RJ. Requirement of mitogen-activated protein kinase kinase 3 (MKK3) for tumor necrosis factor-induced cytokine expression. *Proc Natl Acad Sci USA* 96: 3763–3768, 1999.
392. Xia Y, Makris C, Su B, Li E, Yang J, Nemerow GR, and Karin M. MEK kinase 1 is critically required for c-Jun N-terminal kinase activation by proinflammatory stimuli and growth factor-induced cell migration. *Proc Natl Acad Sci USA* 97: 5243–5248, 2000.
393. Xia Y, Wang J, Xu S, Johnson GL, Hunter T, and Lu Z. MEKK1 mediates the ubiquitination and degradation of c-Jun in response to osmotic stress. *Mol Cell Biol* 27: 510–517, 2007.
394. Xia Z, Dickens M, Raingeaud J, Davis RJ, and Greenberg ME. Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis. *Science* 270: 1326–1331, 1995.
395. Xu LG, Li LY, and Shu HB. TRAF7 potentiates MEKK3-induced AP1 and CHOP activation and induces apoptosis. *J Biol Chem* 279: 17278–17282, 2004.
396. Xu Z, Maroney AC, Dobrzanski P, Kukekov NV, and Greene LA. The MLK family mediates c-Jun N-terminal kinase activation in neuronal apoptosis. *Mol Cell Biol* 21: 4713–4724, 2001.
397. Yamaguchi H and Wang HG. CHOP is involved in endoplasmic reticulum stress-induced apoptosis by enhancing DR5 expression in human carcinoma cells. *J Biol Chem* 279: 45495–45502, 2004.
398. Yamaguchi K, Shirakabe K, Shibuya H, Irie K, Oishi I, Ueno N, Taniguchi T, Nishida E, and Matsumoto K. Identification of a member of the MAPKKK family as a potential mediator of TGF-β signal transduction. *Science* 270: 2008–2011, 1995.
399. Yamaguchi O, Higuchi Y, Hirotani S, Kashiwase K, Nakayama H, Hikoso S, Takeda T, Watanabe T, Asahi M, Taniike M, Matsumura Y, Tsujimoto I, Hongo K, Kusakari Y, Kurihara S, Nishida K, Ichijo H, Hori M, and Otsu K. Targeted deletion of apoptosis signal-regulating kinase 1 attenuates left ventricular remodeling. *Proc Natl Acad Sci USA* 100: 15883–15888, 2003.
400. Yamamoto K, Ichijo H, and Korsmeyer SJ. BCL-2 is phosphorylated and inactivated by an ASK1/Jun N-terminal protein kinase pathway normally activated at G(2)/M. *Mol Cell Biol* 19: 8469–8478, 1999.
401. Yamamoto S, Yang G, Zablocki D, Liu J, Hong C, Kim SJ, Soler S, Odashima M, Thaisz J, Yehia G, Molina CA, Yatani A, Vatner DE, Vatner SF, and Sadoshima J. Activation of Mst1 causes dilated cardiomyopathy by stimulating apoptosis without compensatory ventricular myocyte hypertrophy. *J Clin Invest* 111: 1463–1474, 2003.
402. Yang D, Tournier C, Wysk M, Lu HT, Xu J, Davis RJ, and Flavell RA. Targeted disruption of the MKK4 gene causes embryonic death, inhibition of c-Jun NH2-terminal kinase activation, and defects in AP-1 transcriptional activity. *Proc Natl Acad Sci USA* 94: 3004–3009, 1997.
403. Yang DD, Kuan CY, Whitmarsh AJ, Rincon M, Zheng TS, Davis RJ, Rakic P, and Flavell RA. Absence of excitotoxicity-induced apoptosis in the hippocampus of mice lacking the Jnk3 gene. *Nature* 389: 865–870, 1997.
404. Yang J, Boerm M, McCarty M, Bucana C, Fidler IJ, Zhuang Y, and Su B. Mekk3 is essential for early embryonic cardiovascular development. *Nat Genet* 24: 309–313, 2000.
405. Yao Z, Diener K, Wang XS, Zukowski M, Matsumoto G, Zhou G, Mo R, Sasaki T, Nishina H, Hui CC, Tan TH, Woodgett JP, and Penninger JM. Activation of stress-activated protein kinases/c-Jun N-terminal protein kinases (SAPKs/JNKs) by a novel mitogen-activated protein kinase kinase. *J Biol Chem* 272: 32378–32383, 1997.
406. Yoshida K. Nuclear trafficking of pro-apoptotic kinases in response to DNA damage. *Trends Mol Med* 14: 305–313, 2008.
407. Yoshida K. Role for DYRK family kinases on regulation of apoptosis. *Biochem Pharmacol* 76: 1389–1394, 2008.
408. Yoshida K, Liu H, and Miki Y. Protein kinase Cδ regulates Ser46 phosphorylation of p53 tumor suppressor in the apoptotic response to DNA damage. *J Biol Chem* 281: 5734–5740, 2006.
409. Yoshida K, Yamaguchi T, Natsume T, Kufe D, and Miki Y. JNK phosphorylation of 14–3-3 proteins regulates nuclear targeting of c-Abl in the apoptotic response to DNA damage. *Nat Cell Biol* 7: 278–285, 2005.
410. Youle RJ and Strasser A. The BCL-2 protein family: opposing activities that mediate cell death. *Nat Rev Mol Cell Biol* 9: 47–59, 2008.
411. Yuan ZQ, Feldman RI, Sussman GE, Coppola D, Nicosia SV, and Cheng JQ. AKT2 inhibition of cisplatin-induced

