**SDF-1α Induces PDGF-B Expression and the Differentiation of Bone Marrow Cells into Pericytes**

Randala Hamdan, Zhichao Zhou, and Eugenie S. Kleinerman

**Abstract**

Platelet-derived growth factor B (PDGF-B) and its receptor, PDGFR-β, play a critical role in pericyte maturation; however, the mechanisms by which PDGF-B is upregulated in the tumor microenvironment remain unclear. We previously showed that upregulating stromal-derived factor, SDF-1α, in VEGF165-inhibited Ewing’s sarcoma tumors (TC/siVEGF165) induced PDGF-B mRNA expression, increased infiltration and differentiation of bone marrow cells (BMC) into pericytes and, rescued tumor growth. The purpose of this study was to investigate the mechanism by which SDF-1α increased PDGF-B expression and the role of this pathway in BM-derived pericyte differentiation. We showed that SDF-1α induced expression of PDGF-B mRNA and protein both in vitro and in vivo. In contrast, inhibiting SDF-1α downregulated PDGF-B. We cloned the 2-kb pdgf-b promoter fragment and showed that SDF-1α activates PDGF-B via a transcriptional mechanism. Chromatin immunoprecipitation showed that the ELK-1 transcription factor binds to the pdgf-b promoter in response to SDF-1α. We confirmed the correlation between the SDF-1α/PDGF-B pathway and the differentiation of PDGFR-β+ BMCs into mature pericytes using an in vitro assay. These findings show that SDF-1α regulates PDGF-B expression and that this regulation plays a critical role in the differentiation of PDGF-B+ BMCs into mature pericytes. *Mol Cancer Res;* 9(11); 1462–70. ©2011 AACR.

**Introduction**

Platelet-derived growth factor B (PDGF-B) is a member of the PDGF family of growth factors. When synthesized and secreted in its homodimer form, PDGF-BB binds to its tyrosine kinase receptor, PDGFR-β (1). Both PDGF-B and PDGFR-β are mainly expressed in the developing vasculature (2). Pericytes are recruited by PDGF-B-expressing endothelial cells to remodel, stabilize, and mature the new vascular tube (3). Normally, the expression of PDGF-B is restricted to a limited number of cell types; however, many human tumor cells have been shown to overexpress PDGF-B (4, 5). Although the role of PDGF-B and its receptor in the maturation process of pericytes has been described, the mechanisms by which PDGF-B expression can be induced in areas of vessel maturation remain unclear (1, 2, 4).

PDGFR-β-expressing pericyte progenitor cells (PPP) differentiate into NG2+, desmin+, and α-SMA+ pericytes within tumors (3). Mature pericytes play a role in vascular stabilization, maturation, and survival and surround endothelial cells, which together form the basement membrane of the microvessels (6). Mature pericytes contribute to efficient vascular flow and prevent vascular leakage (3). As PDGF-B plays a critical role in the differentiation process for pericytes, understanding the pathways that regulate PDGF-B in the tumor microenvironment will enable the identification and development of agents that interfere with pericyte maturation which results in vascular reduction during tumor vascular development (7). Several studies have shown that targeting pericytes in addition to endothelial cells is more effective at inhibiting tumor growth than targeting one or the other alone (8, 9).

PDGF-B expression can be regulated by several mechanisms, including transcriptional regulation (10). Previous studies by our group showed that treating VEGF165-inhibited Ewing’s sarcoma tumors (TC/siVEGF165) with an adenoviral vector containing the stromal-derived factor gene (Ad-SDF-1α) increased PDGF-B mRNA levels, increased infiltration and differentiation of bone marrow cells (BMC) into pericytes, and rescued tumor growth (11, 12). The purpose of the current study was to investigate the mechanism by which SDF-1α induced the expression of PDGF-B and examine the role of the SDF-1α/PDGF-B pathway in the differentiation of BMCs into pericytes.

