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Platelet-Derived Growth Factor–Mediated Gliomagenesis and Brain Tumor Recruitment

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Platelet-derived growth factor–mediated gliomagenesis

First identified in 1974, platelet-derived growth factor (PDGF) was initially described as a platelet-dependent serum constituent that enhanced proliferation of arterial smooth muscle cells under in vitro conditions [1]. Several decades later, PDGF was demonstrated to exhibit a broad array of in vivo functions under normal and pathologic conditions and to be expressed in most tissues [2,3]. Potent involvement of PDGF in tumorigenesis was initially demonstrated in the early 1980s, when it was shown to contain extensive homology to *v-sis*, the transforming gene of the simian sarcoma virus [4]. Since then, PDGF ligand or receptor (PDGFR) overexpression has been associated with a variety of cancers, including germ cell tumors, sarcomas, gastrointestinal carcinomas, and tumors of the central nervous system (CNS) [5]. PDGF involvement in gliomagenesis has been demonstrated and well supported through an understanding of the in vivo structure and functions of PDGF, its regulation and effects during normal development, and its importance in mouse modeling of brain gliomagenesis.

Platelet-derived growth factor family: ligands and receptors

The PDGF family, one of the best-characterized growth factor families, is composed of five dimeric secreted PDGF ligands (PDGF-AA, PDGF-AB, PDGF-BB, PDGF-CC, and PDGF-DD) and three interacting tyrosine kinase receptors (PDGFR-$\alpha\alpha$, PDGFR-$\alpha\beta$, and PDGFR-$\beta\beta$) that bind these ligands with different affinities [6]. PDGF ligands are homo- or heterodimeric proteins that arise by formation of disulfide bonds between the 100 amino acid growth factor domains (PDGF/vascular endothelial growth factor [VEGF] homology domains) of four distinct polypeptides (PDGF-A, PDGF-B, PDGF-C, and PDGF-D), which are encoded by four distinct genes (*PDGFA–D*) located on chromosomes 7, 22, 4, and 11, respectively (Fig. 1A, B) [7–10]. Growth factor domain identity across four PDGF polypeptide chains is 25%, whereas PDGF-A and PDGF-B are 50% identical, as are PDGF-C and PDGF-D [6].

Classic PDGF-A and PDGF-B polypeptide chains discovered several decades ago require intracellular proteolytic processing of N-termini for activation, contain C-terminal basic sequences involved in extracellular matrix (ECM) interactions with heparan sulfate proteoglycans limiting ligand range of action, and are secreted as active ligands [11,12]. *PDGFA* gene produces two

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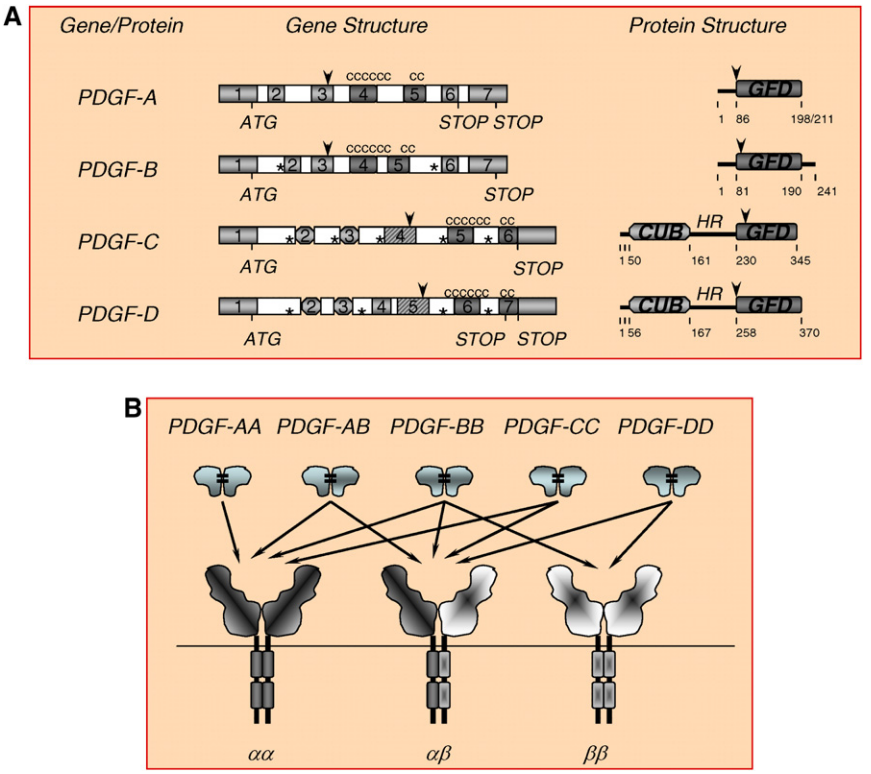

