

PDGFR α -Positive B Cells Are Neural Stem Cells in the Adult SVZ that Form Glioma-like Growths in Response to Increased PDGF Signaling

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Summary

Neurons and oligodendrocytes are produced in the adult brain subventricular zone (SVZ) from neural stem cells (B cells), which express GFAP and have morphological properties of astrocytes. We report here on the identification B cells expressing the PDGFR α in the adult SVZ. Specifically labeled PDGFR α expressing B cells in vivo generate neurons and oligodendrocytes. Conditional ablation of PDGFR α in a subpopulation of postnatal stem cells showed that this receptor is required for oligodendrogenesis, but not neurogenesis. Infusion of PDGF alone was sufficient to arrest neuroblast production and induce SVZ B cell proliferation contributing to the generation of large hyperplasias with some features of gliomas. The work demonstrates that PDGFR α signaling occurs early in the adult stem cell lineage and may help regulate the balance between oligodendrocyte and neuron production. Excessive PDGF activation in the SVZ in stem cells is sufficient to induce hallmarks associated with early stages of tumor formation.

Introduction

The subventricular zone (SVZ) in the walls of the lateral ventricles is the largest germinal center and source of stem cells in the adult brain. The stem cells in the SVZ of the adult rodent brain continue to generate neurons destined for the olfactory bulb (reviewed in Lim and Alvarez-Buylla [2001]). A population of GFAP-expressing cells in the SVZ (B cells) are the primary precursor cells. They first produce a transit-amplifying cell population (C cells) that then give rise to the neuroblasts (A cells) that migrate to the olfactory bulb where they mature into neurons (Doetsch et al., 1999a; Garcia et al., 2004; Parras et al., 2004). Although the SVZ stem cells have characteristics of astrocytes and express GFAP, additional markers that enable further characterization of the stem cells are lacking. New oligodendrocytes are also generated in the SVZ of adult mice (Nait-Oumesmar

et al., 1999), and B cells located in this region also serve as their primary progenitors (Menn et al., 2006). Again, additional markers and factors regulating SVZ oligodendrogenesis have not been well characterized.

During mouse development, the platelet-derived growth factor receptor- α (PDGFR α) is expressed by neuroepithelial cells as early as E8.5 (Andrae et al., 2001). However, the role of PDGF in neuroepithelial stem cells is not understood. Instead, PDGF is considered to function later in development as a potent mitogen of oligodendrocyte precursor cells (OPCs). OPCs were first identified and characterized in cultures from rat perinatal optic nerve (Raff et al., 1983), where they give rise to either oligodendrocytes or type-2 astrocytes. In the rat optic nerve and spinal cord, OPCs express PDGFR α (Hall et al., 1996), and PDGF is important for regulating OPC number and oligodendrocyte production in vivo (Calver et al., 1998; Fruttiger et al., 1999). While most OPCs differentiate into mature oligodendrocytes early in postnatal life, it is believed that a slowly dividing population of OPCs remains widely distributed within the adult brain (Ffrench-Constant et al., 1986; Wolswijk and Noble, 1989). The adult CNS retains the capacity to generate new oligodendrocytes (McCarthy and Leblond, 1988), and here PDGF is also thought to regulate OPC number (Woodruff et al., 2004).

In addition to these developmental roles, PDGF signaling has been widely implicated in the formation of brain tumors. Activation of the pathway has been observed in >80% of oligodendrogliomas and in 50%–100% of astrocytomas and astrocytoma cell lines (Guha et al., 1995; Varela et al., 2004) occurring mainly through overexpression of the ligand and cognate receptor. Overexpression of PDGF/PDGFR occurs with equal frequency in low- and high-grade tumors, suggesting that activation of the pathway may be important for tumor initiation. Thus, understanding which cells in the adult brain normally respond to PDGF may provide insights into the glioma cell of origin and events regulating brain tumor initiation.

Some tumors in the adult human brain have anatomical connections with the ventricular wall (Globus and Kuhlenbeck, 1944), and it has been suggested that brain tumors may arise from neural stem or progenitor cells (Dai et al., 2001; Recht et al., 2003). Support for this hypothesis comes from oncogenic transformation of progenitor cells (Holland et al., 1998) and from a murine astrocytoma model in which tumors appear to initiate in the SVZ (Zhu et al., 2005). Further support comes from work demonstrating the presence of neural stem-like cells within brain tumors (Ignatova et al., 2002; Hemmati et al., 2003; Galli et al., 2004; Singh et al., 2004). However, it has not been shown in vivo that tumor stem cells are derived from normal stem cells or that a specific population of cells with demonstrated stem cell properties is capable of initiating tumor formation.

We report here the identification of PDGFR α -expressing astrocytes (B cells) in the adult SVZ that are neural stem cells. We show that PDGF stimulates growth and self-renewal of SVZ stem cells in neurosphere cultures

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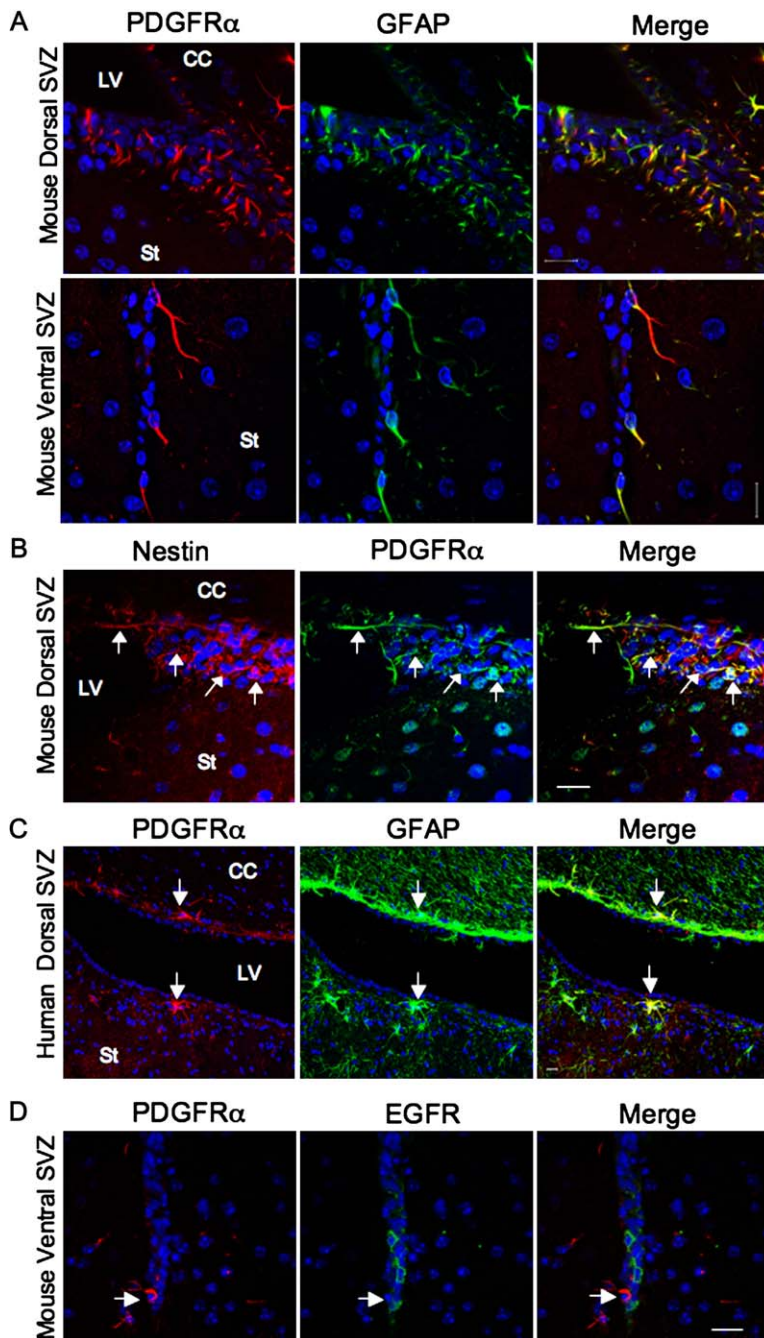


Figure 1. PDGFR α Expression in the Adult SVZ

Confocal optical sections of coronal sections of the mouse SVZ coimmunostained for: (A) PDGFR α (red) and GFAP (green). Numerous cells express both PDGFR α and GFAP in the dorsal wedge region of the SVZ (top panels). The bottom panels show double-positive cells located more ventrally, about midway between the dorsal and ventral SVZ. Note that the staining is mainly localized in the processes and that many of the PDGFR α ⁺ cells have a short radial process. (B) Nestin (red) and PDGFR α (green). Some PDGFR α -expressing cells also express Nestin (arrows). (C) Confocal optical sections of adult human SVZ coimmunostained for PDGFR α (red) and GFAP (green). Note that a subpopulation of the B cells express the PDGFR α (arrows). (D) Confocal optical section of mouse SVZ showing costaining for PDGFR α (red, arrow) and EGFR (green). Note the absence of double-positive cells. Scale bars = 20 μ m.

in vitro and that PDGFR α ⁺ B cells generate new neurons and oligodendrocytes in vivo. In addition, we find that these cells are sensitive to alterations in the PDGF pathway and form atypical hyperplasias in vivo in response to excess PDGF signaling.

