Deletion of the Endothelial Bmx Tyrosine Kinase Decreases Tumor Angiogenesis and Growth

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Abstract

Bmx (Bone marrow kinase in chromosome X), also known as Etk, is a member of the Tec family of nonreceptor tyrosine kinases. Bmx is expressed mainly in arterial endothelia and in myeloid hematopoietic cells. Bmx regulates ischemia-mediated arteriogenesis and lymphangiogenesis, but its role in tumor angiogenesis is not known. In this study, we characterized the function of Bmx in tumor growth using both Bmx knockout and transgenic mice. Isogenic colon, lung, and melanoma tumor xenotransplants showed reductions in growth and tumor angiogenesis in Bmx gene-deleted (–/–) mice, whereas developmental angiogenesis was not affected. In addition, growth of transgenic pancreatic islet carcinomas and intestinal adenomas was also slower in Bmx–/– mice. Knockout mice showed high levels of Bmx expression in endothelial cells of tumor-associated and peritumoral arteries. Moreover, endothelial cells lacking Bmx showed impaired phosphorylation of extracellular signal-regulated kinase (Erk) upon VEGF stimulation, indicating that Bmx contributes to the transduction of vascular endothelial growth factor signals. In transgenic mice overexpressing Bmx in epidermal keratinocytes, tumors induced by a two-stage chemical skin carcinogenesis treatment showed increased growth and angiogenesis. Our findings therefore indicate that Bmx activity contributes to tumor angiogenesis and growth. Cancer Res; 72(14):1–10. ©2012 AACR.

Introduction

Cell differentiation and growth responses are commonly mediated by protein tyrosine phosphorylation, which constitutes a major signaling process in several physiologic states (1). Tyrosine kinases are of particular interest because many of them are activated and play critical roles in the pathogenesis of cancer (2).

Bone marrow kinase in chromosome X (Bmx) belongs to the Tec family of nonreceptor/cytosplasmic tyrosine kinases that also includes Btk, Tec, Tsk, and Itk (3). Tec family kinases are predominantly expressed in cells of hematopoietic origin, and also Bmx was originally identified in bone marrow cells (3). Receptors on bone marrow cells can activate downstream signaling pathways in part via various receptor-associated cytoplasmic tyrosine kinases, including Bmx (4, 5).

Apart from the myeloid cell lineage, abundant Bmx expression is detected in the endocardium and in arterial endothelia starting embryonic day (E)10.5 to E12.5 (6). Bmx deletion does not result in any obvious phenotype (6), suggesting redundancy where other members of this gene family compensate for Bmx functions during development. However, in further studies that aimed at looking into the role of Bmx in pathologic processes, Bmx overexpression in epidermal keratinocytes in the K14-Bmx mice was shown to induce skin hyperplasia, activate a number of inflammatory pathways, and to promote angiogenesis and wound healing (7).

Angiogenesis is needed for tumor growth beyond a few cubic millimeters in size (8). Neovascularization in tumors is promoted by VEGF secreted by tumor cells in response to intratumoral hypoxia (9). Overexpressed Bmx interacts with VEGF and angiopoietin receptors (VEGFR and Tie; ref. 6). Bmx was furthermore shown to regulate tumor necrosis factor receptor 2- (TNFR2-) and VEGFR2-mediated angiogenic signals in mice undergoing ischemia-induced arteriogenesis and angiogenesis (10, 11). A recent study suggested that Bmx is also involved in lymphangiogenesis (12). More detailed analysis of inflammatory signals in Bmx-deficient mice indicated that Bmx was required for full phosphorylation of the mitogen-activated protein kinases (MAPK) p38 and JNK (13).
Although recent reports have suggested that Bmx is involved in prostate cancer and glioblastoma growth (14, 15), little is known about Bmx in tumor biology. Here, we have used Bmx gene–deleted mice (6) and K14-Bmx transgenic syngenic mice (7) to explore the function of Bmx in tumor growth.