SDF-1α or CXCL12 is an alpha chemokine that binds to the 7-transmembrane G-protein coupled receptor CXCR4 (13, 14). Signaling through the SDF-1α/CXCR4 pathway initiates multiple downstream signaling pathways linked to transcription and expression through MEK1/2 and ERK1/2.
ERK can phosphorylate and activate other cellular proteins as well as translocate to the nucleus and phosphorylate and/or activate transcription factors (13). The binding of SDF-1α to CXCR4 induces phosphorylation of the mitogen-activated protein kinases (MAPK) p44 ERK-1 and p42 ERK-2, which subsequently initiates the phosphorylation of the nuclear transcription factor ELK-1 (15). The ELK-1 nuclear transcription factor is a member of the ternary complex factors (TCF), a subfamily of the ETS-domain transcription factors. TCFs are involved in several biological processes; members of this family have been shown to be important in regulating angiogenesis and vasculogenesis (16–20). We show here for the first time that SDF-1α regulates the expression of PDGF-B and that this regulation involves binding of the ELK-1 transcription factor to the pdgf-b promoter. Our data also showed that the SDF-1α/PDGFB pathway plays a critical role in the differentiation of PDGFR-β+BMCs into desmin and NG2 expressing pericytes.

Materials and Methods

Cell lines and culture

We used the human Ewing’s sarcoma cell lines TC71 and VEGF165-inhibited TC/siVEGF7_1 generated by stable transfection of VEGF165 siRNA into TC71 (11). TC/siVEGF7_1 cells express all the VEGF isoforms except VEGF165. Human embryonic kidney 293 (HEK293) cells and C3H/10T1/2 murine embryonic mesenchymal cells were purchased from American Type Culture Collection (CRL-1573, CCL-226). TC/siVEGF7_1 and HEK293 cells express low levels of endogenous SDF-1α and PDGF-B compared with C3H/10T1/2 cells. All cells were cultured in complete Dulbecco’s modified Eagle’s medium (DMEM; ref. 12).

Proteins and antibodies

Recombinant human SDF-1α was purchased from R&D Systems (350-NS-010/CF). The inactive 9SDF-1α recombinant protein used as a negative control was a generous gift from Dr. Qing Ma at The University of Texas MD Anderson Cancer Center. Rabbit polyclonal immunoglobulin (IgG) to human ELK-1 and rabbit polyclonal IgG to mouse desmin were purchased from Abcam (ab28831, ab15-200). ChromoPure rabbit control IgG was purchased from Jackson ImmunoResearch Laboratories (011-000-003). Rabbit polyclonal IgG to mouse PDGF-BB (N-30) and rabbit polyclonal IgG to mouse PDGFR-β (P-20) were purchased from Santa Cruz Biotechnology (sc-127, sc-339). Rabbit polyclonal IgG to mouse NG2 chondroitin sulfate proteoglycan was purchased from Millipore (AB5320).

q-PCR for PDGF-B

TC/siVEGF7_1 and HEK293 cells were cultured in vitro in the absence of growth factors and supplements for 8 hours and then treated with 100 ng/mL of either the recombinant human SDF-1α protein or the inactive 9SDF-1α inactive protein for 24 hours. RNA was collected by the TRIzol method (Invitrogen). Briefly, the medium was removed and the cells were treated with 1 mL of TRIzol for 5 minutes at room temperature (RT). The cells were collected, and 200 μL of chloroform (Fisher Scientific) was added for 3 minutes at RT. The mixture was then centrifuged in the Centrifuge 5415-R (Eppendorf) for 15 minutes at 4°C. The upper clear phase was collected, and 500 μL of isopropanol (Fisher Scientific) was added for 10 minutes at RT. The solution was centrifuged for 10 minutes at 4°C, and the pellet was resuspended and washed 2 times with 75% ethanol for 5 minutes at 4°C. The pellet was air-dried, resuspended in 30 μL of nuclease-free water (Promega), and incubated at 65°C for 5 minutes. cDNA was synthesized using a reverse-transcription system (Promega). Real-time quantitative reverse-transcription PCR (qRT-PCR) was done using iQ-SYBR Green Supermix with the iCycler iQ (Bio-Rad Laboratories). Primers for human pdgf-b were designed and synthesized using Integrated DNA Technologies (IDT). All sequences are available upon request.