Results

PDGFR α Is Expressed by a Subpopulation of SVZ B Cells

The expression of PDGFR α was analyzed by immunostaining in the adult mouse SVZ using a rabbit polyclonal and a mouse monoclonal antibody, which showed very similar staining patterns. Large numbers of PDGFR α -

expressing cells were observed in the dorsal region of the SVZ (Figure 1A), with scattered PDGFR α -positive cells present in the central and ventral portions (Figure 1B). PDGFR α -expressing cells were also observed in the medial wall of the lateral ventricle. PDGFR β was detected in cortical neurons (Smits et al., 1991), but not in the SVZ.

We analyzed sections coimmunostained for the PDGFR α and SVZ cell type-specific markers at the confocal microscope to identify which cells in the adult mouse SVZ expressed the PDGFR α . A subset of GFAP⁺ SVZ astrocytes (B cells) expressed the PDGFR α . The PDGFR α signal was highest in the astrocytic processes, with only a subset of cells exhibiting immunostaining in the cell body (Figure 1A). To determine what

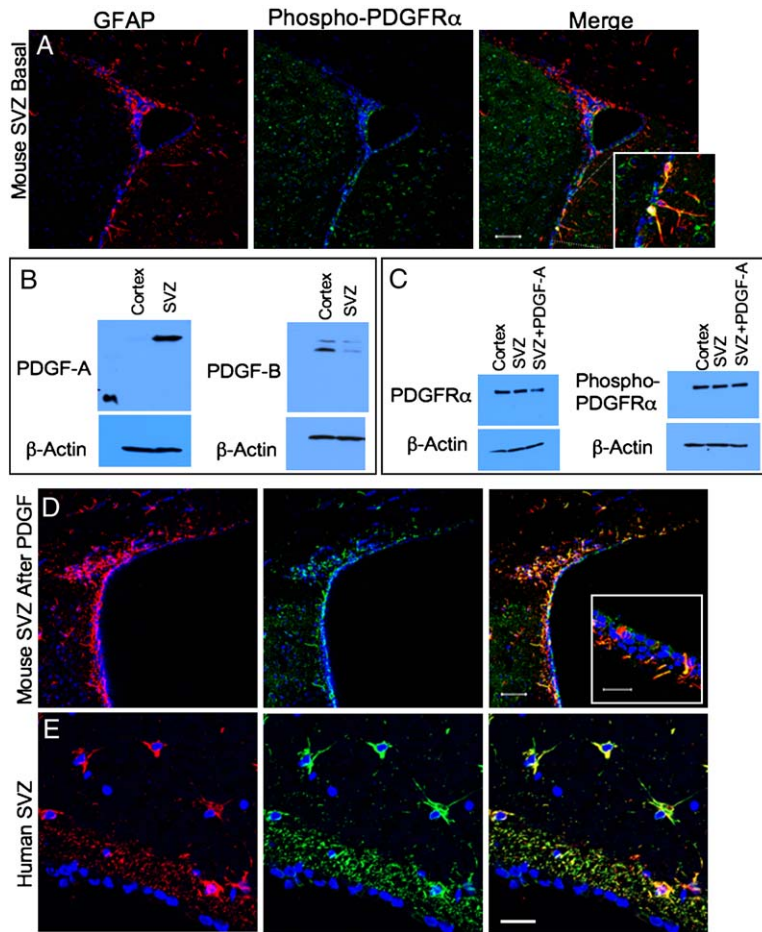


Figure 2. PDGFR α Signaling in Astrocytes of the Adult SVZ

(A) Confocal optical sections showing costaining for GFAP (red) and PDGFR α -phosphoTyr720 (green) on coronal sections of brain from untreated mice. Note the presence of GFAP⁺/phospho-PDGFR α ⁺ astrocytes in the untreated brain ([A], inset).

(B) Western blots of lysates from SVZ and cortex probed for PDGF-A ligand (left) or PDGF-B ligand (right). Note the abundant expression of PDGF-A (~30 kDa band) in the SVZ as compared to the cortex, while the opposite is true for PDGF-B.

(C) Levels of PDGFR α (left) or phospho-PDGFR α -Tyr720 (right) in lysates from cortex, SVZ, and SVZ after injection of PDGF-A ligand into the lateral ventricle. A single band of ~170 kDa is observed.

(D) Confocal optical sections showing costaining for GFAP (red) and phospho-PDGFR α (green) on coronal sections of brain from mice given two injections of PDGF-A ligand into the lateral ventricle separated by 20 min. Many more phospho-PDGFR α ⁺ B cells are present after injection of ligand. Scale bar = 50 μ m. Inset scale bar = 20 μ m.

(E) Costaining for GFAP (red) and phospho-PDGFR α (green) on sections of adult human SVZ demonstrating the occurrence of endogenous PDGF signaling. Scale bar = 20 μ m.

proportion of SVZ B cells express the PDGFR α , we analyzed only those cells for which we could detect GFAP immunostaining in the cell body encompassing the nucleus. Using the mouse anti-PDGFR α 82.5% \pm 4.9% (mean \pm SEM, n = 3 mice) of SVZ B cells were PDGFR α ⁺. This was very similar to results obtained using rabbit anti-PDGFR α in which case 78.1% \pm 0.95% (n = 3) of the B cells were PDGFR α ⁺.

In the SVZ, PDGFR α was not detected on the Dlx2⁺ C cells, nor did the staining colocalize with that of PSA-NCAM or doublecortin, which mark A cells (Bonfanti and Theodosios, 1994; Doetsch et al., 1999a; see Figures S1C and S1D in the Supplemental Data available with this article online). Intriguingly, many of the PDGFR α ⁺ cells also expressed Nestin (Figure 1B), a marker of neural stem and progenitor cells (Lendahl et al., 1990). Furthermore, the PDGFR α was not expressed by EGFR⁺ cells in the SVZ (Figure 1D), most of which are transit-amplifying C cells (Doetsch et al., 2002). To further verify the identity of the PDGFR α ⁺ cells in the murine SVZ, we performed pre-embedding immunostaining in conjunction with electron microscopy (EM). Analysis of semithin sections revealed that SVZ B cells with various morphologies expressed the PDGFR α (Figure S1A). Under the electron microscope, cells expressing the PDGFR α had ultrastructural characteristics of astrocytes including a light cytoplasm and thick bundles of intermediate filaments (Figure S1B).

We next determined whether PDGF-expressing cells existed in the adult human SVZ, where a subpopulation of astrocytes can function as stem cells in vitro (Sanai et al., 2004). Costaining for PDGFR α and GFAP was performed on sections of postmortem adult human brain. Interestingly, a subset of the GFAP⁺ SVZ astrocytes expressed the PDGFR α (Figure 1C).

SVZ B Cells Activate the PDGFR α in Response to Exogenous PDGF-AA

We next investigated whether PDGF signaling occurs in adult SVZ B cells. The presence of the activated receptor was examined by staining with antibodies specific to the phosphorylated PDGFR α (phospho-Tyr720). This analysis revealed a small subpopulation of SVZ B cells in which the PDGFR α was activated (Figure 2A). Interestingly, Western blots of SVZ lysates revealed abundant levels of PDGF-A ligand relative to levels in the cortex (Figure 2B, left). The converse was true of the PDGF-B ligand (Figure 2B, right), and PDGF-C ligand was not detected in Western blots.

