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REGULATION OF STRESS-ACTIVATED MAP KINASE PATHWAYS DURING CELL FATE DECISIONS

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ABSTRACT

Mammalian cells are frequently exposed to a variety of environmental stresses, such as ultraviolet rays, ionizing radiation, genotoxins, heat shock, and oxidative stress. In coping with the barrage of these and other stresses, multi-cellular eukaryotic organisms have developed a strategy as to how damaged cells will respond to stresses. In general, if the intensity of the damage is moderate, the cell will seek to repair the damage. If, however, the damage to a cell is too severe to be repaired, the affected cells are eliminated by apoptosis. This cell death reduces the risk to the organism as a whole, such as development of a cancer. Such a crucial decision between survival and death is, at least in part, mediated by the stress-activated MAP kinase (SAPK) pathways. SAPKs are a group of serine/threonine protein kinases that convert extracellular stress stimuli into diverse cellular responses, including cell cycle arrest, apoptotic cell death, and cytokine production, through phosphorylation of specific target proteins. Recent progress in the identification of molecules that participate in the SAPK pathways, such as GADD45 proteins and Wip1, has provided new insights, not only into the molecular basis of the cellular response to environmental stress, but also into the etiology of human diseases including cancer.

Key Words: SAPK, GADD45, MTK1, Docking interaction, Stress granules

INTRODUCTION

In eukaryotic cells, a variety of extracellular stimuli generate intracellular signals that converge on a limited number of protein kinase cascades, commonly referred to as mitogen-activated protein kinase (MAPK) pathways.[1] The core of any MAPK pathway is composed of three tiers of sequentially activated protein kinases, namely, MAPK kinase kinase (MAPKKK), MAPK kinase (MAPKK), and MAPK. Activation of MAPKs is achieved by phosphorylation of a threonine and a tyrosine residue within a conserved Thr-X-Tyr motif in the activation loop (also called the T-loop), which is catalyzed by MAPKKs. MAPKKs are activated by any of several MAPKKKs, via phosphorylation of serine and/or threonine residues within their activation loop. All eukaryotic cells possess multiple MAPK pathways, each of which is activated by distinct sets of stimuli. In mammalian cells, at least four different subfamilies of MAPKs are present, namely, ERK1/2, JNK1/2/3, p38α/β/γ/δ, and ERK5 (Fig. 1).[2] The ERK1/2 MAPK pathway is preferentially

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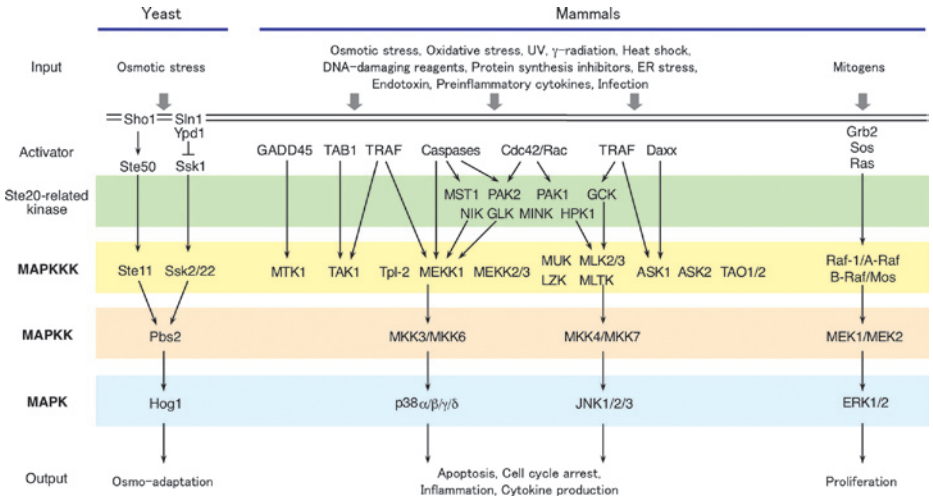

Fig. 1 Overview of MAP kinase signaling cascades. The three defining kinases of all so-called mitogen-activated-protein kinases (MAPK) phosphorelay modules are conserved in all eukaryotic cells from yeast to mammals.

activated in response to mitogenic stimuli such as growth factors and phorbol esters, and plays a central role in cell growth and survival. The ERK1/2 pathway is mainly regulated through the small G-protein Ras, which recruits MAPKKKs of the Raf family to activate the two downstream MAPKKs: MEK1/2. These MAPKKs, in turn, activate the ERK1/2 MAPKs. While the classical ERK MAPK is activated mainly by mitogenic stimuli, two relatively newly identified types of MAPKs, p38 and JNK, are more potently activated by a variety of environmental stresses (e.g., UV and γ irradiation, DNA-damaging reagents, oxidative stress, osmotic stress, heat shock and etc.), and are thus collectively called stress-activated protein kinase (SAPK) pathways.[3] Besides cellular stresses, the SAPK pathways are also activated by cytokines such as IL-1, TNFα, and TGF-β. The JNK subfamily of MAPKs are activated mainly by the MKK4 and MKK7 MAPKKs, while the p38 subfamily MAPKs are activated primarily by the MKK3 and MKK6 MAPKKs, and in some cases, also by MKK4 MAPKKs. In clear contrast to this limited number of MAPKKs in the SAPK pathways, there are numerous MAPKKKs that function upstream of the JNK and p38 MAPKs. These MAPKKKs include MEKK1/2/3, MTK1 (whose mouse homolog is known as MEKK4), TAK1, ASK1/2/3, TAO1/2/3, and MLKs (Fig. 1). This multiplicity at the level of MAPKKK reflects the vastly diverse stress stimuli that can recruit these SAPK pathways.[4]

