Molecular Pathways

Targeting the BMK1 MAP Kinase Pathway in Cancer Therapy
Qingkai Yang and Jiing-Dwan Lee

Abstract
The big mitogen activated protein kinase 1 (BMK1) pathway is the most recently discovered and least-studied mammalian mitogen-activated protein (MAP) kinase cascade, ubiquitously expressed in all types of cancer cells tested so far. Mitogens and oncogenic signals strongly activate this cellular MAP kinase pathway, thereby passing down proliferative, survival, chemoresistance, invasive, and angiogenic signals in tumor cells. Recently, several pharmacologic small molecule inhibitors of this pathway have been developed. Among them, the BMK1 inhibitor XMD8–92 blocks cellular BMK1 activation and significantly suppresses tumor growth in lung and cervical tumor models and is well tolerated in animals. On the other hand, MEK5 inhibitors, BIX02188, BIX02189, and compound 6, suppress cellular MEK5 activity, but no data exist to date on their effectiveness in animals. Clin Cancer Res; 17(11); 3527–32. ©2011 AACR.

Background
The BMK1 signaling cascade
Mitogen activated protein (MAP) kinase pathways are one of the major mechanisms by which cells transduce intracellular signals. These kinase cascades are highly evolutionarily conserved in eukaryotes ranging from yeast to human. Four mammalian MAP kinases have been discovered and are known as extracellular signal regulated kinase 1 and 2 (ERK1/2), Jun N-terminal kinase (JNK), p38, and big mitogen activated protein kinase 1 (BMK1; refs. 1–3). ERK1/2 and BMK1 are activated by growth factors, and JNK and p38 are activated by cytokines or cytotoxic drugs. The core of the MAP kinase module consists of 3 consecutively activated kinases: a MAP kinase kinase kinase, or MEKK; a MAP kinase kinase, or MEK; and a MAP kinase.

In the BMK1 pathway, MEKK2 and MEKK3 are MEKK; MEK5 is MEK; and, BMK1 is the MAP kinase (4–7). However, MEK5 and MEKK3 are not specific for activating the BMK1 pathway, because both are known to modulate the JNK MAP kinase cascades (8). MEK5 is the sole, specific, and nonredundant MEK for the BMK1 pathway. Phosphatase PP2A is known to dephosphorylate MEK1/2 in vitro and plays a role in inhibiting the activation of the ERK1/2 MAPK pathway. Surprisingly, Garcia and colleagues showed that, different from what is observed in other MAPK cascades, PP2A/PP1-like phosphatases are needed for BMK1 activation (9). This result indicates that the ERK1/2 and BMK1 MAP kinase pathways are differentially regulated by phosphatases.

The N-terminal kinase domain of BMK1 is highly homologous to the MAP kinase ERK1/2 (10). However, BMK1 contains a unique large C-terminal nonkinase domain, with about 400 amino acid residues, which does not exist in any other MAP kinase and renders the BMK1 polypeptide twice the size of other MAP kinases (4). The function of the C-terminal nonkinase domain of BMK1 has been implicated in subcellular translocation of BMK1 (11, 12) and in contributing to transactivating activity for transcriptional factors interacting with BMK1 (13). The N-terminal part of BMK1 that is bound to the C-terminal portion leads to the cytoplasmic retention of BMK1. The activation of BMK1 causes phosphorylation of the C-terminal regions of BMK1 resulting in interruption of the binding and subsequent translocation of BMK1 into the nucleus (Fig. 1; ref. 11). Additionally, the C-terminal region of BMK1 not only interacts with myocyte enhancer-binding factor (MEF2), but is also required for maximal MEF2-transactivating activity to activate the endogenous Nur77 gene when BMK1 is recruited to the promoter of Nur77 using the MEF2 binding site (13).

BMK1 activity upregulation in cancer
Mitogens and oncogenic signals are potent stimuli in activating BMK1 (Fig. 1). Most notably, those signals transmit from agonists of the ErbB and RET family of receptor tyrosine kinases such as epidermal growth factor (EGF) and heregulin and glial cell line-derived neurotrophic factor (14–16). Oncogenes such as Her2, Ras, STAT3, and Src are also known to augment BMK1 activity, thereby transmitting signals leading to malignancy including uncontrolled proliferation, transformation, antiapoptosis, and actin reorganization in tumor cells (17–29). Moreover, by a combination of gene expression profiling and subsequent tissue microarray examination by immunohistochemistry, Sticht and colleagues (30) found that...
Figure 1. The activated BMK1 MAPK cascade promotes cell cycle progression of tumor cells induced by mitogens and/or oncogenic signals. The BMK1 pathway is activated by mitogens and oncogenic signals through a 3-level kinase cascade (MEKK2 or MEKK3/MEK5/BMK1). Subsequently, activated BMK1 phosphorylates and suppresses the activity of its downstream effector PML, thereby promoting the S-phase entry of tumor cells. Some tumor cells upregulate BMK1 activity by overexpression of MEK5, which consequently augments their metastatic and chemoresistant potentials. In mitotic tumor cells, it was reported that CDK is involved in phosphorylating and regulating BMK1 in a MEK5-independent manner. PML-NB, PML nuclear body.

