

Mitogen-Activated Protein Kinases in Mammalian Oxidative Stress Responses

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Abstract

All aerobic organisms are exposed to oxidative stress during their lifetime and are required to respond appropriately for maintenance of their survival and homeostasis. Sustained exposure to oxidative stress has devastating effects in organisms, and, not surprisingly, oxidative stress has been implicated in numerous human diseases. Therefore, an understanding of how mammals respond to oxidative stress is crucial both biologically and clinically. Intracellular signaling pathways, which are activated in response to excessive oxygen radicals, play essential roles in overcoming oxidative stress. The mitogen-activated protein kinase (MAPK) signaling pathways are involved in diverse physiological processes, and are critical for induction of oxidative stress responses. In this review, we will discuss the physiological roles of MAPKs in oxidative stress, the upstream signaling pathways leading to MAPK activation, their regulation, and the MAPK downstream substrates, with a focus on mammalian systems. *Antioxid. Redox Signal.* 15, 205–218.

Introduction

Oxidative stress occurs through elevated concentrations of reactive oxygen species (ROS) when the amount of ROS exceeds the capability of the intracellular antioxidant systems. ROS can be produced by several pathways, and one major source is the mitochondria. During respiration, electrons going through the electron transport chain in the inner mitochondrial membrane occasionally reduce O_2 prematurely and incompletely to form superoxide anion, $O_2^{\cdot-}$. $O_2^{\cdot-}$ is a ROS with limited harmful effects. $O_2^{\cdot-}$ is converted to O_2 and H_2O_2 spontaneously or by superoxide dismutases (31). H_2O_2 is a key molecule since it is the most stable ROS, which easily passes the cell membranes and affects surrounding cells. H_2O_2 can react with Fe^{2+} to generate OH^{\cdot} via the Fenton reaction (52). OH^{\cdot} is the most damaging ROS and oxidizes proteins, nucleic acids, carbohydrates, and lipids, which challenges proper cell function (35). $O_2^{\cdot-}$ can react with the secondary messenger, NO, to form peroxynitrite, $ONOO^-$ (117). NO and peroxynitrite are the major types of reactive nitrogen species, which also are involved in and trigger oxidative stress responses. Several antioxidant systems exist to deal with H_2O_2 to prevent cell damage. Key antioxidant proteins include superoxide dismutases, catalases, glutathione peroxidases, and glutathione reductases, and the transcription factor Nrf2, which regulates expression of many of these antioxidant enzymes. Beside its harmful effects, H_2O_2 has also emerged as an important second messenger. Stimu-

lation of cell surface receptors, such as epidermal growth factor (EGF) receptor and tumor necrosis factor α (TNF α) receptor, induce H_2O_2 production (7, 113), which triggers activation of several downstream signaling pathways. Moreover, ROS is also closely associated with hematopoietic cell differentiation (98). For H_2O_2 to function as a secondary messenger, transient and spatial inactivation of the antioxidant systems are required, and this was shown to be mediated by peroxiredoxin I at the plasma membrane, allowing an increase in local H_2O_2 production around the receptors (142). In addition, ROS production can be induced by a number of stimuli, including osmotic stress (141), UV irradiation (47), chemotherapeutic drugs (74), heavy metals (80), and ischemia/reperfusion (137). Thus, ROS has a wide variety of both physiological and toxic effects on cells, and multiple pathways can lead to ROS production. Accordingly, oxidative stress has been shown to be involved in numerous human diseases, such as cancer (133), neurodegenerative diseases (99, 114), and atherosclerosis (10). This emphasizes both biological and clinical importance of a detailed understanding of how cells respond to oxidative stress. Among several intracellular signaling pathways involved in oxidative stress responses, the mitogen-activated protein kinase (MAPK) cascade is one of the most important signaling pathways. MAPKs are activated by H_2O_2 and play pivotal roles in regulating H_2O_2 -induced cell survival or death. This review focuses on the roles of MAPKs in oxidative stress responses and their upstream and downstream signaling pathways.

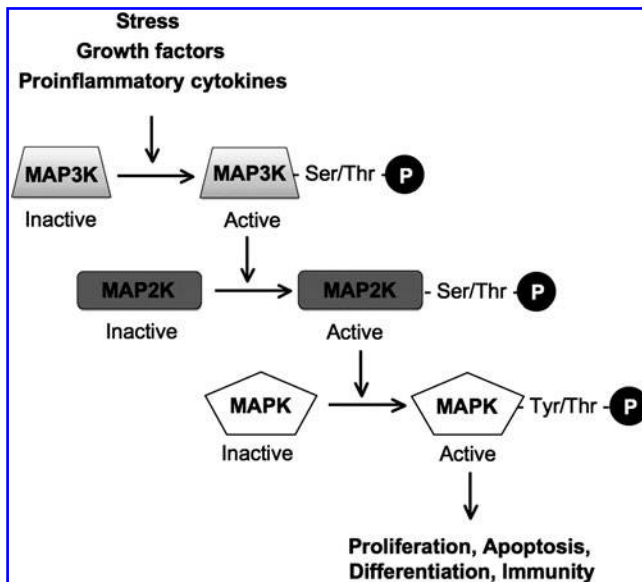


FIG. 1. Activation mechanism of the MAPK cascade. The MAPK cascade is a series of sequentially activated three protein kinases. See the text for details. MAPK, mitogen-activated protein kinase.

The MAPK Cascade

The MAPK cascades are intracellular signaling pathways comprising three components, the MAPK, the MAPK kinase (MAPKK or MAP2K), and the MAPKK kinase (MAPKKK or MAP3K), whose functions and structures have been conserved throughout evolution from unicellular organisms, such as yeast, to complex organisms, including mammals (140). MAPKs are activated by numerous cellular stresses and

ligand–receptor bindings. They have crucial roles in regulating a number of key cellular processes, such as cell proliferation, survival, differentiation, and death, and have been implicated in various human diseases. The MAPK cascade is initiated by activation of the MAP3K, which is induced by either direct self-activation or upstream signaling. Subsequently, the MAP3K phosphorylates and activates the MAP2K, which in turn phosphorylates and activates the MAPK (Fig. 1). The MAPK phosphorylates serine and threonine residues of substrate proteins, including other protein kinases, transcription factors, phospholipases, and cytoskeletal proteins, which regulate various cellular activities. For all three components of the MAPK cascade, there are several different kinases, and currently 21 MAP3K, 7 MAP2K, and 11 MAPK genes have been identified. The MAP3Ks are activated by various signals, and function as signaling hubs, integrating these signals into activation of specific MAP2K/MAPK signaling pathways (Fig. 2).

The response of the MAPK cascade to a specific stimulus is often very complex. One stimulus can activate several different MAP3Ks, and each MAP3K can be activated by several different kinds of stimuli. This means that the response to one stimulus is mediated by several different MAP3Ks and that each MAP3K is usually involved in several different cellular responses. Activation of each MAP3K is integrated into a unique pattern of MAPK activation and substrate phosphorylation, leading to a specific cellular response to the stimulus. The MAPK cascade shows further complexity, since it is extensively regulated by various factors, such as kinases and phosphatases, and there is extensive crosstalk between different MAPK and other signaling pathways. The duration and strength of MAPK activation are also important in determining the specific cellular response. This remarkable complexity ensures appropriate cellular responses to a specific stimulus.

H₂O₂ activates several MAPK signaling pathways, all of which mediate different and specific cellular responses. All of

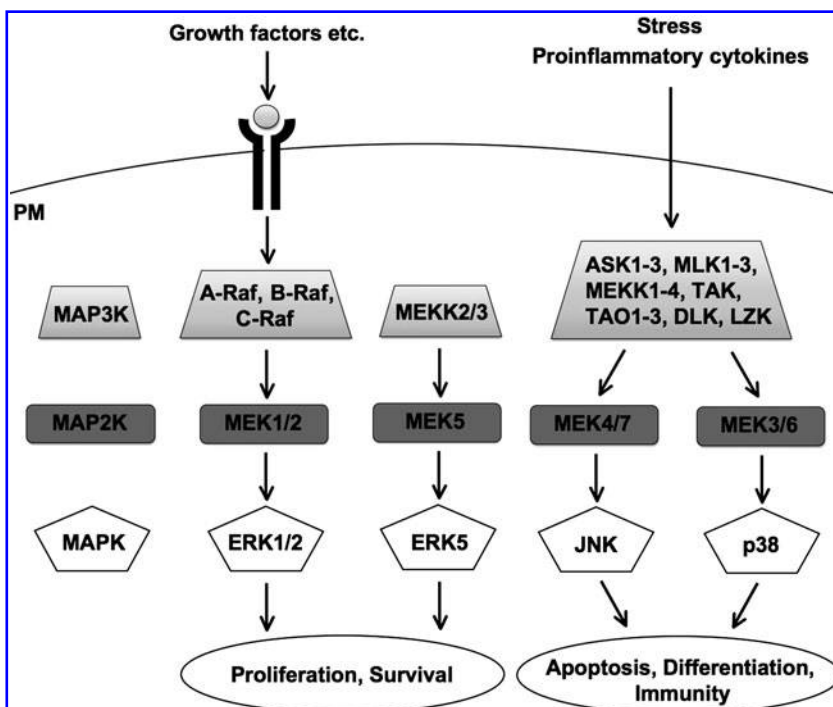


FIG. 2. Overview of the MAPK cascade. Different stimuli, such as growth factors and stresses, initiate the MAPK cascade through the activation of the MAP3K. Each MAP3K activates specific MAP2Ks, which in turn activate specific MAPKs. There are several subgroups of MAPKs, four of which are shown here. ERK1/2 and ERK5 are usually activated by growth factors, whereas JNK and p38 are typically activated by stresses. MAPKs phosphorylate and regulate numerous proteins involved in diverse cellular functions, and thereby determine the cellular response to a specific stimuli. PM, plasma membrane. ERK, extracellular signal-regulated kinase; JNK, c-Jun N-terminal kinase; MAP2K, MAPK kinase; MAP3K, MAPK kinase kinase.

the four conventional MAPK families are activated by H_2O_2 , namely, extracellular signal-regulated kinase 1 (ERK1) and ERK2 (ERK1/2) (91), ERK5/big MAP kinase 1 (BMK1) (2), c-Jun N-terminal kinase (JNK) (85), and p38 (118). The atypical MAPKs, such as ERK3 and ERK4, have not been reported to be activated by H_2O_2 and will not be considered further in this review.

Extracellular Signal-Regulated Kinases 1 and 2

ERK1 and ERK2 (ERK1/2) were the first MAPKs to be identified and have been extensively characterized due to their diverse physiological roles and critical involvement in a number of diseases. As the name implies (extracellular signal-regulated kinases), activation of the canonical ERK pathway is initiated by ligand binding to receptor tyrosine kinases at the plasma membrane, such as the EGF receptor, leading to activation of the small G protein Ras. Ras in turn activates the MAP3K Raf, which phosphorylates and activates MAP2Ks, MEK1 and MEK2, followed by phosphorylation and activation of ERK1/2. Finally, ERK1/2 phosphorylate a large number of substrates leading to an appropriate cellular response. Several key components of other signaling pathways have also been shown to activate ERK1/2, including other receptor tyrosine kinases (39), Src kinases (143, 153), calcium (14), protein kinase C (PKC) (12), and G protein-coupled receptors (59).

