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p38 MAP kinase: a convergence point in cancer therapy

James M. Olson and Andrew R. Hallahan

Fred Hutchinson Cancer Research Center, Seattle, WA 98109, USA, and Department of Pediatrics, University of Washington, Seattle, WA 98195, USA

Recent studies show that activation of p38 mitogen-activated protein kinase (MAPK) results in cancer cell apoptosis initiated by retinoids, cisplatin and other chemotherapeutic agents. The observation that divergent therapies act through a common signal transduction pathway raises the possibility of developing new anti-cancer agents that lack the side-effects caused by events upstream of p38 MAPK. Here, we review p38-MAPK-mediated tumor cell apoptosis and implications for cancer therapeutics.

The mitogen-activated protein kinase (MAPK) superfamily consists of three serine/threonine kinase cascades (Figure 1) [1]. Extracellular-signal-related kinases (ERKs) respond to growth factors or other external mitogenic signals by promoting cell proliferation and opposing cell-death signals. The other two pathways – p38 MAPK and the c-Jun N-terminal kinase (JNK) pathways – are typically described as stress-activated kinases that promote inflammation, or, in certain cases, programmed cell death [2,3]. In addition, several other kinases, including Erk3, Erk5, Erk7 and Erk8, appear to have distinct effectors and functions outside of the classical pathways [4–7]

Pharmaceutical developments related to MAPK pathways have focused on molecules that inhibit MAPKs. For example, p38 MAPK inhibitors are now being evaluated for efficacy in patients with inflammatory diseases, and ERK inhibitors are being tested in cultured cancer cells where activation of the ERK pathway mediates an anti-apoptotic response following treatment with certain chemotherapeutic agents [8]. This review focuses on emerging data showing that p38 MAPK activation is necessary for cancer cell apoptosis induced by therapies previously considered to be mechanistically unrelated.

The p38 MAPK cascade

First described in 1994, the p38 MAPK cascade regulates a variety of cellular responses to stress, inflammation and other signals [9–11]. p38 MAPK is relatively inactive in the non-phosphorylated form and becomes rapidly activated by phosphorylation of two Thr-Gly-Tyr motifs [12,13]. MAPK kinase-3 and -6 phosphorylate p38 MAPK, typically in a matter of minutes following exposure to tumor necrosis factor-α, interleukin-1, heat shock, or other activating stimuli. Inactivation occurs relatively rapidly and is mediated by phosphatases such as protein phosphatase 1, protein phosphatase 2A, or MAPK phosphatase [14–16]. These phosphatases are, in some cases, activated by phospho-p38 MAPK, suggesting a mechanism for tight regulation of active p38 MAPK. There are four isoforms of p38 MAPK, α, β, γ and δ, which differ in their tissue expression and affinity for upstream activators and downstream effectors [17]. The α and β isoforms are expressed in most tissues, whereas the γ and δ isoforms have a more limited expression [18].

Phospho-p38 MAPK activates ATF-2, CHOP-1, MEF-2 and other transcription factors through phosphorylation [19–21]. Other immediate targets include MNK1, MSK2, Elk-1, MAPKAP-2, MAPKAP-K3, MSK1 and hnRNP [22,23]. Of particular note for cancer therapy, p38 MAPK has been demonstrated to phosphorylate directly, thus activating the key cell-cycle regulators p53 [24,25] and p73 [26]. Downstream activities attributed to these phosphorylation events include cell-cycle arrest, apoptosis, cytokine production, regulation of RNA splicing, and cell differentiation. These activities are frequently cell-type specific, with most studies focused on inflammatory cells (reviewed in [27]).

Several p38 MAPK antagonists and dominant–negative constructs provide a means to distinguish activities for which p38 MAPK is necessary from those that simply correlate with p38 MAPK phosphorylation. The bicyclic pyridinyl-imidazole SKF86002 was the first reported inhibitor of p38 MAPK and is also a cyclo-oxygenase and lipoxygenase inhibitor [28]. Since then, SB203580 and other 2,4,5-triaryl imidazoles have been shown to inhibit p38 MAPKα and p38 MAPKβ specifically, without affecting JNK or ERK activity [29]. A series of p38 MAPK inhibitors has since been developed, principally using these agents as a template (reviewed in [27]). In conjunction with pathway-activating and dominant–negative constructs, these drugs have enabled several recent studies clearly showing that p38 MAPK activation is necessary and, in several cases, sufficient, to cause apoptosis in a variety of cancer cell types.

p38-MAPK-mediated apoptosis in cancer cells

Recent studies show that p38 MAPK activation is necessary for cancer cell death initiated by a variety of anti-cancer agents. Recently, bone morphogenetic protein 2 (BMP2) was identified as a paracrine mediator of 13-*cis*

Corresponding author: James M. Olson (jolson@fhcrc.org).

