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# The p38 MAPK stress pathway as a tumor suppressor or more?

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## 1. ABSTRACT

p38 mitogen-activated protein kinases (p38 MAPKs) are a group of serine/threonine protein kinases that together with ERK (extracellular signal-regulated kinases) and JNK (c-Jun N-terminal kinases) MAPKs act to convert different extracellular signals into specific cellular responses through interacting with and phosphorylating downstream targets. In contrast to the mitogenic ERK pathway, mammalian p38 MAPK family proteins (alpha, beta, gamma, and delta), with and without JNK participation, predominantly regulate inflammatory and stress response. Recent emerging evidence suggests that the p38 stress MAPK pathway may function as a tumor suppressor through regulating Ras-dependent and -independent proliferation, transformation, invasion and cell death by isoform-specific mechanisms. A selective activation of a stress pathway to block tumorigenesis may be a novel strategy to control human malignancies.

## 2. INTRODUCTION

MAPKs (mitogen-activated protein kinases) consist of ERK (extracellular signal-regulated kinase), JNK (c-Jun N-terminal kinase), and p38 cascades (1, 2). Classically, MAPKs function by phosphorylating substrates containing consensus sequence Ser/Thr-Pro (3) after they are phosphorylated and activated by upstream kinases (MAPK kinases). Each of these MAPK pathways has several family members but all share the same conservative Thr-*Xaa*-Tyr phosphorylation motif (where *Xaa* is any amino-acid) (1, 2, 4). The ERK activity is mostly frequently activated by mitogens and required for cell proliferation, differentiation and/or transformation (5, 6). JNK and p38 pathways, on the other hand, are predominantly responsive to stress and cytokine signaling and play an in important role in regulating stress response and inflammation (7, 8). MAPKs can be activated almost by all type of stimuli and are consequently involved in

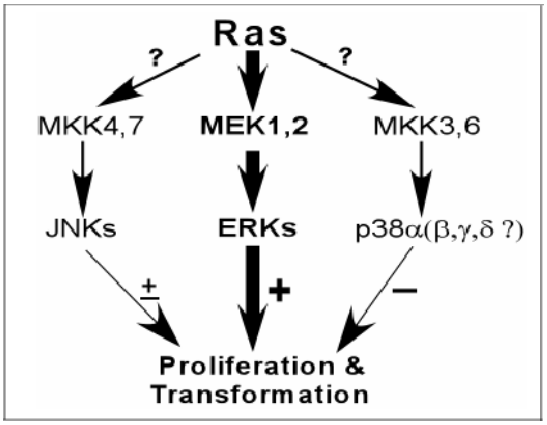


**Figure 1.** An opposing role of the ERK and p38 pathways in Ras transformation. The ERK activity is required for Ras transformation and the ERK activation alone is sufficient to transform cells. The p38 MAPK pathway, as demonstrated with p38alpha, on the other hand, is inhibitory to Ras oncogene signaling. Thus, the ERK and p38 MAPK pathways oppose each other in determining Ras transforming activity. The inconsistent effect of the JNK pathway on Ras transformation was illustrated with the "±" sign, whereas "?" indicates that p38beta, gamma and delta proteins may have a distinct role in regulating Ras oncogene activity.

many critical biological processes such as proliferation, differentiation, cell death and transformation through regulating downstream gene expression and/or interacting with other signaling cascades.

The p38 upstream activators include MAPK kinase 6 (MKK6) and MKK3. Downstream effectors consist of kinases such as MK2 (MAPK-activating protein kinase 2) and PRAK (p38-related/activated protein kinase) as well as transcription factors including ATF-2 (activating transcription factor-2), MEF2 (myocyte enhancement factor 2), and c-Jun (4, 9). The mammalian p38 family consists of four isoform proteins (alpha, beta, gamma, and delta), with p38alpha and p38beta 75% identical in their amino-acid sequence, and p38gamma and p38delta about 60% identical to p38alpha (4, 10). p38alpha and p38beta are susceptible to inhibition by SB drugs (SB203580 and SB202190) whereas p38gamma and p38delta activity is unaffected by these compounds due to their differences in ATP binding pocket (11, 12). While all p38 family proteins can be phosphorylated by MKK6/MKK3 and share many substrates, p38alpha and p38beta have a higher activity to phosphorylate MK2/MK3, whereas p38gamma and p38delta appear to have a selective effect on a microtubule-associated protein Tau and scaffold proteins SAP90 and SAP97 (4, 10). The *in vivo* signaling selectivity of p38 family proteins, however, remain mostly unknown, which may be critical for understanding broad activities of p38 MAPK activation.