Fig. 1. The PDGF family. (*A*) Structures of genes *PDGFA* through *PDGFD* and four full-length PDGF peptides (A–D). *PDGF* gene structures: exons are shown in gray, and introns are shown in white; neither is drawn to scale. *PDGFA* and *PDGFB* genes are approximately 20 kilobytes (kb) in size; *PDGFC* and *PDGFD* genes are close to 200 kb in size. *PDGFA* and *PDGFD* can undergo alternative splicing; during this process, exon 6 is deleted as indicated. ATG, start codon; Cs, conserved cysteine motifs in theVEGF/PDGF domains; STOP, stop codons. Proteolytic cleavage sites are marked with arrowheads. Exons larger than 5 kb are indicated by asterisks. PDGF protein structures: the numbers in the schematics indicate residue numbering; arrows designate proposed sites for proteolytic activation of PDGFs. PDGF-A can undergo alternative splicing and has two stop codons at residues 198 and 211. N-terminal CUB domains of PDGF-C and PDGF-D are separated from the PDGF/VEGF homology growth factor domain (GFD) by the hinge region (HR). PDGF-A and PDGF-B undergo intracellular proteolytic processing, whereas PDGF-C and PDGF-D are modified by extracellular proteases. PDGF-A can be activated by furin, PDGF-C by plasmin and tissue plasminogen activator (tPA), and PDGF-D by plasmin; the processing protease for PDGF-B is unknown. (*B*) PDGF ligand-receptor interactions. PDGF-AA, PDGF-AB, PDGF-BB, PGDF-CC, and PDGF-DD ligands are secreted homo- and heterodimeric proteins composed of four polypeptides, PDGF A through D, joined by disulfide bonds. PDGFRs are tyrosine kinase receptors composed of two subunits (α and β), which are activated and dimerized in response to ligand binding.

PDGF-A splice isoforms, with and without the C-terminal ECM-interacting sequence [13]. The more recently discovered PDGF-C and PDGF-D are secreted as latent factors activated by extracellular proteases, such as tissue plasminogen activator (tPA) or plasmin; they lack C-terminal extensions but contain novel N-terminal CUB domains linked to the growth factor domains by the hinge regions and proposed to be involved in protein-protein or protein-carbohydrate interactions (see Fig. 1A) [14–17]. Incompletely processed PDGF-C and PDGF-D hemidimers, in which the CUB domain is still present on one of the dimer chains, may act as PDGFR antagonists [6].