The mammalian SAPK pathways play pivotal roles in cellular stress responses such as cell cycle arrest and apoptotic cell death. In addition, several lines of evidence have revealed that the SAPK pathways are involved in inflammatory responses as well as in the responses of cancer cells to cytotoxic therapies.[5] Persistent activation of p38 and JNK, especially in the absence of mitogenic stimuli, has been shown to induce apoptotic cell death. In contrast, inhibition of JNK and/or p38 activation, either by genetic inactivation or by the use of a dominant inhibitory mutant, confers resistance to cell death induced by stress stimuli including DNA damage.[6] Thus, cell fate decisions that are influenced by the SAPK pathways might be important for minimizing the chance of carcinogenesis. There is increasing evidence that the stress-activated MAPK pathways are involved in tumor suppression and indeed these pathways are aberrantly regulated in human cancer.[3] In this review, we will discuss recent findings regarding the regulation and

function of the SAPK pathways in cell fate decision and outline some of the major findings from our laboratory.

REGULATION OF THE MAMMALIAN STRESS-RESPONSIVE MTK1 MAPKKK BY GADD45-LIKE PROTEINS

Despite their importance in dictating the fate of cells exposed to stress, the mechanism by which the SAPK pathways are activated by environmental stresses is still ill-defined. We previously identified MTK1, the human homolog of the yeast Ssk2/Ssk22 MAPKKKs, as a specific mediator of stress-induced SAPK activation.[7] The kinase domain of MTK1 is homologous to other MAPKKKs involved in SAPK pathways, and is especially similar to mammalian MEKK1/2/3 and yeast Ssk2/Ssk22. However, the N-terminal non-catalytic domain (regulatory domain) of MTK1 is unique. Using a yeast two-hybrid screen, we identified three growth arrest and DNA damage-inducible 45 (GADD45) family proteins as novel binding partners of MTK1.[8] The human genome encodes three GADD45-like proteins, GADD45α, GADD45β, and GADD45γ, which share 55 to 58% amino acid sequence identity with each other.[8] These proteins are thus collectively referred to as the GADD45 proteins. The three GADD45 genes are all inducible by various environmental stresses, including methyl methanesulfonate (MMS), UV and γ-irradiation, and oxidative stress, although the optimal stimuli for each gene appear to be different. The expression profiles of the three GADD45 genes are also distinct in various tissues. In addition to their binding to MTK1, the GADD45 proteins interact with various intracellular molecules such as proliferating cell nuclear antigen (PCNA), the Cdc2-CyclinB1 complex, p21Waf1/Cip1, and core histones, and they contribute to stress-adaptive processes including growth control, maintenance of genomic stability, DNA repair, and apoptosis.[9,10] Thus, the GADD45 proteins are critical for the signaling of damaged cells. Importantly, ectopic expression of GADD45 genes in mammalian cells strongly activates co-expressed MTK1, as well as p38 and JNK, the MAPKs downstream of MTK1. Activation of the SAPK pathways by GADD45 and MTK1 is temporally a slow process, because it requires induction of GADD45 gene expression prior to activation of MTK1. Thus, GADD45-mediated induction of MTK1 activity mainly provokes delayed and prolonged activation of the SAPK pathways, which is, in some context, crucial for induction of apoptotic cell death. Indeed, expression of each individual GADD45 protein by transient transfection activates p38/JNK and causes apoptosis, which can be partially suppressed by coexpression of a dominant inhibitory MTK1 mutant protein. Thus, the stress-inducible GADD45 proteins mediate activation of the SAPK pathways and apoptotic cell death, through MTK1, in response to environmental stresses.

Recent biochemical data from our laboratory led to the following model of GADD45-mediated activation of MTK1.[11,12] Activation of MTK1 by GADD45 occurs through a series of molecular steps (I through VI) (Fig. 2). In brief, each step is as follows. (I) In unstimulated cells, MTK1 is in a closed (inhibited) conformation in which the N terminal auto-inhibitory domain (AID) blocks the C-terminal kinase catalytic domain (KD). An adaptor protein RACK1 interacts with MTK1 to tether two (or more) inactive MTK1 molecules together (as will be later described in detail). (II) Extracellular stimuli, such as MMS exposure, induce the expression of stress-inducible GADD45 proteins, which bind to the MTK1 N-terminal GADD45-binding domain. (III) GADD45-binding to MTK1 dissociates the latter's AID from the C-terminal kinase catalytic domain. (IV) At the same time, GADD45-binding unmasks the MTK1 dimerization domain, inducing homo-dimer formation. At this stage, the MTK1 kinase domain is in an open conformation (i.e., not actively inhibited), but not yet fully active as a kinase. (V) Dimerized MTK1 becomes fully activated

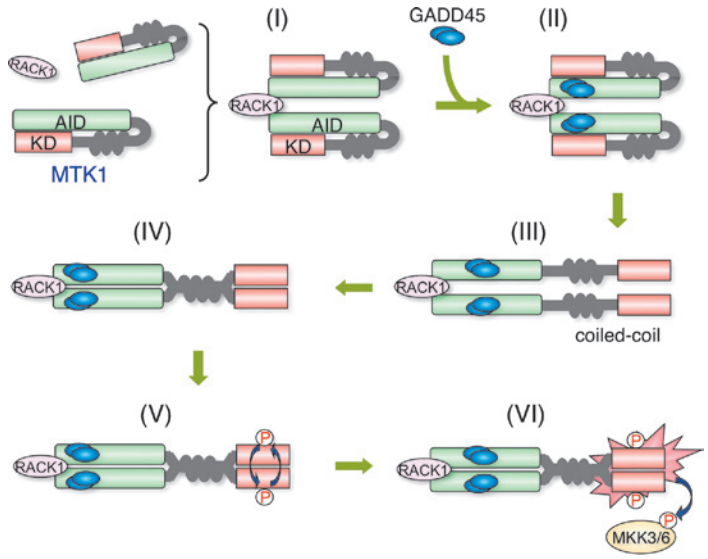

Fig. 2 Schematic model of MTK1 activation. Activation of MTK1 by GADD45 can be dissected into several stages, as indicated by the roman numerals in the scheme (I through VI). See text for details. AID, auto-inhibitory domain; KD, kinase domain.