Physiological roles of ERK1/2 in oxidative stress

ERK1/2 have diverse physiological roles, but are mostly known for their important involvement in stimulating cell survival and cell cycle progression. ERK1/2 are usually activated by growth factors or cytokines, but can also be activated by some stresses. ROS is a well-known potent activator of ERK1/2. Several different kinds of ROS can activate ERK1/2 in several cell lines (91), suggesting that ERK1/2 play important roles in oxidative stress responses in diverse tissues. Consistently with their known pro-survival role, ERK1/2 have been shown to have proliferative and protective effects on cells exposed to oxidative stress. Low and adequate concentration of H_2O_2 is mitogenic (11), which is partly due to activation of ERK1/2. ERK1/2 have also been shown to promote survival and have antiapoptotic effects on cells injured by exposure to oxidative stress (42), and to protect against oxidative stress-induced aging (146). ERK1/2 was thought to only promote proliferation and survival, but recently, it has emerged that ERK1/2 also have pro-apoptotic roles. H_2O_2 -induced apoptosis has been reported to be dependent on ERK1/2 in opossum kidney and human glioma cells (77, 79). Whether ERK1/2 promote cell survival or death is probably in large part cell type specific.

ROS-regulated signaling pathways that activate ERK1/2

H_2O_2 does not directly activate ERK1/2, but instead induces the activation of upstream signaling components. MEK1/2 are required for ERK1/2 activation, since the treatment of cells with MEK1/2 inhibitors blocks H_2O_2 -induced ERK1/2 activation (77, 79). Several major signaling pathways are activated by ROS, finally leading to the activation of ERK1/2 (Fig. 3). EGF receptor is usually activated by EGF ligand binding, causing receptor dimerization and

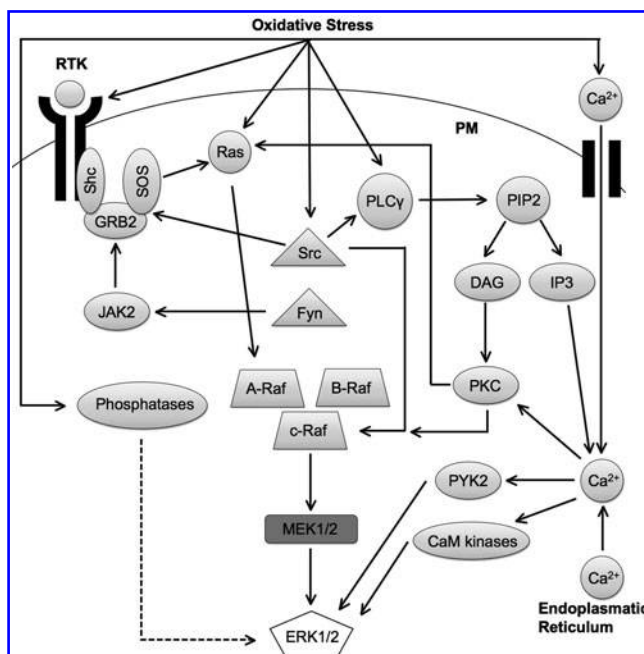


FIG. 3. ERK1/2 signaling pathways in oxidative stress. The Raf family kinases, which are MAP3Ks for ERK1/2, are not activated directly by oxidative stress, but by the activation of upstream signaling pathways. ERK1/2 are activated by oxidative stress *via* several signaling pathways in a complex manner with extensive cross talk between the pathways.

phosphorylation. The EGF receptor-associated protein SOS functions as a guanine nucleotide exchange factor for Ras, thereby activating it and leading to ERK1/2 activation. H_2O_2 induces phosphorylation and activation of the EGF receptor independently of EGF ligand (153). In a similar way, platelet-derived growth factor receptor is also activated by H_2O_2 and induces the activation of ERK1/2 (69). Ras itself can also be a target of ROS. Nitric oxide (NO) has been shown to activate Ras through S-nitrosylation of a cysteine residue of Ras (Cys118) (72). In addition, other oxidants, such as H_2O_2 , S-nitrosoglutathione, diamide, glutathione disulfide, and cystamine, modify reactive cysteines in Ras, and thereby regulate the activity of ERK1/2 (86). Certain members of the Src kinase family have also been shown to be activated by H_2O_2 . H_2O_2 -induced activation of Src leads to the activation of ERK1/2 through different pathways. H_2O_2 -induced activation of EGF receptor has been shown to depend on Src, since a Src inhibitor protein phosphatase (PP)1 inhibits H_2O_2 -induced phosphorylation and activation of EGF receptor and ERK1/2 (153). Src also directly activates C-Raf by phosphorylation of residues Tyr340/341, which is inhibited by treatment with ROS scavengers (143). Fyn, another Src kinase family member, activates JAK2 upon H_2O_2 stimulation, which induces ERK1/2 activation *via* the Shc-Ras signaling pathway (1).

Through the activation of several different signaling pathways, H_2O_2 increases the intracellular concentration of calcium, which is a known activator of ERK1/2 (6). H_2O_2 induces phosphorylation and activation of phospholipase C (PLC)- γ , which causes cleavage of phosphatidylinositol 4,5-bisphosphate (PIP2) into diacylglycerol and inositol

triphosphate (IP₃) (124). IP₃ induces release of calcium from the endoplasmic reticulum. This PLC- γ activation is further enhanced by Src, which has been shown to phosphorylate and activate PLC- γ in a H₂O₂-dependent manner (136). ROS also increases the intracellular concentration of calcium by causing flux through calcium channels (70). Several signaling pathways activated by calcium lead to ERK1/2 activation. Calcium and diacylglycerol activate PKC (156), and the increase of intracellular calcium concentration also activates CaM kinases (27, 122) and PYK2 (81), resulting in ERK1/2 activation.

Another mechanism of ERK1/2 activation by ROS is through the regulation of protein phosphatases. H₂O₂ inhibits several protein phosphatases, such as protein tyrosine phosphatases (PTPs) (116, 139), by the modification of catalytic cysteine residues. H₂O₂ stimulation inhibited several PTPs, including SHP-1, CD45, and hePTP, and overexpression of all three PTPs caused a reduction in ERK1/2 activation, indicating that ROS-induced inhibition of PTPs leads to ERK1/2 activation (78).

ERK1/2 substrates

All signaling pathways leading to ERK1/2 phosphorylation induce conformational changes in ERK1/2, which allows substrate binding and ERK1/2 translocation to the nucleus, enabling activation of nuclear substrates. ERK1/2 have at least around 160 substrates [see (148) for a detailed review on ERK1/2 substrates] and this number is likely to increase. Since ERK1/2 also activate other kinases, such as ribosomal S6 kinases (RSKs), which have their own specific substrates, the ERK signaling cascade probably involves hundreds of proteins. Approximately half of ERK1/2 substrates are nuclear, whereas the other half are located in the cytosol, the plasma membrane, and cellular organelles. ERK1/2 substrates share a consensus motif, Pro-X-Ser/Thr-Pro, where the Ser/Thr residue is phosphorylated by ERK1/2. The phosphorylation domains of ERK1/2 substrates do not provide sufficient binding affinity to ERK1/2 or substrate specificity, so to ensure this, ERK1/2 substrates usually also have an ERK1/2 binding domain, such as a D- or DEF-domain. Phosphorylation by ERK1/2 induces conformational changes in substrates, which have distinct effects, such as either activating or inhibiting their functions, regulating intracellular localization, or altering protein-protein interactions. In addition, ERK1/2 also regulate substrates in a kinase activity-independent manner. For example, ERK1/2 directly interact with and activate topoisomerase II α by induction of its conformational change (123). Well-characterized ERK1/2 substrates include transcription factors such as Elk1, c-Fos, and c-Myc, and kinases such as RSKs and MSK1/2 (148). All ERK1/2 substrates are potentially phosphorylated by oxidative stress, but which ERK1/2 substrates are actually phosphorylated under oxidative stress remains poorly characterized. However, two known substrates are AP-1 and Nrf2. The NADPH oxidase gene has been reported to be induced through ERK1/2-dependent AP-1 activation, which is triggered by angiotensin II (Ang II) and TNF α (87). Even though it is not clear whether Nrf2 is a direct target of ERK1/2, it seems to be an important downstream target of the ERK1/2 signaling pathway. Under stable conditions, Nrf2 interacts with the E3 ligase Keap1, which mediates Nrf2 degradation (55, 92). Exposure to oxi-

dative stress induces Nrf2 dissociation from Keap1 and Nrf2 translocation to the nucleus where it upregulates key antioxidant and detoxifying proteins (49, 103). All MAPKs, especially ERK1/2, have been shown to regulate Nrf2; however, the details of how ERK1/2 and other MAPKs regulate Nrf2 remain unknown. The ERK1/2 signaling pathway has been shown to stabilize Nrf2 (105) and to be required for Nrf2 translocation to the nucleus (155), but the molecular mechanism is not clear.

ERK5 (BMK1)

ERK5, also known as BMK1, is a more recently identified MAPK, and is not as well characterized as ERK1/2, JNK, and p38. ERK5 was cloned in a yeast two-hybrid screen using MEK5 as a bait (151) and was found to be ubiquitously expressed, being especially abundant in heart and muscle. The ERK5 signaling pathway is initiated by the MAP3Ks, MEKK2 and MEKK3. These MAP3Ks phosphorylate and activate a MAP2K, MEK5, which in turn phosphorylates ERK5 on threonine and tyrosine residues, thereby activating it.

Physiological roles and signaling pathways of ERK5 in oxidative stress

ERK5 has similar roles to ERK1/2 and is involved in diverse physiological processes, including cell proliferation (65), survival, and differentiation (13). The ERK5 signaling pathway is activated by various factors, such as cellular stress (3), G protein-coupled receptors (89), and growth factors (65). H₂O₂ strongly activates ERK5 in several different cell lines (2). In H₂O₂-exposed PC12 cells, inhibition of the ERK5 signaling pathway by MEK5 inhibitor treatment enhanced cell death, suggesting that ERK5 has protective effects on H₂O₂-induced apoptosis (126).

Several different pathways lead to oxidative stress-induced ERK5 activation (Fig. 4). Src inhibitors and a kinase negative mutant of Src inhibited H₂O₂-induced activation of ERK5, suggesting that the Src pathway activates ERK5 as well as

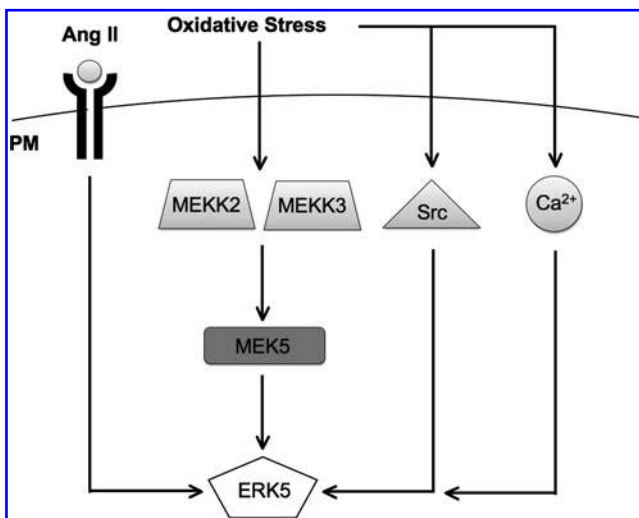


FIG. 4. ERK5 signaling pathways in oxidative stress. ERK5 is activated by oxidative stress *via* several different pathways. The ERK5 MAP3Ks, MEKK2 and MEKK3, are activated indirectly by oxidative stress.