BMK1 in cell cycle regulation

It has been shown that BMK1 regulates cell cycle progression in both G1-S (14, 36, 37) and G2-M phase transitions (38, 39). BMK1 modulates the G1-S phase transition in part through phosphorylation and activation of its downstream effector SGK kinase (36). Recently, BMK1 was shown to inhibit the tumor suppressor activity of PML by blocking PML’s capacity in upregulating the expression of p21, which is critically involved in the G1-S transition of a cell cycle (37). BMK1 is also involved in G2-M phase progression. Activated BMK1 upregulates NF-kB by ribosomal S6 kinase 2 (RSK2)–modulated phosphorylation and degradation of IkB, causing inhibition of transcription of G2-M–specific genes (39). As BMK1 activity is upregulated in some cancer cells, it is possible that the overactivity of BMK1 in tumor cells may help to overcome these 2 checkpoints of the cell cycle and, consequently, promote uncontrolled proliferation of tumor cells.

high BMK1 expression in oral squamous cell carcinoma was associated with an advanced tumor stage and the presence of lymph node metastases. In addition, the BMK1 pathway was found constitutively active in Hodgkin lymphoma (HL) cells lines, and the upregulated BMK1 was shown to be responsible for both proliferation and anti-apoptosis of HL cells through deregulating HOXB9 expression (31). BMK1 activity is also important for the survival of leukemic T cells in vivo, as BMK1 knockdown in leukemic T cells decreased nuclear accumulation of the NF-kB p65 subunit and suppressed the induction of tumors in mice (32). Additionally, it has been shown that the BMK1 activation by the hepatocyte growth factor–scatter factor (HGF) is critical for cell proliferation of human mesothelioma (MM) cells (33). Experimental results suggest that BMK1 is involved in increased MM cell viability and proliferating cell nuclear antigen expression via upregulating the level of Fos-related antigen 1 (Fra-1), which is commonly overexpressed in epithelial cancers and implicated in tumor cell invasiveness.

Several publications have shown that tumor cells can acquire cancerous capacity by increasing expression of MEK5 to activate BMK1 MAP kinase. In breast cancer cells, the activated oncogene STAT3 binds to the promoter regions of MEK5 and induces transcription of MEK5, conferring a critical survival signal (25). In metastatic prostate cancer, strong MEK5 expression is correlated with bony metastases, and less favorable prognosis is caused by upregulated BMK1-induced activator protein-1 (AP-1) activity, a consequent induction of a high level of matrix metalloprotease-9 (MMP-9) and augmented invasive potential (34). Moreover, during chemotherapeutic-induced apoptosis, overexpression of MEK5 in breast cancer cells provides a key survival signal for chemoresistance (35).
BMK1 in tumor-associated angiogenesis

Using an induced knockout (KO) mouse model of the BMK1 gene, BMK1 is shown to be critical for tumor neovascularization by providing proliferation and survival signals to endothelial cells through the RSK-rpS6 signaling module (40). BMK1’s function was also implicated in VEGF-mediated survival and tubular morphogenesis of human endothelial cells, through mediation of VEGF-induced phosphorylation of both AKT and proapoptotic protein BAD, as well as by VEGF-induced increased expression of the antiapoptotic protein BCL2 (41). Recently, Doebele and colleagues (42) discovered that the BMK1 pathway induced inhibitor of differentiation 1 (Id1) and inhibited the expression of thrombospondin-1 (TSP1), thereby antagonizing the effects of Epac/Rap1. Rap1 is a small Ras-related GTPase that regulates cell adhesion, cell-cell junctions, and vascular permeability (43). Rap1 is controlled by several Rap guanine nucleotide exchange factors containing cAMP-activated Epac1 and Epac2 (44). Epac/Rap1 activation is also recognized to significantly block angiogenesis through downregulating Id1 and, consequently, upregulating TSP1. Therefore, the BMK1 pathway and Epac/Rap1 both modulate the expression of TSP1 through Id1 to control neovascularization.