To explore whether SVZ cells respond to increased levels of PDGF ligand, we injected PDGF-AA, which binds specifically to the PDGFR α , or vehicle into the lateral ventricle. The number of phospho-PDGFR α ⁺ B cells was dramatically increased in the PDGF-injected mice (Figure 2D) as compared to vehicle infused or untreated brain (Figure 2A). Together, these data indicate that

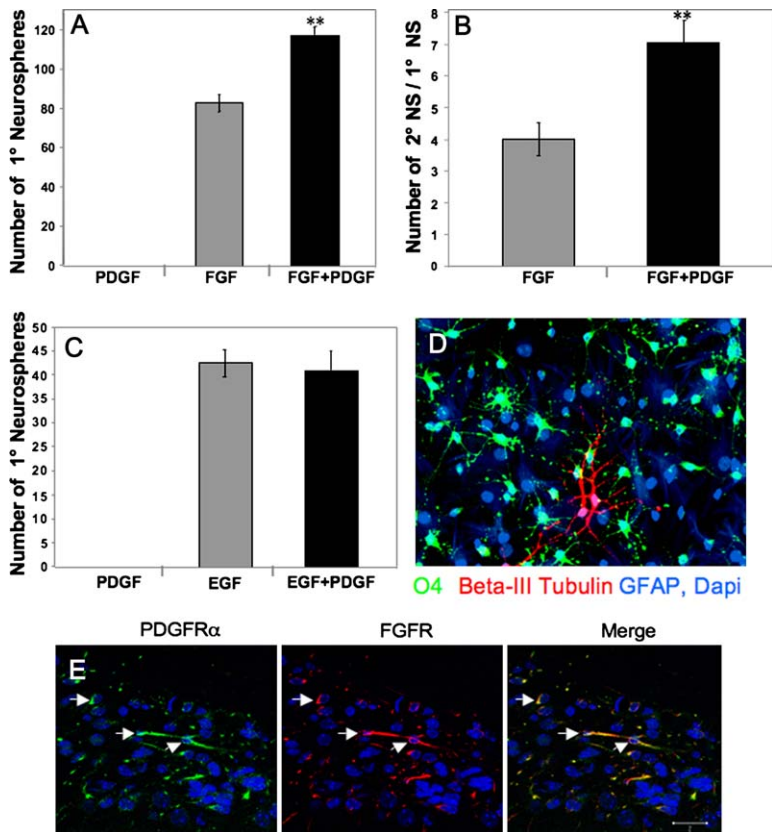


Figure 3. PDGF Cooperates with bFGF to Enhance Neurosphere Formation and Stimulate Stem Cell Self-Renewal

(A) Freshly dissociated SVZ cells were cultured under neurosphere conditions in the presence of PDGF, bFGF, or bFGF + PDGF. No NS grew in PDGF alone, but the addition of PDGF to bFGF containing growth media resulted in an increase in the number of primary neurospheres generated over bFGF alone (data from three independent experiments; mean \pm SEM).

(B) The number of secondary NS derived from the dissociation of a single primary NS increased 76% when cultured in bFGF + PDGF compared to bFGF alone.

(C) Freshly dissociated SVZ cells were cultured under neurosphere conditions in the presence of PDGF, EGF, or EGF + PDGF. PDGF did not enhance NS formation in conjunction with EGF.

(D) Triple labeling of a differentiated NS derived in bFGF + PDGF showing immunoreactivity for astrocyte (GFAP in blue), oligodendrocyte (O4 in green), and neuron-specific (Tuj1 in red) markers.

(E) Confocal optical section of a coronal section of the SVZ showing costaining for FGFR (red) and PDGFR α (green).

PDGF signaling occurs in the normal SVZ as a result of endogenous signals and that signaling through this pathway can be stimulated by the administration of exogenous PDGF-AA.

We also examined the human SVZ for the presence of astrocytes in which the PDGFR α was actively signaling. Again, costaining for phospho-PDGFR α revealed the presence of a subset of GFAP $^+$ astrocytes in which the PDGFR α was phosphorylated (Figure 2E), suggesting that endogenous signaling also occurs in adult human SVZ astrocytes.

PDGF Works in Conjunction with FGF to Stimulate Neurosphere Formation In Vitro

Based on our findings that some B cells expressed the PDGFR α , we hypothesized that PDGF signaling regulates stem cell properties such as survival, proliferation, or self-renewal. Cells from the SVZ grown in vitro in the presence of EGF, bFGF, or both, form neurospheres (NS), which contain multipotent neural stem cells (Morshead et al., 2003). We studied the effects of PDGF on SVZ stem cells in NS assays. Equal numbers of cells dissociated from the SVZ of adult mice were divided into culture media containing PDGF-AA, bFGF, or bFGF and PDGF-AA. Although PDGF-AA alone did not support NS growth, there was a significant increase (45% \pm 9%, $p < 0.001$) in the number of primary NS generated in media containing bFGF and PDGF-AA as compared to bFGF alone (Figure 3A), suggesting that PDGF promotes the survival and/or proliferation of SVZ stem cells. In addition, there was a 76% increase ($p = 0.001$) in the number of secondary NS generated from a single

primary NS in the presence of bFGF and PDGF-AA compared to bFGF alone (Figure 3B), indicating that PDGF-AA also stimulates self-renewal. Upon differentiation, NS derived in bFGF and PDGF-AA were capable of generating neurons, oligodendrocytes, and astrocytes, showing that NS derived in the presence of PDGF-AA retained their multipotency (Figure 3D). The cooperative effects of PDGF-AA and bFGF observed in vitro prompted us to compare the expression patterns of the FGFR and PDGFR in the SVZ. Many of the PDGFR α -expressing cells were also FGFR $^+$ (Figure 3E, arrows). In contrast to the cooperative effects of bFGF and PDGF-AA, there was no difference in the number of NS derived from cells cultured in EGF alone versus EGF and PDGF (Figure 3C). These data, together with the nonoverlapping staining pattern of PDGFR α and EGFR (Figure 1D), suggest that these two growth factors affect different subsets of SVZ cells.

PDGFR α $^+$ B Cells in the Adult SVZ Give Rise to Neurons and Oligodendrocytes

Administration of cytosine- β -D-arabinofuranosidase (Ara-C) kills the rapidly dividing cells, depleting the SVZ of C and A cells but sparing a subpopulation of slowly dividing B cells (Doetsch et al., 1999b). Following Ara-C treatment, B cells divide to repopulate the SVZ, producing C cells which in turn generate A cells. We used this method to determine if the repopulating stem cells express the PDGFR α . Mice were infused with Ara-C for 6 days and sacrificed 12 hr later after receiving a single injection of BrdU 1 hr prior to sacrifice. Costaining for PDGFR α and GFAP revealed PDGFR α $^+$ B cells

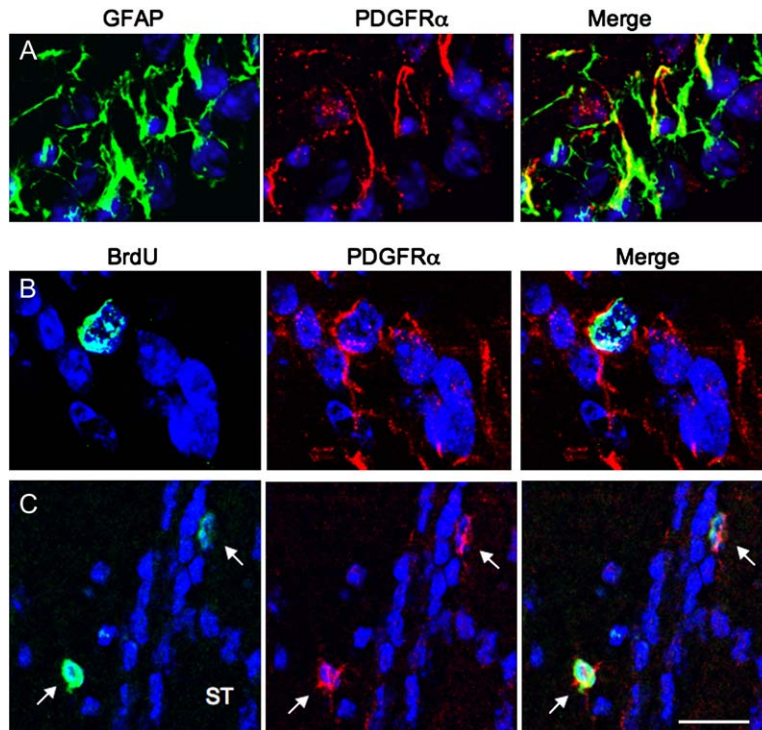


Figure 4. PDGFR α -Expressing B Cells Have Characteristics of Stem Cells

(A and B) PDGFR α ⁺ B cells remain in the SVZ after elimination of C cells and neuroblasts by Ara-C treatment, and some of these cells divide after removal of the drug.

(A) Costaining for GFAP (green) and PDGFR α (red) after 6 day Ara-C infusion.