p38alpha {also called p38} was originally identified in study of protein tyrosine phosphorylation in response to stress (13, 14). Later on, p38beta (15), p38gamma (16) {also called SAPK3 (17) and ERK6 (18)}, and p38delta (19) {also called SAPK4 (20)} were cloned and characterized. p38alpha is ubiquitously expressed, whereas other p38 proteins are detectable in a tissue-specific manner (4). p38alpha knockout turns out to be lethal as a result of extraembryonic effects (21, 22), whereas mice with p38beta (23) or p38gamma and/or p38delta knockout (24) are viable and fertile. Although studies of a p38-interacting protein suggest a redundant role of p38 family proteins in development (25), most published results about the p38 pathway are from analyzing p38alpha (4, 26) and specific effects of other p38 family proteins are just emerging (4, 10). Signaling through the p38 pathway has been shown to be involved in regulating inflammation, cytokine synthesis, cell death and cell differentiation, which has been recently reviewed in detail (4, 9, 10). Below we will review recent studies about roles of the p38 pathway activation in Ras-dependent and -independent malignancies and these results together suggest that a tumor suppressing role of the p38 stress pathway may act by isoform-specific and/or tissue-specific mechanisms. A review about specific effects of p38alpha in tumorigenesis has also been recently published (26).

## 3. RAS ONCOGENE AND THE ERK/JNK MAPK PATHWAYS

Ras proteins play central roles in the control of normal and transformed cell growth, and are among the most frequently mutated and activated genes in human cancers; 30% of Ras mutations occur in codons 12, 13, and 61 (27-29). There are three members of Ras proteins, i.e., H-Ras, K-Ras and N-Ras, and experiments with antisense and knockout studies showed that only K-Ras is required for normal cell growth and/or mouse development (30, 31). In human cancer, 85% of these mutations involve K-Ras whereas less than 15% are from H-Ras (27). K-Ras mutations occur frequently in pancreatic, colon, lung and thyroid cancers, whereas H-Ras mutations mostly arise from bladder and kidney tumors (27-29). In addition to mutations, high levels of normal Ras protein expression also contribute to breast cancer progression downstream of activated membrane tyrosine kinase receptors (29, 32). Targeting Ras oncogene has been therefore an intensive research for cancer therapeutic development for several decades (27, 28, 33).

Ras oncogene signals through multiple pathways or effectors to induce a malignant phenotype in which MAPKs are among the most critical cascades to mediate and modify its activity. The ERK pathway acts downstream of Ras/Raf/MEK to provide a common route by which signals from different growth factor receptors and oncogene Ras converge to activate major transcription factors (34). The ERK MAPK pathway has been shown to be required for Ras-induced transformation (6) (Figure 1). Inhibition of the ERK pathway has consequently become an important tool to screen for novel therapeutics to control Ras-related malignancies (35-37). Activation of the ERK MAPK pathway alone is also known to be sufficient to transform cells, as demonstrated with Raf (38, 39) and constitutively active MEK (5, 40, 41). Recent studies

