Blocking SDF-1α/CXCR4 Downregulates PDGF-B and Inhibits Bone Marrow–Derived Pericyte Differentiation and Tumor Vascular Expansion in Ewing Tumors

Randala Hamdan, Zhichao Zhou, and Eugenie S. Kleinerman

Abstract

Bone marrow cells (BMC) are critical to the expansion of the tumor vessel network that supports Ewing sarcoma growth. BMCs migrate to the tumor and differentiate into endothelial cells and pericytes. We recently demonstrated that stromal-derived growth factor 1α (SDF-1α) regulates platelet-derived growth factor B (PDGF-B) and that this pathway plays a critical role in bone marrow–derived pericyte differentiation in vitro. We investigated the role of SDF-1α/PDGF-B in the tumor microenvironment in vivo in promoting bone marrow–derived pericyte differentiation in Ewing tumors. The CXCR4 antagonist AMD 3100 was used to disrupt the SDF-1α/CXCR4 axis in vivo in two xenograft Ewing tumor models. BMCs from GFP+ transgenic mice were transplanted into lethally irradiated nude mice to track BMC migration to the tumor site. Following BMC engraftment, tumor-bearing mice received daily subcutaneous injections of either PBS or AMD 3100 for 3 weeks. Tumors were resected and tumor sections were analyzed by immunohistochemistry. AMD 3100 inhibited BMC differentiation into desmin+ and NG2+ pericytes, affected the morphology of the tumor vasculature, decreased perfusion, and increased tumor cell apoptosis. We observed smaller vessels with tiny lumens and a decrease in the microvessel density. AMD 3100 also inhibited PDGF-B protein expression in vitro and in vivo. SDF-1α in the tumor microenvironment plays a critical role in promoting pericyte formation and Ewing sarcoma tumor neovascularization by regulating PDGF-B expression. Interfering with this pathway affects tumor vascular morphology and expansion. Mol Cancer Ther; 13(2); 483–91. ©2013 AACR.

Introduction

Ewing sarcoma is the second most common pediatric bone tumor after osteosarcoma (1, 2). Despite a survival of about 65% to 75% in patients with Ewing sarcoma with non-metastatic disease, the overall survival rate of patients with relapsed disease or patients who present with metastasis at the time of diagnosis remains at 20% to 25% (3). Ewing sarcoma depends on a vascular network for growth, invasion, and metastasis (4). Tumor vessel expansion is mediated by angiogenesis and vasculogenesis. Angiogenesis involves the formation of new blood vessels from preexisting capillaries by vessel sprouting or endothelial budding (5), whereas vasculogenesis involves the migration and homing of bone marrow progenitor cells (BMC) from the bone marrow to the tumor site, where they differentiate into cells that participate in blood vessel formation (6, 7).

We previously demonstrated that vasculogenesis plays a critical role in the formation of vessels in Ewing sarcoma tumors. BMCs migrate to TC71 Ewing tumors, differentiate into endothelial cells and pericytes, and participate in the formation of new tumor vessels (8, 9). We further showed that vascular endothelial growth factor 165 (VEGF165) produced by the tumor was the chemotactic stimulus for the BMCs (10, 11). Using genetic manipulation to inhibit BMCs’ participation in vessel formation (12) or inhibiting tumor cell expression of VEGF165 (10, 12, 13) significantly decreased tumor vessel density and inhibited tumor growth. These studies established the participation of BMCs in tumor vessel expansion and their critical role in this process; however, the mechanisms and cytokines responsible for BMC differentiation into endothelial cells and pericytes at the tumor site remain unclear.

Stromal cell-derived growth factor 1α (SDF-1α) plays an important role in the retention and homing of bone marrow progenitor cells to the bone marrow (14) and provides a chemotactic gradient for CXCR4+ bone marrow progenitor cells (15–18). SDF-1α or chemokine (CXC motif) ligand 12 (CXCL12) binds to the G-protein–coupled receptor CXCR4, which only binds SDF-1α (14). We previously showed that using an adenoviral vector containing the SDF-1α gene (Ad-SDF-1α) to induce SDF-1α in the tumor microenvironment rescued both tumor growth and tumor vessel expansion in VEGF165-inhibited TC/siVEGF7,1
Ewing tumors. BMC infiltration into the perivascular area was restored with increased numbers of pericytes surrounding the tumor vessels (19). These findings suggest a link between SDF-1α and the formation of bone marrow–derived pericytes in the tumor and confirm that vasculo-genesis associated pericyte differentiation plays a critical role in the growth of Ewing tumors.