Extracellular portions of α and β PDGFRs share only 31% homology, and therefore exhibit variable affinities to different ligand dimers (see Fig. 1B) [18]. PDGF ligand formation and binding to a receptor monomer preferentially induce homo- or heterotypic receptor dimerization, which, in turn, induces tyrosine phosphorylation of the cytoplasmic receptor domains, allowing for assembly of protein complexes (eg, SH2-containing adaptor proteins or kinases) activating

various signal transduction pathways. These pathways involved in regulation of cell proliferation, growth, survival, and differentiation include mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K), phospholipase Cγ (PLCγ), Src family kinases (SFKs), and Jak family kinases (JFKs) (Fig. 2). Although PDGFRs activate the same signaling pathways, the degree of activation and identity of activated pathways differ; embryologic functions of these receptors diverge as well [18]. Correspondingly, swapping intracellular domains of PDGFRs results in varying degrees of vascular disease and does not recapitulate normal development, whereas in vivo signaling downstream of these receptors in adult tissues seems to be preserved [19].

The signaling pathways listed previously are implicated in glial development or gliomagenesis; yet, it is unclear to what degree PDGFRs play a role in activating these pathways. Ras-MAPK signaling is mediated by PDGFR recruitment of two SH2-containing adaptors Shc and Grb2 and subsequent recruitment of Sos, a guanine exchange factor activating Ras GTPase, which, in turn, signals through the mitogenic MAPK pathway, resulting in upregulation of proliferative target genes (see Fig. 2). Astrocyte-specific expression of activated Ras in transgenic mouse modeling experiments also produces malignant astrocytoma [20]. PDGFR recruitment of PI3K through its SH2 domain results in conversion of PIP2 to PIP3; recruitment of Akt by PIP3; and subsequent activation of bcl2, mTOR, GSK3β, nuclear factor-κB [NFκB], and forkhead transcription factors, which increase cell growth and survival (see Fig. 2). Activated MAPK and Akt pathways characteristic of human glioblastomas are, in fact, sufficient for glioblastoma formation in nestin-positive mouse progenitor cells (PCs), which is partially attributable to their effects on translation (ie, their ability to differentially recruit existing mRNAs to polysomes) [21,22]. The forkhead transcription factors downstream of Akt signaling have also been implicated in controlling glioblastoma cell proliferation [23]. PDGFR recruitment and the phosphorylation of PLCγ necessary for its mitogenic effects cause the intracellular calcium mobilization and activation of protein kinase C (PKC) implicated in glioma cell proliferation and invasion (see Fig. 2) [24–26]. Activation of SH2-containing SFKs by PDGFRs is involved in mediating mitogenic cell responses as well as oligodendrocyte differentiation (by Fyn SFK) and cell migration because of the effects on Rho proteins [27–29]. Finally, PDGFR phosphorylation of JFKs leads to activation of Stat transcription factors, which promote glial differentiation toward astrocytic lineage in response to ciliary neurotrophic factor (CNTF) under normal conditions [30–32]. It should also be noted that PDGFR signaling is potentially mediated by various feedback mechanisms, which might account for the differences

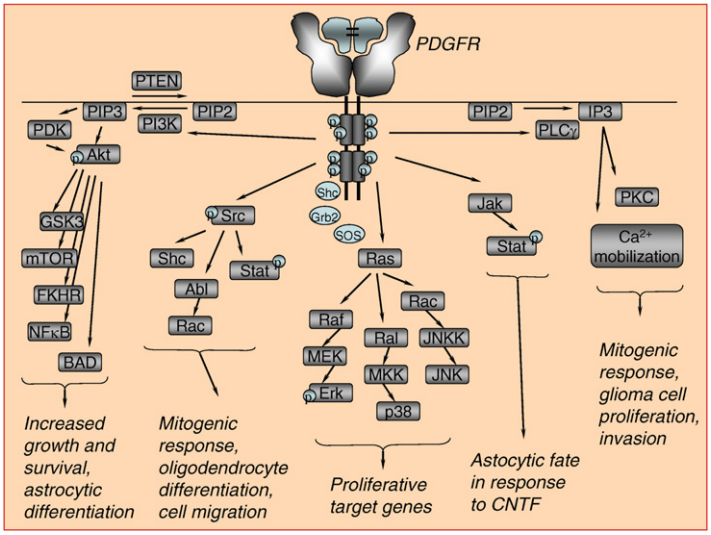

Fig. 2. Signal transduction pathways activated by the PDGFR. MAPK, PI3K, SFK, JFK and PLCγ pathways are activated by the extracellular binding of the PDGF ligands to the PDGFRs, which, in turn, activate their downstream effectors and result in various effects on cellular functions as shown.

observed between transient and chronic PDGF stimulation, the latter of which closely resembles glial tumorigenesis in vivo [33].