when Thr-1493 is trans-autophosphorylated. (VI) GADD45 binding also unmasks a site in the MTK1 kinase domain that interacts with the MAPKK-docking sites, allowing MTK1 to interact with, and phosphorylate its cognate MAPKKs, such as MKK3 and MKK6. Thus, full activation of MTK1 by GADD45 entails four different molecular mechanisms: removal of the autoinhibitory domain; dimerization; phosphorylation of the activation loop; and unmasking of the docking site for MAPKKs. Individually, these mechanisms are used by other MAPKKKs. However, the details of the activation mechanisms are different for each MAPKKK, reflecting their different physiological roles. Our studies revealed how the binding of one protein (GADD45) orchestrated these mechanisms, thereby converting an inert enzyme (MTK1) to a fully active one.

ROLE OF THE GADD45 PROTEINS IN ACTIVATION OF THE p38 PATHWAY BY TRANSFORMING GROWTH FACTOR-β

Transforming growth factor-β (TGF-β) belongs to a family of multifunctional cytokines that regulate essential cellular functions, such as proliferation, apoptosis, extracellular matrix production, angiogenesis and the epithelial-mesenchymal transition. An altered cellular response to TGF-β has been postulated as a mechanism whereby cells undergo neoplastic transformation, because many cancers of epithelial and lymphoid origins are resistant to the negative growth-regulatory effects of TGF-β.[13] TGF-β exerts its biological effects by binding to a cell surface receptor complex composed of type I (TβRI) and type II (TβRII) receptor serine/threonine kinases (Fig. 3). Upon ligand binding, TβRII phosphorylates and activates TβRI, which subsequently phosphorylates the cytoplasmic Smad2 and Smad3 proteins. Phosphorylated Smad proteins form stable complexes with a common partner, Smad4 (also known as deleted in pancreatic carcinoma, locus 4; DPC4). The activated Smad2/4 and Smad3/4 complexes translocate into the nucleus, where the Smad oligomers induce transcriptional activation (or inhibition) of specific target genes

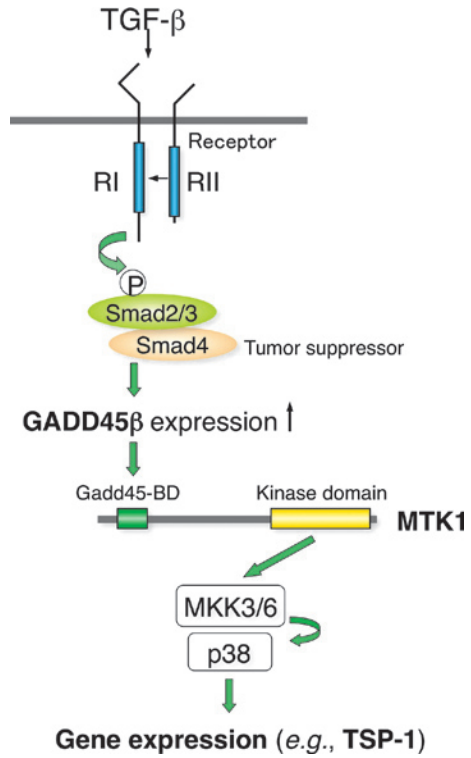

Fig. 3 Model of the signal flow from TGF-β to p38-dependent gene expression. Upon TGF-β binding, TβRII phosphorylates and activates TβRI, which subsequently phosphorylates cytoplasmic Smad2/3, resulting in the formation of a heterodimer with Smad4. The active Smad complex translocates to the nucleus and induces GADD45β expression. The GADD45β protein binds and activates the MTK1 MAPKKK, which leads to p38 activation. Active p38 regulates the expression of specific genes (including TSP-1) via phosphorylation of transcription factors. GADD45-BD, GADD45-binding domain.

in cooperation with other transcription factors. Underscoring the importance of Smad proteins in TGF-β signaling, disruption of Smad pathway components abated transcriptional responses to TFG-β. Furthermore, Smad4 mutations are observed frequently (~50%) in human pancreatic carcinomas.[13)]

Beside activation of the Smad proteins, TGF-β also activates the p38 pathway, which may play an important role in TGF-β-induced gene expression. In this regard, previous studies indicate that TAK1, a member of the MAPKKK family, is involved in TGF-β-induced p38 activation.[14,15)] TβRI interacts indirectly with TAK1 via the bridging proteins XIAP and TAB1. Activation of TAK1 alone, however, seems insufficient to account for the in vivo activation of p38 in response to TGF-β. Thus, TGF-β induces TAK1 activity rapidly but only transiently; TAK1 activity peaks at ~10 min following TGF-β stimulation, and declines to basal levels by 30 min. Rapid activation of p38 by TGF-β, consistent with the kinetics of TAK1 activity, has been reported in some cell lines.[16)] However, in many other cell types (e.g. pancreatic acinar cells, keratinocytes, osteoblasts, gingival fibroblasts and rat hepatocytes), maximal p38 activation occurs 1–2 h following TGF-β stimulation, and this activity persists for several hours.[17)] These findings suggest that there is an additional, TAK1-independent, signaling mechanism that mediates TGF-β-induced p38 activation.

