Oligodendrocyte Progenitor Cells Promote Neovascularization in Glioma by Disrupting the Blood–Brain Barrier

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Abstract
Enhanced platelet-derived growth factor (PDGF) signaling in glioma drives its development and progression. In this study, we define a unique role for stroma-derived PDGF signaling in sustaining tumor homeostasis within the glioma microenvironment. Large numbers of PDGF receptor-α (PDGFRα)–expressing stromal cells derived from oligodendrocyte progenitor cells (OPC) were discovered at the invasive front of high-grade gliomas, in which they exhibited a unique perivascular distribution. In PDGFRα-deficient host mice, in which orthotopic Gl261 tumors displayed reduced outgrowth, we found that tumor-associated blood vessels displayed smaller lumens and normalized vascular morphology, with tumors in host animals injected with the vascular imaging agent gadolinium also being enhanced less avidly by MRI. Notably, glioma-associated OPC promoted endothelial sprouting and tube formation, in part by abrogating the inhibitory effect that perivascular astrocytes exert on vascular endothelial conjunctions. Stromal-derived PDGF-CC was crucial for the recruitment and activation of OPC, insofar as mice genetically deficient in PDGF-CC phenocopied the glioma/vascular defects observed in PDGFRα-deficient mice. Clinically, we showed that higher levels of PDGF-CC in glioma specimens were associated with more rapid disease recurrence and poorer overall survival. Our findings define a PDGFRα/PDGF-CC signaling axis within the glioma stromal microenvironment that contributes to vascular remodeling and aberrant tumor angiogenesis in the brain. Cancer Res; 74(4); 1011–21. © 2013 AACR.

Introduction
The tumor microenvironment is a complex amalgam of tumor cells, intermingling parenchymal cells, infiltrating immune system cells, and a vascular network of endothelial cells, which in concert determine the pathologic and biological features of distinct tumors. Cancer diagnoses and grades significantly vary the composition of the tumor microenvironment (1–3). Gliomas, malignancies arising from glial cells within the central nervous system (CNS), harbor multiple cell types in addition to cells with tumorigenicity. During normal development (4), proliferation, differentiation, and migration of glial cells are tightly controlled and well described (5). However, comparatively little is known about the composition, features, and functions of nontransformed glial cells and neural progenitor cells within the natural progression of glioma.

The involvement of platelet-derived growth factor receptor-α (PDGFRα) in gliomagenesis has been well demonstrated (6). Upregulated PDGF signaling through PDGFRα has been found to be a common feature of low-grade astrocytic and oligodendroglial tumors along with a significant subset of glioblastoma multiforme (7, 8). Interestingly, the adult brain contains a widely distributed, abundant progenitor population known as oligodendrocyte precursor cells (OPC), which have been suggested to have high tumor-initiating potential (9–12). These cells identified by expression of NG2/Olig2 are normally cycling OPCs in the destabilization of vascular architecture and integrity as a major functional contributor to tumor angiogenesis and tumor-grade progression.

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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Materials and Methods

Cells lines and mice lines

PDGF-C–deficient mice have been previously described (16). C57Bl/6-derived glioma cells, GI261 (authenticated as Supplementary Table S1) and KR158, were as described previously (17–19). HCMVEC/D3 have been previously described (20; additional information available in Supplementary Methods).

Intracranial injections and tumor imaging

The intracranial injections have been described previously (21). Biomimetics IVIS-100 (Xenogen) and MRI (Bruker Biospin) were performed to monitor the progression of tumor. Tumor margins in each MRI slice were manually outlined. The area of each region of interest was calculated and then multiplied by the slice thickness. All slice volumes were added up to calculate the volume of each three-dimensional (3D) tumor. For detailed methods, please refer to Supplementary Methods.

Adoptive bone marrow transplantation

Transplantation was carried out as previously described (22) with slight modifications. Recipient mice were lethally irradiated with a single dose of 9.5 Gy to their whole body with their heads shielded. Twenty-four hours after irradiation, 5 × 10^6 total bone marrow cells were injected by tail vein followed by 6 weeks to allow for engraftment of bone marrow cells. The engraftment was confirmed by complete blood count and flow cytometry to ensure adequate hematopoiesis.