Pericytes, also known as smooth muscle cells or mural cells, wrap around endothelial cells and together form the basement membrane of the vessel wall (20, 21). Recent studies have demonstrated that pericytes are important constituents of the tumor vessel wall (20, 22). In a Lewis lung carcinoma tumor model, administering the DNA oligonucleotide aptamer (AX102), which selectively binds to PDGF-B resulted in sequential loss of pericytes and endothelial cells and overall reduction in tumor vascularity (23). PDGF-B is a member of the PDGF family of growth factors that are mitogens for cells of mesenchymal origin including fibroblasts and smooth muscle cells. When synthesized and secreted in its homodimer form, PDGF-BB binds to its tyrosine kinase receptor, PDGFR-β (24). Pericytes are recruited by PDGF-B-expressing endothelial cells to the sites of vessel remodeling, where they play a role in vascular stabilization, maturation, and survival (20, 22). Given that PDGF-B stimulates pericyte maturation and the critical role of pericytes in the vascular development of Ewing tumors, we investigated whether SDF-1α in the tumor microenvironment was inducing bone marrow–derived pericyte differentiation via a PDGF-B–associated mechanism.

The CXCR4 antagonist, AMD 3100, was used to disrupt the SDF-1α/CXCR4 axis in vivo. AMD 3100 is a bicyclam molecule that specifically binds to CXCR4 (25, 26). AMD 3100 inhibited BMC differentiation into pericytes. Inhibiting BMC differentiation into pericytes resulted in smaller tumor vessels with tiny lumens, decreased tumor blood vessel perfusion, and increased tumor cell apoptosis. Furthermore, we confirmed that disrupting the SDF-1α/CXCR4 axis decreased PDGF-B protein expression in the tumor microenvironment.

We are first to demonstrate that SDF-1α modulates the expression of PDGF-B in the tumor microenvironment and that blocking this pathway affects the tumor vasculature. Our findings emphasize the importance of understanding how the tumor microenvironment contributes to the mechanism and milieu that promote vascular expansion and tumor growth. Identifying key growth factors and the potential signaling pathways that promote tumor vascular development will enable us to devise new approaches to disrupt the supportive microenvironment for therapeutic purposes.

Materials and Methods

Mice and murine bone marrow cells

All animal experiments were approved by the Institutional Animal Care and Use Committee at The University of Texas MD Anderson Cancer Center (Houston, TX). Mice were housed in pathogen-free facilities. Female GFP+ transgenic mice (strain c57bl/6-tg (actb-egfp) 1osb/j) were purchased from Jackson Laboratory. Four-to six-week-old T-cell–deficient female BALB/cAnN mice were purchased from the National Cancer Institute (strain 01B70) or Charles River (strain nu/nu 088).

TC71 and A4573 Ewing sarcoma cell lines and bone marrow transplant tumor model

TC71 and A4573 Ewing cells containing the t(11:22) translocation were cultured in complete Dulbecco’s Modified Eagle Medium supplemented with 10% FBS, 2 mmol/L L-glutamine, 1 mmol/L nonessential amino acids, 1 mmol/L penicillin–streptomycin, and 2-fold vitamin solution (Life Technologies). Both cell lines are mycoplasma-free and were authenticated by short terminal repeat fingerprinting. A4573 cells were a gift from Dr. V. Soldatenkov (Georgetown University Medical Center, Washington DC) and were obtained in October of 1997. TC71 cells were a gift from Dr. T. Triche (University of Southern California, Los Angeles, CA) and were obtained in November of 1998. PCR was used to confirm that both cell lines have the EWS/Fli1 fusion. For the in vivo experiments, a bone marrow transplant model in which GFP+ transgenic mice were used as bone marrow donors and nude BALB/cAnN mice served as bone marrow recipients was performed as previously described (9, 11, 19, 27). Four weeks following transplant, engraftment was confirmed, and mice received subcutaneous injections of either TC71 (2 × 106 cells/0.2 mL sterile PBS) or A4573 (6 × 106 cells injected as 2 separate injections of 3 × 106/0.2 mL sterile PBS on days 1 and 3). All mice were euthanized three weeks following tumor cell injection. The tumors were resected and frozen.

