Published in final edited form as:

Clin Cancer Res. 2011 June 1; 17(11): 3527–3532. doi:10.1158/1078-0432.CCR-10-2504.

Targeting the BMK1 MAP Kinase Pathway in Cancer Therapy

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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

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Disclosure of Potential Conflicts of Interest

The authors have no conflicts of interest to disclose.

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- κ B 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 metalloproteinase-9 (MMP-9), and augmented invasive potential (34). Moreover, during chemotherapeutic-induced apoptosis, overexpression of MEK5 in breast cancer cells provides key a survival signal for chemoresistance (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 κ B (NF κ B) by ribosomal S6 kinase 2 (RSK2)-modulated phosphorylation and degradation of I κ B 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 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 (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 IC₅₀ of 4.3 nM and 1.5 nM, respectively (Table 1). These two compounds have some cross-reactivity with CSF1R kinase with IC₅₀ of 280 nM (BIX02188) and IC₅₀ 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 BIX02189. In addition, Flaherty et al. (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 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 IC₅₀ of 240 nM (Table 1) and, with concentration as

high as 5 μ M, 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 μ M 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 XMD8-92 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.

Acknowledgments

Grant Support

This work was supported by grants from the NIH, CA079871 and CA114059, (to J.-D.L.) and by funds from the Tobacco-Related Disease, Research Program of the University of California, 19XT-0084, (to J.-D.L.).

References

1. Chang L, Karin M. Mammalian MAP kinase signalling cascades. *Nature*. 2001; 410:37–40. [PubMed: 11242034]
2. Pearson G, Robinson F, Beers Gibson T, Xu BE, Karandikar M, Berman K, et al. Mitogen-activated protein (MAP) kinase pathways: regulation and physiological functions. *Endocr Rev*. 2001; 22:153–83. [PubMed: 11294822]
3. Raman M, Chen W, Cobb MH. Differential regulation and properties of MAPKs. *Oncogene*. 2007; 26:3100–12. [PubMed: 17496909]

4. Lee JD, Ulevitch RJ, Han J. Primary structure of BMK1: a new mammalian map kinase. *Biochem Biophys Res Commun.* 1995; 213:715–24. [PubMed: 7646528]
5. Zhou G, Bao ZQ, Dixon JE. Components of a new human protein kinase signal transduction pathway. *J Biol Chem.* 1995; 270:12665–9. [PubMed: 7759517]
6. Chao TH, Hayashi M, Tapping RI, Kato Y, Lee JD. MEKK3 directly regulates MEK5 activity as part of the big mitogen-activated protein kinase 1 (BMK1) signaling pathway. *J Biol Chem.* 1999; 274:36035–8. [PubMed: 10593883]
7. Sun W, Kesavan K, Schaefer BC, Garrington TP, Ware M, Johnson NL, et al. MEKK2 associates with the adapter protein Lad/RIBP and regulates the MEK5-BMK1/ERK5 pathway. *J Biol Chem.* 2001; 276:5093–100. [PubMed: 11073940]