We identified this additional signaling mechanism of p38 activation as GADD45β-MTK1 signaling.[18] We initially found that TGF-β-induced p38 activation did not occur in Smad4-deficient pancreatic cancer cell lines, but that reintroduction of Smad4 into these cell lines restored p38 activation. Furthermore, overexpression of Smad3/4 proteins alone, which elicited Smad-dependent transcription, was capable of activating the p38 pathway. Conversely, a dominant-negative Smad4 mutant strongly inhibited p38 activation induced by a constitutively active TGFβRI. These findings imply that Smad-dependent gene expression can provoke p38 activation in response to TGF-β. GADD45β was eventually identified as the TGF-β-inducible gene whose expression activates the p38 pathway. Expression of GADD45β mRNA was efficiently induced by TGF-β in a Smad-dependent manner, and the timing of the TGF-β-induced p38 activation was almost parallel to that of GADD45β induction. Moreover, TGF-β-induced p38 activation was inhibited by expression of dominant-negative MTK1 or of anti-sense GADD45β. These findings indicate that the delayed activation of p38 by TGF-β is mediated mainly by Smad-dependent GADD45β expression and by its subsequent activation of MTK1.

Regarding the physiological roles of TGF-β-induced p38 activation, we identified thrombospondin 1 (TSP-1), a potent inhibitor of tumor cell growth and angiogenesis, as a target for the Smad-GADD45β-MTK1-p38 signaling pathway. TSP-1 mRNA expression was strongly induced by TGF-β in a Smad- and p38-dependent manner. Downregulation or inhibition of any one of the molecules involved in this signaling pathway (i.e., Smad4, GADD45β, MTK1, or p38) abrogated TGF-β-induced TSP-1 expression. Strikingly, in Smad4-deficient pancreatic cancer cells TGF-β did not induce GADD45β expression, p38 activation, or TSP-1 expression. Reintroduction of Smad4 into these Smad4-deficient cancer cells restored TGF-β-induced TSP-1 expression. Because expansion and metastasis of solid tumors is critically dependent on an adequate vascular supply, inefficient activation of p38 in Smad4-deficient tumor cells, and a resulting deficit of TSP-1, might contribute to the etiology of invasive pancreatic cancer. Consistent with this notion, it has been reported that restoration of Smad4 to Smad4-deficient pancreatic carcinoma cells increased the steady-state mRNA levels of TSP-1, and suppressed tumor formation in vivo by repression of tumor angiogenesis.[19] We also confirmed that the TSP-1 protein secreted from TGF-β-stimulated normal cells could inhibit the endothelial cell migration, which is a key step in angiogenesis. These findings suggest that the TSP-1 expression that is mediated by the crosstalk between the Smad and p38 pathways contributes to the tumor-suppressive effect of TGF-β. It should be noted, however, that the role of p38 in the cellular response to TGF-β is by no means limited to TSP-1 expression.

GADD45β/GADD45γ AND MTK1/MEKK4 COMPRISE A GENETIC PATHWAY THAT MEDIATES IFNγ PRODUCTION IN T CELLS

Regulation of SAPK pathway by GADD45 has also been investigated in the immune system, using T helper type 1 (Th1) cells as a model system. Th1 cells produce a specific set of cytokines (so-called Th1 cytokines) in response to antigen receptor challenge or to combined stimulation with Interleukin-12 (IL-12) and IL-18. Interferon γ (IFNγ), the signature Th1 cytokine, is responsible for cell-mediated immunity and for phagocyte-dependent protective responses against microorganisms through the activation of macrophages. Recent studies revealed that *IFN*γ gene transcription requires p38 activation, which is mediated by GADD45 proteins. IL-12/IL-18 stimulation of Th1 cells induced high-level expression of GADD45β and GADD45γ, leading to sustained p38 activation and enhanced IFNγ production in Th1 cells. Dr. Flavell's group also found that GADD45γ was strongly induced during Th1 cell differentiation.[20] Th1

effector cells from *GADD45*γ-/- mice showed reduced p38 activation and IFNγ production in response to T cell receptor (TCR) stimulation. In vivo, *GADD45*γ-/- mice exhibited suppressed contact hypersensitivity, a form of delayed-type hypersensitivity (type IV allergy). These findings demonstrate that GADD45 proteins mediate signaling from both the antigen receptor and the inflammatory cytokines and that they are a prerequisite for p38 activation in Th1 cells, thereby regulating immune function.

To further elucidate the relationship between GADD45 and MTK1, and its functional significance in Th1 regulation, our group created mice deficient in the mouse homolog of MTK1 (MEKK4).[21] Similar to *GADD45*γ-/- mice, CD4-positive T cells from *MEKK4*-/- mice exhibited reduced p38 activity and were therefore defective in IFNγ synthesis. Expression of GADD45β or GADD45γ promoted IFNγ production in *MEKK4*+/+ T cells, but not in *MEKK4*-/- cells, demonstrating that MTK1/MEKK4 mediates the action of GADD45β and GADD45γ on p38 activation and IFNγ production. These results further provide the genetic evidence that GADD45 proteins and MTK1 are in the same signaling pathway in T cells. Furthermore, this study has also presented new insights into the regulation and function of this pathway in immune responses. During Th1 differentiation, the GADD45-MTK1 pathway integrates upstream signals that are transduced by both the TCR and by IL-12/IL-18, leading to augmented production of IFNγ in a process independent of STAT4.