PDGF-B immunohistochemical analysis and microscopy

To investigate the effect of SDF-1α on PDGF-B protein levels in vivo, tumor sections obtained from previous published experiments (12) were evaluated for PDGF-B by immunohistochemical (IHC) analysis with the primary rabbit polyclonal antibody PDGF-B (N-30; Santa Cruz Biotechnology; sc-127). Images were captured using a Nikon Microphot-FXA microscope (Nikon Instruments).

ELISA

TC/siVEGF7_1 and HEK293 cells were cultured in vitro in the absence of growth factors and supplements for 8 hours and then treated with 100 ng/mL of either SDF-1α or the inactive 9SDF-1α protein for 36 hours. The supernatant was collected and concentrated using Amicon Ultra-15, PLLC Ultracel-PL Membrane, 10 kDa (Millipore) by centrifuging at 2,500 rpm for 30 minutes at 4°C in the Centrifuge 5804R (Eppendorf). PDGF-B protein levels were measured by ELISA (R&D Systems; MBB00) according to the manufacturer’s protocol.

ShRNA knockdown of SDF-1α in C3H/10T1/2 cells

ShRNA knockdown of SDF-1α was performed using lentiviral vectors designed and synthesized using Sigma-Genosys.com (Sigma-Genosys). All sequences are available upon request.
**pdgf-b promoter cloning**

DNA was isolated from TC71 Ewing’s sarcoma cells. The 2-kb *pdgf-b* promoter DNA fragment was isolated by PCR. Briefly, primers were designed and synthesized using IDT. All sequences are available upon request. The PCR product was run on a 1% agarose gel, and the 2-kb *pdgf-b* promoter DNA fragment was isolated and purified using the QIAquick Gel Extraction Kit (QIAGEN; 28704). The purified sequence was ligated into the pCR2.1-TOPO expression vector using the TOPO TA Cloning Kit (Invitrogen; K4560-01). The vector was isolated and purified with the QIAprep Spin Miniprep Kit (QIAGEN; 27104) according to the manufacturer’s directions, and the DNA sequence was verified by SeqWright DNA Technology Services. The 2-kb *pdgf-b* promoter DNA fragment was then isolated and cloned into the pGL3-Basic luciferase reporter vector (Promega BioSciences; E1751). The *pdgf-b*/pGL3 reporter vector sequence was verified by SeqWright DNA Technology Services. To test the functionality of the *pdgf-b*/pGL3 construct, we used 50 ng of the construct to transfect TC71 cells and the *pdgf-b* promoter activity was tested by measuring the luciferase signal (data not shown).

**Dual-luciferase reporter assay**

We used 50 ng of either the *pdgf-b*/pGL3 reporter construct or the pGL3-Control vector (Promega BioSciences; E1761) to transfect TC/siVEGF7-1 and HEK293 cells using FuGENE 6 (Roche Applied Science) according to the manufacturer’s directions. Beginning 48 hours after transfection, the cells were cultured in the absence of growth factors and supplements for 24 hours and then treated with 100 ng/mL of either the recombinant SDF-1α or the inactive 9SDF-1α protein for 8 hours. The cells were lysed and the luciferase signal measured using the Dual-Luciferase Reporter Assay System (Promega BioSciences; E1910) according to the manufacturer’s instructions.

**ChIP assay**

Potential ELK-1 binding sites within the 2-kb *pdgf-b* promoter region were identified using the GeneRegulation.com MATCH software. 2 sites at −600 bp and at the transcription start (TS) site which scored more than 85% were analyzed by chromatin immunoprecipitation (ChIP) (Millipore) according to the manufacturer’s instructions. Briefly, TC/siVEGF7-1 and HEK293 cells were incubated with 1:1,000 in 4% formaldehyde for 10 minutes at RT. The cells were sonicated and purified with the ChIP assay. The goat-anti rabbit-Cy5 secondary antibody (Jackson ImmunoResearch Laboratories) was used at 1:1,000 in 4% fish gelatin. The goat-anti rabbit-Cy5 secondary antibody (Jackson ImmunoResearch Laboratories) was used at 1:1,000 in 4% fish gelatin. Nuclei were stained with SytoxGreen (Invitrogen, Molecular Probes). Images were captured using a Zeiss laser confocal microscope (Carl Zeiss MicroImaging).