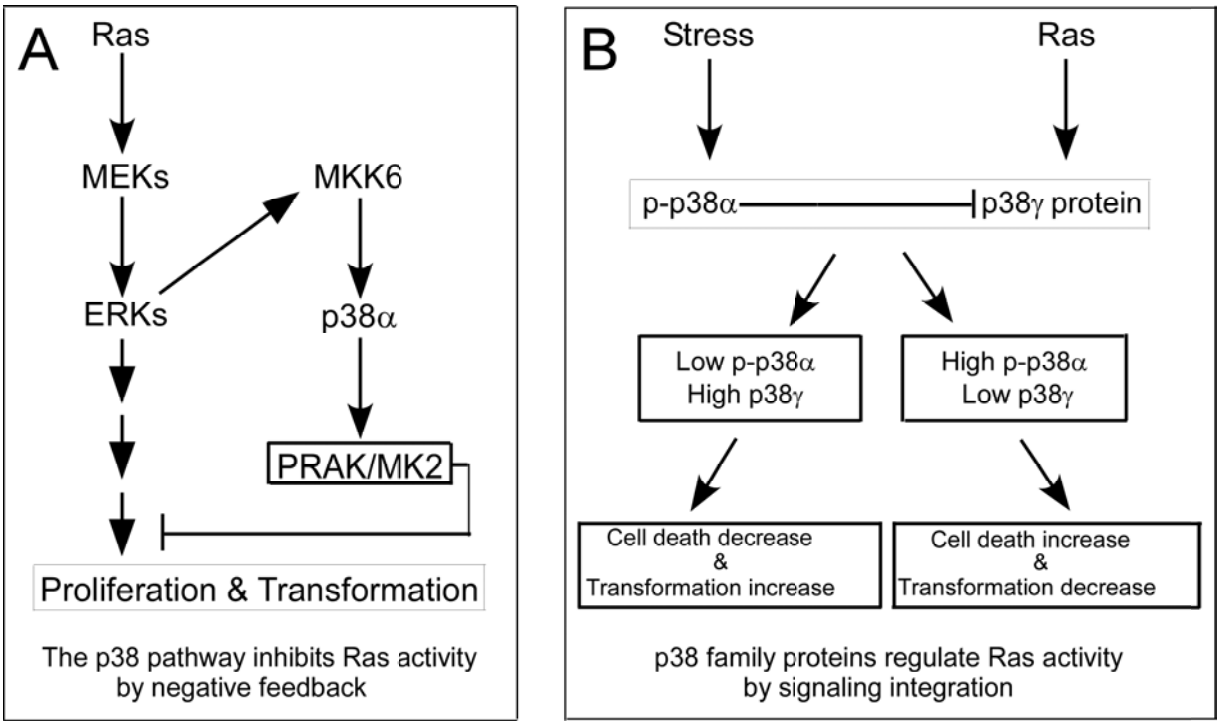


**Figure 2**. p38 pathways regulate Ras oncogene activity by negative feedback (A) and signaling integration (B). Along the p38 pathway, MKK6, p38alpha and MK2/PRAK have been shown to be activated by Ras oncogene through MEK/ERK pathways and in turn suppress Ras activity by negative feedback (A) (60). The p38gamma expression, on the other hand, is induced by Ras that is required for Ras transformation (72). Phosphorylated p38alpha was recently shown to down-regulate p38gamma protein expression by ubiquitin/proteasome pathways (74). In a given system, therefore, the Ras transforming activity will be determined by integrated signaling from the Ras-suppressor p38alpha and the Ras-effector p38gamma (B) (72, 74). Solid lines were used for elucidative purpose and detail pathways for these regulations remain to be established.

further revealed frequent mutations/activations of B-Raf proteins in human melanoma (42-45). Although normal cells also require the ERK activity for proliferation and differentiation in response to growth factors, a selective inhibition of constitutively active proliferative signaling from Ras/Raf/MEK/ERK cascades remains a major research direction for cancer therapeutic developments.

The JNK/MAPK pathway has inconsistent roles in Ras transformation (Figure 1). Early studies using a dominant negative JNK kinase (SEK) showed a required role of the JNK pathway in Ras transformation (46). This observation is further confirmed by Ras transformation experiments in mouse fibroblasts with genetic disrupting c-Jun gene (47) or stably expressing a dominant negative c-Jun protein (Tam67) (48). Additional experiments showed that JNK2 is required for Ras transformation independent of c-Jun/AP1 regulation (49). This conclusion, however, is challenged by an observation in which increased tumor growth was demonstrated when Ras-transformed JNK1/2 null cells were injected into mice as compared to the wild-type cells administration, suggesting a suppressive role of the JNK pathway in Ras transformation (50). Additional studies by application of a specific JNK inhibitor SP600125, however, revealed that the JNK inhibition has no substantial effects on Ras-induced transformion in rat intestine epithelial cells (51). This discrepancy is not clearly understood at the present time and may relate to differences in mouse species or cell lines used resulting in context-dependent effects.