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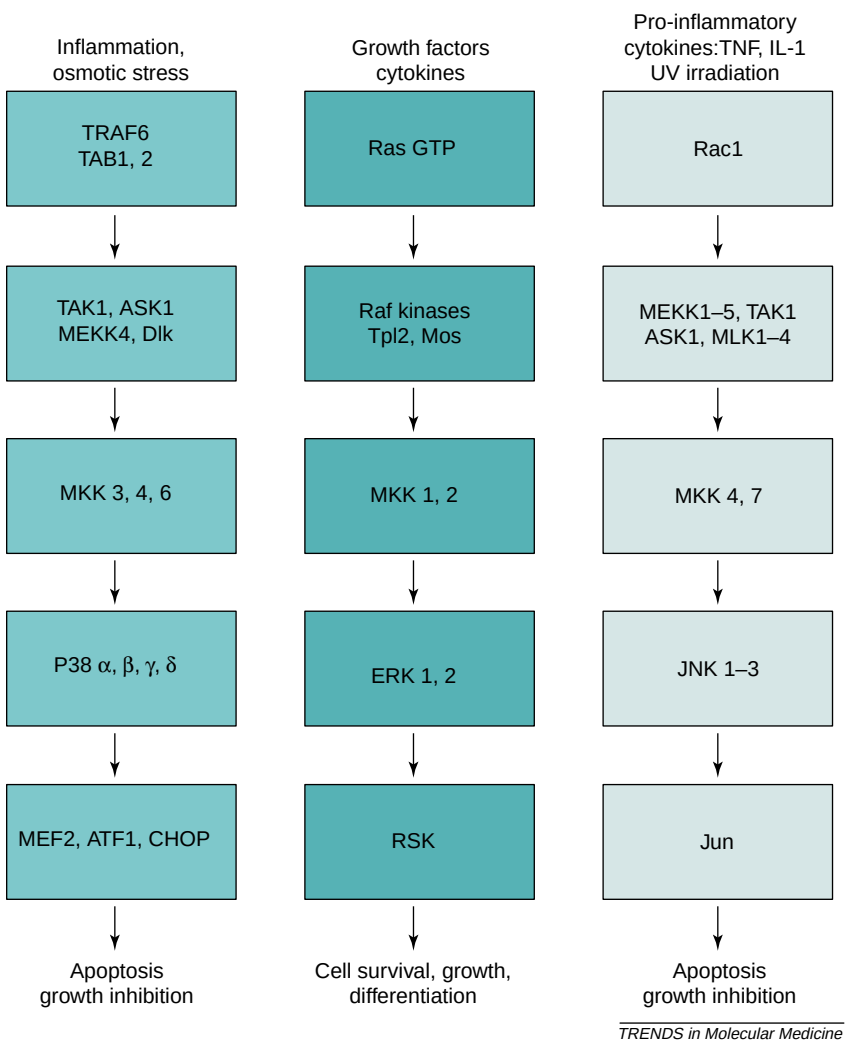

Figure 1. Mitogen-activated protein kinase (MAPK) signaling pathways. The best-described activators and downstream targets are shown. Each step has multiple component kinases and feedback loops, as well as significant interactions, particularly between the p38 and c-Jun N-terminal kinase (JNK) pathways, resulting in many mechanisms to generate specific cellular responses to stimuli. Localization of these enzymes within the cell adds further specificity in signal transduction. Listed pathway endpoints represent a subset of described activities.

retinoic acid or all-*trans* retinoic acid (ATRA)-induced apoptosis in medulloblastoma cells [30]. Retinoids were shown to induce phosphorylation of p38 MAPK through induction of BMP2, and the specific p38 MAPK inhibitor SB203580 blocked both retinoid- and BMP2-mediated apoptosis in established and primary medulloblastoma cell lines (Figure 2).

Apoptosis is induced by another retinoid in cultured ovarian carcinoma cells in a p38-MAPK-dependent way. CD437 completely prevented proliferation of CA-OV-3 cells and induced extensive apoptosis, both of which were completely blocked by three different p38 MAPK antagonists [31]. In these cells, phospho-p38 MAPK phosphorylates the transcription factor MEF-2, which was proposed to lead to mitochondrial depolarization and apoptosis through increased transcription of the orphan nuclear receptor TR3/Nurr77. Interestingly, ATRA does not induce the p38 MAPK cascade in these cells, suggesting an upstream mechanism distinct from that described for medulloblastoma cells.