Platelet-derived growth factor: involvement in normal development

A paracrine mode of action of classic PDGFs is clearly manifested during mouse embryonic development, with PDGF-A and PDGF-B and their receptors, PDGFRα and PDGFRβ, mostly expressed in nonoverlapping patterns [2]. PDGF-A and PDGFRα are characteristic of epithelial-mesenchymal interaction sites; PDGF-A ligand is commonly produced by epithelial cells, whereas α receptor is expressed on underlying mesenchymal cells [34–37]. PDGF-B and PDGFRβ interactions are typical for developing vasculature; PDGF-B is secreted by endothelial cells, and β receptor is found on adjacent pericytes and vascular smooth muscle cells [38]. As far as novel PDGFs are concerned, PDGF-C is widely expressed in a variety of cell types during mouse embryonic development, including CNS cells, whereas PDGF-D has a more limited range of expression [15,39,40].

Two major PDGFs found in the developing brain are the PDGF-A and PDGF-B. *PDGFA* gene is expressed in developing mouse neurons starting from embryonic day (ED) 15 and extending into adulthood, with *PDGFB* expressed at the same time in a more limited set of neurons [41,42]. Each ligand has two known isoforms that result from alternative splicing and undergo translational regulation, whereas the PDGF-A gene is also known to contain alternative promoters [6,43–45]. PDGFRα is expressed more widely during early development and then becomes restricted to oligodendrocyte precursor cells (OPCs) during late neurogenesis [46]. Mice deficient for PDGF-A are characterized by insufficient oligodendrocyte numbers and hypomyelination and demonstrate tremor as a result of insufficient oligodendrocyte expansion and migration but not cell specification [47]. Thus, expression of PDGF ligands by neurons and PDGFRs by the OPCs might reflect normal biology, whereby tropic factor secretion by neurons and paracrine stimulation of oligodendrocytes induce their proliferation and migration. Conversely, PDGF overexpression in neurons of the spinal cord results in initial expansion of OPCs but appropriate numbers of these cells in adult mice, potentially as a result of cell death because of lack of PDGF ligand at increasing distances from neurons [48]. Targeted deletions of phosphorylatable tyrosine residues in PDGFR important for activating SFK and PI3K implicated these signaling pathways in oligodendrocyte proliferation and migration [49]. The critical importance of PDGF-B/β in vascular development is demonstrated by perinatal lethality of mice as a result of edema and massive microvascular hemorrhage [50]. Synergism between PDGF-BB and basic fibroblast growth factor (bFGF), manifested by improvement in angiogenesis and vascular stability on administration after hind limb ischemia, might indicate the importance of this ligand in adult angiogenesis [51].

Platelet-derived growth factor and gliomagenesis: causal relation

PDGFs and PDGFRs are overexpressed in human glioma cell lines and gliomas, with higher levels of expression correlating with increasing tumor grade and a poorer prognosis [52–55]. Overexpression of classic PDGFs in human gliomas and glioma cell lines is well established; recent discovery of novel PDGFs and their expression in glioma cell lines in a nonoverlapping pattern with classic PDGFs might indicate that the role of PDGF in gliomagenesis is greater than previously recognized [56]. Overexpression of PDGF/PDGFR correlates with loss of the TP53 tumor suppressor characteristic of secondary glioblastomas in contrast to primary de novo glioblastomas, which are characterized by epidermal growth factor receptor (EGFR) amplification [57,58]. Most of these cases arise because of deregulated gene expression, whereas some arise because of gene amplification (PDGFRα amplification in glioblastomas and anaplastic astrocytomas; chromosome 7 aneuploidy) or activating mutations (deletion of exons 8 and 9 in PDGFRα) [58–61]. PDGF ligands and PDGFRα are typically expressed in tumor cells, whereas PDGFRβ is primarily expressed on tumor endothelial cells, suggesting autocrine and paracrine modes of action [62,63].