(B) Costaining for BrdU (green) and PDGFR α (red) 12 hr after Ara-C treatment.

(C) Some label-retaining cells express the PDGFR α . Mice received BrdU continuously in the drinking water for 1 week followed by a 3 week survival. Costaining for BrdU (green) and PDGFR α (red).

remaining in the SVZ (Figure 4A), consistent with the hypothesis that they function as primary progenitors not transit-amplifying precursors. We also performed costaining for PDGFR α and BrdU to determine whether the PDGFR α ⁺ cells contribute to regeneration of the SVZ. Because the PDGFR signal is localized to the processes, it was difficult to colocalize this marker with the nuclear BrdU staining. However, we could be certain that a subset of the BrdU⁺ B cells were also PDGFR α ⁺ (Figure 4B). This is consistent with the idea that the PDGFR α ⁺ B cells are induced to re-enter the cell cycle after removal of Ara-C.

In several organs the slowly dividing, label-retaining cells (LRCs) correspond to the resident stem cell population (Bickenbach, 1981; Morris and Potten, 1994). We examined LRCs in the SVZ to see if they were PDGFR α ⁺. BrdU was administered in the drinking water for one week and then removed for 3 weeks, sufficient time for the rapidly dividing C and A cells that were in the SVZ at the time of BrdU administration to complete division and/or migrate to the olfactory bulb (Lois and Alvarez-Buylla, 1994; Luskin and Boone, 1994). Thus, any LRCs remaining in the SVZ at this time are good candidates for stem cells that divided and remained in the germinal zone. Again, we found that a subset of the BrdU-labeled B cells were also PDGFR α ⁺ (Figure 4C).

While the above experiments indicate that PDGFR α ⁺ B cells divide and possibly function as neural stem cells in vivo, they do not directly demonstrate that the PDGFR α ⁺ B cells generate neurons in vivo. To do this, we needed a method to specifically label these cells and follow the fates of their progeny. Recent work identified the PDGFR as a receptor for adeno-associated virus serotype 5 (AAV5; Di Pasquale et al., 2003; Lotery et al., 2003). AAV5 integrates into the host genome and is stably inherited by the daughter cells. We first verified

that AAV5 infection in the SVZ is limited to PDGFR α ⁺ cells. Freshly isolated SVZ cells were infected in vitro with an AAV5-encoding GFP (AAV5-GFP), and coexpression of PDGFR α and GFP was evaluated 24 hr later by costaining. We analyzed all of the cells in 15 random fields. Of 155 cells analyzed, we found 73 GFP⁺ (AAV5-infected) cells, all of which expressed the PDGFR α (Figure 5A). Furthermore, we used conditional ablation of the PDGFR α to confirm that it is required for AAV5 infection of SVZ cells in vivo. Adenovirus encoding Cre recombinase (AdCre, n = 4) or LacZ (AdLacZ, n = 4) as a control was injected stereotactically in the SVZ of mice homozygous for a floxed allele of PDGFR α (PDGFR α ^{Fl/Fl}; Klinghoffer et al., 2002). Nine days later, AAV5-GFP was injected into the SVZ. PDGFR α ^{Fl/Fl} mice that received AdCre had 5-fold fewer GFP⁺ cells than those that received AdLacZ (p = 0.002; Figure S3A). Of the GFP⁺ cells present in the four mice injected with AdCre, only seven cells also expressed Cre; all these cells still exhibited PDGFR α protein expression (Figure S3B). Thus, AAV5-GFP virus provides a method to specifically label B cells expressing PDGFR α .

To confirm that PDGFR α ⁺ B cells are neurogenic stem cells in vivo, we used Ara-C to deplete C and A cells from the SVZ then labeled remaining PDGFR α ⁺ B cells by infection with AAV5-GFP. Twelve days after infection, numerous GFP⁺ neurons were present in the OB, including both granule and periglomerular cells, which were identified by their characteristic morphology (Figure 5B) and by costaining for GFP and doublecortin (Figure 5D). GFP⁺/doublecortin⁺ neuroblasts were also present in the migratory stream between the SVZ and OB (Figure 5C), suggesting the continuing production of neurons by PDGFR α ⁺ B cells. Together, these data demonstrate that the PDGFR α ⁺ B cells function as precursors of new neurons. Since AAV5-GFP infection was done

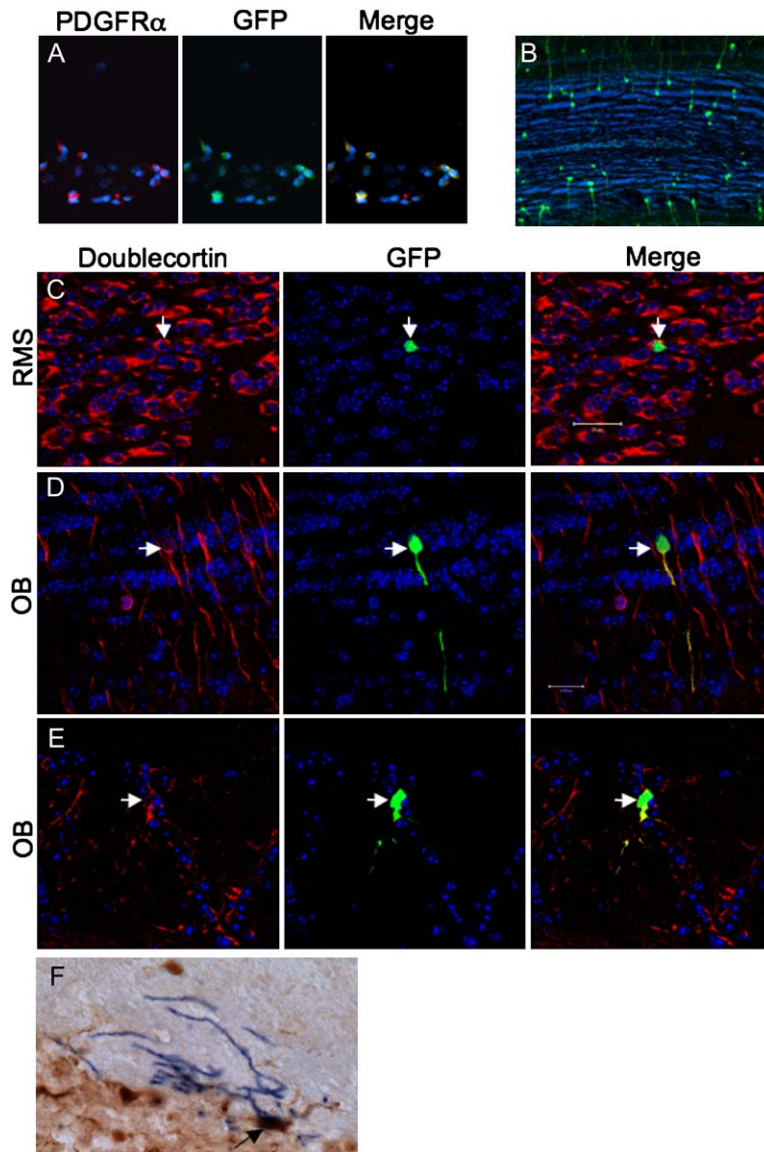


Figure 5. Specific Labeling of PDGFR α -Expressing B Cells Demonstrates that These Cells Generate Neurons and Oligodendrocytes In Vivo

(A) Immunostaining of dissociated SVZ cells after 24 hr AAV5-GFP infection for PDGFR α (red) and GFP (green). The merged image shows that all AAV5-infected GFP $^+$ cells express the PDGFR α . See Figure S4 for additional controls.

(B–E) Mice were treated with Ara-C for 6 days to eliminate C and A cells. Twenty-four hours after Ara-C treatment, the animals were infected with AAV5-GFP in the SVZ and allowed to survive for 12 days.

(B) Section of OB immunostained for GFP showing numerous GFP $^+$ cells.

(C) Confocal optical section of the RMS coimmunostained for doublecortin (red) and GFP (green). The arrow indicates a GFP/doublecortin $^+$ migrating neuroblast.

(D–E) Confocal optical section of OB coimmunostained for doublecortin (red) and GFP (green). The arrow indicates a GFP/doublecortin $^+$ neuron in (D) granule cell layer or (E) a periglomerular cell. Scale bar = 20 μ m.

(F) Section of CC of a mouse 21 days after coinfection with AAV5-GFP and RCAS-AP showing immunostaining for GFP in brown and NBT/BCIP histochemistry for AP in purple. Note the presence of a GFP $^+$ /AP $^+$ cell with characteristic oligodendrocyte morphology (arrow).

after elimination of transit-amplifying C cells in the SVZ, this experiment further suggests that PDGFR α^+ B cells correspond to the primary neural precursors.