## 4. THE p38ALPHA PATHWAY FUNCTIONS AS A RAS ONCOGENE SUPPRESSOR

The p38alpha (p38) pathway generally acts as a suppressor for Ras signaling (Figure 1) (see the review (26, 52). An inhibitory role of the p38 pathway in Ras activity was first suggested by an observation that p38 activation inhibits gene expression of cyclin D1 (53), an essential downstream effector of Ras proliferative signaling (54). Furthermore, application of a dominant negative MKK6 or the SB inhibitor was shown to reverse MEKK3 (an activator of several MAPK pathways (55)) induced cyclin D1 down-regulation and/or cell-growth inhibition (56). Later on, several studies showed that endogenous normal Ras activity is required for the p38 activation by growth factors. For example, treatment of cells with GM-CSF (granulocyte/macrophage colony-stimulating factor) and PDGF (platelet-derived growth factor) requires Ras to activate p38, leading to an increased proliferation (57) or cell motility (58). Furthermore, the Src oncoprotein requires Ras/p38/JNK pathways to induce STAT3

**Table 1.** Studies showing a regulatory role of p38 pathways in tumorigenesis

| Year | Authors | **Results** | **Ref** |
|---|---|---|---|
| 2000 | Chen *et al* | The p38 pathway MKK6/p38alpha/PRAK/MK2 inhibits Ras oncogene-induced proliferation by a negative feedback | 60 |
| 2002 | Pruitt *et al* | **Increased** Ras transformation in cell culture by the p38 inhibitor SB203580 | 51 |
| 2002 | Bulavin *et al* | **Increased** tumor growth of Ras transformed p38alpha$^{-/-}$ MEFs in mice | 131 |
| 2003 | Brancho *et al* | **Increased** tumor growth of Ras transformed MKK3/6$^{-/-}$ MEFs in mice | 63 |
| 2003[1] | Elenitoba-Johnson *et al* | **Decreased** tumor growth of human lymphoma by the p38 inhibitor SB203580 in mice | 85 |
| 2004 | Bulavin *et al* | **Increased** mammary tumor formation by SB203580 | 61 |
| | | in PPM1D null mice carrying MMTV-*Erbb2* transgene | |
| 2005 | Tang *et al* | **Decreased** Ras transformation by p38gamma depletion or p38alpha phosphorylation | 72 |
| 2005 | Timofeev *et al* | **Decreased** tumor growth in nude mice by injecting | 62 |
| | | inducible MKK6-expressing cells | |
| 2006[1] | Matsuo *et al* | **Decreased** lung metastasis of murine tumors without growth alterations in p38alpha$^{+/-}$ mice | 118 |
| 2007 | Sun *et al* | **Increased** Ras transformation in PRAK null MEFs and **increased** DMBA-induced skin tumors in PRAK null mice | 65 |
| 2007 | Hui *et al* | **Increased** carcinogen-induced liver tumors in mice with liver-specific depletion of *p38alpha* gene | 132 |
| 2007 | Demidov *et al* | **Decreased** mammary tumor formation in MMTV-*ErB2/PPM1D* and MMTV-*MKK6* transgenic mice | 66 |

The tumor suppressive activity of the MKK6/p38alpha/PRAK/MK2 pathway was demonstrated in most of these studies except two occasions ([1]) in which the opposite effect was observed likely as a result of involvement of tumor/host/tissue-specific factors and/or other p38 family proteins.

transcriptional activity (59). These results together suggest that endogenous Ras may act upstream of the p38 pathway to regulate cell growth and gene expression.