Dextran infusion assay

10 mg/mL of 70-kD rhodamine-conjugated Dextran (lysin fixable) was intravenously injected into mice (100 µL/mouse) as previously described (23). Two hours after dextran injection, mice were perfused with 10 mL PBS followed by perfusion of 5 mL of 4% paraformaldehyde. Brain tissue was dissected and fixed for the subsequent staining.

Microarray analysis

Microarray data can be accessed from Gene Expression Omnibus (NCBI/GEO) under accession number GSE38283. For detailed procedures of the preparation of samples, please refer to Supplementary Methods.

Quantification

All data are given as mean ± SD or ± SEM. Differences were compared using Student t tests or one-way ANOVA followed by post hoc tests. For more experimental procedures and quantification details, please refer to Supplementary Methods.

Results

Stromal cells in glioma express PDGFRα

PDGF signaling is upregulated in many patients with glioma: 30% of patients harboring high-grade gliomas express an amplified or mutated PDGFRα gene (7). The infiltration of bone marrow–derived cells (BMDC) is also considered as a hallmark of tumor progression (22, 24). To better understand the temporal and spatial variation within the glioma microenvironment of PDGFRα-expressing cells and inflammatory BMDCs, PDGFRα staining was performed on orthotopic GI261 (murine-derived glioma cell line) tumors in mice, which had previously been adoptively transplanted with GFP bone marrow. Large numbers of BMDCs and PDGFRα+ cells were identified at the tumor periphery (Fig. 1A). The number of these peritumoral PDGFRα+ cells increased at day 14 along with the enhanced infiltration of BMDCs (Fig. 1B). We demonstrated that most PDGFRα+ cells, located in the invasive front, were actually nontumor cells. We used orthotopic glioma models with both murine-derived GI261 cells (PDGFRα+) constitutively expressing GFP, as well as human-derived U87-MG and U251 cells also expressing GFP (Fig. 1C and Supplementary Fig. S1A), to show that these were not invading tumor cells. Flow cytometry performed upon whole tumor explants confirmed that PDGFRα-expressing cells are GFP negative in all of the GI261, U87-MG, U251, and KR158 glioma models (Fig. 1D and Supplementary Fig. S1). The existence of peritumoral PDGFRα-expressing cells was also verified in the KR158 glioma model (Supplementary Fig. S1D). Western blot analyses showed enhanced expression of PDGFRα in the tumor margin, compared with tissue isolated from the tumor core, the contralateral hemisphere, or from normal age-matched adult mouse brain (Fig. 1E). Immunostaining of PDGFRα showed more PDGFRα+ cells in tumor periphery than in the contralateral tissue (Supplementary Fig. S2B). Taken together, these results demonstrate that large numbers of PDGFRα stromal cells accumulate in the periphery and invade the front of gliomas.