A CONSERVED DOCKING SITE IS ESSENTIAL FOR ACTIVATION OF MAMMALIAN MAPKKS BY SPECIFIC MAPKKKS

As mentioned above, there are at least four subfamilies of MAPK cascades in mammalian cells, namely p38, JNK, ERK1/2, and ERK5 pathways. Although a number of structurally similar molecules are involved in these cascades, the reactions between kinases and their cognate substrates occur faithfully without erroneous crosstalk. Such accurate signal transduction in individual MAPK cascades is crucial for proper control of cell-fate decisions, as well as for maintenance of human homeostasis. Several mechanisms are likely to be involved in ensuring the fidelity and efficiency of signal flow through these kinase cascades. Naturally, the specific interaction between the kinase catalytic center and the substrate phospho-acceptor site is important. However, recently, equally important contributions of scaffold proteins and docking interactions have been demonstrated.[22,23] A scaffold protein is a molecule that tethers two or more kinases together, thereby enhancing the specificity of the kinases by minimizing extraneous interactions. For example, the scaffold protein, JIP-1, interacts with specific components of the JNK cascade, namely JNK (MAPK), MKK7 (MAPKK), and MLK3 (MAPKKK) (Fig. 4A). In vitro binding assays showed that JNK, MKK7, and MLK3 bind to separate sites on the JIP-1 protein, suggesting that JIP-1 can simultaneously interact with these kinases and thereby preassemble a JNK signaling module in non-stimulated cells. Indeed, JIP-1 proteins can potentiate the activation of JNK when ectopically expressed in cells. The scaffold function of JIP-1, however, is important for JNK activation only in particular types of stress stimuli. In neuronal cells isolated from *JIP1*-/- mice, JIP-1 is necessary for JNK activation when cells are exposed to anoxia or excitotoxic stress, but JIP-1 is dispensable for JNK activation induced by anisomycin or UV radiation.[24] Thus, scaffold proteins regulate both signaling specificity and efficiency, but exert their functions in a cell-type, stimulus-, and molecule-specific manner.

In contrast to the limited role of scaffold proteins, docking interactions are more general mechanisms to ensure signaling fidelity in MAPK cascades. A docking interaction is the formation of a binary complex of a kinase and its substrate through docking sites that are distinct

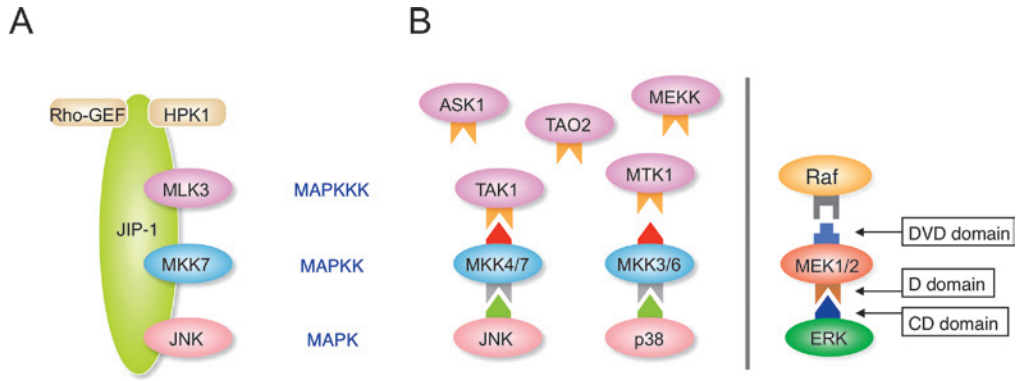

Fig. 4 Scaffolding and docking interactions ensure the fidelity and efficiency of signal flow through MAPK cascades. (A) A scaffold protein JNK-interacting protein 1 (JIP-1) binds to JNK (MAPK), MKK7 (MAPKK), and MLK3 (MAPKKK). JIP-1 also interacts with other JNK pathway regulators, such as p190Rho-GEF and HPK1. (B) A schematic model of the DVD and CD docking interactions in MAPK cascades. Specific DVD docking interactions between MAPKKs and MAPKKs, and CD docking interactions between MAPKKs and MAPKs, ensure proper and efficient signal flow.

from the kinase catalytic center and the substrate phospho-acceptor site (Fig. 4B). Docking interactions mediated by the common docking domain (CD domain) in MAPKs and by the D domain in MAPKKs or in MAPK substrates, have been extensively characterized.[22] In contrast, there has been no convincing evidence for specific docking interactions between MAPKKKs and MAPKKs, although various pairs have been shown to form stable complexes. We recently identified a novel conserved docking site, termed domain for versatile docking (DVD), in the mammalian MAPKKs.[25] These DVD sites bind to specific upstream MAPKKKs, including MTK1, ASK1, TAK1, MEKK1, and Raf-1. The DVD site is a stretch of about 20 amino acids, located at the carboxy-terminal end of MAPKKs, which extends beyond the catalytic core. Interestingly, mutations in the DVD site strongly inhibited MAPKKs from binding to, and being activated by, their specific MAPKKKs, both in vitro and in vivo. Therefore, docking interactions between MAPKKs and MAPKKKs are indispensable not only for signaling specificity but also for efficient activation of MAPKKs by MAPKKKs. We further showed that synthetic oligopeptides containing the MKK6 DVD site were able to competitively inhibit the MAPKKK-MKK6 interaction, thereby attenuating MKK6 activation in vitro and in vivo. Given the well-defined role of the MAPK pathways in inflammation, apoptosis, and carcinogenesis, therapeutic targeting of MAPK signaling components is an area of intense investigation. DVD sites can serve as effective targets for drug design and therapeutic intervention in human diseases, such as cancers and autoimmune diseases, in which the MAPK signaling pathways are involved.