ERK1/2 in response to oxidative stress (3, 126). Activation of ERK5 induced by H_2O_2 was shown to be calcium dependent in several cell lines (2), implicating that the ROS-calcium signaling pathway (discussed for ERK1/2) is also involved in ERK5 activation. This suggests that CaM kinases and PYK2 could be potential activators of ERK5. However, PKC, which is also activated by elevated intracellular calcium levels, was shown to inhibit ERK5 in PC12 cells (33), in contrast to ERK1/2. Another redox-sensitive signaling cascade activating ERK5 is mediated by Ang II. Stimulation of Ang II receptor was found to activate ERK5 by inducing NADPH oxidase-dependent ROS production, partially through the transactivation of insulin-like growth factor-1 receptor (IGF-R1) and EGF receptor. Ang II-induced activation of ERK5 was ROS dependent, since treatment with ROS scavengers inhibited ERK5 activation (132).

Several types of proteins, such as phosphatases and kinases, are known to regulate the ERK5 pathway, but their roles in ROS-mediated regulation of ERK5 are not clear. The serine/threonine phosphatases PP1 and PP2A were shown to regulate ERK5 activity in cells exposed to H_2O_2 . Treatment with PP1 and PP2A inhibitors, such as okadaic acid and calyculin, inhibited H_2O_2 -induced activation of ERK5 in HeLa and PC12 cells, suggesting that PP1 or PP2A, or both are required for H_2O_2 -induced activation of ERK5; however, the mechanism of how they mediate this remains unknown (33).

ERK5 substrates

ERK5 substrates are not as well characterized as for other MAPKs, and only around 10 have so far been identified. ERK5 have both nuclear and cytosolic substrates, and translocates to the nucleus upon its activation. The best characterized ERK5 substrates are the transcription factors MEF2A, MEF2C, and MEF2D, whose transcriptional activities are increased by ERK5-dependent phosphorylation (8, 63, 64). Other ERK5 substrates include transcription factors [c-Myc, Sap1, FOXO3a, PPAR- γ 1 (5, 26, 61, 135)], kinases [p90RSK, SGK (46, 115)], and Bcl-2 family proteins [Bad (135)]. Phosphorylation by ERK5 has different effects on these substrates. Generally, the activity of proliferative proteins is enhanced, such as c-Myc and SGK, whereas that of pro-apoptotic proteins is inhibited, such as Bad and FOXO3a (135).

Among them, only MEF2C has been shown to be regulated by oxidative stress-activated ERK5 (126). Treatment of PC12 cells with H_2O_2 induced ERK5 activation, which correlated with enhanced MEF2C binding affinity to DNA. Inhibition of ERK5 activity increased H_2O_2 -induced apoptosis and decreased MEF2C binding affinity to DNA, suggesting that MEF2C phosphorylated by ERK5 have antiapoptotic functions in oxidative stress-exposed cells.

JNK and p38

JNK and p38 are classified together as stress-responsive kinases, which are involved in numerous physiological processes. Since JNK and p38 often have similar physiological roles and are in many cases activated by similar signaling pathways, they will partly be discussed together in this review.

JNKs were identified as stress-activated kinases in cells treated with protein synthesis inhibitors (22, 71). The JNKs comprise three proteins, JNK1/2/3, encoded by three genes,

which can be alternatively spliced generating at least 10 isoforms. JNK1 and JNK2 are ubiquitously expressed, whereas JNK3 is highly expressed in brain. The JNK signaling pathway is initiated by activation of several MAP3Ks, including apoptosis signal-regulating kinase (ASK)1/2, MEKK1/2/3/4, mixed lineage kinase (MLK)1/2/3, DLK, and TAK1, which phosphorylate and activate the MAP2Ks, MEK4/7. MEK4/7 phosphorylate JNK on critical threonine and tyrosine residues leading to JNK activation.

p38 was identified in a screen as a target molecule of anti-inflammatory drugs (43, 76). The p38 kinases consist of four proteins, p38 α / β / γ / δ . p38 α is ubiquitously expressed, whereas p38 β , in general, shows low expression and its role in MAPK signaling is unclear. p38 γ and p38 δ are expressed in specific tissues. p38 γ is predominantly expressed in muscle, whereas p38 δ is expressed in salivary, pituitary, and adrenal glands, and they both probably have specialized functions. In the literature, p38 mostly refers to p38 α . In a similar way to JNK, the p38 signaling pathway is initiated by activation of several MAP3Ks, including ASK1/2, MEKK1, MLK3, and TAK1, which phosphorylate and activate the MAP2Ks, MEK3/6 (41, 50, 95, 128, 129). MEK3/6 phosphorylate and activate p38 (23, 95).

JNK and p38 are activated by a variety of stressors, such as osmotic stress (32, 43), UV irradiation (22, 58), heavy metals (80), and chemotherapeutic drugs (29). Although JNK and p38 are mostly known for being activated by stressors, several receptors have also been shown to activate JNK and p38. Proinflammatory cytokine receptors, especially TNF α receptor and Fas, strongly activate JNK and p38 (30, 60, 73, 138), and growth factor receptors have also been implicated in JNK and p38 activation (40, 44).

Physiological roles of JNK and p38 in oxidative stress

Exposure of cells to excessive stress is potentially very harmful to multicellular organisms, and an effective defense to this is that the inflicted cells undergo apoptosis. JNK and p38 have critical roles in stress-induced cell death and are in many cases required for stress-induced apoptosis. Treatment with JNK and p38-specific inhibitors or expression of dominant negative JNK and p38 mutants suppresses apoptosis induced by various types of stress (130). Especially, the sustained activation of JNK and p38 is important for their ability to induce apoptosis (88). Beside important roles of JNK and p38 in apoptosis, they are also involved in several other physiological processes, such as immunity and cell differentiation (17, 25). JNK and p38 were thought to oppose the proliferative functions of ERK1/2 (144), but recently, JNK and p38 have also been shown to have proliferative effects. It has been reported that p38 has proliferative roles in retinal pigment epithelial cells exposed to oxidative stress (112), and that JNK phosphorylates Nrf2, thereby enhancing its transcriptional activity and inhibiting oxidative stress-induced apoptosis (109).

JNK and p38 are both strongly activated by H_2O_2 , and are critical mediators of oxidative stress-induced apoptosis. These JNK and p38 signaling pathways are initiated and regulated by the upstream MAP3Ks. Among various MAP3Ks, ASK1/2, MEKK1, and MLK3 have been reported to be closely associated with oxidative stress. ASK1/2 activate both JNK and p38, whereas MEKK1 and MLK3 preferentially activate JNK in response to oxidative stress

(Fig. 5). In the next section, ASK family proteins will be discussed in detail, since they are critically involved in oxidative stress responses, determining whether or not cells commit to apoptosis.

ROS-regulated signaling pathways of ASK family proteins

The ASK family consists of two members, ASK1/2, which are activated by ROS and mediate downstream activation of JNK and p38, leading to apoptosis (50, 121, 128). The first member to be identified was ASK1, which is ubiquitously expressed in various tissues. H_2O_2 -induced cell death was strongly inhibited in ASK1^{-/-} cells (131), providing direct evidence for the requirement of ASK1 in oxidative stress-induced apoptosis. ASK1 interacts with the antioxidant protein thioredoxin (Trx). This ASK1-Trx complex functions as a redox sensor that initiates the activation of JNK and p38 when cells are exposed to excessive oxidative stress. Under normal conditions, the reduced forms of Cys-32 and -35 in the redox active site of Trx mediate noncovalent binding to the N-terminus of ASK1 and suppress ASK1 kinase activity by inhibiting N-terminal oligomerization between ASK1 molecules (121). When cells are exposed to H_2O_2 , the formation of an intramolecular disulfide bond between Cys-32 and -35 of Trx is induced and this mediates Trx dissociation from ASK1. After this, TRAF2/6 are recruited to ASK1, facilitating ASK1 N-terminus interaction and autophosphorylation of Thr845 (106) (Fig. 6). Activated ASK1 directly phosphorylates and activates MKK4/7 and MKK3/6, leading to JNK and p38 activation. In addition, under H_2O_2 stimulation, internal cysteine residues of ASK1 are oxidized and form intermolecular disulfide bonds between different ASK1 molecules, which are required for the downstream activation of JNK and execution of apoptosis (101). By its thiol reductase activity, Trx reduces these internal disulfide bonds in ASK1, thereby inactivating it (100).

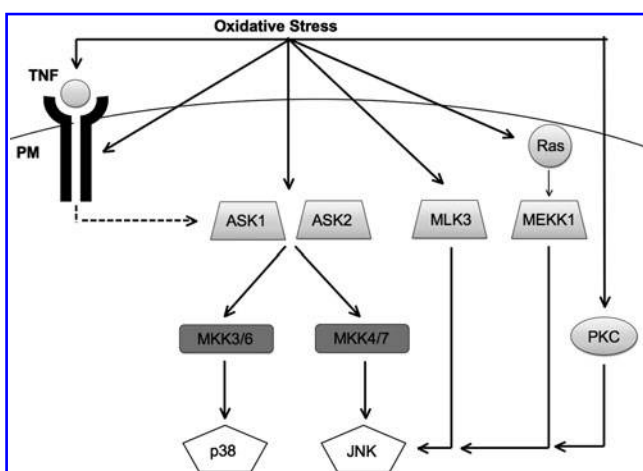


FIG. 5. JNK and p38 signaling pathways in oxidative stress. ASK family proteins have important roles in redox responses and are essential for oxidative stress-induced activation of p38 and JNK. In addition, the MAP3Ks, mixed lineage kinase 3 and MEKK1, are indirectly activated by oxidative stress. ASK, apoptosis signal-regulating kinase; MLK, mixed lineage kinase.

H_2O_2 -dependent activation of ASK1 also plays important roles in receptor signaling. H_2O_2 has dual functions both upstream and downstream of cytokine and growth factor receptors. It has been shown that ROS induces the activation of several receptors independently of ligand binding (110), and that the stimulation of several cytokine and growth factor receptors generates ROS (84, 107), where it functions as a second messenger in downstream signaling pathways. ASK1 has been shown to be activated by the activation of several receptors in a ROS-dependent manner.

TNF α is a well-known proinflammatory cytokine that has crucial roles in inflammation, proliferation, and cell death. TNF α binds to the TNF α receptor and initiates several intracellular pathways, including the ASK1-JNK/p38 pathway. The activation of TNF α receptor induces ROS production (38), and ROS scavengers inhibit TNF α -induced activation of JNK and p38 (45, 125), suggesting that ROS production is required for TNF α -induced activation of JNK and p38. ASK1 is also required for this activation and apoptosis, since TNF α -induced activation of JNK and p38 and apoptosis are reduced in ASK1^{-/-} cells (131). Upon TNF α stimulation, ASK1 is activated in a similar way to oxidative stress, by ROS-dependent dissociation of Trx from ASK1 and subsequent association of TRAF2 (82, 108).