**Immunocytochemistry staining**

BMCs were collected and cultured as described above. The cells were fixed in cold acetone and blocked with 4% fish gelatin in PBS as previously described (12). Primary antibodies for PDGFR-β, desmin, and NG2 expression (Abcam, Inc.) were used at 1:500 in 4% fish gelatin. The goat-anti rabbit-Cy5 secondary antibody (Jackson ImmunoResearch Laboratories) was used at 1:1,000 in 4% fish gelatin. Nuclei were stained with SytoxGreen (Invitrogen, Molecular Probes). Images were captured using a Zeiss laser confocal microscope (Carl Zeiss MicroImaging).

**Results**

SDF-1α regulates the expression of PDGF-B in vitro and in vivo

We previously showed that intratumor injections of an adenoviral vector containing SDF-1α (Ad-SDF-1α) into VEGF165-deficient tumors (TC/siVEGF7-1) increased PDGF-B mRNA levels (12). To determine whether SDF-1α therapy also altered PDGF-B protein, tumor sections were obtained from the Ad-SDF-1α-treated TC/siVEGF7-1 (12) and analyzed by IHC for PDGF-B expression. PDGF-B protein levels were upregulated in Ad-SDF-1α-treated TC/siVEGF7-1 tumors compared with Ad-control (Fig. 1).
SDF-1α or the inactive 9SDF-1α protein for 24 hours and qPCR was done. SDF-1α upregulated PDGF-B mRNA levels in both TC/siVEGF7-1 and HEK293 cells compared with 9SDF-1α (Fig. 2A). To determine whether SDF-1α also upregulated PDGF-B protein levels in vitro, we again treated TC/siVEGF7-1 and HEK293 cells with either SDF-1α or the inactive 9SDF-1α protein for 36 hours. As PDGF-BB, the homodimer protein (∼27 kD), is a secreted protein (5), the cultured supernatant was collected and concentrated and the PDGF-B protein levels analyzed by ELISA. SDF-1α upregulated PDGF-B protein levels compared with 9SDF-1α (Fig. 2B).

We showed that SDF-1α upregulates PDGF-B mRNA and protein levels both in vivo in TC/siVEGF7-1 tumors and in vitro in TC/siVEGF7-1 and HEK293 cells. To further confirm that SDF-1α regulates the expression of PDGF-B, we used an shRNA plasmid specific for SDF-1α (sh-SDF-1) to knockdown its expression levels in 10T1/2 cells and determine whether inhibiting SDF-1α also impacts the expression of PDGF-B. 10T1/2 cells, which endogenously express SDF-1α and PDGF-B were transfected with either the sh-SDF-1 (10T1/2-shSDF-1) or sh-control plasmid (10T1/2-shcontrol). As expected, downregulating SDF-1α resulted in decreased expression of PDGF-B (Fig. 3).

To further elucidate the correlation between SDF-1α and PDGF-B, we examined the effect of recombinant SDF-1α protein on PDGF-B mRNA levels in vitro. TC/siVEGF7-1 and nontumorigenic HEK293 cells express CXCR4 (Supplementary Fig. S1). The cells were incubated with either

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![Image](image_url)

**Figure 1.** SDF-1α upregulates PDGF-B protein expression in TC/siVEGF7-1 tumors. Immunohistochemistry analysis for PDGF-B protein. TC/siVEGF7-1 tumors in vivo were treated with either Ad-SDF-1α or Ad-control 2 times weekly for 3 weeks. On day 23 after tumor cell inoculation, tumors were resected, placed in OCT, and snap frozen in liquid nitrogen. Tumor sections were cut, fixed, and analyzed for expression of PDGF-B by immunohistochemistry (brown staining). Ad-SDF-1α upregulates PDGF-B protein levels compared with Ad-control.