We also noted the presence of GFP $^+$ oligodendrocytes in the CC, suggesting that the PDGFR α^+ B cells in the SVZ also generated oligodendrocytes. However, we could not rule out infection of local PDGFR α^+ OPCs in the CC by the AAV5-GFP because the injection needle passed through the CC. Therefore, to specifically label PDGFR α^+ B cells, we performed a coinfection in GFAP-Tva mice, which express the receptor for RCAS virus only in GFAP-expressing cells. Infection of Gtva mice with an RCAS virus encoding alkaline phosphatase (RCAS-AP) results in specific infection of dividing GFAP-positive cells and labeling of their progeny (Holland and Varmus, 1998; Doetsch et al., 1999a). Costaining showed that expression of tvaR only occurs in GFAP-positive cells in Gtva-a mice (Figure S3C). Injection of RCAS-AP virus into nontransgenic mice did not result in AP labeling. Thus, after confirming the specificity of RCAS infection, we injected a mixture RCAS-AP

and AAV5-GFP into the SVZ. The mice were sacrificed 21 days later, and the CC was analyzed for the presence of AP/GFP-double-positive cells. Indeed, AP $^+$ /GFP $^+$ cells with obvious oligodendrocyte morphology were present in the CC (Figure 5E), corresponding to the progeny of PDGFR α^+ B cells.

These experiments indicate that PDGFR α^+ B cells in the SVZ produce both neurons and oligodendrocytes. In addition, numerous GFP $^+$ /GFAP $^+$ cells were present in the SVZ after AAV5-GFP infection (Figure S4). However, we could not distinguish whether these were the original B cells that were infected by the AAV5 virus or if they were newly generated SVZ astrocytes.

Infusion of PDGF-A into the Lateral Ventricle Blocks Neuroblast Differentiation and Induces the Growth of Atypical Hyperplasias in the SVZ

Having established that the PDGFR α^+ B cells function as primary SVZ progenitors, we examined their sensitivity to alterations in PDGF signaling. PDGF-AA ligand, which specifically binds to the PDGFR α , was infused into the

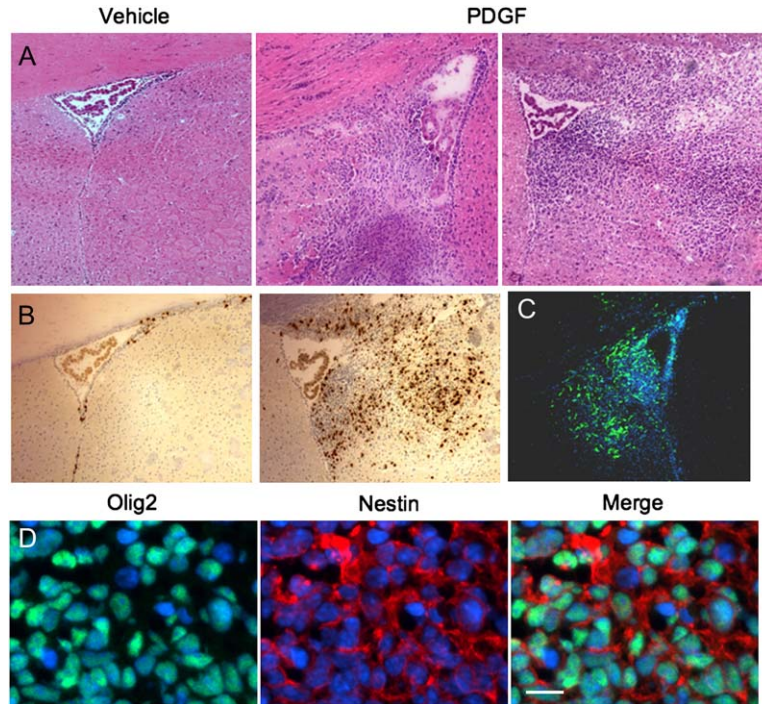


Figure 6. PDGF Induces the Hyperproliferation of B cells Resulting in the Formation of Tumor-like Growths

(A–C) Six day infusion of vehicle or PDGF-A (40 ng/day) into the lateral ventricle.

(A) Hematoxylin and eosin-stained sections of vehicle-infused (left panel) or PDGF-infused brains (two right panels).

(B) BrdU immunostaining of vehicle (left panel) or PDGF-infused brain (right panel). Mice received a single injection of BrdU 1 hr prior to sacrifice.

(C) Photomicrograph of SVZ of mice infected with RCAS-GFP virus at the time of initiation of PDGF infusion showing that a subpopulation of the cells in the tumor-like growths are derived from SVZ B cells.

(D) Section of hyperplasia coimmunostained for Olig2 (green) and nestin (red). The right panel shows the merge of the green and red panels showing that most, but not all, cells are double labeled.

lateral ventricle for 6 days. One hundred percent of the PDGF-AA infused mice ($n = 5$) developed atypical hyperplasias in either the lateral or medial wall of the ipsilateral ventricle (Figure 6A). The hyperplastic nodules showed infiltrating cells invading surrounding tissues including the striatum, CC, and/or septum (Figure 6A), and some also grew inside the lateral ventricles. Furthermore, they contained a large proportion of proliferating cells as determined by BrdU incorporation (Figure 6B). Examination of hematoxylin and eosin-stained sections revealed a heterogeneous population of cells that varied in cytoarchitecture from polygonal cells with short stout processes to more fusiform cells with delicate processes. The cells within the hyperplastic nodules had a high nuclear to cytoplasmic ratio and displayed nuclear pleiomorphism and hyperchromasia.

To ascertain whether the atypical hyperplasias were derived from B cells or their immediate progeny, we labeled a subset of SVZ B cells by infection with RCAS-GFP virus in GFAP-Tva mice, immediately prior to the onset of PDGF-AA infusion. Many cells in the hyperplasias were GFP labeled, indicating that they were derived from SVZ B cells (Figure 6C). Since all the mice studied ($n = 6$) had hyperplasias that included both positive and negative cells, we conclude that these growths have a polyclonal origin, and it is possible that other PDGFR α ⁺ cells contribute to their growth.

Immunohistochemistry was performed for a variety of cell type-specific markers to further characterize the cells comprising the atypical hyperplasias. As expected, the cells showed robust expression of the PDGFR α (Figure S5A). Interestingly, the majority of cells within the hyperplastic nodule did not express the normal markers of cells in the SVZ lineage, such as Dlx2, PSA-NCAM, or doublecortin, suggesting that the cells were no longer undergoing their normal differentiation program. Only a few cells expressed the B cell marker

GFAP, but based on morphology they appeared to be a subpopulation of the hyperproliferating cells rather than reactive astrocytes (Figure S5B). Cells in the hyperplastic nodule did express Nestin, and the majority of cells stained positively for Olig2 (Figure 6D), which is expressed by OPCs and is also a marker of human gliomas of all subtypes (Lu et al., 2001).

While examining marker expression in the hyperplasias, we noted a striking absence of A cells close to the nodule. This suggested that activation of the PDGF pathway interferes with the ability of B cells to generate neurons. To address this, we infused PDGF-AA into the lateral ventricle for 6 days at a higher concentration and faster flow rate (10 ng/ μ l; 1 μ l/hr) to provide enough PDGF-AA to stimulate a larger population of B cells in the SVZ and not just adjacent to the infusion cannula. Mice were given a single injection of BrdU 1 hr prior to sacrifice. Costaining for BrdU and doublecortin revealed a 7.7-fold reduction in the number of proliferating neuroblasts in the SVZ of PDGF-infused mice compared to vehicle-infused controls (Figure 7). Thus, PDGF infusion inhibited neuroblast production and resulted in the formation of large atypical hyperplasias.

Removal of PDGF-AA Results in Regression of Hyperplasias and Increased Oligodendrocyte Production

Next, we determined whether the hyperproliferative cells continued to require exogenous PDGF-AA for their proliferation and survival. We infused PDGF-AA into the lateral ventricle for 14 days and waited 21 days before sacrificing the animals. Strikingly, the SVZ architecture of the PDGF-infused animals looked comparable to that of saline infused controls, indicating a complete regression of the nodule upon growth factor withdrawal (Figure 8A).