AMD 3100 treatment

AMD 3100 octahydrochloride hydrate was purchased from Sigma-Aldrich (A 5602). A stock solution of AMD 3100 was prepared at a concentration of 22 mg/mL in H2O. For in vitro studies, TC71 and A4573 cells were plated and treated with 100 ng/mL rSDF-1α with or without 1 μg/mL of AMD 3100 for 24 hours. The media were collected and PDGF-B protein levels measured by ELISA as previously described (28). For in vivo studies, transplanted mice were subcutaneously injected with TC71 or A4573 cells as described above. Five days following tumor cell inoculation, tumor-bearing mice (10–15 mice/group) received 1 daily subcutaneous injection of AMD 3100 (5 mg/kg body weight in 0.2 mL PBS) or PBS alone (control). The mice were sacrificed 3 weeks later and the tumors were harvested; their final volumes (mm3) were calculated as \( V = \frac{1}{6} \pi a b^2 \), in which a is the longer diameter and b is the shorter diameter, and frozen for further immunohistochemical (IHC) analysis.

Hoechst 33342 perfusion assay

To label perfused tumor vessels at the end of treatment with AMD 3100 or PBS, Hoechst 33342 dye (Molecular Probes Invitrogen; 33342) was reconstituted in PBS per the
manufacturer’s instructions. The mice received intravenous injections of Hoechst 33342 (1.2 mg in 0.1 mL sterile PBS) and were sacrificed 2 minutes later by cervical dislocation.

**Immunohistochemical analysis and microscopy**

Frozen tumor sections were fixed in cold acetone and blocked with 4% fish gelatin in PBS as previously described (9, 27). All primary and secondary antibodies were diluted in 4% fish gelatin according to the concentrations listed in Table 1. Hoechst 33342 dye (Molecular Probes) was used to stain the nuclei. Images were captured using a Zeiss Axioplan fluorescence microscope (Carl Zeiss, Inc.). PDGF-B expression was evaluated in the tumor sections using the primary rabbit polyclonal antibody PDGF-B (H-55), (Santa Cruz Biotechnology; sc-7878) as previously described (19). Frozen tumor sections were fixed with 4% paraformaldehyde in PBS for 20 minutes at room temperature. Apoptosis was measured using the terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay as previously described (29). Images were captured using a Nikon Microphot-FXA microscope (Nikon Instruments).

**Immunohistochemistry quantification and statistical analysis**

Areas of positive staining within the central region of the tumor sections were quantified using the Simple PCI software (Hamamatsu) as previously described (27). The effect of AMD 3100 on tumor growth was statistically evaluated using the Mann–Whitney test. P < 0.05 were considered statistically significant.

**Results**

**AMD 3100 inhibits BMC differentiation into pericytes**

Tumor sections were stained for GFP⁺ bone marrow–derived cells (red staining) and CD31 endothelial cell marker (green staining) to analyze the distribution of BMCs relative to the tumor vasculature. AMD 3100 resulted in changes in the tumor vessel morphology and the distribution of bone marrow–derived cells relative to the tumor vasculature (Fig. 1). In the tumors from the AMD 3100–treated mice, the majority of cells that were GFP⁺ were also CD31⁺, as evidenced by the colocalization of both markers (yellow; Fig. 1A). This indicates that the migrated BMCs predominantly differentiated into endothelial cells. In contrast, in the tumors from the PBS-treated mice, there was a thick layer of GFP⁺/CD31⁻ cells around and separate from the CD31⁺ cells in addition to GFP⁺/CD31⁺ cells (Fig. 1A). This GFP⁺/CD31⁻ layer was absent in the tumors from the AMD 3100–treated mice.