PDGFRα+ stromal cells derive from OPC and predominate perivascularly

Different tumors are supported by unique microenvironments containing distinct and specific stromal cell populations. To better define the origin of PDGFRα+ stromal cells, we studied the localization of PDGFRα within GI261 tumor specimens using other lineage markers commonly encountered within the glioma microenvironment. PDGFRα+ stromal cells did not express markers of neural stem cells (nestin), astrocytes (glial fibrillary acidic protein, GFAP), or endothelium (collagen IV), nor did they colocalize with BMDCs, identified using GFP after GFP+ bone marrow transplant (Supplementary Fig. S2). PDGFRα+ cells did, however, colocalize with NG2, a marker for OPCs. Moreover, PDGFRα+ cells display ramified processes, highly suggestive of OPC morphology, and expressed oligodendritic lineage marker Olig2 (Fig. 2A and Supplementary Fig. S2C). PDGFRα+ glioma-associated OPCs (GA-OPC) can be identified adjacent to vascular endothelium in a sandwich-like configuration (Fig. 2B). Because of permeable features of tumor endothelium and the resolution of microscopy, we did observe limited overlaps between CD31/PDGFRα. The GA-OPCs do not, however, colocalize with pericytes (PDGFRβ+) or perivascular astrocytes (GFAP+; Fig. 2C and D). In addition to their abundance within the tumor periphery, OPCs also exist within tumor cores with perivascular processes as shown by NG2-DsRed (Supplementary Fig. S2D). NG2 is generally used as OPC maker in CNS, but it also marks certain pericytes and microglial cells.
Tumor cells may educate adjacent nontransformed cells into forming tumor-associated stroma, which can be differentiated from the normal tissue (3, 25). In response to malignancy, OPCs may be activated through PDGFRα signaling (9, 26). To understand the functional role of GA-OPCs in the progression of glial malignancies, we created an orthotopic and syngeneic model using Gl261 cells transfected with a luciferase gene. These cells were implanted in PDGFRα−knockout mice to study the importance of PDGFRα signaling between stromal and tumors cell populations, upon glioma progression. In Rosa-cre/PDGFRα−/− mice, growth of Gl261 tumors was inhibited compared with tumor growth in Rosa-cre/PDGFRα+/+ mice (Fig. 3A). MRI analysis of Gl261 tumors in PDGFRα−/− mice demonstrated animals with less tumor burden and tumors with less contrast enhancement on MRI than tumors within control mice (Fig. 3B). Compared with tumors in PDGFRα+/+ mice, tumors in PDGFRα−/− mice had a slightly higher blood vessel density (Supplementary Fig. S3A) and demonstrated distinct vascular morphology. Gliomas within PDGFRα−/− mouse tumors demonstrated considerably smaller diameters of vessel lumens (Fig. 3C), similar to vasculature typical within the normal mouse brain. Importantly, within PDGFRα−/− mouse tumors, pericyte density and architecture was not significantly changed from PDGFRα+/+ mice as visualized by the staining for PDGFRβ (Supplementary Fig. S3B).

Neovascularization of gliomas is distinct from other types of tumors. Perhaps due to the brain’s robust vascular network, brain tumors either remodel preexisting blood vessels or recruit endothelial progenitor cells to undergo microvascular proliferation, a hallmark of high-grade gliomas (1, 27).
Compared with normal brain and low-grade gliomas, grade III and IV gliomas have distorted, enlarged blood vessels but do not necessarily demonstrate higher blood vessel density (27). Blood vessels in high-grade gliomas are distinguishable from those in low-grade gliomas not by their density, but by their morphology. In PDGFRα(fl)/fl mice, Gl261 brain tumor–associated blood vessels were not enlarged or distorted compared with that within tumors in wild-type mice. Tumor cells in PDGFRα(fl)/fl mice have less mitotic activity (decreased Ki67 staining), less hypoxia demonstrated (decreased pimonidazole staining), and less leakiness as indicated by a dextran infusion assay (Supplementary Fig. S3C). Other factors, which could theoretically induce changes in vascular morphology, were studied in both groups of animals by measuring the infiltration of BMDCs and monitoring hematopoiesis; there were moderate decreases of infiltrating BMDCs within Gl261 tumors in PDGFRα(fl)/fl mice (Supplementary Fig. S4A), and hematopoiesis does not appear to be affected (Supplementary Fig. S4B).

GA-OPCs facilitate angiogenesis

To better understand the contribution of GA-OPCs to the vascular remodeling occurring during glioma neovascularization, we performed in vitro assays using primary GA-OPCs cultures. Primary human-derived GA-OPCs and glioma cells were isolated directly from glioma resections (Supplementary Fig. S5A); OPCs were maintained in the progenitor stage within the oligodendritic lineage (Supplementary Fig. S5B and S5C). Using a 3D culture system, we cocultured human brain endothelial cells (HCMEC/D3) with GA-OPCs or human astrocytes, or both, to study their interactions. We labeled the HCMEC/D3...
cells with GFP and allowed them to form tubules. Once a tubule network was established, equal numbers of GA-OPCs and astrocytes were introduced to the endothelial cell cultures. The in vitro 3D coculture system recapitulated the in vivo phenomenon within which the astrocytic foot processes surround endothelium to form the blood–brain barrier. We found that both astrocytes and GA-OPCs were adjacent to the endothelial tubules. Astrocytic foot processes formed an intact and continuous tubule-like structure close to endothelial tubules. In contrast, GA-OPCs appeared to integrate with the endothelial tubules but did not form continuous intercellular interaction with each other as did astrocytes (Fig. 4A). Interestingly, if we allowed astrocytes to establish interaction with HCMEC/D3 first with coculture, and then added GA-OPCs, the GA-OPCs could disrupt the interaction formed between astrocytes (Fig. 4A). We further used in vitro models to test the permeability of these intercellular interactions, which were formed by endothelial cells under differing coculture conditions. These assays demonstrated that astrocytes protect the integrity of the endothelial cell monolayer while bathed in U251 cell–derived conditioned medium, whereas GA-OPCs abrogate the protective effect of astrocytes by creating a more permeable endothelial cell monolayer (Supplementary Fig. S6C).