Use of small-molecule inhibitors of PDGF signaling in vitro resulted in reduced glioma cell proliferation and colony-forming ability, correlating with decreased MAPK and PI3K signaling, as follows from reduced levels of pAkt and pErk [56,64]. Xenograft studies of glioma cell lines have validated the importance of autocrine PDGF signaling, as indicated by intracranial tumor responsiveness to Gleevec (imatinib, STI571), a small-molecule inhibitor of PDGFR that is now in clinical trials for glioma treatment [65–67].

Although in vitro and xenograft approaches provide valuable information on PDGF/PDGFR function, a causal relationship to gliomagenesis can only truly be shown using in vivo models faithfully recapitulating human glioma biology (Fig. 3). Viral delivery of PDGF-B into neonatal mouse brains using Moloney murine leukemia virus (MMLV) induced malignant gliomas characterized by PDGFB/PDGFRα coexpression, and therefore autocrine signaling [68]. Additional p53 loss in the same mouse model resulted in decreased latency and increased tumor frequency, suggesting that PDGF overexpression cooperates with tumor suppressor loss for accelerated glioma progression [69]. Another model using replication-competent ALV-splice acceptor (RCAS)-mediated retroviral delivery of *PDGFB* into neonatal mouse brains further demonstrated its causal role in gliomagenesis, implicating PDGF-B in oligodendroglioma formation on viral targeting to nestin-expressing glial progenitors or mixed oligoastrocytoma formation on viral delivery into differentiated glial fibrillary acidic protein (GFAP)–expressing astrocytes [70]. Because *PDGFB* gene transfer to GFAP-expressing astrocytes in vivo resulted in tumors exhibiting astrocytic and oligodendroglial characteristics, gliomagenesis is possibly mediated by dedifferentiation of infected cells to a more progenitor-like state (eg, bipotent progenitor able to give rise to cells of astrocytic and oligodendrocytic lineages of the tumor) in this case. These observations are supported by in vitro experiments indicating phenotypic dedifferentiation of cultured