Toll-like receptors (TLRs) play key roles in innate immunity and are activated by broadly shared pathogenic markers such as pathogen-associated molecular patterns (PAMPs). Lipopolysaccharide (LPS) is one of the PAMPs that bind to and activate TLR4. Activation of TLR4 leads to association of TRAF6 and other adaptor proteins, triggering intracellular signaling cascades, including JNK and p38 pathways (147). LPS ligation to TLR4 was found to induce ROS production, which activated the TRAF6-ASK1-p38 signaling pathway (90). Treatment with ROS scavengers inhibited LPS-induced

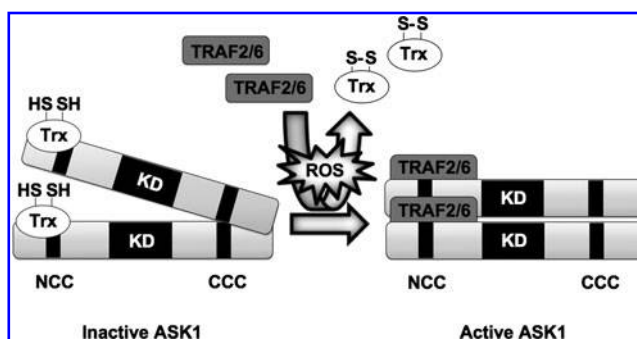


FIG. 6. Activation mechanism of ASK1 by reactive oxygen species. ASK1 contains a central serine/threonine kinase domain and two coiled-coil domains in its N- and C-terminus. Under stable conditions, ASK1 homo-oligomerizes through the C-terminal coiled-coil domain, and forms an inactive complex, including the antioxidant thioredoxin, which blocks N-terminal interaction of ASK1. When cells are exposed to oxidative stress, thioredoxin is oxidized and dissociates from ASK1, and then TRAF2/6 are recruited, facilitating ASK1 N-terminal interaction through the N-terminal coiled-coil domain. This leads to close proximity between ASK1 kinase domains and results in ASK1 activation by autophosphorylation of Thr845. CCC, C-terminal coiled-coil domain; KD, kinase domain; NCC, N-terminal coiled-coil domain; ROS, reactive oxygen species; Trx, thioredoxin.

activation of ASK1 and p38, and prevented association of TRAF6 with ASK1. Probably, LPS-induced ROS production activates ASK1 through the dissociation of Trx from ASK1 followed by the recruitment of TRAF6.

ASK2, a member of the ASK family, has been shown to be a functional binding partner of ASK1, and is also critically involved in oxidative stress-induced apoptosis. ASK2 is highly expressed in tissues that are exposed to the external environment, such as skin, lung, and gastrointestinal tract (54). In contrast to ASK1, ASK2 is intrinsically unstable, and the stability of ASK2 depends on heteromeric complex formation with ASK1 (128). Interestingly, although ASK2 stability depends on ASK1 expression, ASK1 kinase activity is not important for ASK2 activation. When ASK2 was overexpressed with a kinase negative mutant of ASK1, ASK2 was autophosphorylated and induced the activation of p38 and JNK. H₂O₂ was found to induce activation of ASK2, and ASK2 was required for oxidative stress-induced JNK activation in B16 melanoma cells and apoptosis. ROS generated by carcinogenic chemicals also induced ASK2-dependent apoptosis in keratinocytes (54).

ROS-regulated signaling pathways of other kinases that activate JNK

JNK can also be activated by other oxidative stress-activated pathways. As mentioned before, oxidative stress induces the activation of Ras, which leads to activation of Raf and subsequently ERK1/2. However, oxidative stress-activated Ras have also been shown to activate the MAP3K MEKK1. Ras-activated MEKK1 phosphorylates MEK4/7, leading to JNK activation (56). The MEKK1-JNK pathway cooperated with and positively regulated the nuclear factor kappa B signaling pathway, suggesting that, in this case, JNK has proliferative roles. However, MEKK1 can also be negatively regulated by oxidative stress. Menadione-induced oxidative stress inhibited MEKK1 activity by inducing glutathionylation of a crucial residue in the ATP binding domain of MEKK1, Cys1238, thereby probably inhibiting ATP binding (18). Another MAP3K activated by ROS is MLK3. Treatment with genipin, an antidiabetic drug, induced ROS production *via* NADPH oxidase, and activated the MLK3-JNK pathway (48). Transfection with a dominant negative mutant of MLK3 inhibited genipin-induced JNK activation, indicating that MLK3 is required for the genipin-ROS-JNK pathway. A JNK inhibitor suppressed genipin-induced cell death, suggesting that the MLK3-JNK pathway is pro-apoptotic. PKC also has several roles in the activation of JNK. TPA-induced ROS production was found to depend on PKC, and in PKC-deficient cells, the activation of MEKK1 and JNK was abolished (20). PKC also has more direct functions in oxidative stress-induced JNK activation. Ethanol-induced ROS production caused PKC activation, leading to PKC-dependent degradation of MAPK phosphatase 1. This resulted in sustained activation of JNK, which promoted apoptosis in hepatocytes (90).

Regulation of ASK1-JNK/p38 signaling pathway in oxidative stress

The ROS-ASK1-JNK/p38 signaling axis is extensively regulated by different types of proteins, such as kinases and

phosphatases. Especially, ASK1 is strictly regulated, perhaps due to its critical role as a redox-sensor.

Autophosphorylation of ASK1 at Thr845 is critical for ASK1 activation, and several phosphatases have been shown to dephosphorylate ASK1 at this residue, thereby negatively regulating the ASK1-JNK/p38 signaling pathway. The threonine/serine phosphatases, PP5 and PP2C ϵ , negatively regulate ASK1 activation. PP5 recognizes H₂O₂-activated ASK1 and, in a negative feedback mechanism, dephosphorylates Thr845, inhibiting ASK1 activation in response to H₂O₂ (96). PP2C ϵ was found to inhibit basal activity of ASK1. Under basal conditions, PP2C ϵ interacted endogenously with ASK1 and retained ASK1 in its inactive form by dephosphorylation of Thr845. Exposure to H₂O₂ induced the dissociation of PP2C ϵ from ASK1, thereby increasing autophosphorylation and activity of ASK1 (120).

ASK1 activity is also regulated by phosphorylation of other residues than Thr845. The 14-3-3 protein family is composed of highly conserved phospho-threonine/phospho-serine binding proteins that are involved in regulation of diverse intracellular signaling pathways (97). Under stable conditions, 14-3-3 proteins bind to ASK1 by recognizing phosphorylated Ser966, and thereby inhibiting ASK1 kinase activity (150). H₂O₂-induced dephosphorylation of ASK1 at Ser966 correlated with the dissociation of 14-3-3 proteins from ASK1, resulting in enhanced ASK1 activity (37). PP2B was found to be responsible for dephosphorylation of ASK1 at Ser966 and subsequent dissociation of 14-3-3 proteins (83). 14-3-3 Proteins can also dissociate from ASK1 in other ways. Recently, Ste20/oxidant stress response kinase (SOK) 1 has been shown to phosphorylate 14-3-3 ζ at Ser58 in an oxidative stress-dependent manner, thereby inducing the dissociation of 14-3-3 ζ from ASK1 (152). Phosphoglycerate mutases (PGAMs) are involved in glycolysis, but recently, it was shown that PGAM family member, PGAM5, regulates ASK1 activity. PGAM5 interacted endogenously with ASK1 and dephosphorylated ASK1 at inhibitory phosphorylation sites, leading to ASK1 activation (127). The Akt/PKB family consists of threonine/serine kinases that promote cellular proliferation and survival by inactivating pro-apoptotic proteins, such as Bax and Bad (21, 34), *via* their direct phosphorylation. Akt was shown to interact with ASK1 and induced phosphorylation of ASK1 at Ser83 (68). The phosphorylation of Ser83 decreased oxidative stress-induced activation of ASK1 and apoptosis.

ASK1 is also regulated by other types of proteins. It has been reported that DJ-1 is involved in the progression of early onset familial Parkinson's disease, and has cytoprotective effects, which in part are due to its antioxidant activity. DJ-1 was found to interact directly with ASK1 and negatively regulate ASK1 activation. Overexpression of DJ-1 inhibited H₂O₂-induced activation of ASK1 by preventing ASK1 homooligomerization (94). Further, DJ-1 has been shown to block dissociation of Trx from ASK1, thereby retaining ASK1 in its inactive configuration (51). Recently, the deubiquitinating enzyme USP9X was found to be a critical regulator of ASK1 activation during oxidative stress (102). USP9X interacted with ASK1 in a H₂O₂-dependent manner and prevented ubiquitin-mediated degradation of ASK1. In USP9X^{-/-} cells, H₂O₂-induced JNK activation and apoptosis were reduced, suggesting that USP9X enhances the apoptotic response of ASK1 by inhibiting its ubiquitin-dependent degradation.

Other components of the ASK1-JNK/p38 pathway are also regulated by oxidative stress. Several proteins have been shown to bind to and regulate p38 and JNK. The anti-oxidant protein peroxiredoxin I was shown to be required for p38 activation in H_2O_2 -treated macrophages (16). Another antioxidant protein, glutathione *S*-transferase Pi, was found to bind to JNK and inhibit its activity under normal conditions (4). H_2O_2 induced the dissociation of glutathione *S*-transferase Pi from JNK, allowing JNK activation. As mentioned earlier, sustained activation of JNK is required for stress-induced apoptosis. JNK-inactivating phosphatases were found to be inhibited by ROS, resulting in sustained JNK activation and cell death (62). Ferritin heavy chain, an iron storage factor, was found to inhibit TNF α -induced ROS production, thereby blocking sustained activation of JNK and cell death (111). Similar to ERK1/2, JNK and p38 pathways are also regulated by H_2O_2 -induced inhibition of PTPs, since overexpression of PTPs inhibits JNK and p38 activation by H_2O_2 stimulation (78).

JNK substrates

JNK has at least around 60 substrates (9), and just as for ERK1/2, approximately half of the substrates are nuclear, whereas the other half are located in the cytosol. In fact, many of the details discussed in the section of ERK1/2 substrates also apply to JNK, including an identical phosphorylation consensus motif. However, the binding domain of JNK substrates to JNK is different from that of ERK1/2 substrates, and is termed JNK binding domain. The effect of JNK phosphorylation on substrates is also similar to ERK1/2 effects. Theoretically, all JNK substrates are candidates for JNK phosphorylation induced by oxidative stress, and in contrast to ERK1/2, several substrates have been shown to be phosphorylated by oxidative stress-activated JNK (Fig. 7).

JNK has been shown to regulate several pro-apoptotic and anti-apoptotic proteins in oxidative stress. c-Jun is one of the best characterized substrates of JNK. c-Jun functions as a transcription factor, which regulates cell proliferation and death, and forms the AP-1 complex together with other transcription factors, such as c-Fos and ATF-2. c-Jun has been shown to be involved in oxidative stress-induced cell death. In hepatocytes exposed to menadione-induced oxidative stress, JNK activation and cell death were increased. Adenoviral expression of a dominant negative mutant of c-Jun blocked menadione-induced apoptosis, suggesting that c-Jun is required for oxidative stress-induced apoptosis in hepatocytes (19). JNK regulates the pro-apoptotic adaptor protein p66^{ShcA}, which has important roles in oxidative stress-induced cell death. Mice lacking p66^{ShcA} showed decreased sensitivity to oxidative stressors, such as H_2O_2 and UV irradiation, suggesting that p66^{ShcA} is required for oxidative stress-induced cell death (93). Further, pro-apoptotic signals induced ROS production in the mitochondria, which depended on p66^{ShcA} (36). Phosphorylation of p66^{ShcA} at Ser36 was shown to be critical for oxidative stress-induced cell death, and JNK was identified as the responsible kinase for Ser36 phosphorylation in response to UV irradiation (75). c-Abl is a pro-apoptotic tyrosine kinase, which is indirectly regulated by JNK. Nuclear c-Abl is activated by diverse genotoxic agents and induces apoptosis. Under stable conditions, c-Abl is localized in the cytosol and binds to 14-3-3

proteins. Oxidative stress-activated JNK phosphorylates 14-3-3 proteins, which are dissociated from c-Abl, allowing c-Abl translocation to the nucleus (149). JNK also regulates anti-apoptotic proteins. Mcl-1, an anti-apoptotic member of the Bcl-2 family, was found to be phosphorylated on Ser121 and Thr163 by JNK in H_2O_2 -stimulated cells (53). Transfection with an unphosphorylatable Mcl-1 mutant decreased oxidative stress-induced apoptosis compared to Mcl-1 wild-type, indicating that JNK-mediated phosphorylation inhibits Mcl-1 anti-apoptotic activity.