Materials and Methods

Mice

Eight- to 12-week-old Bmx knockout (KO), heterozygous (HT), and wild-type (WT) female mice, or KO and WT male mice of C57Bl/6J and BALB/c backgrounds as well as transgenic K14-Bmx (TG) male mice and the FVB/N WT littermates were used in the experiments. ApoB(–/–) mice were obtained from the Jackson Laboratory (Bar Harbor; stock no. 002020). RIP1-Tag2 mice (16) were a kind gift from Dr. Gerhard Christofori, University of Basel, Switzerland. The National Board for Animal Experiments at the Provincial State of Southern Finland approved all experiments conducted in Finland. All mouse studies conducted in the United States were approved by the Institutional Animal Care and Use Committee of the Yale University School of Medicine. All experiments were conducted in accordance with the legislation about the humane care and use of laboratory animals.

Tumor cell lines and cell culture

Luciferase tagged B16-F10 melanoma cells were obtained from Caliper Life Sciences Inc., and the cells (used in passages below 30) were maintained in Dulbecco’s Modified Eagle’s Medium (DMEM) supplemented with 2 mmol/L L-glutamine, penicillin (100 U/mL), streptomycin (100 μg/mL), and 10% FBS (PromoCell). Lewis lung carcinoma (hereafter LLC) and CT26. WT colon carcinoma cells were purchased from American Type Culture Collection (Manassas, VA) and maintained in complete DMEM and RPMI-1640 medium, correspondingly.

Syngenic tumor assays

CT26.WT cells (5 × 10^5 cells/mouse, n = 8–10 tumors/group, 4 experiments), syngenic in the BALB/c background, or B16-F10 (1 × 10^6 cells/mouse, n = 8–10 tumors/group, 4 experiments) or LLC cells (3 × 10^5 cells/mouse, n = 8–10 tumors/group, 4 experiments), syngenic in the C57Bl/6J background, were injected into the abdominal subcutis of Bmx(–/–) and age-matched control mice under anesthesia with ketamine (Ketalar; Pfizer) and xylazine (Rompun vet; Bayer Healthcare). After the establishment of the tumors during the first few days, the primary tumor growth rates were followed by measurements of the height, width, and depth of the tumors with a digital caliber every second day. During the first few days, the primary tumor growth rates were followed by measurements of the height, width, and depth of the tumors with a digital caliber every second day. At the end point, the tumors and the regional (inguinal) lymph nodes were excised, weighed, and processed for histology. The tumor volumes were then calculated with the formula (height × width × depth)/2 and statistically quantified. For bioluminescent imaging analysis, tumor cells were injected bilaterally s.c. into the abdominal flanks of the Bmx(–/–) and age-matched Bmx(+/+) mice, and the bioluminescence was followed thereafter for 10 days by in vivo bioluminescent optical imaging as described in the Supplementary Materials.

Analysis of tumor vascular perfusion

Fluorescently conjugated Lycopersicon esculentum lectin (100 μL, 1 mg/mL; Vector Laboratories) was administered intravenously. After 5 minutes, the mice were sacrificed and the tumors were excised, fixed with 4% paraformaldehyde (PFA), and processed for further histologic analysis.

Immunohistochemical staining and β-galactosidase staining of tumors

The protocols are detailed in Supplementary Materials.

Microscopy

Bright-field sections were viewed with a Leica DM LB microscope (Leica Microsystems) and images were captured with an Olympus DP50 color camera (Olympus Soft Imaging Solutions GMBH). Immunofluorescent images were taken with a Zeiss Axioplan 2 microscope (Carl Zeiss AG) as detailed in the Supplementary Materials.

Isolation and stimulation of murine blood vascular endothelial cells

The murine blood vascular endothelial cells were isolated from cardiac auricles of 8 mice from each genotype, as described (17).