8. Blank JL, Gerwins P, Elliott EM, Sather S, Johnson GL. Molecular cloning of mitogen-activated protein/ERK kinase kinases (MEKK) 2 and 3. Regulation of sequential phosphorylation pathways involving mitogen-activated protein kinase and c-Jun kinase. *J Biol Chem.* 1996; 271:5361–8. [PubMed: 8621389]
9. Garcia L, Garcia F, Llorens F, Unzeta M, Itarte E, Gomez N. PP1/PP2A phosphatases inhibitors okadaic acid and calyculin A block ERK5 activation by growth factors and oxidative stress. *FEBS Lett.* 2002; 523:90–4. [PubMed: 12123810]
10. Nishimoto S, Nishida E. MAPK signalling: ERK5 versus ERK1/2. *EMBO Rep.* 2006; 7:782–6. [PubMed: 16880823]
11. Kondoh K, Terasawa K, Morimoto H, Nishida E. Regulation of nuclear translocation of extracellular signal-regulated kinase 5 by active nuclear import and export mechanisms. *Mol Cell Biol.* 2006; 26:1679–90. [PubMed: 16478989]
12. Borges J, Pandiella A, Esparis-Ogando A. Erk5 nuclear location is independent on dual phosphorylation, and favours resistance to TRAIL-induced apoptosis. *Cell Signal.* 2007; 19:1473–87. [PubMed: 17317102]
13. Kasler HG, Victoria J, Duramad O, Winoto A. ERK5 is a novel type of mitogen-activated protein kinase containing a transcriptional activation domain. *Mol Cell Biol.* 2000; 20:8382–9. [PubMed: 11046135]
14. Kato Y, Tapping RI, Huang S, Watson MH, Ulevitch RJ, Lee JD. Bmk1/Erk5 is required for cell proliferation induced by epidermal growth factor. *Nature.* 1998; 395:713–6. [PubMed: 9790194]
15. Ostrander JH, Daniel AR, Lofgren K, Kleer CG, Lange CA. Breast tumor kinase (protein tyrosine kinase 6) regulates heregulin-induced activation of ERK5 and p38 MAP kinases in breast cancer cells. *Cancer Res.* 2007; 67:4199–209. [PubMed: 17483331]
16. Hayashi Y, Iwashita T, Murakamai H, Kato Y, Kawai K, Kurokawa K, et al. Activation of BMK1 via tyrosine 1062 in RET by GDNF and MEN2A mutation. *Biochem Biophys Res Commun.* 2001; 281:682–9. [PubMed: 11237712]
17. Esparis-Ogando A, Diaz-Rodriguez E, Montero JC, Yuste L, Crespo P, Pandiella A. Erk5 participates in neuregulin signal transduction and is constitutively active in breast cancer cells overexpressing ErbB2. *Mol Cell Biol.* 2002; 22:270–85. [PubMed: 11739740]
18. English JM, Pearson G, Baer R, Cobb MH. Identification of substrates and regulators of the mitogen-activated protein kinase ERK5 using chimeric protein kinases. *J Biol Chem.* 1998; 273:3854–60. [PubMed: 9461566]
19. Kamakura S, Moriguchi T, Nishida E. Activation of the protein kinase ERK5/BMK1 by receptor tyrosine kinases. Identification and characterization of a signaling pathway to the nucleus. *J Biol Chem.* 1999; 274:26563–71. [PubMed: 10473620]
20. English JM, Pearson G, Hockenberry T, Shivakumar L, White MA, Cobb MH. Contribution of the ERK5/MEK5 pathway to Ras/Raf signaling and growth control. *J Biol Chem.* 1999; 274:31588–92. [PubMed: 10531364]
21. Abe J, Takahashi M, Ishida M, Lee JD, Berk BC. c-Src is required for oxidative stress-mediated activation of big mitogen-activated protein kinase 1. *J Biol Chem.* 1997; 272:20389–94. [PubMed: 9252345]
22. Sun W, Wei X, Kesavan K, Garrington TP, Fan R, Mei J, et al. MEK kinase 2 and the adaptor protein Lad regulate extracellular signal-regulated kinase 5 activation by epidermal growth factor via Src. *Mol Cell Biol.* 2003; 23:2298–308. [PubMed: 12640115]