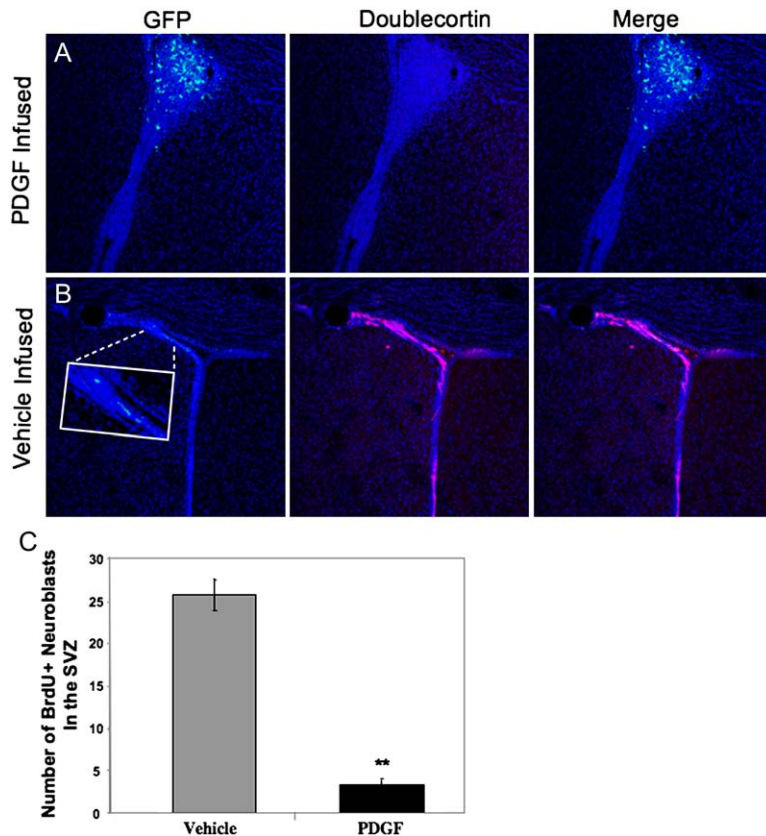


Figure 7. PDGF Arrests Neuroblast Formation

(A and B) Coronal sections of SVZ from animals labeled with RCAS-GFP before 6 day infusion with (A) PDGF-A or (B) vehicle showing immunostaining for GFP (green) and doublecortin (red). Note the increased number of GFP⁺ cells and absence of doublecortin⁺ cells in the SVZ of PDGF-infused mice.

(C) Histogram showing a 7.7-fold decrease in the number (mean ± SEM) of doublecortin⁺ neuroblasts in the SVZ of PDGF infused mice (n = 3; p < 0.001).

To follow the fate of SVZ cells after exposure to PDGF-AA, we infected the SVZ with RCAS-GFP virus immediately prior to a 6 day infusion of high concentration PDGF-AA into the lateral ventricle and analyzed brains 21 days later. GFP-positive neurons were detected in the OBs of both saline-infused and PDGF-infused mice, however the PDGF-infused mice had significantly fewer (99.6 ± 13.4) OB cells than vehicle-infused mice (191.3 ± 26.5 , $p = 0.005$; Figures 8B and 8E). In contrast, there were significantly more GFP-positive cells in the CC of the PDGF-infused animals (178 ± 71.3) than in the vehicle infused mice (92.7 ± 38 , $p = 0.004$; Figures 8C–8E). These cells had the typical branched morphology of nonmyelinating and myelinating oligodendrocytes. This provides further evidence that the PDGFR α ⁺ B cells also generate oligodendrocytes and suggests that PDGF stimulates oligodendrocyte production by these cells.

PDGFR α Signaling Is Required for SVZ Oligodendrogenesis but Not for Neurogenesis

To further elucidate the role of PDGFR α signaling in the SVZ, we performed a loss-of-function analysis using Cre-mediated gene ablation in PDGFR α ^{F1/F1} mice. These mice were crossed to the Z/EG reporter strain (Novak et al., 2000) that expresses GFP upon Cre-mediated recombination to enable permanent GFP labeling of cells that lose PDGFR α . Adeno-Cre infection was targeted to a small subpopulation of radial glia in neonatal (P0) mice (Merkle et al., 2004). These cells function as the progenitors of adult neural stem cells. PDGFR α ^{Flox/Flox}; Z/EG and PDGFR α ^{+/+}; Z/EG littermates were infected

as controls. The animals were sacrificed at 8 weeks of age. While GFP⁺ neurons were detected at similar numbers in the olfactory bulbs of PDGFR α ^{Flox/Flox} (155.8 ± 34.8) and control mice (181.4 ± 36.3 ; Figure 8F), there was a 19-fold reduction ($p = 0.044$) in the number of GFP⁺ oligodendrocytes (Figure 8G) present in the CC of PDGFR α ^{Flox/Flox} mice (n = 4) compared to control mice (n = 4).

Discussion

We have identified a subpopulation of PDGFR α -expressing B cells in the adult murine SVZ and have demonstrated that these cells function as progenitors of neurons and oligodendrocytes in vivo. We describe the effects of PDGF stimulation on neurosphere formation in vitro and show that PDGFR α ⁺ B cells act as primary progenitors during SVZ regeneration in vivo after elimination of transit-amplifying precursors and migrating neuroblasts. Furthermore, we show that increased PDGF signaling in these cells stimulates their proliferation and blocks their ability to give rise to differentiated progeny, causing them to form tumor-like growths resembling astrocytomas. Interestingly, we also found that similar PDGFR α -expressing astrocytes exist in the adult human SVZ.

Identification of PDGFR α ⁺ SVZ Type B Cells in the Adult Brain

Several lines of evidence indicate that oligodendrocytes are produced from the adult SVZ. Studies of demyelinating lesions of the CC suggest that progenitors in the SVZ

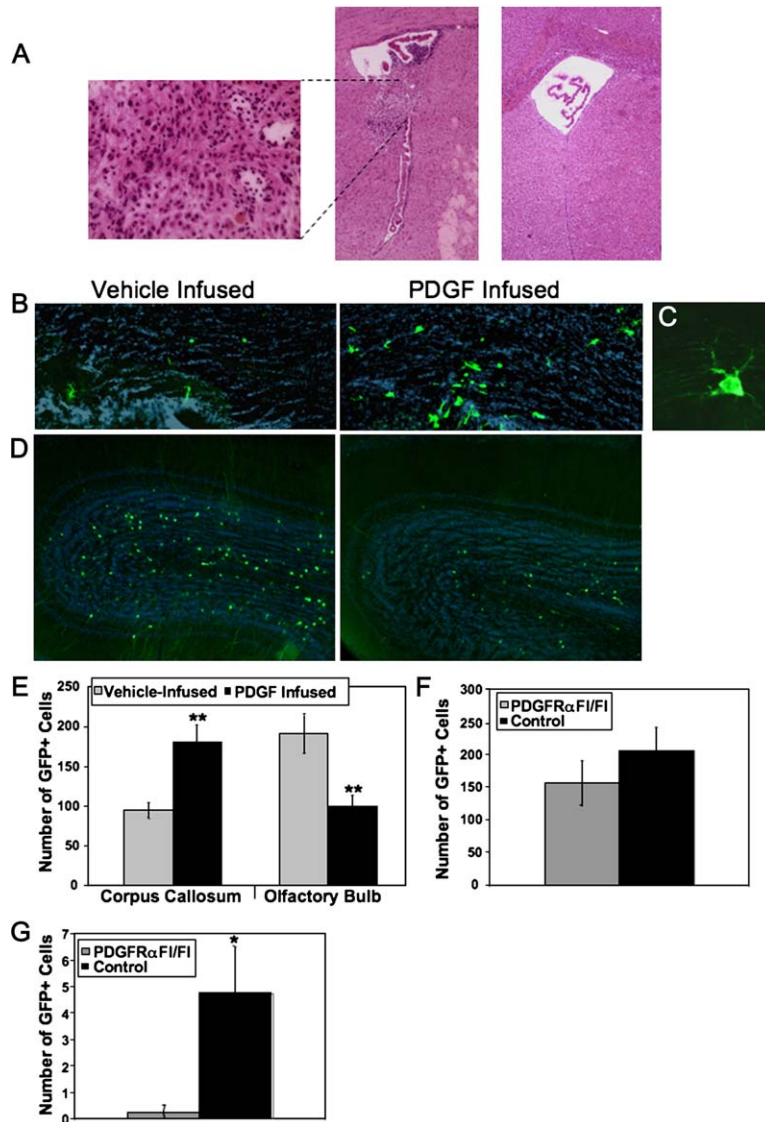


Figure 8. PDGF Infusion Results in Increased Production of Oligodendrocytes, and Decreased Production of Neurons from the SVZ and PDGFR α Is Required for SVZ-Derived Oligodendrogenesis

(A) H&E-stained section of brain immediately after (left) and 21 days after (right) 14 day PDGF infusion. No atypical hyperplasia is observed in the SVZ 21 days after infusion.