Chemotherapy induces p38 MAPK

In HeLa human cervical carcinoma cells, four chemotherapeutic agents were shown to induce p38 activation and mitotic cell-cycle arrest by de-polymerizing (nocodazole, vincristine and vinblastine) or stabilizing (taxol) microtubules [32]. The p38 MAPK inhibitors SB203580 and SB202190 inhibited nocodazole-induced apoptosis by 46% and 42%, respectively. To further assess the role of p38 MAPK, dominant activated MAPK kinase (DAMKK6) was transfected into HeLa cells, resulting in an increase in apoptosis from 2% in the control population to 54% in DAMKK6-transfected cells. In this model, DAMKK6 induced translocation of the pro-apoptotic protein Bax from the cytoplasm to the mitochondria, an event that triggers apoptosis. The activity of DAMKK6 on Bax translocation and HeLa cell apoptosis was inhibited by both p38 MAPK inhibitors. In this study, DAMKK6 caused apoptosis in 54% of the cells, but nocodazole-induced p38-MAPK-mediated apoptosis was measurable in only 25% of the cells and no data were provided for apoptosis induction

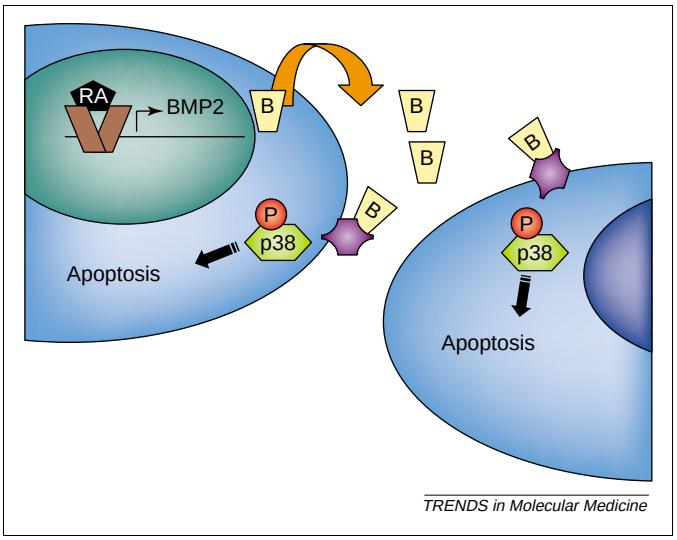

Figure 2. Diagram of p38 mitogen-activated protein kinase (MAPK) activation in medulloblastoma cells by retinoids. Retinoid agonists bind to retinoic acid receptors to induce transcription of bone morphogenetic protein 2 (BMP2). BMP2 protein (designated 'B') is secreted by cells and acts in both an autocrine and paracrine way to induce phosphorylation of p38 MAPK, which, in turn, leads to apoptosis [25].

by the other chemotherapeutic agents. However, the authors of this study provide data to explain this paradox. Nocodazole, vincristine, vinblastine and taxol induce p21-activated kinase (PAK) as well as p38 MAPK in mitotically arrested HeLa cells, and PAK phosphorylates Bad, which is an anti-apoptotic protein when phosphorylated. Thus, the simultaneous activation of pro- and anti-apoptotic pathways results in less apoptosis than observed in response to direct activation of p38 MAPK. It will be interesting to learn whether PAK inhibitors increase the efficacy of anti-mitotic agents.

Induction of phosphorylated p38 by cisplatin was observed in multiple transformed cell lines in a study that also showed p38 MAPK activation by taxol and doxorubicin in several tumor lines [33]. Cisplatin is an inorganic heavy metal coordination complex that contains platinum surrounded by two chloride and two ammonia molecules. Its anti-cancer mechanism correlates with the formation of DNA adducts and intra-strand crosslinks. Doxorubicin is a *Streptomyces*-derived agent that intercalates between DNA bases and inhibits DNA and RNA synthesis. The mechanism by which these agents induce apoptosis has been poorly understood. This study focused on cisplatin, which induced phosphorylation of p38 in a dose- and time-dependent way. Two inhibitors of p38 MAPK – SKF86002 or SB203580 – showed a modest effect on cisplatin-mediated reduction of HaCaT cell survival. However, it is difficult to assess the effect of p38 MAPK or p38 MAPK inhibitors on apoptosis in this study because the endpoint was the fraction of control cells that survived, which is influenced by cell-cycle kinetics.