The first systemic analyses about the role of p38 pathways in Ras oncogene activity was reported in 2000 (Figure 2A and Table 1) (60). In this study, transient expression of Ras oncogene in NIH 3T3 cells stimulated the kinase activity of MKK6, p38alpha, and its downstream kinases PRAK/MK2. This stimulation was blocked by co-expressing their respective upstream dominant negative and/or the non-phosphorable ERK, indicating an activation of the entire p38 cascade downstream of the ERK pathway. Moreover, dominant negative forms of each of these molecules inhibit Ras-induced proliferation and gene expression, indicating a negative feedback property of the p38 pathway activation (60). Additional analyses revealed that this suppressive effect of the p38 pathway may occur through its inhibitory activity on Ras-induced JNK activation. Importantly, the p38 activation was only growth-inhibitory in human bladder cancer cells that harbor a mutated but not a normal Ras gene. These results together suggest that the entire p38 pathway from MKK6 through p38alpha to PRAK/MK2 may function as a Ras suppressor by negative feedback and its activation may inhibit Ras-dependent malignant growth.

The inhibitory effect of the p38 pathway on Ras oncogene activity was further confirmed and extended by a series of *in vitro* and *in vivo* studies (Table 1). First, inhibition of the p38 pathway by SB203580 increases Ras-induced soft agar growth whereas JNK inhibition with SP600125 does not have substantial effect, indicating a suppressive activity of endogenous p38 in Ras transformation (51). The suppressive effect of the p38 on tumorigenesis was further established in mice by a SB203580-mediated increase (61) and MKK6-induced decrease in tumor growth (62). Furthermore, knockout of several endogenous genes along the p38 pathway was shown to increase Ras-induced transformation and resultant tumor growth in mice. These include MKK3/6 (63), p38alpha (61, 64), and PRAK (65). In these studies, wild-type and knockout mouse embryonic fibroblasts were typically expressed with Ras oncogene and resultant tumorigenesis was assessed by *in vitro* transformation and/or by *in vivo* tumor growth following their injection into mice. Studies by Sun *et al* further showed that PRAK knockout also renders mice prone to chemically induced skin tumors (65). In addition, the MMTV-*MKK6* transgene reduces the mammary tumor formation in MMTV-*ErbB2/PPM1D* transgenic mice (66). These studies together establish an inhibitory role of the p38 pathway in Ras transformation in experimental cancers.

## 5. THE p38 PATHWAY REGULATES RAS ACTIVITY BY ISOFORM-SPECIFIC MECHANISMS

The mammalian p38 pathway consists of four isoform proteins with distinct biological activities that can act cooperatively or antagonistically in executing various functions. p38gamma, but not its other family proteins, was first shown to bind and phosphorylate alpha1-syntrophin through its unique C-terminal PDZ-binding motif (67). Further studies showed that p38beta increases whereas p38gamma and p38delta decrease or have no effects on stress-induced AP-1 transcriptional activity in human breast cancer cells (68). In another separate study using rat primary hepatocytes, however, p38alpha, p38beta, and p38delta decreased while p38gamma increased the gene expression of HO-1 (heme oxygenase-1) (69). In mouse mesangial cells, on the other hand, TGF-beta1 was shown to signal through p38alpha and p38delta, but not p38beta, downstream of MKK3 (70). Moreover, in human keratinocytes, p38alpha increases whereas p38delta decreases MKK6-induced gene expression (71). These results together indicate that p38 family proteins may play a distinct role in regulating gene expressions by a cell- or tissue-specific mechanism. Most of these analyses, however, have been performed by over-expressing p38 proteins and roles of endogenous p38 MAPKs in these regulations remain mostly un-established.

In rat intestinal epithelial cells (IEC-6), Ras oncogene was found to increase p38gamma RNA and protein expression with concurrently stimulated p38alpha phosphorylation and decreased p38gamma phosphorylation in which p38beta and p38delta proteins were undetectable (72). Further analysis using the p38alpha inhibitor SB and