RIP1-Tag2 pancreatic tumor model

The RIP1-Tag2 mice deficient of Bmx expression were obtained by crossing the RIP1-Tag2 mice (16) with Bmx(–/–) mice. A more extensive description of the analysis is provided in Supplementary Materials. Briefly, the RIP1-Tag2(+/–); Bmx(–/–) (n = 15) and control RIP1-Tag2(+/+); Bmx(+/+) mice (n = 27) were analyzed at 13.5 weeks of age by measuring the diameters of the macroscopic tumor foci above 1 mm in size. The tumor volume was subsequently quantified, assuming a spherical tumor shape. Tumor burden in each mouse was calculated as a sum of its tumor volumes.

ApcMin/+ tumor model

The gastrointestinal tract was opened longitudinally, rinsed, and spread flat on a filter paper. A more extensive description of the analysis is provided in Supplementary Materials. After overnight fixation with 4% PFA and staining with 0.1% methylene blue, the diameter of each macroscopically visible intestinal adenoma was scored. Tumor volumes were calculated as hemispheres. The ApcMin/+; Bmx(−/−) mice (n = 8) and ApcMin/+; BmxMin/+ control mice (n = 18) were analyzed at 20 weeks of age.

Chemical skin carcinogenesis in the K14-Bmx mice

Transgenic mice overexpressing Bmx in keratinocytes (n = 16; ref. 7) and their FVB/N control littermates (n = 18) were subjected to the chemical skin carcinogenesis involving 7,12-dimethylbenz[a]anthracene (DMBA; Sigma-Aldrich) and 12-O-tetradecanoyl phorbol-13-acetate (TPA; Sigma-Aldrich) treatments, and tumor development was monitored once a week for...
Bioluminescent imaging of the tumors of Bmx development showed also a trend for decreased signal in melanoma and CT26.WT colon carcinoma xenografts excised of the tumors was indeed attenuated in the Bmx us. As Bmx has been shown to mediate pathologic angiogenesis, significance was not obtained, for reasons that are as yet unknown to Bmx (Fig. 1A, B). More heterogenous results were obtained for the ways ANOVA followed by Tukey post hoc test for multiple comparisons. Fisher exact test was used for statistical analysis as described in Supplementary Materials.

Statistical analysis
Values are indicated as mean ± SEM. Statistical analysis was conducted with unequal n test for comparison of 2 groups or 2-way ANOVA followed by Tukey post hoc test for multiple comparisons. Fisher exact test was used for statistical analysis of metastasis occurrence. All statistical tests were 2-tailed. Differences were considered statistically significant at P<0.05.

Results
Impaired growth of isogenic B16-F10 melanoma in Bmx+/− mice
To analyze Bmx expression in tumor vessels and to evaluate the effect of Bmx on primary tumor growth, isogenic B16-F10 melanoma cells were implanted subcutaneously into the abdominal flanks of female Bmx+/− mice and Bmx+/+ control mice. Bmx deficiency in the host led to slower growth of the B16-F10 tumors, seen as impaired tumor volume development (Bmx+/− mice 982 mm³ ± 123 mm³, n = 10; Bmx+/− mice 555 mm³ ± 49 mm³, n = 8, P = 0.009). Analysis of early tumor development showed also a trend for decreased signal in bioluminescent imaging of the tumors of Bmx+/− mice (Fig. 1A, B). More heterogenous results were obtained for the Bmx−/− male mice; in these experiments, statistical significance was not obtained, for reasons that are as yet unknown to us. As Bmx has been shown to mediate pathologic angiogenesis (7, 10), we evaluated whether the loss of Bmx altered the vasculature of xenotransplanted melanomas. Neovascularization of the tumors was indeed attenuated in the Bmx−/− mice (Fig. 1C).

Bmx is expressed in the peritumoral arteries
To localize Bmx expression in tumor-bearing mice, B16-F10 melanoma and CT26.WT colon carcinoma xenografts excised from the Bmx+/− mice were stained for β-galactosidase. The blue-stained β-galactosidase marker is expressed in place of Bmx, indicating the sites of native Bmx expression. As shown by analysis of both whole mounts and histologic sections, Bmx was abundantly expressed in the preexisting peritumoral arteries surrounding the subcutaneously implanted tumor tissue in the abdominal area (Fig. 2A). The colocalization of the β-galactosidase signal with antibody staining of PECAM-1 confirmed the Bmx expression in tumor-associated blood vasculature.