Implicated role of BMK1 in tumor metastasis

BMK1’s role in tumor metastasis has been implicated in several studies (30, 34, 45–47). BMK1 is capable of promoting integrin-mediated cell adhesion and motility in cancer cells through regulating focal adhesion kinase signaling (45). BMK1 activity is also known to be critical for forming Src-induced invasive adhesions, podosomes, in tumor cells by inducing RhoGAP7 and consequently limiting Rho activation (46). Notably, BMK1 upregulation is detected in human metastatic prostate tumors and is involved in augmenting the invasive potential of prostate tumor cells by increasing their production and secretion of MMP-9 (34). Increased BMK1 expression is also correlated with lymph node metastases in oral squamous cell carcinoma (30). Additionally, in breast tumor cells, it has been shown that the BMK1 pathway mediated HGF-induced cell migration through the MET receptor/breast tumor kinase (Brk) signaling module, indicating that the BMK1 cascade contributing to breast cancer progression to metastasis is activated via the HGF/MET/Brk pathway (47).

BMK1 downstream substrates in cancer development

Activated BMK1 phosphorylates and activates many transcription factors (TF) such as MEF2A, MEF2C, MEF2D, and Sap1A (19, 48–51). The activated MEF2 family of TFs (MEF2A, 2C, and 2D) subsequently regulates the expression of oncogene c-Jun, which is the putative transforming gene of avian sarcoma virus 17 (48). SAP-1a, the serum response elements, and serum response factor have been shown to form a ternary complex on the c-Fos promoter to modulate c-Fos expression (52, 53). BMK1 activated by HGF is also known to upregulate the expression level of transcription factor Fra-1 (33). Fra-1 is an immediate early gene encoding a transcription factor involved in cell propagation, differentiation, migration, and death. Moreover, Fra-1 gene overexpression also contributes to cellular transformation (54). Interestingly, c-Jun, c-Fos, and Fra-1 are components of the AP-1 transcription factor. As such, it has been reported that BMK1 upregulated AP-1 activity at the MMP-9 promoter and increased the invasive potential of high-grade prostate cancer (34). In addition, BMK1 was implicated in the phosphorylation and activation of the cAMP response element-binding transcription factor (CREB) in the insulin-like growth factor-II–induced antiapoptosis effect in lung tumor cells (55). CREB is a nuclear transcription factor activated by phosphorylation by serine-threonine kinases, and upregulation of CREB was observed in patients with breast, prostate, and non–small cell lung tumor as well as acute leukemia (56). Most recently, BMK1 was also implicated in suppressing the tumor suppressor activity of PML by direct phosphorylation (Fig. 1; ref. 37). PML is a tumor suppressor that was initially identified for its involvement in the translocation (15, 17) of acute promyelocytic leukemia (57). BMK1–mediated PML modification reduces the capacity of PML to upregulate p21 expression and the consequent proliferation inhibition of tumor cells.

Clinical-Translational Advances

Small compound inhibitors for MEK5

Recently, Tatake and colleagues (58) did high-throughput screening of the Boehringer Ingelheim compound collection against MEK5 and found that the indolinone-6-carboxamides, BIX02188 and BIX02189, block MEK5 activity with IC_{50} of 4.3 nmol/L and 1.5 nmol/L, respectively (Table 1). These 2 compounds have some cross-reactivity

Table 1. Small molecule inhibitors for the BMK1 pathway

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Target</th>
<th>IC_{50} in vitro (nmol/L)</th>
<th>IC_{50} in vivo</th>
<th>Clinical development</th>
</tr>
</thead>
<tbody>
<tr>
<td>BIX02188</td>
<td>MEK5</td>
<td>4.3</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>BIX02189</td>
<td>MEK5</td>
<td>1.5</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Compound 6</td>
<td>MEK5</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>XMD8-92</td>
<td>BMK1</td>
<td>240</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Abbreviation: NA, not available.
with CSF1R kinase with IC₅₀ of 280 nmol/L (BIX02188) and IC₅₀ of 46 nmol/L (BIX02189). These 2 compounds are very effective in inhibiting cellular BMK1 activation induced by osmotic shock and in blocking MEK2C activation by ectopic-expressed BMK1 in HeLa and HEK293 cells. However, the inhibition efficiency of these 2 MEK5 blockers in growth factor–induced activation of BMK1 still needs to be examined. Currently, no data are available on pharmacokinetics and pharmacodynamics for BIX02188 and BIX02819. In addition, Flaherty and colleagues (59) also produced a series of benzimidazole-based MEK5 inhibitors (no IC₅₀ data available). Among these small molecule compounds, compound 6 inhibits EGF-induced BMK1 activation and slows down the growth of MCF-7 breast cancer cells (59). Nevertheless, compound 6 is not specific to MEK5 because it also blocks ERK1/2 activation induced by EGF. To date, no data are available on pharmacokinetics and pharmacodynamics for compound 6.