In addition to defining interactions between endothelial cells, astrocytes, and GA-OPCs, we performed a series of angiogenesis assays to study the mechanism through which GA-OPCs impact vascular remodeling. We used a sprouting assay to investigate the influence of perivascular stromal cells upon the migration of endothelial cells. Astrocytes suppressed sprouting from endothelial spheres made from HCMEC/D3; GA-OPCs reversed this inhibitory effect when cocultured with astrocytes (Fig. 4B). We also analyzed the number of endothelial cells in coculture with astrocytes and GA-OPCs. Both astrocytes and GA-OPCs only minimally affected the number of endothelial cells (Supplementary Fig. S5D), suggesting that the regulatory effect of stromal cells on endothelial sprouting was proliferation independent. This function is PDGFRα dependent; a neutralizing PDGFRα-specific antibody reversed the effect (Supplementary Fig. S6A). Tubule formation assays similarly demonstrated that astrocytes inhibited the formation of new endothelial tubules and GA-OPCs reversed this in a PDGFRα-signaling–dependent manner (Fig. 4C and Supplementary Fig. S6B).

How these interactions between endothelial cells and OPCs directly affect the tumor progression is difficult to precisely define. We have demonstrated that inhibiting OPCs clearly affects angiogenesis, which might then inhibit tumor progression. More interestingly, there may also exist a direct link between endothelial and tumor cells. We hypothesized that OPCs may direct endothelial cells into a more active state through upregulation of angiocrine factors, thereby promoting malignancy. We cocultured endothelial cells with Gl261 cells in the presence or absence of VEGF (to stimulate endothelial cells). We found that tumor cells proliferated more when cocultured with activated endothelial cells than when cocultured with normal endothelial cells (Supplementary Fig. S7). Considering the complex nature of the glioma microenvironment, interaction between OPCs and endothelial cells may be
even more complicated than that hypothesized with multiple pathways independently promoting malignancy.

BMDCs and stromal-derived PDGF-CC are key mediators to GA-OPCs

In the context of the glioma microenvironment, OPCs exponentially increase in number and in the expression of PDGFRα (Fig. 1). Signaling that originates from tumor-initiating or alternative stromal cells might trigger OPC activation and redirect them into protumoral phenotypes. As described above, the number of PDGFRα⁺ cells dramatically increased around day 14 after injection of tumor cells, which mirrored the pattern of immune cell infiltration (Fig. 1A and B). Therefore, we sought to test the hypothesis that infiltrating BMDCs contribute to the recruitment and activation of GA-OPCs during tumor progression. In addition, BMDCs are detected not only intratumorally, but also within the tumor periphery (Fig. 1A), a localization that would