JNK/p38 and Bax activation by phosphorylation of ASK1: implication of AKT2 in chemoresistance. *J Biol Chem* 278: 23432–23440, 2003.
412. Yujiri T, Sather S, Fanger GR, and Johnson GL. Role of MEKK1 in cell survival and activation of JNK and ERK pathways defined by targeted gene disruption. *Science* 282: 1911–1914, 1998.
413. Yustein JT, Xia L, Kahlenburg JM, Robinson D, Templeton D, and Kung HJ. Comparative studies of a new subfamily of human Ste20-like kinases: homodimerization, subcellular localization, and selective activation of MKK3 and p38. *Oncogene* 22: 6129–6141, 2003.
414. Zalckvar E, Berissi H, Mizrachy L, Idelchuk Y, Koren I, Eisenstein M, Sabanay H, Pinkas-Kramarski R, and Kimchi A. DAP-kinase-mediated phosphorylation on the BH3 domain of beclin 1 promotes dissociation of beclin 1 from Bcl-XL and induction of autophagy. *EMBO Rep* 10: 285–292, 2009.
415. Zhang DW, Shao J, Lin J, Zhang N, Lu BJ, Lin SC, Dong MQ, and Han J. RIP3, an energy metabolism regulator that switches TNF-induced cell death from apoptosis to necrosis. *Science* 325: 332–336, 2009.
416. Zhang J, Pho V, Bonasera SJ, Holtzman J, Tang AT, Hellmuth J, Tang S, Janak PH, Tecott LH, and Huang EJ. Essential function of HIPK2 in TGFβ-dependent survival of midbrain dopamine neurons. *Nat Neurosci* 10: 77–86, 2007.
417. Zhang Q, Yoshimatsu Y, Hildebrand J, Frisch SM, and Goodman RH. Homeodomain interacting protein kinase 2 promotes apoptosis by downregulating the transcriptional corepressor CtBP. *Cell* 115: 177–186, 2003.
418. Zhao B, Wei X, Li W, Udan RS, Yang Q, Kim J, Xie J, Ikenoue T, Yu J, Li L, Zheng P, Ye K, Chinnaiyan A, Halder G, Lai ZC, and Guan KL. Inactivation of YAP oncoprotein by the Hippo pathway is involved in cell contact inhibition and tissue growth control. *Genes Dev* 21: 2747–2761, 2007.
419. Zhou D, Conrad C, Xia F, Park JS, Payer B, Yin Y, Lauwers GY, Thasler W, Lee JT, Avruch J, and Bardeesy N. Mst1 and Mst2 maintain hepatocyte quiescence and suppress hepatocellular carcinoma development through inactivation of the Yap1 oncogene. *Cancer Cell* 16: 425–438, 2009.
420. Zhou D, Medoff BD, Chen L, Li L, Zhang XF, Praskova M, Liu M, Landry A, Blumberg RS, Boussiotis VA, Xavier R, and Avruch J. The Nore1B/Mst1 complex restrains antigen receptor-induced proliferation of naive T cells. *Proc Natl Acad Sci USA* 105: 20321–20326, 2008.
421. Zhou G, Bao ZQ, and Dixon JE. Components of a new human protein kinase signal transduction pathway. *J Biol Chem* 270: 12665–12669, 1995.
422. Zhu Y, Mao XO, Sun Y, Xia Z, and Greenberg DA. p38 Mitogen-activated protein kinase mediates hypoxic regulation of Mdm2 and p53 in neurons. *J Biol Chem* 277: 22909–22914, 2002.
423. Zihni C, Mitsopoulos C, Tavares IA, Baum B, Ridley AJ, and Morris JD. Prostate-derived sterile 20-like kinase 1-α induces apoptosis. JNK- and caspase-dependent nuclear localization is a requirement for membrane blebbing. *J Biol Chem* 282: 6484–6493, 2007.
424. Zinszner H, Kuroda M, Wang X, Batchvarova N, Lightfoot RT, Remotti H, Stevens JL, and Ron D. CHOP is implicated in programmed cell death in response to impaired function of the endoplasmic reticulum. *Genes Dev* 12: 982–995, 1998.