23. Scapoli L, Ramos-Nino ME, Martinelli M, Mossman BT. Src-dependent ERK5 and Src/EGFR-dependent ERK1/2 activation is required for cell proliferation by asbestos. *Oncogene*. 2004; 23:805–13. [PubMed: 14737115]
24. Sirvent A, Boureux A, Simon V, Leroy C, Roche S. The tyrosine kinase Abl is required for Src-transforming activity in mouse fibroblasts and human breast cancer cells. *Oncogene*. 2007; 26:7313–23. [PubMed: 17533370]
25. Song H, Jin X, Lin J. Stat3 upregulates MEK5 expression in human breast cancer cells. *Oncogene*. 2004; 23:8301–9. [PubMed: 15378007]
26. Marchetti A, Colletti M, Cozzolino AM, Steindler C, Lunadei M, Mancone C, et al. ERK5/MAPK is activated by TGFbeta in hepatocytes and required for the GSK-3beta-mediated Snail protein stabilization. *Cell Signal*. 2008; 20:2113–8. [PubMed: 18760348]
27. Yan C, Takahashi M, Okuda M, Lee JD, Berk BC. Fluid shear stress stimulates big mitogen-activated protein kinase 1 (BMK1) activity in endothelial cells. Dependence on tyrosine kinases and intracellular calcium. *J Biol Chem*. 1999; 274:143–50. [PubMed: 9867822]
28. Rovida E, Spinelli E, Sdelci S, Barbetti V, Morandi A, Giuntoli S, et al. ERK5/BMK1 is indispensable for optimal colony-stimulating factor 1 (CSF-1)-induced proliferation in macrophages in a Src-dependent fashion. *J Immunol*. 2008; 180:4166–72. [PubMed: 18322228]
29. Suzaki Y, Yoshizumi M, Kagami S, Koyama AH, Taketani Y, Houchi H, et al. Hydrogen peroxide stimulates c-Src-mediated big mitogen-activated 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. [PubMed: 11782488]
30. Sticht C, Freier K, Knopfle K, Flechtenmacher C, Pungs S, Hofele C, et al. Activation of MAP kinase signaling through ERK5 but not ERK1 expression is associated with lymph node metastases in oral squamous cell carcinoma (OSCC). *Neoplasia*. 2008; 10:462–70. [PubMed: 18472963]
31. Nagel S, Burek C, Venturini L, Scherr M, Quentmeier H, Meyer C, et al. Comprehensive analysis of homeobox genes in Hodgkin lymphoma cell lines identifies dysregulated expression of HOXB9 mediated via ERK5 signaling and BMI1. *Blood*. 2007; 109:3015–23. [PubMed: 17148583]
32. Garaude J, Cherni S, Kaminski S, Delepine E, Chable-Bessia C, Benkirane M, et al. ERK5 activates NF-kappaB in leukemic T cells and is essential for their growth in vivo. *J Immunol*. 2006; 177:7607–17. [PubMed: 17114430]
33. Ramos-Nino ME, Blumen SR, Sabo-Attwood T, Pass H, Carbone M, Testa JR, et al. HGF mediates cell proliferation of human mesothelioma cells through a PI3K/MEK5/Fra-1 pathway. *Am J Respir Cell Mol Biol*. 2008; 38:209–17. [PubMed: 17872495]
34. Mehta PB, Jenkins BL, McCarthy L, Thilak L, Robson CN, Neal DE, et al. MEK5 overexpression is associated with metastatic prostate cancer, and stimulates proliferation, MMP-9 expression and invasion. *Oncogene*. 2003; 22:1381–9. [PubMed: 12618764]
35. Weldon CB, Scandurro AB, Rolfe KW, Clayton JL, Elliott S, Butler NN, et al. Identification of mitogen-activated protein kinase kinase as a chemoresistant pathway in MCF-7 cells by using gene expression microarray. *Surgery*. 2002; 132:293–301. [PubMed: 12219026]
36. Hayashi M, Tapping RI, Chao TH, Lo JF, King CC, Yang Y, et al. BMK1 mediates growth factor-induced cell proliferation through direct cellular activation of serum and glucocorticoid-inducible kinase. *J Biol Chem*. 2001; 276:8631–4. [PubMed: 11254654]
37. Yang Q, Deng X, Lu B, Cameron M, Fearn C, Patricelli MP, et al. Pharmacological inhibition of BMK1 suppresses tumor growth through promyelocytic leukemia protein. *Cancer Cell*. 2010; 18:258–67. [PubMed: 20832753]
38. Zen K, Yasui K, Nakajima T, Zen Y, Zen K, Gen Y, et al. ERK5 is a target for gene amplification at 17p11 and promotes cell growth in hepatocellular carcinoma by regulating mitotic entry. *Genes Chromosomes Cancer*. 2009; 48:109–20. [PubMed: 18973138]
39. Cude K, Wang Y, Choi HJ, Hsuan SL, Zhang H, Wang CY, et al. Regulation of the G2-M cell cycle progression by the ERK5-NFkappaB signaling pathway. *J Cell Biol*. 2007; 177:253–64. [PubMed: 17452529]