![Image](image_url)

**Figure 2.** SDF-1α upregulates PDGF-B mRNA and protein levels in vitro. A, TC/siVEGF7-1 and HEK293 cells were plated, incubated in the absence of growth factors and supplements for 8 hours, and then treated with 100 ng/mL of either SDF-1α or the inactive 9SDF-1α protein for 24 hours. RNA was collected and analyzed by qPCR for the expression of PDGF-B. Each bar graph shows pooled data from 3 independent experiments, each done in triplicate ± SD. $P < 0.05$ was considered significant. B, ELISA for PDGF-B protein levels. TC/siVEGF7-1 and HEK293 cells were plated, cultured in the absence of growth factors and supplements for 8 hours, and then treated with 100 ng/mL of either SDF-1α or the inactive 9SDF-1α protein for 36 hours. The cultured supernatant was collected and concentrated and the levels of the ∼27 kDa soluble PDGF-B protein levels quantified by ELISA. Each bar graph shows pooled data from 3 independent experiments, each done in triplicate, ± SD. $P < 0.05$ was considered significant.
SDF-1α regulates the expression of PDGF-B via transcription

We next investigated whether SDF-1α regulates PDGF-B through a transcriptional mechanism. We cloned the 2-kb human pdgf-b promoter fragment into a luciferase reporter vector. DNA was collected from TC71 cells and used as a template to isolate the 2-kb promoter fragment. The purified fragment was cloned into the PGL3 reporter vector, and the luciferase signal was measured. A luciferase signal indicates promoter activity. The transfected cells were treated with either SDF-1α or the inactive 9SDF-1α protein for 8 hours. SDF-1α activated the pdgf-b promoter in both the TC/siVEGF7.1 and HEK293 cells compared with 9SDF-1α (Fig. 4).

SDF-1α induces binding of the ELK-1 transcription factor to the pdgf-b promoter

The SDF-1α chemokine binds to its G-protein coupled receptor CXCR4 expressed on the surface of cells (21). Once the SDF-1α ligand binds to CXCR4, the signaling cascade activates several MAPKs, including ERK1/2, which phosphorylates and activates the ELK-1 transcription factor (15). TC/siVEGF7.1 and HEK293 cells express CXCR4 (Supplementary Fig. S1). Stimulation with SDF-1α activated the ERK1/2(p44/42)/ELK-1 pathway and induced phosphorylation of both ERK1/2(p44/42) and ELK-1 (S383) in TC/siVEGF7.1 (Supplementary Fig. S2). We next investigated whether SDF-1α induced binding of ELK-1 to the pdgf-b promoter. Potential ELK-1 binding sites within the pdgf-b promoter region were identified (Fig. 5A). Using ChIP, we investigated 2 of the identified sites that scored above 85% at −600 bp and at the TS site. TC/siVEGF7.1 and HEK293 cells were treated with SDF-1α for 15, 30, or 60 minutes, and ChIP was done. The ELK-1 transcription factor bound to the pdgf-b promoter with a peak binding at 30 minutes (P < 0.05) and with a higher affinity at the −600 bp site (Fig. 5B) than at the TS site (P > 0.05; data not shown).

PDGFR-β+ BMCs differentiate into pericytes that express PDGFR-β, desmin, and NG2 in response to the SDF-1α/PDGF-B pathway

As PDGF-B is required for the recruitment and integration of pericytes in the vascular wall (22), we established an in vitro model to study the effect of the SDF-1α/PDGF-B pathway on the differentiation of BMCs into pericytes.
Expression of the pericyte markers PDGFR-β, NG2, and desmin were used to indicate pericyte differentiation. Although fresh BMCs express PDGFR-β, they do not express the mature pericyte markers desmin and NG2 (Fig. 6A). After incubation for 2 weeks in conditioned medium from 10T1/2 cells, PDGFR-β+ BMCs attached to the plate, acquired a pericyte-like morphology (Fig. 6B), and expressed desmin and NG2 (Fig. 6C, and Table 1). The cells cultured in DMEM alone did not attach or acquire a pericyte-like morphology (Fig. 6B).