p38gamma siRNA showed that induced phospho-p38alpha inhibits while resultant elevated p38gamma proteins promote Ras transformation, indicating an opposite role of two p38 family proteins in Ras transformation (Figure 2B). More importantly, p38gamma mRNA was unanimously increased in a group of primary human colon cancer tissues as compared to the matched normal tissues, pointing to its potential role in colon cancer development. Ras also increases p38gamma protein expression (but not p38alpha phosphorylation) in human breast cancer (73). In this case, p38gamma protein acts to mediate Ras-invasive signaling without affecting its proliferative activity, which was inhibited by estrogen receptor alpha (ER) through their direct interactions. These results together indicate that increased p38gamma gene expression is required for Ras oncogenic activity. Recent studies further showed that phospho-p38alpha can down-regulate p38gamma protein expression through c-Jun dependent ubiquitin/proteasome pathways (74). Because p38alpha is ubiquitously expressed and its activating signals are more abundant, stress signaling may act through both stimulating p38alpha phosphorylation and p38gamma down-regulation to inhibit Ras oncogene activity. However, an increased p38gamma gene expression in human colon and breast cancers suggests its potential role in Ras-dependent and perhaps also in Ras-independent human malignancies.

## 6. THE ROLES OF THE p38 PATHWAY IN REGULATING MALIGNANT GROWTH

In normal tissues, p38 activation is known to lead to an inhibition of liver cell proliferation, as demonstrated by decreased growth by MKK6 that was reversed by the SB inhibitor (75). Moreover, studies with the organ-specific p38alpha knockout and over-expression also showed that p38alpha is inhibitory to fetal cardiomyocyte proliferation (76). In macrophages, however, p38 activation is involved in promoting cell proliferation in response to GM-CSF (77) and inhibiting cell death (78). This distinct p38 activity may relate to its regulatory effects on the biosynthesis as well as the stability of pro-inflammatory molecules, which may play an important role in regulating cancer initiation and progression {see the recent review (10, 79)}.

A moderate activation of the p38 pathway also frequently leads to growth-inhibitory response in cancer. Studies from Pramanik *et al*, for example, showed that the MKK6 expression inhibits DNA synthesis in human MCF-7 breast cancer cells and this inhibitory effect is enhanced by the co-expressed p38gamma but decreased by the p38beta, indicating isoform-specific mechanisms involved (68). The p38 activation was further shown to inhibit tumor growth *in vivo*, as adenoviral-mediated MKK6 over-expression completely abolished the tumor formation of human breast cancer in nude mice, although the involved p38 family protein(s) was not identified in this study (80). In addition, p38 activation with MKK6 and inhibition with SB 203580 were found to decrease and increase tumor growth in chick embryos (81). The p38 activation has also been reported to inhibit proliferation of K-Ras mutated pancreatic cancer cells (82) and is required for the growth inhibition by activin, a member of the TGFbeta family proteins (83). In some cases, however, the p38 activity can be growth-stimulatory: inhibition of p38alpha/beta activity with SB203580 suppresses DNA synthesis in human thyroid carcinoma cells *in vitro* (84) and blocks the growth of human lymphoma *in vitro* and in mice (85). Whether these different effects result from specific tumor lines or are due to non-specific activities of SB and/or different contributions of p38alpha versus p38beta proteins remains unknown (Table 1).

The growth inhibitory activity of the p38 MAPK may be associated with its role in promoting differentiation. The best example for this is that activation of the p38 pathway by MKK6 induces terminal differentiation of human rhabdomyosarcoma cells that couples with a reduced proliferation (86). Further studies also showed that p38 activity is required for neuronal differentiation in PC12 cells (87, 88). Moreover, p38 activity is required for muscle differentiation (89) and knockout studies revealed that myoblasts lacking p38alpha, but not p38beta and p38delta, are deficient in differentiation that couples with a continuous proliferation (90). Studies from Uddin *et al* further showed that there is a differentiation-associated expression of p38alpha and p38gamma in primary human erythroid cells (91). Moreover, p38gamma and p38delta were also demonstrated to be involved in myoblasts (18) and keratinocytes differentiation respectively (92). These results together indicate that the p38 pathway may induce cellular differentiation through isoform- and -tissue specific mechanisms, which could contribute to its growth-inhibitory activity.