Homzygous Bmx deletion delays LLC lung carcinoma and CT26.WT colon cancer growth
The growth of LLC tumor in Bmx−/− targeted female mice was statistically significantly inhibited in Bmx−/− mice when compared Bmx control mice in 4 of 4 experiments (Fig. 2B in right, and data not shown, P values of the tumor weight differences in the experiments: P = 0.0038, P = 0.0067, P = 0.0285, and P = 0.0489). Furthermore, Bmx deficiency resulted in the inhibition of CT26.WT colon carcinoma growth in the Bmx-deficient female mice to a statistically significant extent in 3 of 4 experiments (Fig. 2B in left, significant P values of tumor weight analysis as follows: P = 0.0001, P = 0.0057, and P = 0.0418). Together, these data suggest that host Bmx deficiency delays primary tumor growth in the Bmx−/− mice.

Bmx deficiency in host reduces tumor angiogenesis and vascular perfusion
To analyze if Bmx in host-derived tissues influences tumor perfusion via blood vessels, fluorescently labeled Lycopersicon esculentum lectin was systemically administered into the tumor-bearing mice before the excision of the CT26.WT colon carcinoma tumors. Comparison of the lectin and PECAM-1 staining showed a moderate reduction of tumor vascular perfusion and decreased vascular density in the Bmx−/− mice (Fig. 3A and B). The intratumoral vascular lumen area was decreased also in B16-F10 melanomas excised from the Bmx−/− mice (data not shown). Increased tumor necrosis and decreased density of smooth muscle actin positive cells lining the vessels was observed in the Bmx−/− mice (Fig. 3C and D). The density of Ki67 antigen–positive proliferating cells was reduced in the Bmx−/− mice as well (Fig. 3C and D). However, no change was detected in the infiltration of CD45, Gr-1, CD11b, or F4/80 positive inflammatory cells (Fig. 3C and D and data not shown). Taken together, these results suggested that host Bmx contributes to signals for efficient tumor angiogenesis, but not inflammatory cell recruitment into the tumor.

Increased skin tumor growth and angiogenesis in transgenic K14-Bmx mice
We also evaluated the effects of Bmx on tumor growth by subjecting the transgenic K14-Bmx mice expressing Bmx in basal epidermal cells to the chemical skin carcinogenesis model involving DMBA and TPA treatments. The K14-Bmx mice developed increased numbers of tumors when compared with their WT littermates (Fig. 4A). Tumor development was somewhat accelerated in the TG mice and the proportion of large tumors (3–10 mm in diameter) was slightly higher in the TG mice at late stage of carcinogenesis (Fig. 4A). However, both mouse strains reached 100% tumour incidence by week 13. Tumor angiogenesis was augmented in the papillomas and SCCs of the TG mice in comparison with
their WT littermates (Fig. 4B and C). Consistent with the data obtained from CT26.WT colon carcinomas in Bmx<sup>−/−</sup> mice, inflammatory cells did not show differences between the K14-Bmx and the WT tumors (data not shown). In this model, Bmx probably not only regulates tumor angiogenesis, but also tumor growth through its expression in the keratinocytes. Taken together, the results from the DMBA-TPA model support the view that Bmx contributes to tumor growth and angiogenesis.