To determine whether SDF-1α in the 10T1/2 conditioned medium played a role in inducing PDGFR-β+ BMCs to express NG2 and desmin, we cultured PDGFR-β+ BMCs in conditioned medium from 10T1/2-shSDF-1 or 10T1/2-shcontrol cells for 2 weeks. We already showed that transfection of 10T1/2 cells with shSDF-1 decreased PDGF-B expression (Fig. 3). PDGFR-β+ BMCs cultured in 10T1/2-shSDF-1 conditioned medium behaved in a similar manner to those cultured in DMEM alone: their morphology did not change, and they failed to express NG2 and desmin (Fig. 7A). In contrast, PDGFR-β+ BMCs cultured in 10T1/2-shcontrol conditioned medium stained positive for desmin, and NG2 and had a similar pericyte-like morphology to those cultured in conditioned medium from 10T1/2 cells (Fig. 7B). These results confirm that the SDF-1α in the conditioned medium was responsible for inducing BMCs to differentiate into pericyte-like cells and that the SDF-1α/PDGFB pathway participates in the differentiation of PDGFR-β+ BMCs into pericytes.

Discussion

The regulation of PDGF-B expression during vascular remodeling is poorly understood. We previously showed that the inhibition of VEGF165 in TC71 tumor cells (TC/ siVEGF7-1) resulted in smaller tumors with decreased perivascular coverage and decreased bone marrow (BM)-derived tumor vascular pericytes (12, 23, 24). Intratumoral injections with an adenoviral vector containing the SDF-1α gene (Ad-SDF-1α) upregulated the expression of SDF-1α (but not of VEGF165), increased BM-derived pericytes surrounding the tumor vessels, and rescued tumor growth (12). In this study, we evaluated tumor sections from our previous study (12) for the expression of PDGF-B and found that SDF-1α gene therapy increased PDGF-B protein expression as well as SDF-1α. These findings suggested an association between SDF-1α, PDGF-B, and the differentiation of BMCs into pericytes.

Here, we showed that SDF-1α regulates PDGF-B. Treating 2 different cell lines (TC/siVEGF7-1 and HEK293) with SDF-1α increased the expression of PDGF-B mRNA and protein levels in vitro. In contrast, an shRNA specific for SDF-1α downregulated both SDF-1α and PDGF-B. A luciferase assay and ChIP indicated that SDF-1α regulates PDGF-B via a transcriptional mechanism and that the ELK-1 transcription factor binds to the pdgf-b promoter in response to SDF-1α stimulation. Furthermore, our findings showed that BMCs cultured in 10T1/2-sh-SDF-1 conditioned medium in which expression of both SDF-1α and PDGF-B is downregulated, failed to differentiate into pericytes as defined by morphology and the expression of NG2 and desmin mature pericyte markers. Taken together, our data support the concept of an SDF-1α/PDGFB pathway that plays a critical role in the differentiation of PDGFR-β+ BMCs into mature pericytes.

PDGF-B, a member of the PDGF family of growth factors, is a mitogen for cells of mesenchymal origin such as fibroblasts and smooth muscle cells (1, 5). When secreted in its homodimer form, PDGF-BB binds to its tyrosine kinase receptor, PDGFR-B. PDGF-B and PDGFR-B are expressed in the developing vasculature, in which PDGF-B is produced by endothelial cells and PDGFR-B is expressed by pericytes and vascular smooth muscle cells (1). Many cancers, including colorectal cancer, pancreatic cancer, glioma, and sarcoma, overexpress PDGF-B (4, 5, 25). However, the mechanisms by
which PDGF-B is upregulated are unclear. VEGF-C has been shown to regulate vascular stabilization by controlling PDGF-B expression (26). Our data shows that SDF-1 is shown to regulate vascular stabilization by controlling PDGF-B, which PDGF-B is upregulated are unclear. VEGF-C has been shown to regulate vascular stabilization by controlling PDGF-B expression (26). Our data shows that SDF-1 can regulate vascular stabilization by controlling PDGF-B expression. Our results further show that this regulation is mediated via a transcriptional mechanism which involves the ELK-1 transcription factor.

The ELK-1 transcription factor is a member of the 3 TCFs which is a subfamily of the E twenty-six (Ets) domain transcription factors (19). SDF-1 binds to the 7-transmembrane G-protein coupled receptor CXCR4 and induces phosphorylation of the MAPKs p44 ERK-1 and p42 ERK-2, which further leads to the phosphorylation of the nuclear transcription factor ELK-1 (13–15). We identified potential ELK-1-binding sites within the pdgf-b promoter region and found that SDF-1α stimulates binding of ELK-1 to the pdgf-b promoter at the −600 bp site in both TC/siVEGF7.1 and HEK293 cells. We are the first to report the regulation of PDGF-B by SDF-1α and binding of the ELK-1 transcription factor to the pdgf-b promoter in response to SDF-1α stimulation.