ROLES OF PROTEIN PHOSPHATASE TYPE 2C (PP2C) IN DOWN REGULATION OF THE STRESS-RESPONSIVE MAPK PATHWAYS

Since MAPK pathways transmit their signals through sequential phosphorylation by three protein kinases, inactivation of MAPK pathway signaling can be accomplished by phospho-protein phosphatases. Following activation, p38 and JNK are downregulated by several phospho-protein phosphatases.[26] We previously identified important roles for PP2Cα, a member of the Ser/Thr-specific protein phosphatase type 2C (PP2C) family, in down-regulation of the mammalian SAPK

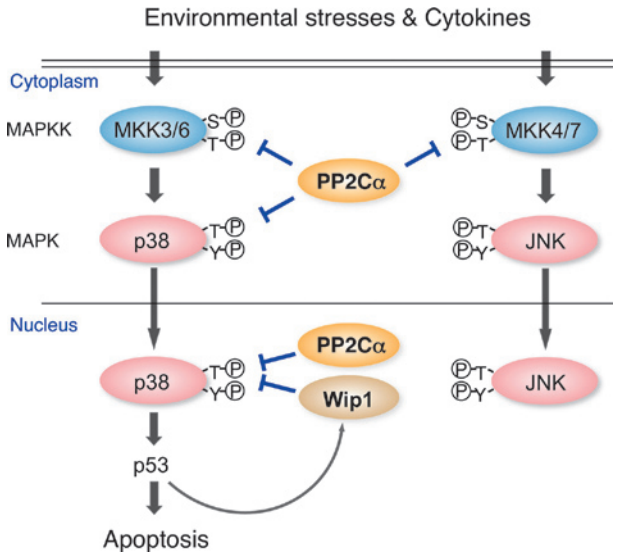

Fig. 5 The PP2C-type Ser/Thr protein phosphatases, PP2Cα and Wip1, negatively regulate SAPK pathways. PP2Cα is constitutively expressed both in the cytoplasm and in the nucleus, and dephosphorylates and inactivates the stress-responsive MAPKKs as well as the p38 MAPK. Wip1 is transcriptionally induced by p53 and localizes exclusively in the nucleus, where it dephosphorylates its nuclear target proteins including p38.

pathways.[27] PP2Cα dephosphorylates the phospho-Ser and/or Thr residues in the activation-loop of stress responsive MAPKKs (i.e., MKK3, MKK4, and MKK6), and the phospho-Thr residue in the p38 activation-loop, thereby inactivating these kinases (Fig. 5). Because PP2Cα is constitutively expressed and is equally localized to the cytoplasm and the nucleus, the major function of PP2Cα must be to maintain the SAPK activities low in the absence of any external stimulus.

In contrast to PP2Cα, the expression of another member of the PP2C family, wild-type p53-inducing phosphatase 1 (Wip1), is induced by diverse environmental stresses, and is predominantly localized in the nucleus. Wip1 was originally identified as a human gene whose expression is induced by the p53 tumor-suppressor protein in response to γ-irradiation. Shortly thereafter, Wip1 expression was found to be induced also by UV-radiation, H_2O_2, and a DNA-alkylating reagent (MMS) in a wild type p53-dependent manner. The first substrate identified for this intriguing phosphatase was p38 MAPK, which was discovered by our group.[28] UV radiation causes p38 activation by inducing its dual phosphorylation at Thr180 and Tyr182 residues. This activated p38, in turn, phosphorylates p53 at several residues, including Ser33, and thereby increases the transcriptional activity of p53. Activated p53 then induces apoptosis in cells that have suffered extensive DNA damage. When the intensity of the damage is moderate, however, the cell can repair the damage and escape apoptotic cell death by downregulating p38–p53 signaling. Under these conditions, p53-induced Wip1 phosphatase interacts with activated p38 and selectively dephosphorylates p38 at the phospho-Thr180 residue, thereby reducing its kinase activity towards p53 (Fig. 5). As a consequence, Wip1 attenuates the stress-induced, p38-mediated phosphorylation of p53, resulting in suppression of p53-mediated transcription and apoptosis. Thus, p53 induction of Wip1 phosphatase mediates a negative feedback regulation of p38–p53 signaling and contributes to suppression of DNA damage-induced apoptosis.

Recent studies have revealed that Wip1, not only dephosphorylates p38, but also directly de-

phosphorylates and regulates several other key molecules that function in DNA damage responses, such as ATM, Chk1, Chk2, Mdm2, and p53 itself. It should be noted that Wip1 dephosphorylation of these molecules eventually inhibits p53 function and apoptosis. Therefore, Wip1 has been proposed to function as a homeostatic regulator of the DNA damage response, which facilitates the recovery of the cell to a normal pre-stressed state following repair of the DNA damage, through restraint of the p53 apoptotic pathway. A corollary of this proposition is that aberrant activation of Wip1 promotes tumorigenesis by inhibition of p53 tumor suppressor function. Indeed, gene amplification and the subsequent overexpression of the Wip1 protein have been observed in various human cancers.[29] For instance, Wip1 overexpression was detected in approximately 15% of primary breast cancers and correlated with poor prognosis. The *p53* locus is rarely mutated in cancers with amplified and overexpressed Wip1, suggesting that the inhibition of p53 activity that results from Wip1 amplification is sufficient for the promotion of tumorigenesis. In genetically engineered mouse models, Wip1 overexpression in the mammary gland accelerated mammary tumorigenesis induced by ErbB2 expression. Conversely, reduced expression of Wip1 suppressed malignant transformation in mouse embryonic fibroblasts, consistent with the notion that Wip1 inactivation is associated with p53 activation. Furthermore, *Wip1*-deficient mice exhibited a marked reduction in spontaneous and oncogene-induced tumor formation compared to their wild-type counterparts.[29] These findings indicate that Wip1 can act as a bona fide oncogene, at least in part through its ability to suppress p53 as well as p38.