Address correspondence to:
Dr. Kohsuke Takeda
Laboratory of Cell Signaling
Graduate School of Pharmaceutical Sciences
The University of Tokyo
7-3-1 Hongo, Bunkyo-ku
Tokyo 113-0033
Japan

E-mail: takeda@mol.f.u-tokyo.ac.jp

Prof. Hidenori Ichijo
Laboratory of Cell Signaling
Graduate School of Pharmaceutical Sciences
The University of Tokyo
7-3-1 Hongo, Bunkyo-ku
Tokyo 113-0033
Japan

E-mail: ichijo@mol.f.u-tokyo.ac.jp

Date of first submission to ARS Central, June 16, 2010; date of final revised submission, October 21, 2010; date of acceptance, October 24, 2010.

Abbreviations Used

Aβ = amyloid-β
ABD = actin-binding domain
AD = Alzheimer's disease
ALS = amyotrophic lateral sclerosis
Ang II = angiotensin II
ANK = ankyrin repeats
ASK1 = apoptosis signal-regulating kinase 1
ATM = ataxia telangiectasia mutated
ATR = ATM and Rad3-related
BCR = breakpoint cluster region
BMK = big MAP kinase 1
CaM = Ca^{2+}/calmodulin
CARD = caspase recruitment domain
CCC = C-terminal coiled-coil
CDK = cyclin-dependent kinase
CHOP = cyclic AMP response element binding protein homologous protein
CRIB = Cdc42/Rac-interactive binding
CtBP = C-terminal binding protein
DA = dominant active
DAG = diacylglycerol
DAPK = death-associated protein kinase
DASK1 = *Drosophila* ASK1
DBD = DNA-binding domain
DD = death domain
DLC1 = dynein light chain 1
DLK = dual-leucine-zipper-bearing kinase
DRAK = DAPK-related apoptosis-inducing protein kinase
DRP1 = DAPK-related protein-1
ER = endoplasmic reticulum
ERAD = ER-associated degradation
ERK = extracellular signal-regulated kinase
FADD = Fas-associated death domain
FFA = free fatty acids
GADD45 = growth arrest and DNA damage inducible 45
GCK = germinal center kinase
H2B = histone H2B
HCC = hepatocellular carcinoma
HD = Huntington's disease
HDAC = histone deacetylase
HFD = high-fat diet
HIPK = homeodomain-interacting protein kinase
HPK1 = hematopoietic progenitor kinase 1
IFN = interferon
IL = interleukin
JNK = c-Jun N-terminal kinase
KD = kinase domain
LPS = lipopolysaccharide
LZ = leucine-zipper
LZK = leucine-zipper kinase
MAP = mitogen-activated protein
MAPK = MAP kinase
MAP2K = MAP kinase kinase
MAP3K = MAP kinase kinase kinase
Mcl-1 = myeloid cell leukemia-1
MDM2 = murine double minute 2
MEFs = mouse embryonic fibroblasts
MEF2 = myocyte enhancer factor 2