To determine whether the decrease in the perivascular layer observed in the tumors from the AMD 3100–treated mice corresponded to a decrease in bone marrow–derived pericytes, tumor sections were stained for GFP⁺ cells (red staining) and the pericycle markers desmin or NG2 (Fig. 1B and C respectively; green staining). AMD 3100 decreased bone marrow–derived pericytes in both TC71 and A4573 tumors as indicated by the lack of colocalization (yellow staining) between GFP and both desmin and NG2. The percentage of BMCs that differentiated into pericytes was determined by quantifying green and red pixels and then calculating the ratio of green to red. There was a 79% and 74% decrease in desmin⁺ bone marrow–derived pericytes and a 74% and 73% decrease in NG2⁺ bone marrow–derived pericytes in TC71 and A4573 tumors from AMD 3100–treated mice, respectively, compared with the tumors from PBS-treated mice.

**AMD 3100 decreases PDGF-B protein expression**

Next, we analyzed the impact of AMD 3100 on PDGF-B protein expression in response to SDF-1α stimulation both in vitro and in vivo. For in vitro studies, TC71 and A4573 Ewing sarcoma cells were plated and treated with 100 ng/mL rSDF-1α with or without 1 μg/mL AMD 3100 for 24 hours. PDGF-B levels were measured by ELISA. We found that whereas SDF-1α induces secretion of PDGF-B in both TC71 and A4573, AMD 3100 inhibits PDGF-B secretion in response to SDF-1α (Fig. 2A). To demonstrate the effect of AMD 3100 on PDGF-B in vivo, tumor sections were stained for PDGF-B expression (red staining). The total amount of positive red pixels for PDGF-B was quantified.

<table>
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<tr>
<th>Primary antibody</th>
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<tr>
<td>Chicken anti-GFP</td>
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<td>Abcam</td>
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<td>Cy5 goat anti-rabbit IgG (H+L)</td>
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PDGF-B protein levels in TC71 and A4573 tumors from AMD 3100–treated mice were significantly lower than those in tumors from PBS-treated mice (Fig. 2B). Because AMD 3100 is a specific CXCR4 antagonist, in that it disrupts the SDF-1α/CXCR4 axis (26), and combined with our previous findings that SDF-1α regulates PDGF-B expression (19, 28), these findings establish a connection between SDF-1α signaling and the induction of PDGF-B in the tumor microenvironment and suggest that SDF-1α promotes bone marrow–derived pericyte differentiation via a PDGF-B–dependent mechanism.

AMD 3100 changes the tumor vascular morphology, decreases the microvessel density, and decreases tumor vessel perfusion

The decrease in PDGF-B expression and bone marrow–derived tumor vessel pericytes prompted us to investigate the effect of AMD 3100 on the tumor vascular morphology. CD31 was used as the endothelial cell marker to identify the tumor vasculature. Tumors from the AMD 3100–treated mice had small punctuate vessels with tiny lumens compared with those from the PBS-treated mice (Fig. 3A). The microvessel density in the tumors from the PBS and AMD 3100–treated mice was compared by quantifying positive red pixels for CD31. All CD31+ cells were included in the quantification. The total microvessel density was reduced by 50% and 55% in TC71 and A4573 tumors, respectively, compared with those from the PBS-treated mice (Fig. 3B).

To assess the effect of AMD 3100 on tumor vessel perfusion, mice were intravenously injected with Hoechst 33342 nuclear dye, which stains cells blue (Fig. 3C). Tumors were excised, fixed, and the total amount of positive blue pixels for Hoechst 33342 was quantified. Vessel perfusion was reduced by 71% and 47% in TC71 and A4573 tumors, respectively, from the AMD 3100–treated mice compared with the PBS-treated mice.

AMD 3100 decreases tumor cell viability but does not inhibit tumor growth

The observed changes in the tumor vasculature morphology, density, and efficiency following AMD 3100 prompted us to investigate tumor cell viability. Tumor cell apoptosis was analyzed by TUNEL. AMD 3100 increased tumor cell apoptosis by 3.4- and 3.8-fold in TC71 tumors and A4573 tumors, respectively, compared with PBS (Fig. 4). Although the tumors from the AMD 3100–treated mice were smaller, the difference was not statistically significant (Fig. 5).