40. Hayashi M, Fearn C, Eliceiri B, Yang Y, Lee JD. Big mitogen-activated protein kinase 1/extracellular signal-regulated kinase 5 signaling pathway is essential for tumor-associated angiogenesis. *Cancer Res.* 2005; 65:7699–706. [PubMed: 16140937]
41. Roberts OL, Holmes K, Muller J, Cross DA, Cross MJ. ERK5 is required for VEGF-mediated survival and tubular morphogenesis of primary human microvascular endothelial cells. *J Cell Sci.* 2010; 123:3189–200. [PubMed: 20736307]
42. Doebele RC, Schulze-Hoepfner FT, Hong J, Chlenski A, Zeitlin BD, Goel K, et al. A novel interplay between Epac/Rap1 and mitogen-activated protein kinase kinase 5/extracellular signal-regulated kinase 5 (MEK5/ERK5) regulates thrombospondin to control angiogenesis. *Blood.* 2009; 114:4592–600. [PubMed: 19710505]
43. Bos JL. Linking Rap to cell adhesion. *Curr Opin Cell Biol.* 2005; 17:123–8. [PubMed: 15780587]
44. Bos JL. Epac proteins: multi-purpose cAMP targets. *Trends Biochem Sci.* 2006; 31:680–6. [PubMed: 17084085]
45. Sawhney RS, Liu W, Brattain MG. A novel role of ERK5 in integrin-mediated cell adhesion and motility in cancer cells via Fak signaling. *J Cell Physiol.* 2009; 219:152–61. [PubMed: 19089993]
46. Schramm M, Ying O, Kim TY, Martin GS. ERK5 promotes Src-induced podosome formation by limiting Rho activation. *J Cell Biol.* 2008; 181:1195–210. [PubMed: 18573916]
47. Castro NE, Lange CA. Breast tumor kinase and extracellular signal-regulated kinase 5 mediate Met receptor signaling to cell migration in breast cancer cells. *Breast Cancer Res.* 2010; 12:R60. [PubMed: 20687930]
48. Kato Y, Kravchenko VV, Tapping RI, Han J, Ulevitch RJ, Lee JD. BMK1/ERK5 regulates serum-induced early gene expression through transcription factor MEF2C. *Embo J.* 1997; 16:7054–66. [PubMed: 9384584]
49. Hayashi M, Kim SW, Imanaka-Yoshida K, Yoshida T, Abel ED, Eliceiri B, et al. Targeted deletion of BMK1/ERK5 in adult mice perturbs vascular integrity and leads to endothelial failure. *J Clin Invest.* 2004; 113:1138–48. [PubMed: 15085193]
50. Kato Y, Zhao M, Morikawa A, Sugiyama T, Chakravorty D, Koide N, et al. Big mitogen-activated kinase regulates multiple members of the MEF2 protein family. *J Biol Chem.* 2000; 275:18534–40. [PubMed: 10849446]
51. Marinissen MJ, Chiariello M, Pallante M, Gutkind JS. A network of mitogen-activated protein kinases links G protein-coupled receptors to the c-jun promoter: a role for c-Jun NH2-terminal kinase, p38s, and extracellular signal-regulated kinase 5. *Mol Cell Biol.* 1999; 19:4289–301. [PubMed: 10330170]
52. Hipskind RA, Rao VN, Mueller CG, Reddy ES, Nordheim A. Ets-related protein Elk-1 is homologous to the c-fos regulatory factor p62TCF. *Nature.* 1991; 354:531–4. [PubMed: 1722028]
53. Price MA, Rogers AE, Treisman R. Comparative analysis of the ternary complex factors Elk-1, SAP-1a and SAP-2 (ERP/NET). *Embo J.* 1995; 14:2589–601. [PubMed: 7540136]
54. Young MR, Colburn NH. Fra-1 a target for cancer prevention or intervention. *Gene.* 2006; 379:1–11. [PubMed: 16784822]
55. Linnerth NM, Baldwin M, Campbell C, Brown M, McGowan H, Moorehead RA. IGF-II induces CREB phosphorylation and cell survival in human lung cancer cells. *Oncogene.* 2005; 24:7310–9. [PubMed: 16158061]
56. Xiao X, Li BX, Mitton B, Ikeda A, Sakamoto KM. Targeting CREB for cancer therapy: friend or foe. *Curr Cancer Drug Targets.* 2010; 10:384–91. [PubMed: 20370681]
57. de The H, Chen Z. Acute promyelocytic leukaemia: novel insights into the mechanisms of cure. *Nat Rev Cancer.* 2010; 10:775–83. [PubMed: 20966922]
58. Tataka RJ, O'Neill MM, Kennedy CA, Wayne AL, Jakes S, Wu D, et al. Identification of pharmacological inhibitors of the MEK5/ERK5 pathway. *Biochem Biophys Res Commun.* 2008; 377:120–5. [PubMed: 18834865]
59. Flaherty PT, Chopra I, Jain P, Yi S, Allen E, Cavanaugh J. Identification of benzimidazole-based inhibitors of the mitogen activated kinase-5 signaling pathway. *Bioorg Med Chem Lett.* 2010; 20:2892–6. [PubMed: 20382528]
60. Mody N, Leitch J, Armstrong C, Dixon J, Cohen P. Effects of MAP kinase cascade inhibitors on the MKK5/ERK5 pathway. *FEBS Lett.* 2001; 502:21–4. [PubMed: 11478941]

61. Inesta-Vaquera FA, Campbell DG, Tournier C, Gomez N, Lizcano JM, Cuenda A. Alternative ERK5 regulation by phosphorylation during the cell cycle. *Cell Signal*. 2010; 22:1829–37. [PubMed: 20667468]
62. Hayashi M, Lee JD. Role of the BMK1/ERK5 signaling pathway: lessons from knockout mice. *J Mol Med*. 2004; 82:800–8. [PubMed: 15517128]

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.

Table 1

Small molecule inhibitors for the BMK1 pathway.

Inhibitor	Target	IC ₅₀ <i>in vitro</i> (nM)	IC ₅₀ <i>in vivo</i>	Clinical development
BIX02188	MEK5	4.3	NA	NA
BIX02189	MEK5	1.5	NA	NA
Compound 6	MEK5	NA	NA	NA
XMD8-92	BMK1	240	NA	NA