As discussed earlier, under some conditions, JNK also has anti-apoptotic roles in response to oxidative stress. SIRT1 is a nicotinamide adenine dinucleotide-dependent deacetylase that is involved in cellular protection against stress and apoptosis. SIRT1 deacetylates a number of nuclear substrates, including histones, FOXOs, PGC-1 α , and p53. JNK was shown to interact with and induce phosphorylation of SIRT1 in an oxidative stress-dependent manner (104). JNK-mediated phosphorylation induced SIRT1 translocation to the nucleus and increased its enzymatic activity. This result partly accounts for the anti-apoptotic response of JNK. The transcription factors of the FOXO protein family have also been implicated in cellular protection against oxidative stress, by the upregulation of antioxidants such as MnSOD and catalase. H_2O_2 -activated JNK was found to phosphorylate FOXO4 on Thr447 and Thr451 and facilitate FOXO4 translocation to the nucleus and an increase in transcriptional activity, thereby enhancing the protective response to oxidative stress (28). Similar to ERK1/2, JNK seems to regulate Nrf2 activity. Expression of JNK increased transcriptional activity of Nrf2, which was inhibited by overexpression of a JNK dominant negative mutant (66). JNK was also found to induce translocation of Nrf2 to the nucleus (145). In addition, JNK phosphorylated c-Jun inter-

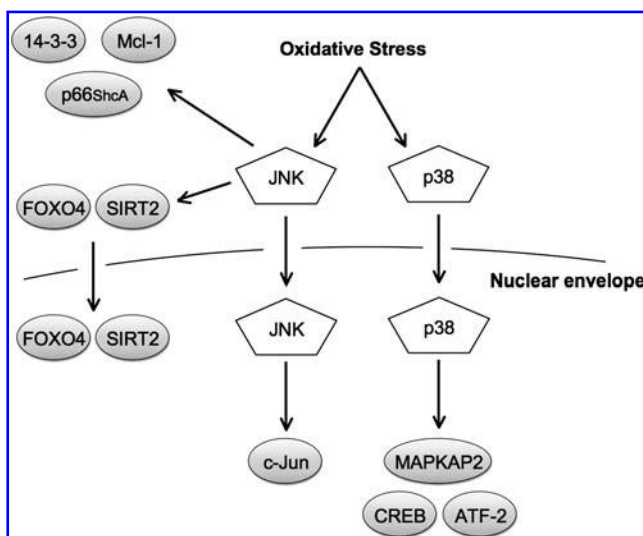


FIG. 7. JNK and p38 substrates in oxidative stress. The figure shows the reported substrates of JNK and p38 under oxidative stress. JNK and p38 have both cytosolic and nuclear substrates. JNK and p38 phosphorylate both pro- and antiapoptotic substrates, and regulate them in a way to promote apoptosis. The details are described in the text.

acts with Nrf2, which induced transcription of several anti-oxidant genes (57, 134).

p38 substrates

p38 has numerous and diverse substrates, and the regulatory mechanisms of p38 substrates share most of the characteristics discussed in the section of ERK1/2 and JNK substrates. p38 substrates specifically activated by oxidative stress are poorly characterized, but both pro- and anti-apoptotic proteins have been identified (Fig. 7).

The transcription factors CREB and ATF-2 are among the best characterized substrates of p38, and both have been shown to be phosphorylated by p38 under oxidative stress conditions. Cadmium-induced oxidative stress resulted in sustained activation of p38 and phosphorylation of CREB and ATF-2 (119). p38 inhibitors blocked phosphorylation of CREB and ATF-2 and cadmium-induced cell death, suggesting that p38 mediates oxidative stress-induced apoptosis in part by the activation of CREB and ATF-2. Another well-characterized substrate of p38 is the kinase MAPKAPK2. Treatment of neonatal rat ventricular myocytes with H₂O₂ induced MAPKAPK2 phosphorylation. Activated MAPKAPK2 subsequently phosphorylated the small heat shock protein HSP25/27, which may be important for cardioprotection. These effects were blocked by p38 inhibitors, suggesting a protective effect of p38 on myocytes exposed to oxidative stress (15). The p38 pathway also regulates Nrf2. p38 has been reported to be required for Nrf2 binding to promoters by an unknown mechanism (154). However, p38 has also been shown to inhibit Nrf2 activity by inducing formation of the Nrf2 and Keap1 complex (67), thereby inhibiting Nrf2 nuclear translocation. Whether p38 activates or inhibits Nrf2 is probably both cell type and cell context specific.

Conclusion

ROS is produced by numerous chemicals and stresses and plays roles in diverse pathologies. Especially in cancer cells, ROS seems to be a key player in mediating tumor development by diverse mechanisms. Recently, MAPKs were shown to have crucial roles in inhibiting the oncogenic potential of ROS by inducing apoptosis (24). This emphasizes the importance of understanding the effects of ROS and their link to MAPKs in both normal and pathological conditions.

The big question is how cells determine the appropriate response to a given ROS stimuli. H₂O₂ functions as a proliferatory signal, a second messenger, or as a signal for apoptosis by the activation of different MAPKs. When cells are exposed to H₂O₂, often all MAPKs are activated and send conflicting signals to determine cell fate. This necessitates strict regulation to ensure that inappropriate responses are avoided. Low concentrations and local cellular distribution of ROS mediates its role as a second messenger, whereas high concentrations of ROS through out the cell mediate its role as a death signal. Perhaps by keeping the ROS levels low and local, the cell ensures that the ROS-induced apoptotic stimuli by activation of p38 and JNK are not sufficient to initiate the apoptotic program. As ROS levels increase, a certain threshold is reached, and apoptosis is induced. In the end, what determines the cell response is the availability of MAPK substrates, and this is largely cell type specific. Which substrates are

specifically activated by ROS in different cells remains poorly characterized and will be an important future area of research.

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References

1. Abe J and Berk BC. Fyn and JAK2 mediate Ras activation by reactive oxygen species. *J Biol Chem* 274: 21003–21010, 1999.
2. Abe J, Kusuvara M, Ulevitch RJ, Berk BC, and Lee JD. Big mitogen-activated protein kinase 1 (BMK1) is a redox-sensitive kinase. *J Biol Chem* 271: 16586–16590, 1996.
3. Abe J, Takahashi M, Ishida M, Lee JD, and Berk BC. c-Src is required for oxidative stress-mediated activation of big mitogen-activated protein kinase 1. *J Biol Chem* 272: 20389–20394, 1997.
4. Adler V, Yin Z, Fuchs SY, Benezra M, Rosario L, Tew KD, Pincus MR, Sardana M, Henderson CJ, Wolf CR, Davis RJ, and Ronai Z. Regulation of JNK signaling by GSTp. *EMBO J* 18: 1321–1334, 1999.
5. Akaike M, Che W, Marmarosh NL, Ohta S, Osawa M, Ding B, Berk BC, Yan C, and Abe J. The hinge-helix 1 region of peroxisome proliferator-activated receptor gamma1 (PPARGgamma1) mediates interaction with extracellular signal-regulated kinase 5 and PPARGgamma1 transcriptional activation: involvement in flow-induced PPARGgamma activation in endothelial cells. *Mol Cell Biol* 24: 8691–8704, 2004.
6. Atherfold PA, Norris MS, Robinson PJ, Gelfand EW, and Franklin RA. Calcium-induced ERK activation in human T lymphocytes. *Mol Immunol* 36: 543–549, 1999.
7. Bae YS, Kang SW, Seo MS, Baines IC, Tekle E, Chock PB, and Rhee SG. Epidermal growth factor (EGF)-induced generation of hydrogen peroxide. Role in EGF receptor-mediated tyrosine phosphorylation. *J Biol Chem* 272: 217–221, 1997.
8. Barsyte-Lovejoy D, Galanis A, Clancy A, and Sharrocks AD. ERK5 is targeted to myocyte enhancer factor 2A (MEF2A) through a MAPK docking motif. *Biochem J* 381: 693–699, 2004.
9. Bogoyevitch MA and Kobe B. Uses for JNK: the many and varied substrates of the c-Jun N-terminal kinases. *Microbiol Mol Biol Rev* 70: 1061–1095, 2006.
10. Bonomini F, Tengattini S, Fabiano A, Bianchi R, and Rezzani R. Atherosclerosis and oxidative stress. *Histol Histopathol* 23: 381–390, 2008.
11. Burdon RH. Superoxide and hydrogen peroxide in relation to mammalian cell proliferation. *Free Radic Biol Med* 18: 775–794, 1995.
12. Burgering BM, de Vries-Smits AM, Medema RH, van Weeren PC, Tertoolen LG, and Bos JL. Epidermal growth factor induces phosphorylation of extracellular signal-regulated kinase 2 via multiple pathways. *Mol Cell Biol* 13: 7248–7256, 1993.
13. Cavanaugh JE, Ham J, Hetman M, Poser S, Yan C, and Xia Z. Differential regulation of mitogen-activated protein