### Variable effects on cutaneous tumor growth and angiogenesis in Bmx-deficient mice

To analyze the influence of Bmx deficiency on the development of chemically induced epidermal tumors, Bmx-deficient female and male mice with the age- and gender-matched control mice were subjected to the DMBA-TPA treatments. The rate of tumor initiation was similar between Bmx<sup>+/−</sup> and Bmx<sup>+/+</sup> female mice, and slightly, but not significantly, delayed in the Bmx<sup>−/−</sup> mice (Supplementary Fig. S1A). However, surprisingly, Bmx<sup>−/−</sup> mice developed significantly increased numbers of tumors when compared with Bmx<sup>+/+</sup> mice, whereas Bmx<sup>+/−</sup> mice showed a trend for slightly decreased tumor multiplicity. The tumor size was also statistically significantly reduced in the Bmx<sup>−/−</sup> mice compared with Bmx<sup>+/+</sup> mice, but comparable with Bmx<sup>+/−</sup> mice at 20 weeks. At the end point at 25 weeks, a marked decrease in the tumor size was evident in the Bmx<sup>−/−</sup> group compared with the Bmx<sup>−/−</sup> and Bmx<sup>+/−</sup> groups. In histologic analysis,
tumor angiogenesis was attenuated especially in the SCCs of Bmx−/− mice in comparison with the Bmx+/+ and Bmx+/− control mice, whereas papillomas showed more heterogeneous results (Supplementary Fig. S1B). This suggests that the genetic loss of Bmx results in a significant growth delay of chemically induced skin tumors in female mice, with suppression of angiogenesis in the tumors that have undergone malignant conversion. However, in the analysis of the same parameters, the Bmx−/−/− and Bmx+/+ male mice did not show any significant changes between the genotypes in this tumor model (Supplementary Fig. S2A–S2C). These data indicate that the haploid and homozygous Bmx deletions have different effects on tumor initiation and growth.

Altered tumor metastasis in Bmx-modified mice

We further assessed if Bmx deletion impairs the dissemination of tumor cells from the primary tumor. The lymph nodes of tumor-bearing mice were excised and macroscopically examined for metastases at various time points. The occurrence of melanoma metastases was significantly decreased in Bmx-deficient mice when compared with wild-type mice at 3 and 6 weeks after tumor cell implantation (Fig. 5A). Lymph nodes from the K14-Bmx and Bmx−/− mice and controls bearing macroscopically malignant SCCs were also analyzed at 15, 20, and 25 weeks after the DMBA-TPA treatment. The frequency of tumor metastasis was significantly increased in the K14-Bmx mice when compared with the WT control mice at 20 and 25 weeks (Fig. 5B), whereas it was decreased in the homozygous Bmx−/− and, unexpectedly, increased in the heterozygous Bmx+/− female mice in comparison with the Bmx+/+ mice (data not shown). Consistent with other analyses, no differences were detected in the analysis of dissemination to lymph nodes in the Bmx−/− and Bmx+/+ groups (data not shown). These data further support our observations that homozygous and haploid deletion of Bmx has different effects on tumor development.

Delayed growth of ApcMin/+ intestinal adenomas in Bmx−/− mice

VEGF inhibition has been reported to delay the growth of intestinal adenomas in the transgenic ApcMin/+ colon carcinoma model (19). Recently, Toll-like receptor (TLR) signaling and extracellular signal–regulated kinase (Erk) activation were also shown to be essential for the intestinal tumorigenesis in the ApcMin/+ mice (20). As Bmx has been shown to mediate TLR signals, in particular signals downstream of TLR-4 (21), and interact with MyD88 (22), an important mediator in Erk signaling (20), we analyzed the ApcMin/+ phenotype in the Bmx−/− mice. The intestinal adenoma growth was reduced in Bmx-deficient female mice to a statistically significant extent (Fig. 6A and B). However, intestinal adenoma growth
was unaltered in the Bmx-deficient male mice when compared with their age-matched ApcMin/+; Bmx+/0 controls (data not shown).

Inhibition of transgenic RIP1-Tag2 insulinoma growth in Bmx−/− mice
To further analyze the effect of Bmx deficiency on tumor growth, we assessed Bmx−/− mice in the context of RIP1-Tag2 pancreatic islet carcinomas (16, 23). A reduction of pancreatic tumor burden was observed in the RIP1-Tag2+/−; Bmx−/− mice when compared with the RIP1-Tag2+/−; Bmx+/0 controls at the age of 13.5 weeks (Fig. 6C). However, Bmx deficiency did not alter RIP1-Tag2-driven oncogenic tumor growth in Bmx−/0 male mice when compared with the RIP1-Tag2+/−, Bmx−/0 control mice (Supplementary Fig. S2D).