MEKK = MAP/ERK kinase kinase
MK = MAP kinase-activated protein (MAPKAP) kinase
MLK = mixed-lineage kinase
MNK = MAP kinase-interacting kinase
MSK = mitogen- and stress-activated protein kinase
MST = mammalian ste20-related kinase
NBs = nuclear bodies
NCC = N-terminal coiled-coil
NES = nuclear export signal
NF-κB = nuclear factor-kappa B
NGF = nerve growth factor
PAK = p21-activated kinase
PBD = p21-binding domain
PKC = protein kinase C
PKD = protein kinase D
PP5 = protein phosphatase 5
PRAK = p38-regulated/activated kinase
PTP-PEST = protein tyrosine phosphatase proline-, glutamine-, serine-, and threonine-rich sequence
Puma = p53-upregulated modulator of apoptosis
RACK1 = receptor for activated C-kinase 1
RHIM = RIP homotypic interaction motif
RIP = receptor interacting kinase
RNS = reactive nitrogen species
ROS = reactive oxygen species
SAM = sterile-α motif
SCG = superior cervical ganglion
Siah-1 = seven in absentia homolog-1
SLK = Ste20-like kinase
SOD1 = superoxide dismutase
SOK1 = oxidative stress response kinase 1
SH2 = Src-homology 2
SH3 = Src-homology 3
Ste20p = sterile 20 protein
TAB = TAK1-associated binding protein
TAK1 = TGF-β activated kinase 1
TAO = thousand and one amino acid
TGF-β = transforming growth factor-β
TLR = Toll-like receptor
TNF = tumor necrosis factor
TNFR1 = TNF receptor 1
TRADD = TNFR-associated death domain
TRAF = TNF receptor-associated factor
TRAIL = TNF-related apoptosis-inducing ligand
Trx = thioredoxin
UV = ultraviolet
VEGF = vascular endothelial growth factor
VV = vaccinia virus
WSB-1 = WD-repeat and suppressor of cytokine signaling (SOCS) box-containing-1
WT = wild-type
YAP1 = yes-associated protein
ZAK = zipper sterile-α-motif kinase

This article has been cited by:

1. Magdalena L. Circu, Tak Yee Aw. 2012. Glutathione and modulation of cell apoptosis. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research* **1823**:10, 1767-1777. [CrossRef]
2. Mayumi Soga, Atsushi Matsuzawa, Hidenori Ichijo. 2012. Oxidative Stress-Induced Diseases via the ASK1 Signaling Pathway. *International Journal of Cell Biology* **2012**, 1-5. [CrossRef]
3. Lucia Biasutto , Ildiko' Szabo' , Mario Zoratti . 2011. Mitochondrial Effects of Plant-Made Compounds. *Antioxidants & Redox Signaling* **15**:12, 3039-3059. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]