(B–E) Mice were infected with RCAS-GFP virus immediately prior to 6 day infusion of high concentration PDGF followed by a 21 day recovery period.

(B) IF for GFP (green) on the CC of vehicle-infused (left) and PDGF-infused (right) mice.

(C) Example of characteristic morphology of GFP⁺ cells in the CC.

(D) GFP immunostaining (green) of olfactory bulb of vehicle-infused (left) and PDGF-infused mice.

(E) Histogram showing the number (mean \pm SEM) of GFP⁺ cells in the CC (total cells counted) and OB (cells per section) of vehicle-infused (n = 4) or PDGF-infused (n = 4) mice. Note the decreased number of neurons (p = 0.005) and increased number of oligodendrocytes (p = 0.004) in the PDGF-infused mice.

(F and G) PDGFR α is required for oligodendrogenesis but not neurogenesis. PDGFR α ^{Flox/Flox}; Z/EG mice and their littermates were infected with Adeno-Cre virus at P0 to delete the PDGFR α and GFP label a sub-population of stem cells.

(F) Histogram showing the number (mean \pm SEM) of GFP⁺ OB neurons in PDGFR α ^{Flox/Flox} (n = 4) and control mice (n = 4).

(G) Histogram showing the number (mean \pm SEM) of GFP⁺ oligodendrocytes in the CC of PDGFR α ^{Flox/Flox} mice and their littermate controls. Note the reduction in the number of GFP⁺ oligodendrocytes in the PDGFR α ^{Flox/Flox} mice (p = 0.044).

produce oligodendrocytes after injury (Nait-Oumesmar et al., 1999) and that PDGF promotes this process (Lachapelle et al., 2002). We have recently determined that B cells are the primary progenitors of SVZ-derived oligodendrocytes (Menn et al., 2006). PDGF has long been recognized as a mitogen for OPCs that gives rise to oligodendrocytes and type 2 astrocytes in vitro (Raff et al., 1983; French-Constant et al., 1986). Although OPCs have been viewed as glial-restricted progenitors, it was recently demonstrated that they can be reprogrammed to a neural stem cell-like cell (NSLC) in vitro after first being induced to form type 2 astrocytes (Kondo and Raff, 2000). However, the relevance of this finding to in vivo neurogenesis was not clear.

Our analysis of PDGF receptor expression in the adult SVZ revealed for the first time the presence of a PDGFR α -expressing GFAP⁺ B cell population present in both the murine and human brain. Furthermore, we demonstrate that these PDGFR α ⁺ B cells are not only precursors of oligodendrocytes, but also of neurons. Depleting SVZ C and A cells and labeling the remaining

PDGFR α ⁺ B cells with AAV5-GFP resulted in many labeled granule and periglomerular neurons. In addition, by coinfecting the SVZ with RCAS-AP virus and AAV5-GFP, we showed that PDGFR α ⁺ B cells in the SVZ also generate oligodendrocytes. The PDGFR α is commonly used as a marker of OPCs or glial-restricted progenitors (GRPs) in the CNS (Grinspan and Franceschini, 1995; Redwine et al., 1997). Our work demonstrates that these PDGFR α ⁺ B cells are not GRPs but instead function as the primary progenitors of both oligodendrocytes and new neurons produced throughout adult life. A common progenitor population of neurons and oligodendrocytes also exists during development. Oligodendrocytes and neurons are derived from the same population of progenitors in the ventral region of the embryonic spinal cord (Lu et al., 2002; Takebayashi et al., 2002; Zhou and Anderson, 2002) and in the developing cerebral cortex (He et al., 2001). Furthermore, it has recently been shown that PDGF-responsive progenitors capable of generating multipotent neurospheres can be isolated from the embryonic medial ganglionic eminence (Chojnacki and Weiss, 2004).

Our data suggest that PDGF signaling may be involved in the normal regulation of oligodendrogenesis and neurogenesis in the SVZ. We confirmed the presence of PDGF-A and to a lesser extent PDGF-B ligand in the SVZ by Western blot, and cells actively signaling from the PDGFR α were revealed by immunostaining for phospho-PDGFR α . Analysis of PDGFR $\alpha^{F1/F1}$; Z/EG animals demonstrated that the PDGFR α is required for SVZ-derived oligodendrogenesis, and prelabeling B cells with RCAS-GFP demonstrated that PDGF infusion increased oligodendrocyte production from the SVZ. In contrast, loss of the PDGFR α did not interfere with the production of neurons, and PDGF infusion blocked neuroblast production during infusion and resulted in decreased numbers of GFP $^{+}$ neurons present in the OB 21 days after treatment. Together, these data are consistent with a role for PDGF signaling in balancing neuron and oligodendrocyte production from PDGFR α^{+} SVZ B cells. The robust expression of the bHLH transcription factor Olig2 by the cells responding to PDGF stimulation suggests a mechanism for this regulation. The Olig family of transcription factors is required for oligodendrocyte development (Lu et al., 2002; Zhou and Anderson, 2002). Olig2 repressor function is sufficient to repress neuronal differentiation in the postnatal (Marshall et al., 2005) and adult SVZ (Hack et al., 2005). Thus, PDGF may function upstream of Olig2 in regulating fate choice in the SVZ. It will be important to determine the source of PDGF *in vivo*.

In contrast to our findings, previous *in vitro* studies addressing the effects of PDGF stimulation on embryonic CNS stem/progenitor cells indicate that PDGF directs neuronal differentiation from these cells (Johe et al., 1996; Williams et al., 1997). A similar study showed that PDGF increased proliferation of immature neurons and delayed their differentiation (Erlandsson et al., 2001). This discrepancy could be explained by differences in growth factor responsiveness between embryonic and adult cells. It is also likely that cells *in vivo* encounter a complex network of secreted, ECM-associated, and cell surface-associated factors that in combination elicit responses different to that of any single factor *in vitro*. Our findings that PDGF alone does not support neurosphere growth but robustly stimulates B cell proliferation *in vivo* are consistent with this notion.

PDGF Blocks the Differentiation of SVZ Stem Cell Progeny and Induces the Formation of Tumor-like Growths

The “blocked differentiation” model of cancer proposes that malignant transformation interferes with a cell's ability to differentiate, holding the cell in a proliferative state (Buick et al., 1979). Human gliomas often express the progenitor marker Nestin (Tohyama et al., 1992), supporting the notion that the tumor cells do not fully differentiate. Moreover, differentiation state is one criteria used to distinguish among low-grade astrocytoma, anaplastic astrocytoma, and GBM. Previous studies also suggested that blocking the differentiation of postnatal glial progenitor cells by increased PDGF signaling contributed to the formation of oligodendrogliomas in mice (Uhrbom et al., 1998; Dai et al., 2001). Our data suggest that an uncoupling of proliferation and differentia-

tion by increased PDGF signaling may be important for glioma initiation.

Activation of the PDGF pathway is a common event in gliomagenesis and has been implicated in tumor initiation, since PDGF/PDGFR overexpression occurs with equal frequency in both low- and high-grade gliomas (Varela et al., 2004). Our work provides compelling evidence that activation of the PDGF pathway in adult murine SVZ stem cells (B cells) is sufficient to induce the formation of atypical hyperplasias. Importantly, we show that PDGF stimulation blocks the ability of B cells to give rise to differentiated progeny, resulting in a dramatic reduction in the number of doublecortin $^{+}$ neuroblasts in the SVZ. This leads to the accumulation of a large number of rapidly proliferating cells in the SVZ that invade the adjacent parenchyma. Interestingly, we found that most SVZ C and A cells do not normally express the PDGFR α . Together these data suggest that PDGF signaling may be involved in the regulation of primary precursors, being downregulated during neuronal differentiation but maintained in the early stages oligodendrocyte formation (Hart et al., 1989; Barres et al., 1992; Ellison and De Vellis, 1994).

