Targeting the BMK1 MAP Kinase Pathway in Cancer Therapy

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Running Title: BMK1 as Cancer Drug Target

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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, chemo-resistance, invasive and angiogenic signals in tumor cells. Recently, several pharmacological small molecule inhibitors of this pathway have been developed. Among them, 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 yet on their effectiveness in animal.
Background

**The BMK1 signaling cascade.** 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 BMK1 (1-3). ERK1/2 and BMK1 kinase 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 three 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, MEKK2 and MEKK3 are not specific for activating the BMK1 pathway, since both are known to modulate the JNK MAP kinase cascades (8). MEK5 is the sole, specific and non-redundant 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 et al demonstrated 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 MAP kinase ERK1/2 (10). However, BMK1 contains a unique large C-terminal non-kinase 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 non-kinase 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 (Figure 1)(11). Additionally, the C-terminal region of BMK1 not only interacts with myocyte enhancer-binding factor (MEF2), but also is 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 (Figure 1). Most notably, those signals transmit from agonists of the ErbB and RET family of receptor tyrosine kinases (RTK) such as epidermal growth factor (EGF), and heregulin and glial cell line-derived neurotrophic factor (GDNF) (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, anti-apoptosis and actin-reorganization in tumor cells (17-29). Moreover, by a combination of gene expression profiling and subsequent tissue microarray examination by immunohistochemistry, Sticht et al (30) found that 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 the expression of HOXB9 (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-κB p65 subunit and suppressed the induction of tumors in mice (32). Additionally, it has been demonstrated 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 (PCNA) 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 demonstrated 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 chemotherapeutically-induced apoptosis, overexpression of MEK5 in breast cancer cells provides key a survival signal for chemoresistence (35).

**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 nuclear factor κB (NFκB) by ribosomal S6 kinase 2 (RSK2)-modulated phosphorylation and degradation of IκB causing inhibition of transcription of G2-M-specific genes (39). As BMK1 activity is upregulated in some cancer cells, it is possible that the over activity of BMK1 in tumor cells may help to overcome these two checkpoints of the cell cycle, and consequently promote uncontrolled proliferation of tumor cells.

**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 vascular endothelial growth factor (VEGF)-mediated survival and tubular morphogenesis of human endothelial cells through mediation of VEGF-
induced phosphorylation of both AKT and pro-apoptotic protein BAD, as well as by VEGF-induced increased expression of the anti-apoptotic protein BCL2 (41). Recently, Doebele et al (42) discovered that the BMK1 pathway induced inhibitor of differentiation 1 (Id1) and inhibited the expression of thrombospondin-1 (TSP1), thereby antagonized the effects of Epac/Rap1. Rap1 is a small Ras-related guanine triphosphatase (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 down-regulating Id1 and consequently up-regulating 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 (FAK) 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 demonstrated that the BMK1 pathway mediated HGF-induced cell migration though 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 (TFs) such as MEF2A,
MEF2C, MEF2D and Sap1A (19, 48-51). Activated MEF2 family of TFs (MEF2A, 2C and 2D) subsequently regulate 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 anti-apoptosis effect in lung tumor cells (55). CREB is a nuclear transcription factor activated by phosphorylation by serine/threonine kinases, and upregulation of CREB were 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 (37) (Figure 1). PML is a tumor suppressor that was initially identified for its involvement in the (15;17) translocation of acute promyelocytic leukemia (57). BMK1-mediated PML modification reduces the capacity of PML to upregulate p21 expression, and in the consequent proliferation inhibition of tumor cells.

Clinical-Translational Advances

**Small compound inhibitors for MEK5.** Recently, Tatake et al. (58) performed 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 nM and 1.5 nM, respectively
These two compounds have some cross-reactivity with CSF1R kinase with IC$_{50}$ of 280 nM (BIX02188) and IC$_{50}$ of 46 (BIX02189). These two compounds are very effective in inhibiting cellular BMK1 activation induced by osmotic shock as well as in blocking MEF2C activation by ectopic-expressed BMK1 in HeLa and HEK293 cells. However, the inhibition efficiency of these two MEK5 blockers in growth factor-induced activation of BMK1 still needs to be examined. For now, there is no data on pharmacokinetics and pharmacodynamics for BIX02188 and BIX02819. In addition, Flaherty et al. (59) also produced a series of benzimidazole-based MEK5 inhibitors (no IC$_{50}$ 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 since it also blocks ERK1/2 activation induced by EGF as well. So far, there is no data on pharmacokinetics and pharmacodynamics for compound 6.

Two common MEK1 inhibitors, PD98059 and U0126, were generally used in numerous preclinical experiments to demonstrate the efficacy of targeting the ERK1/2 pathway for treating diseases (10). As there is high-sequence homology between MEK1 and MEK5, it has been discovered that these two pharmacological 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, one 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 (Figure 1), suggesting that targeting MEK5 alone in mitotic tumor cells may not be sufficient to completely block the BMK1 pathway (61).
Pharmacological inhibitor of BMK1. Yang et al. (37) found that modification of adenosine triphosphate (ATP)-competitive polo kinase 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 as well as in the KiNativ method against all detectable kinases in HeLa cell lysates. XMD8-92 blocks EGF-induced activation of BMK1 with IC_{50} of 240 nM (Table 1) and, with 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 hr 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 nM were observed by 30 min, with 34 nM remaining 8 hr postdose. Tolerability experiments were performed with high plasma concentrations of XMD8-92 (10 μM after intraperitoneal dosing of 50 mg/kg) which was kept during 14 days. The drug appeared 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 anti-tumor 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. To this point, 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 are viable and has no obvious effect on their development, general health, mating and aging (62) (unpublished data). 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 different outcome between BMK1 KO and BMK1 activity inhibition by XMK8-92 in the vasculature integrity of animals may be due to the following reasons. First, BMK1 KO in mice leads to complete and irreversible removal of the BMK1 protein, while 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 lack of the C-terminal non-kinase domain of BMK1, which is still present during XMD8-92 treatment of animals. Nonetheless, future preclinical and clinical tests of pharmacological 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 demonstrated 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 to currently approved treatments that affect tumor cell proliferation, metastasis and tumor-associated angiogenesis.
Disclosure of Potential Conflicts of Interest

The authors have no conflicts of interest to disclose.

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Figure legend

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 three-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 chemo-resistant 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.
References


protein kinase 1 (BMK1) and the MEF2C signaling pathway in PC12 cells: potential role in cell survival following oxidative insults. J Biol Chem. 2002;277:9614-21.


Table 1. Small molecule inhibitors for the BMK1 pathway.

<table>
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Mitogens and oncogenic signals

MEKK2, MEKK3

MEK5

MEK5 overexpression

CDK?

Cytosol

Nucleus

BMK1

NKD

PML-NB

PML

p21

G1/S cell cycle progression

Phosphorylation

KD Kinase domain

NKD Non-kinase domain

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