Discussion

The cure rates for patients with Ewing sarcoma, especially those who present with metastasis, have remained unchanged for over 20 years, emphasizing the urgency for
new therapies (30). We previously demonstrated that vasculogenesis is essential for the expansion of Ewing sarcoma tumor vasculature and that inhibiting this process, inhibits tumor growth (12, 13). VEGF165 was the chemotactic stimulus for the BMCs (9, 10, 13). In the absence of VEGF165, introducing SDF-1α partially restored vasculogenesis, increased tumor vessel pericytes, and rescued tumor growth (19). In the present study, we demonstrate for the first time that SDF-1α in the tumor microenvironment not only provides a chemotactic stimulus for BMC migration into the tumor, but also stimulates the differentiation of the migrated BMCs into pericytes by regulating PDGF-B production. We found that disrupting the SDF-1α/CXCR4 axis by blocking CXCR4 with the AMD 3100 antagonist downregulated PDGF-B protein expression, inhibited bone marrow–derived pericyte differentiation, and decreased the total number of tumor vessel pericytes.

GFP+ BMCs were transplanted into lethally irradiated nude mice to track BMC migration and differentiation in vivo. IHC analysis showed that the thick GFP+ perivascular layer around the vessels in the control tumors was absent in the tumors from AMD 3100–treated mice. There was a significant decrease in the number of desmin+ and NG2+ pericytes relative to the CD31+ endothelial cell population. However, we did see some GFP+ pericytes in the tumor vessels, indicating that these pericytes were derived from local cells. We also showed that the vessels in the tumors from AMD 3100–treated mice were smaller than those from the PBS-treated mice and had tiny lumens. There was also decreased microvessel density and increased tumor cell apoptosis demonstrating that the tumor vascular integrity and blood flow had been compromised in response to blocking CXCR4 leading to tumor cell death.

SDF-1α provides a chemotactic gradient for CXCR4+ bone marrow progenitor cells and its implication in tumor angiogenesis has been extensively described (15, 16, 31). Hypoxia-inducible factor 1-α, a direct effector of hypoxia, induced recruitment of bone marrow–derived myeloid cells, endothelial cells, and pericyte progenitor cells to promote neovascularization in glioblastoma by increasing SDF-1α (32). Previous findings from our group using VEGF165–downregulated TC71 tumors demonstrated that

![Figure 2. AMD 3100 decreases PDGF-B expression. A, TC71 or A4573 cells were plated and treated with 100 ng/mL rSDF-1α with or without 1 μg/mL AMD 3100 for 24 hours. PDGF-B levels were measured by ELISA. B, TC71 or A4573 cells were injected into GFP+ BMC-transplanted mice. Tumor-bearing mice were treated with AMD 3100 or PBS as described in Fig. 1. Tumors were resected and analyzed by IHC using anti-PDGF-B (red). ×100 magnification.](mct.aacrjournals.org)
SDF-1α gene therapy upregulated SDF-1α and restored BMC infiltration to the tumor perivascular area without affecting VEGF165. The SDF-1α-treated tumors had a huge increase in the perivascular layer, even more than that seen in the wild-type TC71 tumors (19). The striking difference in bone marrow–derived pericyte formation seen following SDF-1α gene therapy prompted us to investigate whether there was a link between SDF-1α and bone marrow–derived pericyte differentiation in vivo.

The notion that SDF-1α binds exclusively to CXCR4 and has CXCR4 as its only receptor was accepted for many years (33); however, recent studies such as the ones by Balabanian and colleagues (34) and Burns and colleagues (35) have demonstrated the existence of a new receptor for CD31 microvessel density in TC71 tumors.

Figure 3. AMD 3100 changes the tumor vascular morphology, decreases the microvessel density, and decreases tumor vessel perfusion. TC71 (top) or A4573 (bottom) cells were injected into GFP+ BMC-transplanted mice. Tumor-bearing mice were treated with AMD 3100 or PBS as described in Fig. 1. A and B, Tumors were resected and analyzed by IHC using anti-CD31 (red). ×100 magnification. Microvessel density was quantified by counting red pixels and P values < 0.05 were considered significant. C, two minutes before sacrificing, mice received intravenous injections of Hoechst 33342 to identify perfused vessels (blue). ×100 magnification.