We do not propose that the PDGFR α^{+} B cells undergo neoplastic or malignant transformation upon PDGF infusion. However, increasing the levels of PDGF ligand available to the B cells is sufficient to induce atypical hyperplasias with a similar degree of cellular atypia and pleomorphism as that found in low- to intermediate-grade human gliomas. This suggests that these cells may be particularly susceptible to neoplastic transformation. Activation of PDGFR signaling may provide a first step in this process. There is a long-held belief that cells must accumulate numerous genetic alterations to be transformed. For example, cultured human astrocytes require overexpression of hTERT, Ras, and human papillomavirus E6/E7 to form xenograft tumors (Sonoda et al., 2001). However, the initial growth events that allow for the accumulations of these mutations are poorly understood, and new evidence suggests that physiological levels of oncogene expression may be sufficient to initiate transformation (Tuveson et al., 2004). Targeting mutations to the appropriate cell of origin may be crucial for understanding requirements for transformation. This study identifies a subpopulation of cells that under epigenetic stimulation can give rise to large hyperplasias that may serve as a substrate for accumulation of transforming mutations.

The robust expression of the bHLH transcription factor Olig2 by a large proportion of the cells responding to PDGF stimulation is also intriguing. In addition to its physiological roles in specifying oligodendrocyte development, overexpression of Olig2 is also a common event in gliomas of all grades and classes (Lu et al., 2001; Ligon et al., 2004), and Olig2 has also been directly implicated in tumorigenesis in other tissues (Wang et al., 2000; Chatterjee et al., 2002). Future studies examining if Olig2 is a critical mediator of PDGF-induced hyperproliferation may yield new insights into the putative growth-promoting properties of this transcription factor.

We have identified a population of PDGFR α^{+} B cells in the adult SVZ and shown that they give rise to both neurons and oligodendrocytes *in vivo*. These findings are significant due to our limited knowledge of surface

markers for neural stem cells. Our data also provide evidence of a link between these PDGFR α ⁺ B cells and the early changes associated with tumor initiation, suggesting they may be targets of neoplastic transformation. The regression of atypical hyperplasia after PDGF removal described here suggests that inhibition of PDGF signaling could provide a useful therapy for those gliomas in which the pathway is upregulated, especially given the recovery of the normal architecture after regression of the hyperplasia.

Experimental Procedures

Animals Used

For all adult experiments, we used 2- to 4-month-old CD-1 or GFAP-Tva mice. For PDGFR loss-of-function experiments, we used P0 PDGFR α ^{Flox/Flox}; Z/EG mice. All animal procedures were approved by the Institutional Animal Care and Use Committee.

Human Postmortem Specimens

Three brains (ages 18, 50, and 80 years) were collected at autopsy. All specimens were obtained within 12 hr of death from patients without clinical or postmortem evidence of brain pathology and processed as described in Quinones-Hinojosa et al. (2006). Collection of pathology specimens was in accordance with the guidelines of the UCSF Committee on Human Research (Approval # H11170-19113-03B).

Immunohistochemistry

Mice were perfused with 4% paraformaldehyde, and the brains were postfixed overnight at 4°C. Fifty micrometer sections were cut on the Vibratome, blocked in 5% goat serum in PBS (except where noted), and incubated overnight at 4°C in primary antibody. Polyclonal PDGFR α (ready-to-use no dilution; Spring Biosciences), monoclonal PDGFR α (required antigen retrieval by boiling in 10 mM citrate buffer for 10 min, 1:50 dilution; Spring Biosciences), polyclonal PDGFR α -phosphoTyr720 (Blocked in 5% donkey serum, 1:50 dilution in PBS containing 1.5% donkey serum; Santa Cruz Biotechnology), monoclonal FGF-R1 and 2 (1:500; Chemicon), monoclonal GFAP (1:1000; Chemicon), polyclonal GFAP (1:500; DAKO), PSA-NCAM (1:400; AbCys), Doublecortin (1:500 + 0.5% Triton-X 100; Chemicon), Nestin (1:500 + 0.5% Triton-X 100; Chemicon), Olig2 (1:10,000; a kind gift of Charles Stiles), O4 (1:100; Chemicon), Tuj1 (1:500; Covance), and GFP (1:500; Aves Labs). Controls in which primary antibody was omitted resulted in no immunostaining. Preincubation of the rabbit polyclonal PDGFR α antibody with blocking peptide resulted in no immunostaining for this antibody.

PDGF Administration

For receptor activation analysis, two injections of PDGF-AA (R&D Systems; 6.6 ng) in vehicle (2 μ l) or vehicle (1 mg/ml BSA/0.9% saline) alone, separated by 20 min, were administered and animals perfused immediately after the last injection. For infusions, PDGF-AA (40 ng/day for low-concentration and 240 ng/day for high-concentration experiments) in vehicle or vehicle alone was infused into the lateral ventricle for 6 or 14 days with a miniosmotic pump (Alzet). At least three mice per group were infused for all experiments.

Ara-C Infusion

Two percent Ara-C (Sigma) was infused as previously described (Doetsch et al., 1999b).

Neurosphere Experiments

Neurospheres were prepared from the SVZ of adult mice (five mice per experiment; Doetsch et al., 1999a). Cells were plated at a density of 25 cells/ μ l in the presence of bFGF (10 ng/ml), PDGF-AA (20 ng/ml), or EGF (20 ng/ml) as indicated. Primary neurospheres were counted 9 days after plating, and secondary neurospheres were counted 7 days after passaging. Neurosphere differentiation was performed as described Doetsch et al. (1999a).

AAV5-GFP Infection

0.1 μ l of AAV5-GFP (10^{12} PFU/ml) was injected into the SVZ of CD-1 mice 24 hr after Ara-C treatment at three coordinates: anterior, lateral, depth (mm) relative to bregma, the midline, and the surface of the brain, respectively (0, 1.4, 1.6; 0.5, 1.1, 1.7; and 1, 1, 2.3). (See Supplemental Experimental Procedures for in vivo controls for the specificity of AAV5-GFP infection in PDGFR α -expressing cells).

RCAS Infection with PDGF Infusion and Analysis

0.2 μ l of RCAS-GFP virus ($\sim 2 \times 10^7$ PFU/ml) was injected into the SVZ of GFAP-Tva mice at three coordinates: (0, 1.4, 1.6; 0.5, 1.1, 1.7; and 1, 1, 2.3). PDGF or vehicle was infused at the time of retrovirus injection, and mice were sacrificed 6 days (n = 3) or 27 days (n = 4) after infection. For quantification of GFP⁺ cells 21 days after pump removal, 50 μ m coronal vibratome sections from four saline-infused and four PDGF-infused mice, were immunostained for GFP. The total number of green cells present in the CC was counted in three sets of six sections spanning the SVZ from anterior to posterior. For quantification of GFP⁺ cells present in the olfactory bulb, all green cells present in three equivalent OB sections were counted per mouse.

Coinfection with AAV5-GFP and RCAS-AP

0.2 μ l of a 1:1 mixture of AAV5-GFP (10^{12} PFU/ml) and RCAS-AP virus ($\sim 10^7$ PFU/ml) was injected into the SVZ of GFAP-Tva mice at three coordinates: (0, 1.4, 1.6; 0.5, 1.1, 1.7; and 1, 1, 2.3). Mice were sacrificed 21 days after infection. Mice were perfused with 4% paraformaldehyde and postfixed overnight. The brains were cryoprotected in 30% sucrose then frozen in OCT prior to cutting (12 μ m sections) on a cryostat. Endogenous AP was inactivated by heating at 65°C in PBS for 30 min. The sections were then washed in AP detection buffer (100 mM Tris-HCl [pH 9.5], 100 mM NaCl, 5 mM MgCl₂) then incubated in AP buffer containing NBT/BCIP (200 μ l/10ml; Roche) overnight at room temperature. The reaction was halted by washing in PBS and IHC for GFP was performed.

P0 Infection of PDGFR α ^{F1/F1}; Z/EG Mice with AdCre

P0 infections were performed as previously described (Merkle et al., 2004). Brains were analyzed at 8 weeks of age. For quantification of GFP⁺ cells, 50 μ m coronal vibratome sections from four control and four PDGFR α ^{F1/F1} mice were immunostained for GFP. The total number of green cells present in the CC was counted in six sections spanning the SVZ from anterior to posterior. For quantification of GFP⁺ neurons, all green cells present in three equivalent OB sections were counted per mouse.

Western Blots

Tissues were lysed in RIPA buffer containing protease and phosphatase inhibitors. SDS-PAGE was performed using 20 μ g total protein per lane. Western blotting was performed using the SuperSignal West Pico kit (Pierce Biotechnology) as per manufacturer's instructions. The primary antibodies used were as follows: PDGF-A (1:200; Santa Cruz), PDGF-B (1:200; Santa Cruz); rabbit anti-PDGFR α (1:200; Spring Bioscience); phospho-PDGFR α (1:100; Santa Cruz).

Supplemental Data

Supplemental Data for this article can be found online at <http://www.neuron.org/cgi/content/full/51/2/187/DC1/>.

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