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**Figure 4.** GA-OPCs promote opening of the endothelial–astrocytic junction and angiogenic activity of endothelial cells. A, GFP⁺ HCMEC/D3 cells (green) were seeded in Matrigel to assess tubule formation. Rodamine labeled (red), human astrocytes (HA), and GA-OPC were added subsequently. For the combination of human astrocytes and OPCs, labeled human astrocytes were allowed to seed with endothelial tubules first, followed by nonlabeled GA-OPCs 12 hours later. Scale bar of the first left panel, 100 μm. Scale bar of right three panels, 50 μm. GFP⁺ endothelial tubules’ length and coverage were quantified. Means ± SEM, n = 5; one-way ANOVA, *, P < 0.05. B, coculture with human astrocytes, GA-OPC (glioma associated OPCs), or both. The coculture system was maintained in U251-conditioned medium. Endothelial sprouting was quantified by the area of endothelial cells beyond the primary spheroid (dashed lines). Means ± SEM, n = 6; one-way ANOVA, ***, P < 0.001. C, GFP-HCMEC/D3 cells were seeded on Matrigel to form tubules, cocultured with GA-OPC, human astrocytes, or both, and maintained in U251-conditioned medium. The GFP⁺ endothelial tubules in each group were quantified by length. Means ± SEM, n = 6; one-way ANOVA, **, P < 0.01. All graphs were acquired from two independent experiments. N, the number of independent wells of treatment per condition.
C57BL/6 mice with bone marrow from into tumor-supporting phenotypes, we transplanted wild type derived macrophages or microglial cells might push GA-OPCs PDGF-C deletion recapitulates PDGFR PCR (RT-PCR; Fig. 5A).

Isolated from murine gliomas was confirmed by real-time PCR (RT-PCR; Fig. 5A).

To examine whether PDGF-CC derived from bone marrow–derived macrophages or microglial cells might push GA-OPCs into tumor-supporting phenotypes, we transplanted wild type C57BL/6 mice with bone marrow from PDGF-C−/− donor mice. Six weeks after bone marrow engraftment, luciferase-labeled Gl261 cells were injected intracranially into recipient mice. PDGF-C−/− mice did not show any developmental defects in their hematopoietic system (Supplementary Fig. S9); however, we found that tumors implanted within mice transplanted with PDGF-C−/− bone marrow grew more slowly at an early stage of tumorigenesis (day 14) than tumors in mice transplanted with littermate PDGF-C+/− bone marrow. However, in later stages (day 21) of tumor growth, PDGF-C−/− bone marrow–transplanted animals no longer demonstrated a statistically significant reduction of tumor size (Fig. 5B). Within the tumor periphery specifically, we found significantly fewer PDGFRα+ cells in the PDGF-C−/− bone marrow–transplanted group at D14 (P = 0.0051), but not at a later stage (D21; P = 0.32; Fig. 5C). These results suggest that microphage–microglia-derived PDGF-CC did contribute to the recruitment and activation of GA-OPCs, a process not necessarily dependent upon bone marrow–derived macrophages/microglia at later stages of tumor development.

Soluble PDGF-CC may not be solely secreted by BMDCs; thus, we inferred that other stromal cells within the glioma microenvironment such as resident microglia, activated astrocytes, or angiogenic endothelial cells could be candidates as sources of soluble PDGF-CC. Therefore, we tested PDGF-C expression in astrocytes and endothelial cells in response to tumor cells. Primary cultures of astrocytes and endothelial

Figure 5. Stromal derived PDGF-CC serves as an activator of OPCs in glioma. A, PDGF-C is upregulated in glioma-associated macrophages. Quantitative RT-PCR of PDGF-C on GFP+/F4/80+ macrophage/microglia sorted from orthotopic Gl261 tumors or normal brain. Means ± SEM, n = 6 mice; t test, ***, P < 0.001. B, the growth of Gl261 tumors in mice transplanted with PDGF-C−/− bone marrow or littermate PDGF-C+/− bone marrow was measured by photon flux. Means ± SD, n = 8 mice; t test, **, P < 0.01. C, PDGFRα+ cells were quantified in the tumor periphery of Gl261 tumors at D14 and D21, respectively, from both PDGF-C−/− and PDGF-C+/− BMT animals. Means ± SEM, n = 5 mice; one-way ANOVA, ***, P < 0.01. D, endothelial cells and astrocytes isolated from cortical tissue of C57BL/6 mice were treated with Gl261 conditioned medium or control medium. Quantitative RT-PCR of PDGF-C was performed, demonstrating the upregulation of PDGF-C in both endothelial and astrocytic cells in response to glioma conditions. Means ± SEM, n = 6; t test, ***, P < 0.001. The graph was acquired from two independent experiments. N, the number of independent wells of treatment per condition.
cells were isolated from mouse brain, and treated with or without conditioned medium derived from Gl261 cell culture. RT-PCR from both primary cultures showed that the expression of PDGF-C was upregulated by tumor-conditioned medium (Fig. 5D), implying that PDGF-CC might be expressed and secreted by glioma-associated astrocytes and endothelial cells. However, we found that other types of tumor cells, including B16/F10 and KR158, could both upregulate the PDGF-C expression within stromal cells (Supplementary Fig. S10A), suggesting that the upregulation of PDGF-C may be specific to glioma stroma, but not to tumor cells. The stromal-derived PDGF-CC may also be related to other cancers and proinflammatory states.