FORMATION OF STRESS GRANULES INHIBITS APOPTOSIS BY SUPPRESSING STRESSRESPONSIVE MAPK PATHWAYS

When confronted with environmental stress, cells either activate defense mechanisms to survive, or initiate apoptosis, depending on the type and the extent of the stress. Certain stresses such as genotoxic reagents and γ-irradiation induce apoptosis at least partly through the SAPK pathways. In contrast, other types of stresses, such as hypoxia, viral infection, and arsenite, induce cells to assemble large cytoplasmic aggregates of non-translated mRNPs, the so-called stress granules (SGs) (Fig. 6).[30] SGs are discrete cytoplasmic structures that harbor small ribosomal (40S) subunits, selected mRNA, and numerous RNA-binding proteins such as TIA-1/TIAR, G3BP, and several translation initiation factors. Because SGs do not contain large ribosomal (60S) subunits, SG assembly is postulated to inhibit the translation of housekeeping mRNAs, thereby preventing the accumulation of misfolded proteins and allowing cell survival under adverse conditions. These two opposite reactions to stress, namely SAPK-induced apoptosis and SG-mediated survival, are of biological importance in the determination of cell fate. However, the functional relationship between SG formation and SAPK responses, if any, remains obscure. We have recently found that the signaling adaptor protein RACK1 serves as a mediator between these two responses (Fig. 7).[12]

We initially identified RACK1 as a novel interactor of MTK1 by mass spectrometric analysis of proteins that co-immunoprecipitated with MTK1 from human cells. RACK1 selectively interacts with the N-terminal regulatory domain of MTK1. Depletion of endogenous RACK1 by using short hairpin RNA inhibition (shRNAi) strongly inhibited stress-induced activation of MTK1, but not of other MAPKKKs such as TAK1, whereas RACK1 overexpression led to moderate increases in MTK1 activation only at lower RACK1 concentrations. Thus, RACK1 is necessary, but not sufficient, for MTK1 activation. Through a series of binding experiments, we eventually determined that RACK1 maintains MTK1 in a dimeric, but yet inactive, form (i.e. in a primed state) in the absence of any stress (Figs. 1 and 7). Because the activating auto-phosphorylation

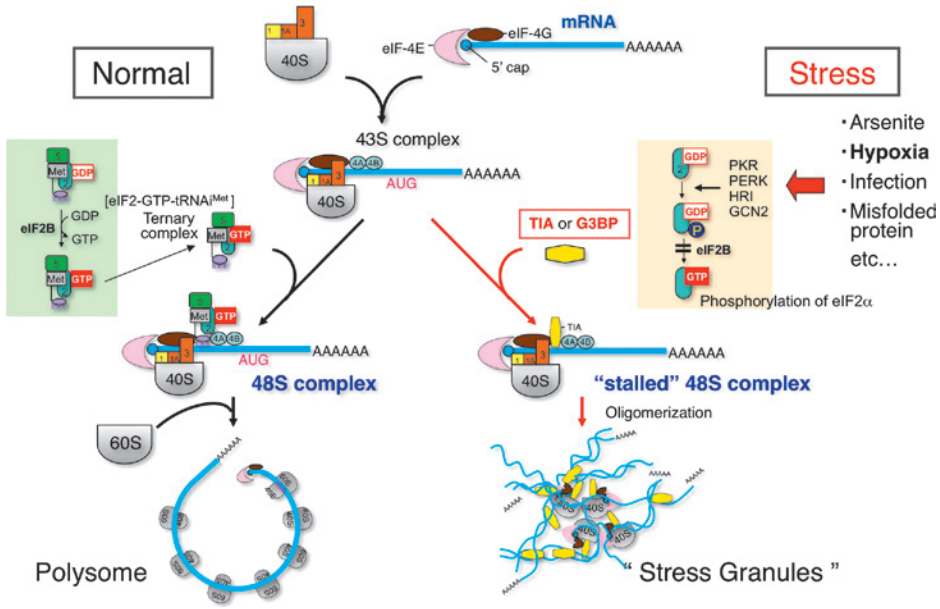

Fig. 6 Specific stress stimuli induce the formation of cytoplasmic stress granules.[30] In the absence of stress, eIF2B promotes assembly of the ternary complex (eIF2-GTP-tRNA^{Met}), which in turn binds to the 43S pre-initiation complex (consisting of the 40S ribosomal subunit, mRNA and initiation factors) to form the 48S pre-initiation complex at the 5' end of capped mRNAs (black arrows: Normal). Upon recognition of the initiation codon by the anticodon of tRNA^{Met}, early initiation factors are displaced by the 60S subunit. The resulting active 80S ribosomes eventually form polysomes on mRNAs to facilitate protein synthesis (bottom left). In stressed cells (red arrows: Stress), phosphorylation of eIF2α by the eIF2 kinases (GCN2, HRI, PKR, and PERK) depletes the cellular store of the ternary complex. Under these conditions, specific mRNA-binding proteins, such as TIA-1 and/or G3BP, interact with the 43S complex, leading to the assembly of a stalled 48S complex that is translationally silent. Self-oligomerization of TIA-1 or G3BP then promotes the aggregation of these complexes at discrete cytoplasmic foci known as stress granules (bottom right). Modified from [30].