## 7. THE ROLES OF THE p38 PATHWAY IN REGULATING CELL DEATH

The p38 pathway activity is usually pro-apoptotic. An inducible expression of ASK1 (apoptosis signal-regulating kinase 1), an upstream kinase that activates both the JNK and p38 pathways, was first shown to induce cell death (93). Later studies showed that the p38 activation by adenovirus-mediated MKK6 gene delivery or a chemical stimulus arsenite induces cell death in estrogen receptor negative (ER-) but not in ER+ breast cancer cells through c-Jun activation (80). In human colon cancer cells, however, the p38 activation only leads to a cell-death response when K-Ras gene is mutated, and this selective K-Ras dependent apoptotic effect was shown to be due to a down-regulation of anti-apoptotic vitamin D receptor (VDR) protein expression (94). Knockout studies further revealed a pro-apoptotic property of the p38 pathway by demonstrating a decreased cell death in cells lacking MKK6 (95), p38alpha (64, 96) and MK2 (97). These results together suggest a sufficient role of the p38 pathway in inducing cell death. As previously demonstrated (80, 85), the pro-apoptotic activity of the p38 pathway may be an important component of its tumor-inhibitory activity *in vitro* and *in vivo*.

Additional studies with genetic and/or chemical inhibitors showed that the p38 pathway can mediate various upstream signaling to induce cell death. For example,

activation of p38 and/or JNK stress MAPK pathways has been shown to be required for a cell-death response after treatment with cancer therapeutic agents such as adriamycin (98), etoposide (99), cisplatin (100), UV radiation (7, 101), gamma-irradiation (102) and even ligand epidermal growth factor (EGF) (103) in various systems. p38 activation, however, was also found to be anti-apoptotic in melanoma (104) and glioma cells (105) in response to UV radiation. In addition to cell-types, some of these effects may be due to involvements of distinct p38 family proteins in this process. Indeed, recent evidence suggests that in contrast to p38alpha, p38beta appears to be anti-apoptotic in some systems (106-109). Furthermore, p38gamma, by both over-expression and depletion, was recently shown to be anti-apoptotic through antagonizing the pro-apoptotic p38alpha signaling (74). The pro-apoptotic activity of p38alpha is likely integrated by anti-apoptotic effects of its family proteins, leading to either a cell-survival or cell-death response by a cell-type and/or stimuli specific mechanism.

Multiple mechanisms have been shown to be involved in p38 regulating cell death. Early studies showed that that p38 activation may be required for UV-induced cell death through phosphorylating p53 protein at Ser33/46 and thereby increasing its stability and pro-apoptotic activity (101). A serine/threonine specific protein phosphatase Wip1 (PPM1D) of the PP2C family was later found to be induced by a p38-dependent and p53-dependent pathway and in turn dephosphorylate p38, thereby preventing p53-mediated apoptosis (110). Recent studies further showed that p38 can promote apoptosis by directly phosphorylating Bcl-2 family proteins: it phosphorylates $Bim_{EL}$ protein at Ser65 to enhances its pro-apoptotic functions (111) but phosphorylates Bcl-2 at Thr56 and Ser87 to inhibits its anti-apoptotic potential (112). Additional experiments showed that the p38 pathway can increase cell death through activating a downstream target $p18^{Hamlet}$, a transcriptional co-activator (113), or cross-talking with the PI3K pathway (102, 114). Therefore, dissecting signaling interactions of the p38 pathway with different pro-apoptotic and anti-apoptotic cascades may additionally contribute to understanding its pleiotropic cell-death regulatory activities.

## 8. THE ROLES OF THE p38 PATHWAY IN REGULATING CELL INVASION AND CANCER METASTASIS

p38 activity has been shown to increase cancer cell invasion/migration in many systems. Levels of phospho-p38 proteins, for example, are positively correlated with breast cancer invasive activity and its inhibition with the SB compound was shown to decrease the invasion likely through decreasing the mRNA stability of uPA (urokinase plasminogen activator) and its receptor uPAR (115). Inhibition of the p38 activity with different dominant negative mutants further showed that it is p38alpha, but not p38beta, activation that is responsible for increasing the uPA and uPAR RNA expression. Additional studies revealed that the p38 acts downstream of MKK3 and upstream of MK2 to maintain the uPA stability and the invasive phenotype (116). Of interest, in human umbilical vein endothelial cells (HUVECs) expression of dominant negative forms of p38alpha and p38gamma as well as its downstream kinase MK2, but not p38beta or p38delta, blocks VEGF (vascular endothelial growth factor) induced endothelial migration (117). However, a recent study showed a resistance of $p38alpha^{+/-}$ mice to experimental lung metastasis as compared to the normal mice following an intravenous injection of mouse melanoma F10 and Lewis lung carcinoma cells (118). These results indicate that p38alpha activity in tumor versus host may play an opposite role in regulating tumor invasion and metastasis.