Phosphorylation of signaling proteins downstream of Bmx in endothelial cells and tumor xenografts
VEGF, by binding to its cognate receptor, VEGFR2, mediates angiogenesis during development and in pathologic
conditions, including cancer (24). Although Bmx has been shown to interact with VEGFR2 in cultured endothelial cells (25), little is known about the Bmx downstream signals in the vascular endothelium in vivo. To elucidate the cellular mechanisms responsible for the effect of Bmx deficiency on the tumor-associated vascular phenotype, we analyzed signaling mediators downstream of Bmx. For this purpose, vascular endothelial cells of Bmx null and control mice were isolated from cardiac atria, where Bmx expression is highest (6), and subjected to proteomic profiling of 46 intracellular

Figure 4. Increased growth and angiogenesis of cutaneous tumors in the K14-Bmx mice. A, representative images of DMBA-TPA–induced skin tumors in K14-Bmx (TG) mice and their WT littermates at week 15. Cumulative tumor multiplicity in TG mice, n = 16, and in WT mice, n = 18 mice, P = 0.048 at week 25. Tumor incidence; tumor size presented as the percentage of tumors in 3 different size categories, with tumor diameter of 1 to 3 mm, 3 to 7 mm, or 7 to 10 mm. B, representative images of immunohistochemical staining for PECAM-1 in skin papillomas (PAP) and squamous cell carcinomas (SCC) harvested from the TG and WT mice. C, quantification of tumor blood vessel densities. Scale bar, 100 μm; *, P < 0.05; **, P < 0.01; ***, P < 0.001.

Figure 5. Bmx expression correlates with the frequency of tumor metastasis. A, the occurrence of melanoma metastasis in WT and Bmx KO mice at 3 and 6 weeks after tumor cell implantation. The images in the inset show regional inguinal lymph nodes excised at 6 weeks after implantation; note the black melanoma metastases. B, lymph nodes from K14-Bmx and WT mice bearing SCCs analyzed 15, 20, and 25 weeks after the DMBA-TPA treatment. The values indicate the percentage of metastatic lymph nodes versus all lymph nodes analyzed; *, P < 0.05.
phosphoproteins, with and without VEGF stimulation. Bmx-deficient cells displayed decreased phosphorylation of the extracellular signal regulated kinase Erk when compared with the Bmx expressing cells isolated from wild-type mice (data not shown). The reduced Erk phosphorylation in cells lacking Bmx was verified in Western blot analysis (Fig. 7A).

To further analyze the downstream signaling of Bmx and the molecular mechanisms of the reduced tumor growth in Bmx-deficient mice, equal-sized pieces of tumors and surrounding skin tissue from B16-F10 melanomas in Bmx null and control mice were excised 0, 2, 3, or 5 weeks after tumor implantation and subjected to analysis with phosphospecific Akt and Erk antibodies. Representative results, shown in Fig. 7B, indicate decreased phosphorylation of Akt and Erk in the 2- and 3-week tumor samples from the Bmx-deficient hosts.

**Normal postnatal retinal arteriogenesis in Bmx-deficient mice**

To investigate if the Bmx tyrosine kinase influences the growth of retinal vessels in the early postnatal period, retinas of Bmx+/−, Bmx−/−, Bmx−+/+, and Bmx+/+ mice were sacrificed at postnatal day 5 and stained for isoelectin B4 to visualize the blood vasculature and for β-galactosidase to identify the sites of Bmx expression. The Bmx-deficient mice had normal retinal vasculature architecture, including the pericyte coating, and Bmx expression was detected in the endothelium of proximal parts of the retinal arterial network (Supplementary Fig. S3A–S3C).

**Discussion**

Bmx, a member of Tec family of nonreceptor tyrosine kinases, has been implicated in several cellular processes, including cell differentiation and growth signaling (5, 26), but a definitive function of Bmx in tumor growth, particularly in tumor angiogenesis, has remained thus far elusive. In this study, we characterized the contribution of Bmx to tumor growth by using genetically modified mice that either lack or overproduce Bmx and a variety of tumor models. By using the Bmx-LacZ knock-in mice, we localized host Bmx expression to arterial vasculature and inflammatory cells associated with the tumor, whereas very little or no expression was detected in the capillaries. These findings are in agreement with previously published data (27, 28).