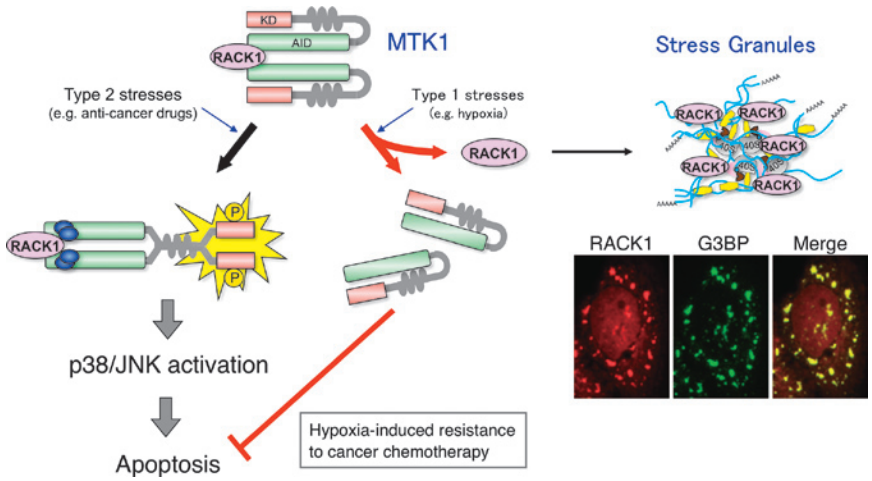

Fig. 7 RACK1 mediates crosstalk between SG formation and SAPK pathways. See text for details.

of MTK1 occurs intermolecularly, preformation of an inactive dimer facilitates MTK1 activation. Therefore, RACK1 serves as an enhancer of MTK1 activation.

Consistent with their interaction, MTK1 was found to co-localize with endogenous RACK1 in the cytoplasm of unstimulated cells. However, induction of SG assembly by specific cellular stresses, such as arsenite, led to dissociation of RACK1 from MTK1, and to incorporation of RACK1 into SGs (Fig. 7). DNA-damaging reagents including etoposide and MMS, which are strong activators of MTK1-SAPK pathways, do not induce SG formation. Thus, we could recognize two types of stresses: type 1 stresses that preferentially induce SG formation, and type 2 stresses that preferentially activate the SAPK cascades. Importantly, SG formation strongly inhibits MTK1 activation due to sequestration of RACK1 into SGs. SG assembly was also found to inhibited GADD45- and MTK1-induced apoptosis. Thus, SGs interact with and sequester the signaling molecule RACK1, resulting in the suppression of both MTK1 activation and subsequent apoptosis.

In solid tumors, cancer cells rapidly outgrow their vascular supply and develop hypoxic microenvironments. Tumor hypoxia is a critical therapeutic problem as it renders tumor cells resistant to apoptotic cell death induced by radiation and chemotherapeutics. Because MTK1 mediates both the apoptosis and the activation of p38 and JNK that are induced by anti-cancer drugs such as etoposide, we determined if inhibition of MTK1 by SG assembly might be involved in hypoxia-induced resistance to etoposide. Exposure of cells to hypoxia induced formation of SGs and the recruitment of RACK1 into SGs. Concurrently, hypoxia reduced etoposide-induced activation of p38 and JNK, and the subsequent activation of caspase-3, which is a hallmark of apoptosis. Ectopic expression of a RACK1 mutant, (RACK1-DE) which is not recruited into SGs, reversed the inhibitory effect of hypoxia on etoposide-induced SAPK activation and apoptosis. Our results thus suggest that resistance to etoposide-induced apoptosis in hypoxic cells is mediated, at least in part, by the sequestration of RACK1 into SGs. RACK1 is incorporated into SGs following specific stresses, and thus mediates a novel crosstalk between the pro-survival SG response and the pro-apoptotic SAPK pathways. When cells are exposed to type 2 stresses such as etoposide, MTK1 activates SAPK pathways, leading to apoptosis. In contrast, when cells are exposed to type 1 stresses, such as hypoxia, SGs are formed, thereby promoting cell survival. When both types of stresses are simultaneously applied, sequestration of RACK1 into SGs by a type 1 stress suppresses the apoptosis induced by a type 2 stress. Cancer cells may exploit this regulatory system to acquire resistance to anticancer therapies (Fig. 7).

CONCLUSION REMARKS

Significant progress towards understanding physiological and pathological functions of SAPK signaling pathways has been achieved over the past few years. Identification of novel molecules that are involved in the SAPK signaling pathways has provided new insights into roles of p38 and JNK in cell fate decisions and in the development of human diseases. Besides their well-established roles in inflammation and stress responses, recent evidence indicates that SAPK pathways function as tumor suppressors by controlling cell cycle arrest, cell death, transformation, and angiogenesis.[3] Indeed, aberrant regulation of SAPK pathways has been identified in various human cancers. For instance, loss-of-function mutations in the MKK4 gene have been detected at a fairly consistent rate (5%) in primary cancers of the pancreas, bile ducts, breast, lung, colon, and testis. Mutational inactivation of MKK4 in certain human cancers has been linked to poor prognosis and increased invasiveness. Epigenetic gene silencing of the three GADD45 family members due to promoter hyper-methylation has also been frequently observed in several types

of human cancers. The GADD45α promoter is methylated in breast cancers and prostate cancers, resulting in reduced GADD45α expression.[31] The GADD45β as well as the GADD45γ genes are methylated and silenced in hepatocellular carcinoma. Epigenetic GADD45γ silencing has also been observed in 85% of non-Hodgkin, 50% of Hodgkin lymphoma, 73% of nasopharyngeal, 50% of cervical, 29% of esophageal, and 40% of lung carcinoma cell lines.[32] Thus, GADD45-meditaed signaling is frequently impaired in a variety of human cancers and represents a novel class of targets for therapeutic intervention in cancer. Additional research regarding the regulatory mechanisms of SAPK pathways is needed to comprehensively understand cellular responses to environmental stress, and to open new avenues for therapy of currently intractable human disorders such as cancer and autoimmune diseases.

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