- kinases ERK1/2 and ERK5 by neurotrophins, neuronal activity, and cAMP in neurons. *J Neurosci* 21: 434–443, 2001.
14. Chao TS, Byron KL, Lee KM, Villereal M, and Rosner MR. Activation of MAP kinases by calcium-dependent and calcium-independent pathways. Stimulation by thapsigargin and epidermal growth factor. *J Biol Chem* 267: 19876–19883, 1992.
 15. Clerk A, Michael A, and Sugden PH. Stimulation of multiple mitogen-activated protein kinase sub-families by oxidative stress and phosphorylation of the small heat shock protein, HSP25/27, in neonatal ventricular myocytes. *Biochem J* 333 (Pt 3): 581–589, 1998.
 16. Conway JP and Kinter M. Dual role of peroxiredoxin I in macrophage-derived foam cells. *J Biol Chem* 281: 27991–28001, 2006.
 17. Craig EA, Stevens MV, Vaillancourt RR, and Camenisch TD. MAP3Ks as central regulators of cell fate during development. *Dev Dyn* 237: 3102–3114, 2008.
 18. Cross JV and Templeton DJ. Oxidative stress inhibits MEKK1 by site-specific glutathionylation in the ATP-binding domain. *Biochem J* 381: 675–683, 2004.
 19. Czaja MJ, Liu H, and Wang Y. Oxidant-induced hepatocyte injury from menadione is regulated by ERK and AP-1 signaling. *Hepatology* 37: 1405–1413, 2003.
 20. Datta R, Yoshinaga K, Kaneki M, Pandey P, and Kufe D. Phorbol ester-induced generation of reactive oxygen species is protein kinase cbeta-dependent and required for SAPK activation. *J Biol Chem* 275: 41000–41003, 2000.
 21. Datta SR, Dudek H, Tao X, Masters S, Fu H, Gotoh Y, and Greenberg ME. Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. *Cell* 91: 231–241, 1997.
 22. 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.
 23. Derijard B, Raingeaud J, Barrett T, Wu IH, Han J, Ulevitch RJ, and Davis RJ. Independent human MAP-kinase signal transduction pathways defined by MEK and MKK isoforms. *Science* 267: 682–685, 1995.
 24. Dolado I, Swat A, Ajenjo N, De Vita G, Cuadrado A, and Nebreda AR. p38alpha MAP kinase as a sensor of reactive oxygen species in tumorigenesis. *Cancer Cell* 11: 191–205, 2007.
 25. Dong C, Davis RJ, and Flavell RA. MAP kinases in the immune response. *Annu Rev Immunol* 20: 55–72, 2002.
 26. English JM, Pearson G, Baer R, and Cobb MH. Identification of substrates and regulators of the mitogen-activated protein kinase ERK5 using chimeric protein kinases. *J Biol Chem* 273: 3854–3860, 1998.
 27. Enslen H, Tokumitsu H, Stork PJ, Davis RJ, and Soderling TR. Regulation of mitogen-activated protein kinases by a calcium/calmodulin-dependent protein kinase cascade. *Proc Natl Acad Sci U S A* 93: 10803–10808, 1996.
 28. Essers MA, Weijzen S, de Vries-Smits AM, Saarloos I, de Ruiter ND, Bos JL, and Burgering BM. FOXO transcription factor activation by oxidative stress mediated by the small GTPase Ral and JNK. *EMBO J* 23: 4802–4812, 2004.
 29. Fan M and Chambers TC. Role of mitogen-activated protein kinases in the response of tumor cells to chemotherapy. *Drug Resist Updat* 4: 253–267, 2001.
 30. Fiers W, Beyaert R, Boone E, Cornelis S, Declercq W, Decoster E, Denecker G, Depuydt B, De Valck D, De Wilde G, Goossens V, Grooten J, Haegeman G, Heyninck K, Penning L, Plaisance S, Vancompernelle K, Van Crielinge W, Vandenabeele P, Vanden Berghe W, Van de Craen M, Vandevoorde V, and Vercammen D. TNF-induced intracellular signaling leading to gene induction or to cytotoxicity by necrosis or by apoptosis. *J Inflamm* 47: 67–75, 1995.
 31. Fridovich I. The biology of oxygen radicals. *Science* 201: 875–880, 1978.
 32. Galcheva-Gargova Z, Derijard B, Wu IH, and Davis RJ. An osmosensing signal transduction pathway in mammalian cells. *Science* 265: 806–808, 1994.
 33. Garcia L, Garcia F, Llorens F, Unzeta M, Itarte E, and Gomez N. PP1/PP2A phosphatases inhibitors okadaic acid and calyculin A block ERK5 activation by growth factors and oxidative stress. *FEBS Lett* 523: 90–94, 2002.
 34. Gardai SJ, Hildeman DA, Frankel SK, Whitlock BB, Frasch SC, Borregaard N, Marrack P, Bratton DL, and Henson PM. Phosphorylation of Bax Ser184 by Akt regulates its activity and apoptosis in neutrophils. *J Biol Chem* 279: 21085–21095, 2004.
 35. Gille G and Sigler K. Oxidative stress and living cells. *Folia Microbiol (Praha)* 40: 131–152, 1995.
 36. Giorgio M, Migliaccio E, Orsini F, Paolucci D, Moroni M, Contursi C, Pelliccia G, Luzi L, Minucci S, Marcaccio M, Pinton P, Rizzuto R, Bernardi P, Paolucci F, and Pelicci PG. Electron transfer between cytochrome c and p66Shc generates reactive oxygen species that trigger mitochondrial apoptosis. *Cell* 122: 221–233, 2005.
 37. 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.
 38. Goossens V, Grooten J, De Vos K, and Fiers W. Direct evidence for tumor necrosis factor-induced mitochondrial reactive oxygen intermediates and their involvement in cytotoxicity. *Proc Natl Acad Sci U S A* 92: 8115–8119, 1995.
 39. Graves LM, Bornfeldt KE, Sidhu JS, Argast GM, Raines EW, Ross R, Leslie CC, and Krebs EG. Platelet-derived growth factor stimulates protein kinase A through a mitogen-activated protein kinase-dependent pathway in human arterial smooth muscle cells. *J Biol Chem* 271: 505–511, 1996.
 40. Grill B and Schrader JW. Activation of Rac-1, Rac-2, and Cdc42 by hemopoietic growth factors or cross-linking of the B-lymphocyte receptor for antigen. *Blood* 100: 3183–3192, 2002.
 41. Guan Z, Buckman SY, Pentland AP, Templeton DJ, and Morrison AR. Induction of cyclooxygenase-2 by the activated MEKK1 → SEK1/MKK4 → p38 mitogen-activated protein kinase pathway. *J Biol Chem* 273: 12901–12908, 1998.
 42. Guyton KZ, Liu Y, Gorospe M, Xu Q, and Holbrook NJ. Activation of mitogen-activated protein kinase by H2O2. Role in cell survival following oxidant injury. *J Biol Chem* 271: 4138–4142, 1996.
 43. 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.
 44. Hashimoto A, Kurosaki M, Gotoh N, Shibuya M, and Kurosaki T. Shc regulates epidermal growth factor-induced activation of the JNK signaling pathway. *J Biol Chem* 274: 20139–20143, 1999.
 45. Hashimoto S, Gon Y, Matsumoto K, Takeshita I, and Horie T. N-acetylcysteine attenuates TNF-alpha-induced p38 MAP kinase activation and p38 MAP kinase-mediated IL-8

- production by human pulmonary vascular endothelial cells. *Br J Pharmacol* 132: 270–276, 2001.
46. Hayashi M, Tapping RI, Chao TH, Lo JF, King CC, Yang Y, and Lee JD. BMK1 mediates growth factor-induced cell proliferation through direct cellular activation of serum and glucocorticoid-inducible kinase. *J Biol Chem* 276: 8631–8634, 2001.
47. Herrling T, Fuchs J, Rehberg J, and Groth N. UV-induced free radicals in the skin detected by ESR spectroscopy and imaging using nitroxides. *Free Radic Biol Med* 35: 59–67, 2003.
48. Hong HY and Kim BC. Mixed lineage kinase 3 connects reactive oxygen species to c-Jun NH2-terminal kinase-induced mitochondrial apoptosis in genipin-treated PC3 human prostate cancer cells. *Biochem Biophys Res Commun* 362: 307–312, 2007.
49. Huang HC, Nguyen T, and Pickett CB. Regulation of the antioxidant response element by protein kinase C-mediated phosphorylation of NF-E2-related factor 2. *Proc Natl Acad Sci U S A* 97: 12475–12480, 2000.
50. 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.
51. Im JY, Lee KW, Junn E, and Mouradian MM. DJ-1 protects against oxidative damage by regulating the thioredoxin/ASK1 complex. *Neurosci Res* 67: 203–208, 2010.
52. Imlay JA and Linn S. DNA damage and oxygen radical toxicity. *Science* 240: 1302–1309, 1988.
53. 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.
54. 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.
55. Itoh K, Wakabayashi N, Katoh Y, Ishii T, Igarashi K, Engel JD, and Yamamoto M. Keap1 represses nuclear activation of antioxidant responsive elements by Nrf2 through binding to the amino-terminal Neh2 domain. *Genes Dev* 13: 76–86, 1999.
56. Janssen-Heininger YM, Macara I, and Mossman BT. Cooperativity between oxidants and tumor necrosis factor in the activation of nuclear factor (NF)-kappaB: requirement of Ras/mitogen-activated protein kinases in the activation of NF-kappaB by oxidants. *Am J Respir Cell Mol Biol* 20: 942–952, 1999.
57. Jeyapaul J and Jaiswal AK. Nrf2 and c-Jun regulation of antioxidant response element (ARE)-mediated expression and induction of gamma-glutamylcysteine synthetase heavy subunit gene. *Biochem Pharmacol* 59: 1433–1439, 2000.
58. Jinlian L, Yingbin Z, and Chunbo W. p38 MAPK in regulating cellular responses to ultraviolet radiation. *J Biomed Sci* 14: 303–312, 2007.
59. Johnson GL, Gardner AM, Lange-Carter C, Qian NX, Russell M, and Winitz S. How does the G protein, Gi2, transduce mitogenic signals? *J Cell Biochem* 54: 415–422, 1994.
60. 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.
61. Kamakura S, Moriguchi T, and Nishida E. Activation of the protein kinase ERK5/BMK1 by receptor tyrosine kinases. Identification and characterization of a signaling pathway to the nucleus. *J Biol Chem* 274: 26563–26571, 1999.
62. Kamata H, Honda S, Maeda S, Chang L, Hirata H, and Karin M. Reactive oxygen species promote TNFalpha-induced death and sustained JNK activation by inhibiting MAP kinase phosphatases. *Cell* 120: 649–661, 2005.
63. Kasler HG, Victoria J, Duramad O, and Winoto A. ERK5 is a novel type of mitogen-activated protein kinase containing a transcriptional activation domain. *Mol Cell Biol* 20: 8382–8389, 2000.
64. Kato Y, Kravchenko VV, Tapping RI, Han J, Ulevitch RJ, and Lee JD. BMK1/ERK5 regulates serum-induced early gene expression through transcription factor MEF2C. *EMBO J* 16: 7054–7066, 1997.
65. Kato Y, Tapping RI, Huang S, Watson MH, Ulevitch RJ, and Lee JD. Bmk1/Erk5 is required for cell proliferation induced by epidermal growth factor. *Nature* 395: 713–716, 1998.
66. Keum YS, Owuor ED, Kim BR, Hu R, and Kong AN. Involvement of Nrf2 and JNK1 in the activation of antioxidant responsive element (ARE) by chemopreventive agent phenethyl isothiocyanate (PEITC). *Pharm Res* 20: 1351–1356, 2003.
67. Keum YS, Yu S, Chang PP, Yuan X, Kim JH, Xu C, Han J, Agarwal A, and Kong AN. Mechanism of action of sulforaphane: inhibition of p38 mitogen-activated protein kinase isoforms contributing to the induction of antioxidant response element-mediated heme oxygenase-1 in human hepatoma HepG2 cells. *Cancer Res* 66: 8804–8813, 2006.
68. 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.
69. Knebel A, Rahmsdorf HJ, Ullrich A, and Herrlich P. Dephosphorylation of receptor tyrosine kinases as target of regulation by radiation, oxidants or alkylating agents. *EMBO J* 15: 5314–5325, 1996.
70. Kourie JJ. Interaction of reactive oxygen species with ion transport mechanisms. *Am J Physiol* 275: C1–C24, 1998.
71. Kyriakis JM, Banerjee P, Nikolakaki E, Dai T, Rubie EA, Ahmad MF, Avruch J, and Woodgett JR. The stress-activated protein kinase subfamily of c-Jun kinases. *Nature* 369: 156–160, 1994.
72. Lander HM, Milbank AJ, Tauras JM, Hajjar DP, Hempstead BL, Schwartz GD, Kraemer RT, Mirza UA, Chait BT, Burk SC, and Quilliam LA. Redox regulation of cell signalling. *Nature* 381: 380–381, 1996.
73. Latinis KM and Koretzky GA. Fas ligation induces apoptosis and Jun kinase activation independently of CD45 and Lck in human T cells. *Blood* 87: 871–875, 1996.
74. Lau AT, Wang Y, and Chiu JF. Reactive oxygen species: current knowledge and applications in cancer research and therapeutic. *J Cell Biochem* 104: 657–667, 2008.
75. Le S, Connors TJ, and Maroney AC. c-Jun N-terminal kinase specifically phosphorylates p66ShcA at serine 36 in response to ultraviolet irradiation. *J Biol Chem* 276: 48332–48336, 2001.