Figure 4. AMD 3100 decreases tumor cell viability. TC71 or A4573 Ewing sarcoma cells were injected into GFP+ BMC-transplanted mice. Tumor-bearing mice were treated with AMD 3100 or PBS as described in Fig. 1. Tumors were resected and analyzed by IHC for apoptosis using TUNEL. The total amount of brown pixels was quantified and P values < 0.05 were considered significant.
SDF-1α, CXCR7. Although the SDF-1α/CXCR4 pathway has been extensively described, the role of SDF-1α/CXCR7 remains an area of study and the signaling pathways modulated by SDF-1α binding to CXCR7 remain under investigation (33). CXCR7 has been described to lack ligand-induced calcium mobilization and a role in cell migration. On the other hand, CXCR7 has been shown to be preferentially expressed by transformed cells and during embryonic development to confer cell growth and survival advantages and to increase adhesiveness of cells (35). The AMD 3100 bicyclam molecule has been widely used as a pharmacologic tool to demonstrate the involvement of CXCR4 in the action of SDF-1α in various settings (36). In their study, Kalatskaya and colleagues (36) demonstrated that although AMD 3100 is an allosteric ligand for both CXCR4 and CXCR7, it is an allosteric agonist of CXCR7 acting as a positive allosteric modulator with potential intrinsic activity on several signaling pathways. To address the possibility that AMD 3100 may be binding CXCR7 and promoting tumor cell growth and survival in our current tumor model, we tested both TC71 and A4573 cells for CXCR4 and CXCR7 protein expression by Western blot analysis. We found that although TC71 and A4573 express high levels of CXCR4, CXCR7 expression was low (Supplementary Fig. S1). These findings suggest that our current results using AMD 3100 are primarily mediated through its negative impact on SDF-1α/CXCR4, and not through SDF-1α/CXCR7.

PDGF-B and its receptor, PDGFR-β, induce pericyte maturation. PDGFR-β+ pericyte progenitor cells migrate to sites of tumor growth, where they differentiate into NG2+, desmin+, and α-SMA+ pericytes (22). We have shown that SDF-1α regulates PDGF-B and that the SDF-1α/PDGF-B pathway plays a critical role in bone marrow–derived pericyte differentiation in vitro (28). Here, we show that the tumor sections from the AMD 3100–treated mice have lower PDGF-B expression, fewer bone marrow–derived pericytes, and less tumor vessel pericyte coverage. These data confirm a correlation between SDF-1α and the production of PDGF-B in the tumor microenvironment and suggest that SDF-1α induces bone marrow–derived pericyte differentiation by upregulating PDGF-B.

Pericytes appear sparse and detached from the vessel wall in tumors; however, targeting pericytes in addition to endothelial cells more efficiently reduces tumor vascularization than targeting one cell type alone (37). We found that tumors from the AMD 3100–treated mice had smaller vessels with tiny lumens and a statistically significant decrease in the tumor microvessel density compared with tumors from the PBS-treated mice. Tumor vessel perfusion was also decreased in response to AMD 3100, suggesting that the vessels from the AMD 3100–treated mice were less efficient in delivering blood to the tumor. TUNEL staining confirmed these observations as we saw an increase in tumor cell apoptosis in the tumors from the AMD 3100–treated mice. Thus, interfering with BMC differentiation into pericytes at the tumor site resulted in both morphologic and functional changes, which compromised the tumor vascular integrity and efficiency, resulting in decreased blood flow into the tumor. Our data demonstrate that bone marrow–derived pericytes play a critical role in the proper formation and function of blood vessels in Ewing sarcoma, further emphasizing the importance of understanding how the tumor microenvironment contributes to and participates in the formation of vessels that can support tumor growth.