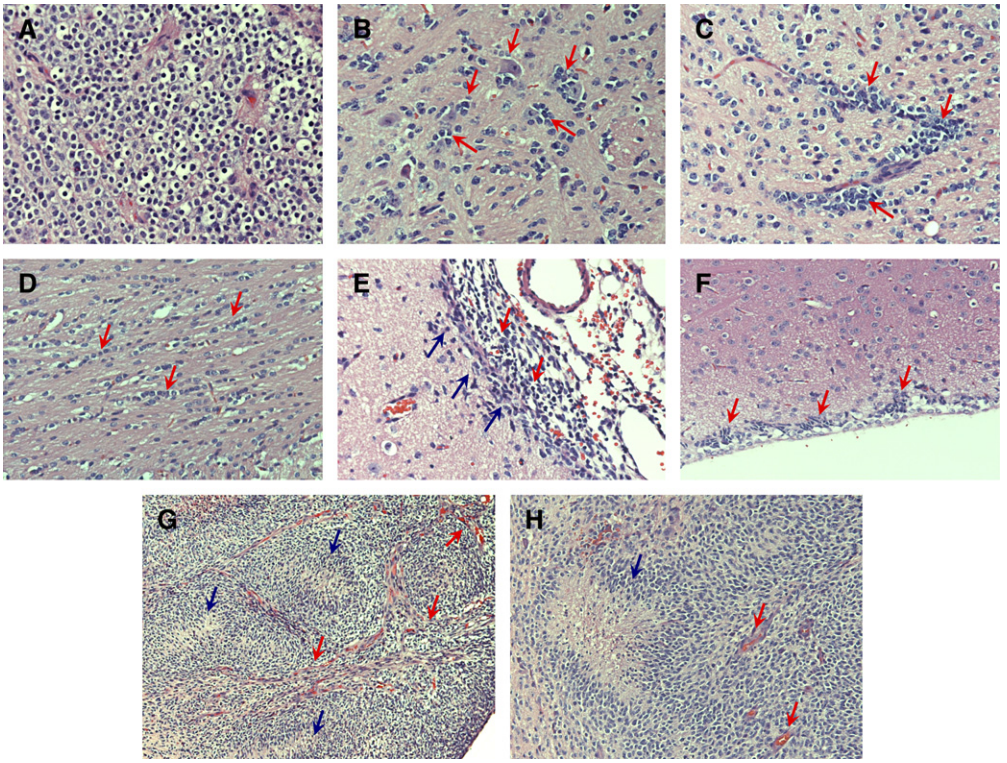

Fig. 3. Histologic features of low-grade and high-grade PDGF-induced oligodendrogliomas in the RCAS-tv-a mouse model of somatic cell gene transfer–mediated gliomagenesis. (*A–F*) Histologic features typically found in mouse low-grade oligodendrogliomas, as indicated by red and blue arrows: general chicken-wire appearance of the PDGF-induced mouse oligodendroglioma (*A*), perineuronal satellitosis (*B*), perivascular satellitosis (*C*), white matter tracking (*D*), pial (*red arrows*) and subpial (*blue arrows*) accumulations of tumor cells (*E*), and periventricular accumulations of tumor cells (*F*). (*G, H*) Histologic features characteristic of high-grade mouse oligodendrogliomas. (*G*) Low-power view of the mouse PDGF-induced oligodendroglioma containing areas of microvascular proliferation (*red arrows*) and pseudopalisading necrosis (*blue arrows*). (*H*) High-power view of tumor microvasculature (*red arrows*) and pseudopalisading necrosis (*blue arrow*).

astrocytes to a progenitor-like morphology [70]. Retroviral *PDGDB* transfer into nestin-positive progenitors of *ink4a/arf* null mice in the same system results in higher grade gliomas, confirming PDGF's ability to cooperate with tumor suppressor loss during gliomagenesis [69]. Although activation of Erk and Akt pathways is low in these gliomas, activation of Akt in combination with PDGFB overexpression during glioma initiation and progression results in astrocytic tumors, indicating the contribution of PDGF to gliomagenesis from nestin-expressing progenitors with not only oligodendrocytic but also astrocytic characteristics [33].

The RCAS model of PDGF-mediated gliomagenesis unravels interesting parallels between amounts of PDGF and glioma biology. Deletion of PDGF-B mRNA 5′ untranslated region (UTR) results in elevated protein levels in vivo, indicating potential regulation of PDGF by translational mechanisms during gliomagenesis in human beings [71,72]. Elevated levels of PDGF correlated with induction of higher grade tumors with reduced latency as well as higher order tumor structures (eg, microvascular proliferation, pseudopalisading necrosis), and recruitment of vascular smooth muscle cells necessary for tumor angiogenesis. Furthermore, use of small-molecule PDGF inhibitors indicated that PDGF signaling is necessary to maintain high-grade tumor characteristics in this model [71].