76. Lee JC, Laydon JT, McDonnell PC, Gallagher TF, Kumar S, Green D, McNulty D, Blumenthal MJ, Heys JR, Landvatter SW, *et al.* A protein kinase involved in the regulation of inflammatory cytokine biosynthesis. *Nature* 372: 739–746, 1994.
77. Lee JS, Kim SY, Kwon CH, and Kim YK. EGFR-dependent ERK activation triggers hydrogen peroxide-induced apoptosis in OK renal epithelial cells. *Arch Toxicol* 80: 337–346, 2006.
78. Lee K and Esselman WJ. Inhibition of PTPs by H₂O₂ regulates the activation of distinct MAPK pathways. *Free Radic Biol Med* 33: 1121–1132, 2002.
79. Lee WC, Choi CH, Cha SH, Oh HL, and Kim YK. Role of ERK in hydrogen peroxide-induced cell death of human glioma cells. *Neurochem Res* 30: 263–270, 2005.
80. Leonard SS, Harris GK, and Shi X. Metal-induced oxidative stress and signal transduction. *Free Radic Biol Med* 37: 1921–1942, 2004.
81. Lev S, Moreno H, Martinez R, Canoll P, Peles E, Musacchio JM, Plowman GD, Rudy B, and Schlessinger J. Protein tyrosine kinase PYK2 involved in Ca²⁺-induced regulation of ion channel and MAP kinase functions. *Nature* 376: 737–745, 1995.
82. Liu H, Nishitoh H, Ichijo H, and Kyriakis JM. Activation of apoptosis signal-regulating kinase 1 (ASK1) by tumor necrosis factor receptor-associated factor 2 requires prior dissociation of the ASK1 inhibitor thioredoxin. *Mol Cell Biol* 20: 2198–2208, 2000.
83. 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.
84. Lo YY and Cruz TF. Involvement of reactive oxygen species in cytokine and growth factor induction of c-fos expression in chondrocytes. *J Biol Chem* 270: 11727–11730, 1995.
85. Lo YY, Wong JM, and Cruz TF. Reactive oxygen species mediate cytokine activation of c-Jun NH₂-terminal kinases. *J Biol Chem* 271: 15703–15707, 1996.
86. Mallis RJ, Buss JE, and Thomas JA. Oxidative modification of H-ras: S-thiolation and S-nitrosylation of reactive cysteines. *Biochem J* 355: 145–153, 2001.
87. Manea A, Manea SA, Gafencu AV, Raicu M, and Simionescu M. AP-1-dependent transcriptional regulation of NADPH oxidase in human aortic smooth muscle cells: role of p22phox subunit. *Arterioscler Thromb Vasc Biol* 28: 878–885, 2008.
88. Mansouri A, Ridgway LD, Korapati AL, Zhang Q, Tian L, Wang Y, Siddik ZH, Mills GB, and Claret FX. Sustained activation of JNK/p38 MAPK pathways in response to cisplatin leads to Fas ligand induction and cell death in ovarian carcinoma cells. *J Biol Chem* 278: 19245–19256, 2003.
89. Marinissen MJ, Chiariello M, Pallante M, and Gutkind JS. A network of mitogen-activated protein kinases links G protein-coupled receptors to the c-jun promoter: a role for c-Jun NH₂-terminal kinase, p38s, and extracellular signal-regulated kinase 5. *Mol Cell Biol* 19: 4289–4301, 1999.
90. 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.
91. McCubrey JA, Lahair MM, and Franklin RA. Reactive oxygen species-induced activation of the MAP kinase signaling pathways. *Antioxid Redox Signal* 8: 1775–1789, 2006.
92. McMahon M, Itoh K, Yamamoto M, and Hayes JD. Keap1-dependent proteasomal degradation of transcription factor Nrf2 contributes to the negative regulation of antioxidant response element-driven gene expression. *J Biol Chem* 278: 21592–21600, 2003.
93. Migliaccio E, Giorgio M, Mele S, Pelicci G, Reboldi P, Pandolfi PP, Lanfrancone L, and Pelicci PG. The p66shc adaptor protein controls oxidative stress response and life span in mammals. *Nature* 402: 309–313, 1999.
94. Mo JS, Jung J, Yoon JH, Hong JA, Kim MY, Ann EJ, Seo MS, Choi YH, and Park HS. DJ-1 modulates the p38 mitogen-activated protein kinase pathway through physical interaction with apoptosis signal-regulating kinase 1. *J Cell Biochem* 110: 229–237, 2010.
95. Moriguchi T, Kuroyanagi N, Yamaguchi K, Gotoh Y, Irie K, Kano T, Shirakabe K, Muro Y, Shibuya H, Matsumoto K, Nishida E, and Hagiwara M. A novel kinase cascade mediated by mitogen-activated protein kinase kinase 6 and MKK3. *J Biol Chem* 271: 13675–13679, 1996.
96. 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.
97. Morrison DK. The 14-3-3 proteins: integrators of diverse signaling cues that impact cell fate and cancer development. *Trends Cell Biol* 19: 16–23, 2009.
98. Motohashi H, Kimura M, Fujita R, Inoue A, Pan X, Takayama M, Katsuoka F, Aburatani H, Bresnick EH, and Yamamoto M. NF-E2 domination over Nrf2 promotes ROS accumulation and megakaryocytic maturation. *Blood* 115: 677–686, 2010.
99. Multhaup G, Ruppert T, Schlicksupp A, Hesse L, Beher D, Masters CL, and Beyreuther K. Reactive oxygen species and Alzheimer's disease. *Biochem Pharmacol* 54: 533–539, 1997.
100. Nadeau PJ, Charette SJ, and Landry J. REDOX reaction at ASK1-Cys250 is essential for activation of JNK and induction of apoptosis. *Mol Biol Cell* 20: 3628–3637, 2009.
101. Nadeau PJ, Charette SJ, Toledano MB, and Landry J. Disulfide Bond-mediated multimerization of Ask1 and its reduction by thioredoxin-1 regulate H₂O₂-induced c-Jun NH₂-terminal kinase activation and apoptosis. *Mol Biol Cell* 18: 3903–3913, 2007.
102. 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 USP9X and oxidative stress-induced cell death. *Mol Cell* 36: 805–818, 2009.
103. Nakaso K, Yano H, Fukuhara Y, Takeshima T, Wada-Isoe K, and Nakashima K. PI3K is a key molecule in the Nrf2-mediated regulation of antioxidative proteins by hemin in human neuroblastoma cells. *FEBS Lett* 546: 181–184, 2003.
104. Nasrin N, Kaushik VK, Fortier E, Wall D, Pearson KJ, de Cabo R, and Bordone L. JNK1 phosphorylates SIRT1 and promotes its enzymatic activity. *PLoS One* 4: e8414, 2009.
105. Nguyen T, Sherratt PJ, Huang HC, Yang CS, and Pickett CB. Increased protein stability as a mechanism that enhances Nrf2-mediated transcriptional activation of the antioxidant response element. Degradation of Nrf2 by the 26 S proteasome. *J Biol Chem* 278: 4536–4541, 2003.

106. 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.
107. Niwa Y, Ozaki Y, Kanoh T, Akamatsu H, and Kurisaka M. Role of cytokines, tyrosine kinase, and protein kinase C on production of superoxide and induction of scavenging enzymes in human leukocytes. *Clin Immunol Immunopathol* 79: 303–313, 1996.
108. 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.
109. Owuor ED and Kong AN. Antioxidants and oxidants regulated signal transduction pathways. *Biochem Pharmacol* 64: 765–770, 2002.
110. Ozsoy HZ, Sivasubramanian N, Wieder ED, Pedersen S, and Mann DL. Oxidative stress promotes ligand-independent and enhanced ligand-dependent tumor necrosis factor receptor signaling. *J Biol Chem* 283: 23419–23428, 2008.
111. Pham CG, Bubici C, Zazzeroni F, Papa S, Jones J, Alvarez K, Jayawardena S, De Smaele E, Cong R, Beaumont C, Torti FM, Torti SV, and Franzoso G. Ferritin heavy chain upregulation by NF-kappaB inhibits TNFalpha-induced apoptosis by suppressing reactive oxygen species. *Cell* 119: 529–542, 2004.
112. Pocrnich CE, Liu H, Feng M, Peng T, Feng Q, and Hutnik CM. p38 mitogen-activated protein kinase protects human retinal pigment epithelial cells exposed to oxidative stress. *Can J Ophthalmol* 44: 431–436, 2009.
113. Radeke HH, Meier B, Topley N, Floge J, Habermehl GG, and Resch K. Interleukin 1-alpha and tumor necrosis factor-alpha induce oxygen radical production in mesangial cells. *Kidney Int* 37: 767–775, 1990.
114. Radunovic A, Porto WG, Zeman S, and Leigh PN. Increased mitochondrial superoxide dismutase activity in Parkinson's disease but not amyotrophic lateral sclerosis motor cortex. *Neurosci Lett* 239: 105–108, 1997.
115. Ranganathan A, Pearson GW, Chrestensen CA, Sturgill TW, and Cobb MH. The MAP kinase ERK5 binds to and phosphorylates p90 RSK. *Arch Biochem Biophys* 449: 8–16, 2006.
116. Rao RK and Clayton LW. Regulation of protein phosphatase 2A by hydrogen peroxide and glutathionylation. *Biochem Biophys Res Commun* 293: 610–616, 2002.
117. Ricciardolo FL, Di Stefano A, Sabatini F, and Folkerts G. Reactive nitrogen species in the respiratory tract. *Eur J Pharmacol* 533: 240–252, 2006.
118. Robinson KA, Stewart CA, Pye QN, Nguyen X, Kenney L, Salzman S, Floyd RA, and Hensley K. Redox-sensitive protein phosphatase activity regulates the phosphorylation state of p38 protein kinase in primary astrocyte culture. *J Neurosci Res* 55: 724–732, 1999.
119. Rockwell P, Martinez J, Papa L, and Gomes E. Redox regulates COX-2 upregulation and cell death in the neuronal response to cadmium. *Cell Signal* 16: 343–353, 2004.
120. 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 2Cepsilon. *Biochem J* 405: 591–596, 2007.
121. 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.
122. Schmitt JM, Wayman GA, Nozaki N, and Soderling TR. Calcium activation of ERK mediated by calmodulin kinase I. *J Biol Chem* 279: 24064–24072, 2004.
123. Shapiro PS, Whalen AM, Tolwinski NS, Wilsbacher J, Froelich-Ammon SJ, Garcia M, Osheroff N, and Ahn NG. Extracellular signal-regulated kinase activates topoisomerase IIalpha through a mechanism independent of phosphorylation. *Mol Cell Biol* 19: 3551–3560, 1999.
124. Shasby DM, Yorek M, and Shasby SS. Exogenous oxidants initiate hydrolysis of endothelial cell inositol phospholipids. *Blood* 72: 491–499, 1988.
125. Shrivastava A and Aggarwal BB. Antioxidants differentially regulate activation of nuclear factor-kappa B, activator protein-1, c-jun amino-terminal kinases, and apoptosis induced by tumor necrosis factor: evidence that JNK and NF-kappa B activation are not linked to apoptosis. *Antioxid Redox Signal* 1: 181–191, 1999.
126. Suzuki Y, Yoshizumi M, Kagami S, Koyama AH, Taketani Y, Houchi H, Tsuchiya K, Takeda E, and Tamaki T. Hydrogen peroxide stimulates c-Src-mediated big mitogen-activated protein kinase 1 (BMK1) and the MEF2C signaling pathway in PC12 cells: potential role in cell survival following oxidative insults. *J Biol Chem* 277: 9614–9621, 2002.
127. Takeda K, Komuro Y, Hayakawa T, Oguchi H, Ishida Y, Murakami S, Noguchi T, Kinoshita H, Sekine Y, Iemura S, Natsume T, and Ichijo H. Mitochondrial phosphoglycerate mutase 5 uses alternate catalytic activity as a protein serine/threonine phosphatase to activate ASK1. *Proc Natl Acad Sci U S A* 106: 12301–12305, 2009.
128. Takeda K, Shimozone 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 in a heteromeric complex with ASK1. *J Biol Chem* 282: 7522–7531, 2007.
129. Tibbles LA, Ing YL, Kiefer F, Chan J, Iscove N, Woodgett JR, and Lassam NJ. MLK-3 activates the SAPK/JNK and p38/RK pathways via SEK1 and MKK3/6. *EMBO J* 15: 7026–7035, 1996.
130. Tibbles LA and Woodgett JR. The stress-activated protein kinase pathways. *Cell Mol Life Sci* 55: 1230–1254, 1999.
131. 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.
132. Touyz RM, Cruzado M, Tabet F, Yao G, Salomon S, and Schiffrin EL. Redox-dependent MAP kinase signaling by Ang II in vascular smooth muscle cells: role of receptor tyrosine kinase transactivation. *Can J Physiol Pharmacol* 81: 159–167, 2003.
133. Toyokuni S. Novel aspects of oxidative stress-associated carcinogenesis. *Antioxid Redox Signal* 8: 1373–1377, 2006.
134. Venugopal R and Jaiswal AK. Nrf2 and Nrf1 in association with Jun proteins regulate antioxidant response element-mediated expression and coordinated induction of genes encoding detoxifying enzymes. *Oncogene* 17: 3145–3156, 1998.
135. Wang X and Tournier C. Regulation of cellular functions by the ERK5 signalling pathway. *Cell Signal* 18: 753–760, 2006.