We hypothesized that the increased pericyte coverage with BM-derived cells seen in the TC/siVEGF7.1 tumors after SDF-1α gene therapy was a result of the upregulation of PDGF-B by SDF-1α. PDGF-B and PDGFR-β have been shown to play a critical role in the process of pericyte maturation (4). Overexpression of PDGF-B in colorectal and pancreatic cancer increased pericyte content (4) whereas lack of PDGFR-β signaling led to pericyte loss, which in turn resulted in endothelial cell changes and capillary dilation and rupture (2, 5). PPPs have been shown to migrate to sites of tumor growth, where they differentiate into NG2+, desmin+, and α-SMA+ pericytes (3). HIF1-α has also been shown to induce recruitment of BM-derived myeloid cells, as well as endothelial and pericyte progenitor cells, and to promote neovascularization in glioblastoma through increases in SDF-1α (13, 27). To determine whether SDF-1α played a role in BM-derived pericyte differentiation, we investigated the effect of SDF-1α on PDGFR-β+ BMCs collected from the hind femurs of mice. Whereas fresh BMCs are positive for PDGFR-β, they are negative for the mature pericyte markers NG2 and desmin. Conditioned medium containing SDF-1α induced PDGFR-β+ BMCs

### Table 1. Summary of pericyte marker staining

<table>
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<th>Markers</th>
<th>Expression</th>
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<tr>
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<td>Before Tx</td>
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<td>PDGFR-β</td>
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<td>NG2</td>
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Note: Freshly isolated BMCs (before Tx) and BMCs that were cultured for 2 weeks in conditioned medium from 10T1/2 cells (after Tx) were stained for the expression of PDGFR-β, NG2, and desmin pericyte markers. Fresh BMCs express PDGFR-β, but they do not express the mature pericyte markers, desmin and NG2.
to express both NG2 and desmin, with a change in morphology that indicated pericyte differentiation. This effect was abolished when SDF-1α was inhibited. As mentioned above, downregulating SDF-1α expression also decreased PDGF-B expression. Taken together, these observations suggest that SDF-1α−induced differentiation of BMCs into mature pericytes which express desmin and NG2 is mediated via its regulation of PDGF-B.

Pericytes, also known as smooth muscle cells or mural cells, wrap around endothelial cells which together form the basement membrane of the vessel wall (28). 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 (3, 6). As pericytes seem sparse and detached from the vessel wall in tumors (3), they have been neglected as a potential target for antivascular therapy. However, pericytes are important constituents that support the tumor vasculature (3) and studies have shown that targeting pericytes in addition to endothelial cells is more effective in inhibiting tumor growth than targeting one or the other alone (8, 9); inhibiting PDGF-B with the AX102 aptamer in combination with bevacizumab in human ovarian carcinoma resulted in increased tumor growth inhibition (9). Altogether, our current findings showed that SDF-1α induces the differentiation of PDGF-R−BMDCs into mature pericytes in vitro and our previous findings showed that upregulating SDF-1α in the tumor microenvironment led to increased BM-derived pericyte coverage and tumor growth (12). Therefore, we anticipate that inhibiting SDF-1α will inhibit BMDC differentiation into pericytes, resulting in smaller and less perfused tumor vessels, which, in combination with other antiangiogenic factors, could subsequently negatively impact tumor growth and metastasis.

In summary, we have shown that SDF-1α regulates PDGF-B via a transcriptional mechanism involving binding of the ELK-1 transcription factor to the pdgf-b promoter. Our findings also suggest that this SDF-1α/PDGF-B pathway plays an important role in the differentiation of PDGF-R−BMDCs into mature pericytes which express desmin and NG2. All together, our findings show the importance of SDF-1α not only as a chemotactic factor for BMDCs (29, 30), but also as a signaling molecule that can regulate the expression of growth factors such as PDGF-B. Our findings emphasize the importance of understanding the different growth factors and signaling pathways that can control the differentiation of BMDCs into pericytes which can ultimately affect tumor vascular morphology and function.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References


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