Brain tumor recruitment

Understanding mechanistic aspects of gliomagenesis is indispensable for identifying molecular targets for glioma treatment. The difficulty in successfully treating brain tumors lies in the lack of understanding of their origins, encompassing the diversity of cell types composing the tumor, and proper targeting of complex tumor cell interactions with each other and their environment. Although many still view brain tumors as separate clonal and somewhat alien entities arising in the brain, consuming all resources and negatively interacting with the adjacent brain, the tumor microenvironment has recently been shown to play a crucial role in tumorigenesis and to behave as a positive active player in tumor progression and metastasis [73,74]. The origins of brain tumors are still under debate; nonetheless, some propose that gliomas most likely arise from transformed neural or bone marrow stem cells or from transdifferentiating somatic cells, a process potentially mediated by horizontal gene transfer, cell fusion, or aneuploidy [75]. The existence of various normal cell types migrating into the tumor and documentation of their extensive differences when compared with their non–tumor-stimulated normal counterparts as well as careful study of the cellular composition of modeled gliomas suggest additional nonclonal complexity characteristic of gliomagenesis.

Glioma microenvironment: stromal brain cell recruitment

Interactions between a tumor and its microenvironment normally include contributions from the ECM, fibroblasts (a major stromal component in sites other than the CNS), and macrophages and other infiltrating cells of the immune system as well as tumor vasculogenesis and lymphangiogenesis. Brain tissue differs from other tissues, however, in that it contains a distinct ECM and specialized immune-like phagocytic cells called microglia that are involved in various developmental processes (apoptosis, axon growth, and vasculogenesis) but lacks fibroblasts and a lymphatic system (Fig. 4A, B). Some functions normally performed by fibroblasts in other tissues are fulfilled by astrocytes in the brain; because of the absence of a lymphatic system, brain tissue is considered immunoprivileged; lacks the immune response found in other tissues; and depends on the blood-brain barrier (BBB) to protect its environment from foreign invaders and toxic substances, efflux waste products and regulate ionic traffic (see Fig. 4A) [76]. ECM components have profound effects on a variety of cellular functions, such as motility, survival, and proliferation; disruption of integrin-mediated ECM adhesion triggers signaling pathways leading to cell apoptosis or anoikis if not followed by readhesion [77,78]. During normal brain development, joint PDGF and integrin signaling promotes proliferation of OPCs; vitronectin binding of $\alpha v\beta 3$ integrin results in PI3K signaling, which is enhanced by PDGF signaling to integrin receptors by way of the PKC pathway [79]. In addition, integrin signaling that depends on the composition of the ECM modulates growth factor receptor signaling in tumor cells. ECM remodeling immediately adjacent to the invading tumor cells is necessary for local invasion and is mostly mediated by inhibitors and enzymes produced by the host cells [80–82]. In addition, some integrins have been proposed