136. Wang XT, McCullough KD, Wang XJ, Carpenter G, and Holbrook NJ. Oxidative stress-induced phospholipase C-gamma 1 activation enhances cell survival. *J Biol Chem* 276: 28364–28371, 2001.
137. Waxman K. Shock: ischemia, reperfusion, and inflammation. *New Horiz* 4: 153–160, 1996.
138. Westwick JK, Weitzel C, Minden A, Karin M, and Brenner DA. Tumor necrosis factor alpha stimulates AP-1 activity through prolonged activation of the c-Jun kinase. *J Biol Chem* 269: 26396–26401, 1994.
139. Whisler RL, Goyette MA, Grants IS, and Newhouse YG. Sublethal levels of oxidant stress stimulate multiple serine/threonine kinases and suppress protein phosphatases in Jurkat T cells. *Arch Biochem Biophys* 319: 23–35, 1995.
140. 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.
141. Winter-Vann AM and Johnson GL. Integrated activation of MAP3Ks balances cell fate in response to stress. *J Cell Biochem* 102: 848–858, 2007.
142. Woo HA, Yim SH, Shin DH, Kang D, Yu DY, and Rhee SG. Inactivation of peroxiredoxin I by phosphorylation allows localized H₂O₂ accumulation for cell signaling. *Cell* 140: 517–528, 2010.
143. Wu HW, Li HF, Wu XY, Zhao J, and Guo J. Reactive oxygen species mediate ERK activation through different Raf-1-dependent signaling pathways following cerebral ischemia. *Neurosci Lett* 432: 83–87, 2008.
144. 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.
145. Xu C, Yuan X, Pan Z, Shen G, Kim JH, Yu S, Khor TO, Li W, Ma J, and Kong AN. Mechanism of action of isothiocyanates: the induction of ARE-regulated genes is associated with activation of ERK and JNK and the phosphorylation and nuclear translocation of Nrf2. *Mol Cancer Ther* 5: 1918–1926, 2006.
146. Yan L, Vatner DE, O'Connor JP, Ivessa A, Ge H, Chen W, Hirotani S, Ishikawa Y, Sadoshima J, and Vatner SF. Type 5 adenylyl cyclase disruption increases longevity and protects against stress. *Cell* 130: 247–258, 2007.
147. Yang H, Young DW, Gusovsky F, and Chow JC. Cellular events mediated by lipopolysaccharide-stimulated toll-like receptor 4. MD-2 is required for activation of mitogen-activated protein kinases and Elk-1. *J Biol Chem* 275: 20861–20866, 2000.
148. Yoon S and Seger R. The extracellular signal-regulated kinase: multiple substrates regulate diverse cellular functions. *Growth Factors* 24: 21–44, 2006.
149. 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.
150. Zhang L, Chen J, and Fu H. Suppression of apoptosis signal-regulating kinase 1-induced cell death by 14-3-3 proteins. *Proc Natl Acad Sci U S A* 96: 8511–8515, 1999.
151. Zhou G, Bao ZQ, and Dixon JE. Components of a new human protein kinase signal transduction pathway. *J Biol Chem* 270: 12665–12669, 1995.
152. Zhou J, Shao Z, Kerkela R, Ichijo H, Muslin AJ, Pombo C, and Force T. Serine 58 of 14-3-3zeta is a molecular switch regulating ASK1 and oxidant stress-induced cell death. *Mol Cell Biol* 29: 4167–4176, 2009.
153. Zhuang S and Schnellmann RG. H₂O₂-induced transactivation of EGF receptor requires Src and mediates ERK1/2, but not Akt, activation in renal cells. *Am J Physiol Renal Physiol* 286: F858–F865, 2004.
154. Zipper LM and Mulcahy RT. Inhibition of ERK and p38 MAP kinases inhibits binding of Nrf2 and induction of GCS genes. *Biochem Biophys Res Commun* 278: 484–492, 2000.
155. Zipper LM and Mulcahy RT. Erk activation is required for Nrf2 nuclear localization during pyrrolidine dithiocarbamate induction of glutamate cysteine ligase modulatory gene expression in HepG2 cells. *Toxicol Sci* 73: 124–134, 2003.
156. Zou Y, Komuro I, Yamazaki T, Aikawa R, Kudoh S, Shiojima I, Hiroi Y, Mizuno T, and Yazaki Y. Protein kinase C, but not tyrosine kinases or Ras, plays a critical role in angiotensin II-induced activation of Raf-1 kinase and extracellular signal-regulated protein kinases in cardiac myocytes. *J Biol Chem* 271: 33592–33597, 1996.

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Abbreviations Used

Ang II = angiotensin II
ASK = apoptosis signal-regulating kinase
BMK = big MAP kinase
CCC = C-terminal coiled-coil domain
EGF = epidermal growth factor
ERK = extracellular signal-regulated kinase
JNK = c-Jun N-terminal kinase
KD = kinase domain
MAP2K = MAPK kinase
MAP3K = MAPK kinase kinase
MAPK = mitogen-activated protein kinase
MLK = mixed lineage kinase
NCC = N-terminal coiled-coil domain
PGAM = phosphoglycerate mutase
PKC = protein kinase C
PLC = phospholipase C
PM = plasma membrane
PP = protein phosphatase
ROS = reactive oxygen species
TNF α = tumor necrosis factor α
Trx = thioredoxin

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2. 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](#)]
3. Zhe-Ren Shao, Qi Wang, Xiao-Fang Xu, Zhuang Zhang, Yun-Bi Lu, Gang Shen, Ming Wu. 2012. Phospholipase D participates in H₂O₂-induced A549 alveolar epithelial cell migration. *Experimental Lung Research* **38**:8, 427-433. [[CrossRef](#)]
4. Mark William Lopes, Flávia Mahatma Schneider Soares, Nelson Mello, Jean Costa Nunes, Fabiano Mendes Cordova, Roger Walz, Rodrigo Bainy Leal. 2012. Time-Dependent Modulation of Mitogen Activated Protein Kinases and AKT in Rat Hippocampus and Cortex in the Pilocarpine Model of Epilepsy. *Neurochemical Research* **37**:9, 1868-1878. [[CrossRef](#)]
5. Mohammad K. Mohammad, Diana Avila, Jingwen Zhang, Shirish Barve, Gavin Arteel, Craig McClain, Swati Joshi-Barve. 2012. Acrolein cytotoxicity in hepatocytes involves endoplasmic reticulum stress, mitochondrial dysfunction and oxidative stress. *Toxicology and Applied Pharmacology* . [[CrossRef](#)]
6. Ji-Yeon Yu, Seung-Youp Lee, Young-Ok Son, Xianglin Shi, Soon-Sun Park, Jeong-Chae Lee. 2012. Continuous presence of H₂O₂ induces mitochondrial-mediated, MAPK- and caspase-independent growth inhibition and cytotoxicity in human gingival fibroblasts. *Toxicology in Vitro* **26**:4, 561-570. [[CrossRef](#)]
7. Y. Chen, C. P. Curran, D. W. Nebert, K. V. Patel, M. T. Williams, C. V. Vorhees. 2012. Effect of vitamin C deficiency during postnatal development on adult behavior: functional phenotype of Gulo(-/-) knockout mice. *Genes, Brain and Behavior* no-no. [[CrossRef](#)]
8. Hayato Nakagawa, Shin Maeda. 2012. Molecular Mechanisms of Liver Injury and Hepatocarcinogenesis: Focusing on the Role of Stress-Activated MAPK. *Pathology Research International* **2012**, 1-14. [[CrossRef](#)]
9. Tadesse Yayeh, Mei Hong, Qi Jia, Young-Chul Lee, Hyun-Jin Kim, Eujin Hyun, Tae-Wan Kim, Man Hee Rhee. 2012. Pistacia chinensis Inhibits NO Production and Upregulates HO-1 Induction via PI-3K/Akt Pathway in LPS Stimulated Macrophage Cells. *The American Journal of Chinese Medicine* **40**:05, 1085-1097. [[CrossRef](#)]
10. O. Arican, P. Ozturk, E.B. Kurutas, V. Unsal. 2011. Status of oxidative stress on lesional skin surface of plantar warts. *Journal of the European Academy of Dermatology and Venereology* no-no. [[CrossRef](#)]
11. Stephanie Krifka, Karl-Anton Hiller, Carola Bolay, Christine Petzel, Gianrico Spagnuolo, Franz-Xaver Reichl, Gottfried Schmalz, Helmut Schweikl. 2011. Function of MAPK and downstream transcription factors in monomer-induced apoptosis. *Biomaterials* . [[CrossRef](#)]
12. Elizabeth Veal , Alison Day . 2011. Hydrogen Peroxide as a Signaling Molecule. *Antioxidants & Redox Signaling* **15**:1, 147-151. [[Abstract](#)] [[Full Text HTML](#)] [[Full Text PDF](#)] [[Full Text PDF with Links](#)]