The observed decrease in bone marrow–derived pericytes in response to AMD 3100 may have been secondary to an effect of the drug on the endothelial cell layer. However, other investigators have found that pericyte loss reduces tumor vascularity and that the pericyte layer is required for the endothelial cell layer to form (23). These findings are consistent with those of the current study and
support the hypothesis that disrupting the SDF-1α/CXCR4 axis decreases PDGF-B expression in the tumor microenvironment, which in turn inhibits bone marrow–derived pericyte differentiation and disrupts efficient tumor vasculature formation. Furthermore, we found cells in the tumors from AMD 3100–treated mice that were both GFP⁺ and CD31⁺, indicating that the BMCs were able to differentiate into endothelial cells. Indeed, the majority of the GFP⁺ cells were also CD31⁺ in the tumors from AMD 3100–treated mice.

Although SDF-1α/CXCR4 plays an important role in the retention and homing of bone marrow progenitor cells to the bone marrow, and provides a chemotactic gradient for CXCR4⁺ bone marrow progenitor cells (14), determining the net effect of AMD 3100 on BMC migration to the tumor site in our current study cannot be achieved. Whether BMCs migrated to the tumor and then exited due to a lack of stimulus to stay and differentiate, or whether they did not migrate at all in response to AMD 3100 cannot be validated. We quantified the amount of GFP⁺ BMCs in tumor sections from both PBS- and AMD 3100–treated mice and found that treating the mice with AMD 3100 significantly decreased GFP⁺ BMCs in both TC71 and A4573 tumors at the time the tumors were collected and sectioned (Supplementary Fig. S2). However, these sections and results represent a snapshot in time; we are unable to quantify the amount of initial bone marrow cells that migrated into the tumor area and to account for cells that exited over the period of treatment in real time. Therefore, our current findings demonstrate that using AMD 3100 to disrupt the SDF-1α/CXCR4 axis inhibits BMC differentiation into pericytes and their participation in tumor vascular expansion, which leads to increased tumor cell apoptosis and decreased tumor vessel perfusion.

Although this observed effect on tumor vascular morphology and efficiency did not significantly reduce tumor size, it did increase tumor cell apoptosis. Our findings coincide with those of others showing that while targeting PDGF-B with the selective aptamer AX102 reduced tumor vascularity, it did not decrease tumor size (23). In contrast, several studies have demonstrated the effectiveness of combining antiangiogenic agents with chemotherapy in inhibiting tumor growth and inducing tumor regression (38, 39). Combining the chemotherapeutic agent, gemcitabine, with the anti-VEGF monoclonal antibody, bevacizumab, resulted in a strong antitumor effect in a model of pancreatic neuroendocrine carcinoma (38). We propose that blocking BMC differentiation into pericytes may potentially augment the activity and effect of cytotoxic chemotherapy when used in combination. The compromised state of the tumor vasculature may render the tumor cells more susceptible to the toxicity of cytotoxic chemotherapeutic agents by blocking blood flow to the tumor and thus interfering with the ability of the damaged tumor cells to repair themselves. This combined approach may therefore be synergistic and result in a more effective therapeutic outcome. However, because vascular changes may induce hypoxia that could induce a survival response in the tumor microenvironment (40), future studies examining the effects of combination therapies on Ewing sarcoma tumor vascular expansion and growth will be needed before translation to the clinic.

In conclusion, we are first to identify a link between SDF-1α, PDGF-B expression, and the differentiation of tumor-associated BMCs into pericytes. Our study shows how the tumor microenvironment helps promote efficient tumor vessel formation by producing specific cytokines that contribute to the migration and differentiation of BMCs, the formation of functional, open tumor blood vessels and rich tumor perfusion. Interfering with tumor vascular development and function may provide increased therapeutic benefit to the cytotoxic actions of standard chemotherapy by depriving the damaged tumor cells from oxygen and nutrients.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Authors' Contributions

Conception and design: R. Hamdan, Z. Zhou, E.S. Kleinerman
Development of methodology: R. Hamdan, Z. Zhou, E.S. Kleinerman
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): R. Hamdan, Z. Zhou, E.S. Kleinerman
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): R. Hamdan, Z. Zhou, E.S. Kleinerman
Writing, review, and/or revision of the manuscript: R. Hamdan, E.S. Kleinerman
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): Z. Zhou, E.S. Kleinerman
Study supervision: E.S. Kleinerman

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References


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