To assess the contribution of total stromal PDGF-CC to gliomagenesis, we intracranially injected Gl261 cells into PDGF-C−/− knockout mice. Gl261 tumor growth in PDGF-C−/− mice was significantly slower, and tumor burden was less than the tumors in littermate PDGF-C−/− mice (Fig. 6A and B). MRI demonstrated strikingly less tumor enhancement in PDGF-C−/− mice. These results recapitulated the phenotype of Gl261 tumor progression in PDGFRα knockout mice. More importantly, we found many fewer PDGFRα+ cells in the tumor periphery in PDGF-C−/− mice and significantly smaller vascular lumens (Fig. 6C). Taken together, these results demonstrate that stromal-derived PDGF-CC is a key mediator in the recruitment and activation of OPCs during the progression of gliomas.

PDGF-CC, of which the primary receptor is PDGFRα, has been implicated in various cell types and pathologic conditions. A recent study showed that PDGF-CC knockout mice showed abnormal cerebral vascularization (28), supporting the role of PDGF-CC in regulating the vascular architecture under physiologic conditions in the CNS. In the postnatal brain, PDGFRα is exclusively expressed on OPCs (29). Thus, the stromal PDGF-CC/PDGFRα axis may be an important intrinsic angiogenic signaling pathway regulating angiogenesis in gliomas—indeed of the classic VEGF-mediated pathway. To further understand the role of this signaling axis in regulating angiogenesis, we used an angiogenesis antibody array (R&D systems) to profile the angiogenic factors derived from OPCs after the exposure to PDGF-CC. PDGF-CC upregulated proangiogenic factors such as CXCL10, CX3CL1, and IGFBP-1 in OPCs (Supplementary Fig. S10B). PDGF-AA, PDGF-AB, PDGF-BB, and VEGF remained unchanged in the same assay. Among upregulated factors, OPC-derived MMP9 was recently found to mediate blood–brain barrier opening in response to white matter injury (26). These results indicate that GA-OPCs might have a direct impact upon activating endothelial cells and recruiting proangiogenic myeloid cells.
GA-OPCs in patients with glioma

The brain tumor microenvironment in patients with glioma is very complex. The signaling within undefined stromal populations and tumor cells is intricate. This is further complicated by the molecular heterogeneity between subpopulations of actual glial tumor cells. Therefore, we sought to confirm and quantify the existence of PDGFRα+ stromal cells directly from samples resected from patients diagnosed with a high-grade glioma. Among established markers for glioma cells, isocitrate dehydrogenase 1 (IDH1) mutation is extremely reliable in distinguishing tumor cells from stromal cells as it has been used to detect even single disseminated tumor cells (30, 31). Grade III astrocytomas bearing IDH1 mutations were chosen, and stromal cells were characterized as negative for the IDH mutation. Doubly staining for PDGFRα and IDH (R231H), we demonstrated a population of cells positive for PDGFRα and negative for IDH (R231H; Fig. 7A). Interestingly, this region was actively undergoing microvascular proliferation evidenced by enlarged vascular lumens (Supplementary Fig. S11).