Interactions with other MAPK cascades

Park and colleagues evaluated the mechanism of phytosphingosine-mediated apoptosis in Jurkat human T cell lymphoma and NCI-H460 human non-small-cell lung cancer cells [34]. In both cell lines, phytosphingosine induced phosphorylation of p38 MAPK within 5 mins, and the effect lasted for 3–6 h. Cells underwent apoptosis after: (i) translocation of Bax from the cytosol to the mitochondria, (ii) loss of mitochondrial membrane potential, (iii) cleavage of caspase-8, -3 and -9, (iv) mitochondrial cytochrome *c* release, (v) DNA laddering, and (vi) chromatin condensation. The p38 MAPK inhibitor SB203580 prevented phytosphingosine-induced loss of mitochondrial transmembrane potential and increase in plasma membrane permeabilization. Both SB203580 and a dominant–negative p38 MAPK blocked phytosphingosine-induced Bax translocation, mitochondrial cytochrome *c* release and cleavage of caspase 9, but had no effect on caspase 8 cleavage. p38 inhibition markedly reduced apoptosis in these systems. Additional studies showed that phytosphingosine also reduced ERK activity and that this activity was responsible for caspase 8 and caspase 3 cleavage. Together, these data indicate that simultaneous induction of P38 MAPK activation and suppression of ERK activity mediates phytosphingosine-induced apoptosis in these cancer cell models.

Another report showing interaction between two MAPK cascades also showed that 2-methoxyestradiol (2-ME) uses both p38 activation and JNK-mediated BCL2 phosphorylation for apoptosis induction in the LNCaP prostate cancer cell line [35]. In this case, 2-ME treatment leads to p53 induction and inactivation of the anti-apoptotic protein BCL2 by phosphorylation. Antisense experiments revealed that p53 induction was necessary for apoptosis of these cells. The p38 antagonist SB203580, antisense to JNK, or a dominant–negative form of the specific kinase of JNK [MAPK kinase 7 (MKK7-KL)] inhibited p53 induction and reduced apoptosis. Inhibition of apoptosis was incomplete with SB203580 or JNK antisense, but was nearly complete with MKK7-KL. The authors conclude that MKK7-KL was more effective because it uniquely blocked 2-ME-induced phosphorylation of BCL2. BCL2 is an anti-apoptotic protein that is inactivated through phosphorylation. However, this interpretation is problematic because the majority of BCL2 remains in the active form under any of the conditions tested. In addition, there is less non-phosphorylated BCL2 in the MKK7-KL clones than the other conditions. Furthermore, no explanation is provided to account for why MKK7-KL prevents BCL2 phosphorylation and JNK antisense does not; both conditions were found to cause similar inhibition of JNK as measured by AP-1 activity.

It is evident that the effects of p38 MAPK activation vary between specific cell types and might be different in Ras-transformed cells. Weijzen and colleagues showed that oncogenic Ras activates Notch signaling in part through a p38-dependent pathway [36]. Kim and colleagues showed that p38 MAPK is a mediator of H-ras-induced cell motility and invasion in tissue culture models of a human breast epithelial cell line [37]. p38 activation did not affect cell death in the MCF10A cells used in this study. ERK-1/2 appeared to act in concert with p38 signaling to increase cell migration and invasion partly through upregulation of matrix metalloproteinase 2. Both studies relied on tissue culture models and thus require further investigation into the effect of modulation of p38

MAPK activity on oncogenic-Ras signaling *in vivo*. One study showed that p38 MAPK can activate the pro-survival PI3K/AKT pathway in fibroblasts [38]. However, other studies show that PI3K/AKT downregulates p38 MAPK [39], implying that this is more likely to be a potential mechanism of resistance to pathway activation than a target of p38 MAPK. By contrast, sustained activation of p38 MAPK in human rhabdomyosarcoma cells resulted in terminal differentiation through activation of MEF-2 and restoration of MyoD function [40].

Using patient-derived specimens from 20 liver cancer cases, Iyoda and colleagues showed that both MKK6 and p38 MAPK levels are lower in hepatocellular carcinoma tumors than adjacent non-neoplastic liver [41]. They propose that reduction of p38 MAPK in these tumors represents an anti-apoptotic mechanism that provides a growth advantage to these cells. They also show that activated MKK6 induces apoptosis in HepG2 and HuH7 human hepatoma cell lines, and that this activity is blocked by SB203580.

Taken together, these reports indicate that a variety of anti-cancer agents induce phosphorylation of p38 MAPK, and that this event is a key component for cancer cell death in many tumors.