In our experiments, loss of Bmx in the host resulted in variably decreased tumor growth in syngeneic tumor models of colon carcinoma, melanoma and non–small cell lung cancer in female mice. Tumor growth in RIP1-Tag2 and ApcMin/+ transgenic mice was clearly reduced upon homozygous Bmx deletion, suggesting that Bmx expressed in the tumor cells also contributes to tumor growth. On the other hand, when Bmx was overexpressed in epidermal cells, the development of cutaneous carcinomas induced by the DMBA-TPA was accelerated. This is in agreement with studies where Bmx expression in tumor cells was shown to promote the growth of human tumor xenografts in mice (14). The reason for the failure to reproduce the results obtained in Bmx−/− female mice in
Bmx−/− male mice is as yet unknown to us, but we speculate that Bmx may have haploinsufficient function in male mice that is compensated by some other genetic pathway that we are currently trying to identify. It is interesting to note that Bmx knockdown was recently shown to potently inhibit the STAT3 signaling protein activation, expression of glioblastoma stem cell transcription factors, and growth of glioblastoma stem cell–derived intracranial tumors (15), and that the STAT5 pathway downstream of Bmx is regulated by female steroid hormones (4, 29).

Bmx is involved in the activation of the NF-κB pathway (13) and in angiogenesis and arteriogenesis downstream of TNFR2 (10, 11, 25, 30). In cultured endothelial cells lacking Bmx, TNF-α–induced phosphorylation of VEGFR2 was attenuated (25). In light of these data, the recent finding of the coordinated VEGFR2-Bmx activation in TNFR2-mediated growth of renal cell cancer is of interest and warrants further analysis of Bmx in tumor growth and angiogenesis (31).

To look into the cellular mechanisms underlying the reduced angiogenesis in mice lacking Bmx, we conducted proteomic profiling of a variety of intracellular phosphokinases in isolated vascular endothelial cells stimulated with VEGF. Phosphorylation of Erk was reduced in cells lacking Bmx, especially upon VEGF treatment. This result suggests that the interaction between VEGF/VEGFR2 and Bmx promotes an angiogenic tumor phenotype, perhaps in part via Erk as a downstream target in the vascular endothelium.

In addition to vascular endothelium, myeloid cells form another primary site of endogenous Bmx expression (3, 27). Because of this, and because of the possibility that Bmx mediates inflammatory signals in the tumors, bone marrow derived cells were investigated in the tumors. The recruitment of leukocytes or macrophages to the tumors was not altered by Bmx-decient cells were investigated in the tumors. The recruitment of leukocytes or macrophages to the tumors was not altered by Bmx deficiency or excess, suggesting that inflammatory signals were not responsible for the tumor phenotype.

We also detected decreased dissemination of B16-F10 melanoma cells into lymph nodes Bmx-deficient mice as well as decreased dissemination of SCCs into lymph nodes in Bmx−/− the DMBA-TPA model, and an increase of lymph node metastasis in the K14-Bmx mice subjected to chemical carcinogenesis protocol. Although the abrogation of tumor cell dissemination may be secondary to the decreased tumor growth or vascularization, previous data have implicated Bmx signals in cell migration (32–34). Bmx promotes integrin signaling via binding to focal adhesion kinase (FAK; ref. 33), and this crosstalk is required for integrin-mediated cell motility (33). Indeed, Bmx protein levels have been reported to be elevated in carcinoma cells prone to metastasis (33, 35).

Our present data with oncogenic tumor models demonstrate the contribution of Bmx kinase in tumor growth and angiogenesis in vivo. Interestingly, molecular inhibitors targeting Bmx are available (36), and one of these was reported to inhibit Src-induced cellular transformation in culture (36). Further studies should evaluate Bmx as a possible target of cancer therapy.

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

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