Two common MEK1 inhibitors, PD98059 and U0126, were generally used in numerous preclinical experiments to show the efficacy of targeting the ERK1/2 pathway for treating diseases (10). Because there is high-sequence homology between MEK1 and MEK5, it has been discovered that these 2 pharmacologic compounds also inhibit MEK5 (19, 60). Therefore, experimental results generated by PD98059 and U0126 need to be reevaluated by more specific inhibitors to MEK1 and to MEK5, respectively.

As MEK5 is thought to be the sole upstream regulator for BMK1, it is logical to assume that blocking MEK5 activity can be an effective means of inhibition of the cellular BMK1 pathway. However, 1 report recently indicated that cyclin-dependent kinase (CDK) is also involved in phosphorylation and the regulation of BMK1 in mitosis in an MEK5-independent manner (Fig. 1), suggesting that targeting MEK5 alone in mitotic tumor cells may not be sufficient to completely block the BMK1 pathway (61).

Pharmacologic inhibitor of BMK1
Yang and colleagues (37) found that modification of ATP-competitive polo kinase inhibitor BI-2536 resulted in the loss of polo kinase inhibition activity, but led to compounds with high selectivity toward BMK1. Subsequent structure activity-guided optimization resulted in the synthesis of XMD8–92. XMD8–92 shows high selectivity to BMK1 in an in vitro ATP site competition–binding assay against 402 kinases and in the KiNativ method against all detectable kinases in HeLa cell lysates. XMD8–92 blocks EGF-induced activation of BMK1 with IC₅₀ of 240 nmol/L (Table 1) and, with a concentration as high as 5 µM, XMD8–92 has no inhibitory effect on ERK1/2 activation by EGF. The pharmacokinetics of XMD8–92 was found to have a 2.0-hour half-life clearance of 26 ml/min/kg. The tissue distribution of XMD8–92 was reasonable with a calculated volume of distribution of 3.4 L/kg. XMD8–92 had high oral bioavailability with 69% of the dose absorbed. After a single oral dose of 2 mg/kg, maximal plasma concentration of approximately 500 nmol/L was observed by 30 min, with 34 nmol/L remaining 8 hours postdose. Tolerability experiments were done with high plasma concentrations of XMD8–92 (10 µM after intraperitoneal dosing of 50 mg/kg), which was kept during 14 days. The drug seemed to be well tolerated, and the mice appeared healthy with no sign of distress. XMD8–92 treatment in both immunocompetent and immunodeficient mice blocked the growth of lung and cervical xenograft tumors, respectively, by 95% (37). This remarkable antitumor effect of XMD8–92 in lung and cervical xenograft tumor models was due to XMD8–92’s capacity to inhibit tumor cell proliferation through the PML suppression–induced p21 checkpoint protein and by blocking of BMK1’s contribution in tumor-associated angiogenesis. Consequently, XMD8–92 is still at preclinical development stage.

Anticipated side effects
Conditional deletion of the BMK1 gene in various tissues (cardiomyocyte, neuron, hepatocyte, smooth muscle, and T cell) in mice is viable and has no obvious effect on their development, general health, mating, and aging (Unpublished data; ref. 62). However, a selective BMK1 KO in endothelial cells of adult mice induces vasculature instability (49). On the other hand, XMD8–92 treatment in mice has no adverse effect on the integrity of blood vessels and is well tolerated by mice (37). The difference in outcome between BMK1 KO and BMK1 activity inhibition by XMK8–92 in the integrity of animal vasculature may be due to the following reasons: First, BMK1 KO in mice leads to complete and irreversible removal of the BMK1 protein, whereas XMD8–92 treatment in mice only suppresses the activity of BMK1, which is reversible; Second, the vasculature instability observed in BMK1 KO mice may be due to the lack of the C-terminal nonkinase domain of BMK1, which is still present during XMD8–92 treatment of animals. Nonetheless, future preclinical and clinical tests of pharmacologic inhibitors for the BMK1 pathway need extra scrutiny to examine their impact on the integrity of blood vessels in tested subjects.

In conclusion, preclinical data showed that the BMK1 pathway is a promising target for effectively suppressing tumor growth. Future clinical trials should reveal whether blocking the activity of this cascade can bring greater efficacy and better tolerability to cancer patients compared with currently approved treatments that affect tumor cell proliferation, metastasis, and tumor-associated angiogenesis.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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