Effect of p38-MAPK-activation on non-neoplastic cells

The value of p38-MAPK-activating drugs depends on whether cancer cells are more susceptible to p38-mediated apoptosis than non-neoplastic tissue. Cancer patients tolerate retinoids, cisplatin, anti-mitotic agents and the other p38-MAPK-inducing agents fairly well, suggesting that an acceptable therapeutic window exists. Nevertheless, several studies demonstrate p38-MAPK-mediated apoptosis of non-neoplastic cells in tissue culture, raising the question of whether p38-mediated apoptosis is responsible for some dose-limiting toxicity.

Shou and colleagues showed that cyanide-induced apoptosis in neurons is accompanied by increased phosphorylated p38 MAPK, and that cyanide effects are blocked by SB202474 [42]. Similarly, ceramide-induced neuronal apoptosis was blocked by SB203580. In a model of pro-atherosclerotic vascular smooth muscle proliferation, estrogen and raloxifene induced apoptosis, and this was blocked by SB203580. The same antagonist inhibited apoptosis in human umbilical vein endothelial cells. Interestingly, blockade of apoptosis could also be achieved by treatment with vascular endothelial growth factor (VEGF), which inhibited p38 MAPK and caspase-3 activity [43]. Although p38 MAPK might mediate VEGF activity in some systems [44,45], this does not correlate with previous studies and a study by Issbrucker and colleagues [46] in which inhibition of p38 MAPK was found to enhance VEGF-induced angiogenesis. Therefore, the effect of p38 pathway activation on angiogenesis requires further studies using *in vivo* models. Several other examples provide evidence that, in certain laboratory models, p38 MAPK is involved in non-neoplastic cell apoptosis. However, we were unable to find studies that address the role of p38-MAPK-mediated apoptosis of non-neoplastic cells in response to anti-cancer agents.

Implications for therapeutics

Reports showing mechanistic p38 MAPK mediation of drug-induced apoptosis in cancer cells were all published in the past few months. These probably represent the tip of the iceberg in terms of p38 MAPK being a convergence point for a wide-range of anti-cancer therapies. Although many effective chemotherapeutic agents do not act via the p38 pathway, these recent findings provide an opportunity to re-evaluate proven therapies in a new light, and look for strategies to increase efficacy and decrease toxicity.

It is important to note that the studies outlined here rely on cell culture models and require verification using *in vivo* models. In addition, it is not known whether these drugs preferentially activate p38 MAPK in cancer cells, or whether p38 MAPK is activated in many non-neoplastic cells but that cancer cells are more susceptible to p38-MAPK-mediated apoptosis. Such questions could easily be answered using established mouse cancer models. Because the side-effects of some p38-MAPK-activating agents are quite different, it will be important to focus on the tissues in which dose-limiting toxicity occurs. For example, does cisplatin, which causes deafness, pancytopenia and nephrotoxicity, induce p38-MAPK-mediated apoptosis in inner-ear hair cells, hematopoietic progenitors, or kidney? Similarly, retinoid activity on p38 MAPK in liver, skin and mucous membranes also needs to be assessed because these are the sites where dose-limiting toxicity occurs.

Many of the details of p38-induced apoptosis in tumors remain to be elucidated. For example, what is the relative expression of the four isoforms of p38 in different tumor types and their relative contributions to apoptosis? Such information might also shed light on tumor pathogenesis and potential toxicities to non-neoplastic cells. If different isoforms can be independently regulated pharmacologically, the therapeutic window for p38 activators could be significantly improved. Although researchers in academia and industry have identified several drugs that induce p38 MAPK phosphorylation, many drugs probably act further upstream. For example, in the case of retinoid treatment of medulloblastoma, transcriptionally induced BMP2 acting in a paracrine fashion through BMP2 receptors is downstream of retinoid receptor binding and upstream of p38 MAPK induction. Discovery of drugs that induce p38 phosphorylation downstream of retinoids, cisplatin or other anti-cancer agents would potentially circumvent off-target or upstream toxicities.

Concluding remarks

The use of any cancer therapy depends on the prevention of drug resistance, and more needs to be learnt about how tumors protect themselves from pro-apoptotic activation of p38 MAPK. Emerging data suggest that some cancer cells simultaneously induce anti-apoptotic kinase pathways, such as PAK or ERK, in parallel with pro-apoptotic signals. Because PAK and ERK can be antagonized pharmacologically with ease, the possibility of treating patients with available p38-MAPK-activating agents and antagonists of anti-apoptotic pathways is a reasonable avenue to explore. An alternative strategy would be to use combinations of p38-activating drugs to overcome these anti-apoptotic

signals. A complementary strategy would be to inhibit the breakdown of phosphorylated p38 MAPK using phosphatase inhibitors specific to this pathway. The potential value of activating p38 MAPK provides a therapeutic opportunity that needs to be exploited by thoughtful mechanistic preclinical studies in multiple tumor models.

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