In experimental cancer, p38 activity was shown to be required for H-Ras, but not N-Ras, induced cell invasion in breast epithelial MCF10A cells (119). This effect may involve Rac1/PI3K activation and up-regulation of MMP-2/9 (matrix metalloproteinase 2 and 9) (120). Additional studies also showed that p38 phosphorylation is required for K-Ras dependent invasion in pancreatic cancer (121) as well as for prostate (122) and lung cancer invasion (123). Although studies with over-expressed dominant negative p38 proteins showed that only p38alpha (but not other isoforms) is required for H-Ras-induced invasion in 3T3 cells (124), p38gamma proteins were recently shown to promote breast cancer invasion downstream of Ras (73). Furthermore, p38alpha, but not p38beta, can phosphorylate pro-invasive EGFR (epidermal growth factor receptor) at Y1045 and T669, leading to its internalization and destruction (125-127). Although the relationship between the p38-mediated EGFR down-regulation and the p38 invasion-stimulatory effect remains to be established further, outcomes of the p38 pathway activation on cancer invasion and/or metastasis will likely be determined by the integrated signaling from its associated pro-invasive and anti-invasive pathway activities.

## 9. CONCLUSION REMARKS

Activation of the entire p38 stress MAPK pathway (MKK6-p38alpha-PRAK) has been systemically demonstrated to suppress Ras-dependent and -independent malignant growth through inhibition of cell proliferation, induction of differentiation and/or cell-death. It was further shown that the tumor-suppressive and/or cell-death promoting effect of p38alpha couples with its activity to disrupt its antagonistic family protein p38gamma. These results together indicate a general tumor suppressor activity of the p38 MAPK stress pathway. It should be pointed out that the p38 pathway activation by phosphorylation may only act as a tumor suppressor during early stages of tumorigenesis, with the final outcomes likely different in different types of cancers and different with the systemic versus the neoplastic p38 activation by isoform-specific mechanisms. It is therefore not surprising to note that different than other tumor suppressors there exists an inversed correlation between levels of phospho-p38 proteins (and JNK) and breast cancer patient survival (128, 129). Perhaps, an altered expression of p38 family proteins may be more relevant to clinical cancer progression, as demonstrated with an increased p38gamma expression in human colon and breast cancers (72), increased p38beta

transcripts in human lymphomas (85) and increased p38alpha expression and phosphorylation in thyroid cancers (84). Since a high rate of Ras mutations and/or over-expression has been observed in all these clinical malignancies (27, 85), studies of Ras activation and p38 pathway regulations may provide a unique angle to understand roles of this stress pathway in tumorigenesis. Moreover, with the well established role of the p38 MAPKs in regulating cytokine signaling and inflammatory response that can affect tumorigenesis through multiple pathways (130), investigating systemic effects of the p38 MAPK activation may be particularly needed for understanding its roles in malignant progression.

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**Abbreviations:** p38 MAPKs: p38 mitogen-activated protein kinases; ERK: extracellular signal-regulated kinases; JNK: c-Jun N-terminal kinases; MAPKs: mitogen-activated protein kinases; MKK: MAPK kinase; MK: MAPK-activating protein kinase; PRAK: p38-related/activated protein kinase; ATF-2: activating transcription factor-2; MEF2: myocyte enhancement factor 2; MEK: MAP kinase/ERK kinase; SEK: dominant

negative JNK kinase; GM-CSF: granulocyte/macrophage colony-stimulating factor; PDGF: platelet-derived growth factor; HO-1: heme oxygenase-1;
ER: estrogen receptor alpha; ASK1: apoptosis signal-regulating kinase 1; VDR: vitamin D receptor; uPA: urokinase plasminogen activator; HUVECs: human umbilical embryonic cells; VEGF: vascular endothelial growth factor;
MMP: matrix metalloproteinase; EGFR: epidermal growth factor receptor

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