As we demonstrated in our animal glioma models, PDGF-CC is a potential ligand through which the activation of GA-OPCs may occur, thereby contributing to angiogenesis and glioma progression. In PDGF-C null mice, glioma progression was slowed due to a lack of activated GA-OPCs. We examined the expression of PDGF-C in different types of gliomas within Rembrandt. We observed that expression levels of PDGF-C were higher in high-grade gliomas as compared with low-grade gliomas or nontumor diseases (Supplementary Fig. S12). To further study the role of PDGF-CC in human high-grade gliomas, data from The Cancer Genome Atlas (TCGA) for different subsets of patients with glioblastoma were analyzed based on their expression of PDGF-C. Samples were subgrouped into those with higher PDGF-C expression (top 10–14%) with the remainder classified as PDGF-C low. Analyses of survival and progression were performed within four recognized subsets of glioblastoma, including classic, proneural, mesenchymal, and neural. Within the classic subset of glioblastoma, but not in other subsets, expression of PDGF-C was significantly correlated with disease progression ($P = 0.0060$), and inversely correlated with survival of patients ($P = 0.0081$; Fig. 7B and Supplementary Fig. S13). This demonstration within the classic subset of human glioblastoma is consistent with our demonstrated slowing of orthotopic Gl261 tumor progression in PDGD-C null mice. Surprisingly, even other members of the PDGF family, including PDGF-A and PDGF-B, commonly upregulated in glioblastoma, were not found to be statistically significantly ($P > 0.05$) correlated with patients’ survival or disease progression (Supplementary Fig. S14).

Discussion

OPCs are the most abundant neural progenitor cells in postnatal brains. They can differentiate into oligodendrocytes or astrocytes in response to different stimuli to aid in myelination and wound healing, among other roles in the CNS (32–36). Recent studies also suggest that OPCs have a higher degree of plasticity and may be more sensitive to transformation (9, 10). In certain rodent brain tumor models, it has been demonstrated that recruited stromal OPCs can be tumorigenic. However, their transformation of OPCs may require a preexisting oncogenic mutation such as Ink4a/Arf or P53 as indicated by these studies (37).
Invasive glioma cells frequently migrate along myelinated white matter fiber tracts (38), a phenomenon whose underlying mechanism is not clearly elucidated. It has also been established that OPCs preferentially reside along identical pathways of dissemination (39, 40). Many studies have examined and demonstrated that glioma progression and invasion follows a perivascular pattern (25, 41, 42), particularly at disease recurrence. In this study, we demonstrated angiogenic endothelial cells, perivascular macrophages/microglia, and OPCs working in concert to form a proangiogenic and proinvasive niche at the invading front of glioma. Our data suggest that stromal OPCs are a key element not only in initiating angiogenesis but also in driving glioma invasion. To extend our hypotheses derived from mouse models to humans, we selected IDH1R132H mutant grade III astrocytomas within which to analyze stromal PDGFRα+ cells from human specimens. The IDH1 R132H mutation is an ideal marker to distinguish between tumor cells and stromal cells in grade III astrocytomas. In addition, grade III astrocytomas are by definition in the transition stage, during which we suggest that OPCs play a crucial role in both microvascular proliferation and tumor invasion.

In the previous work, PDGF-CC was found to induce the loss of the blood–brain barrier and induce vascular leakiness in a stroke model, and PDGF-C deficiency leads to abnormal cerebral vascularization (28, 43). In our study in tumor-bearing mice, the number of OPCs with activated PDGFRα was increased compared with non–tumor-bearing mice. However, similar tumors could not increase the number of OPCs in PDGF-C−/− mice. Interestingly, PDGF-CC has been identified as a key factor allowing tumors to recur following the anti-VEGF treatment in glioblastoma and other types of cancer (44, 45). All of this evidence suggests that PDGF-CC plays an important role in the vascular remodeling occurring in gliomas, which may be independent of the classic VEGF pathway. When studying survival using the glioblastoma cohort in TCGA, we demonstrated a survival advantage correlating with the PDGF-C expression only in the classic subgroup of glioblastoma. We hypothesize that the correlation within the classic subgroup might be due to differences of the origin of tumor cells between the subgroups of glioblastoma. Mesenchymal, or proneural subtypes may be derived directly from OPCs where it has been suggested that tumor cells may inherit certain features directly from OPCs. In this class of glioblastoma, the tumor cells may overshadow stromal OPCs with respect to their proangiogenic role. Recent studies have demonstrated that tumor cells may transdifferentiate into endothelial cells and pericytes (46–48). This finding suggests that tumor heterogeneity along with the complex nature of the tumor microenvironment can result in the utilization of multiple angiogenic pathways.

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

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