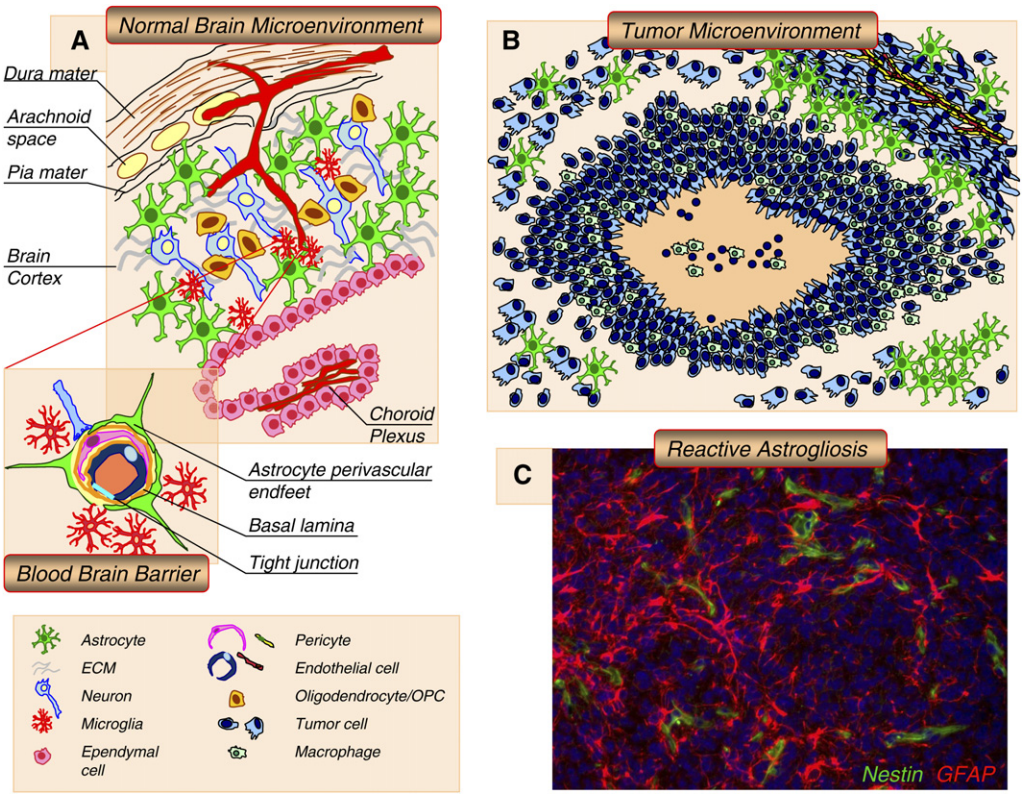

Fig. 4. Variety of cell types present in the normal brain and characteristic of the brain tumor microenvironment. (*A*) Normal brain microenvironment schematic indicates a variety of cell types characteristic of the normal brain, as defined in the legend located at the lower left of the figure. OPC, oligodendrocyte precursor cell. The figure inset indicates cellular constituents of the blood-brain barrier formed by capillary endothelial cells, surrounding basal lamina, and astrocyte perivascular endfeet as well as pericytes and microglial cells present in the same niche. (*B*) Brain tumor microenvironment schematic shows an area of pseudopalisading necrosis (*middle*) with infiltrating macrophages, vasculature-associated tumor cells (*top right*), and a tumor blood vessel. (*C*) Reactive astrogliosis: immunofluorescent staining for GFAP (*red*) and nestin (*green*) of the PDGF-induced mouse oligodendroglioma, indicating the presence of "reactive" astrocytes in the tumor area.

to be involved in a particular subtype of multidrug resistance (MDR) called cell adhesion–mediated drug resistance (CAM-DR) [83].

It is well accepted that tumor cells of various cancers migrate into adjacent normal tissue. In gliomas, diffuse infiltration of brain parenchyma is directly evident from T2-weighted MRI, involves trapping of normal neurons and astrocytes in the tumor, and results in the formation of secondary Scherer structures. The presence of reactive astrocytes in glioma tissue (eg, astrogliosis, glial scarring), as shown in Fig. 4C, occurs as a homotypic injury response of astrocytes regardless of their location in the brain or time in development, and it was first documented almost 50 years ago [84]. Recent evidence has indicated that formation of these rigid growth-inhibitory glial scars might be mediated by the S100A4 function and that some of the genes expressed in reactive astrocytes might show specific antiglioma effects on targeted delivery to the tumor cells [85,86]. Conversely, in response to everlasting stimulation by trophic signals produced by malignant cells, host cells are likewise continuously induced to invade the tumor and become a part of its microenvironment; in regions of diffuse invasion, the distinction between invading and being invaded and between tumor and tumor stimulated becomes blurred [73,87]. In other cancers, fibroblasts are known to be active players at the invading tumor front, producing tumor cell chemoattractants (scatter factor [SF]/hepatocyte

growth factor [HGF]) and latent enzymes (matrix metalloproteinases [MMPs] and urokinase plasminogen activator [uPA]) that modify secreted growth factors, degrade ECM, and release ECM-trapped growth factors (epidermal growth factor [EGF] and transforming growth factor [TGF]-β) and cryptic Arg-Glu-Asp RGD protein interaction sites involved in integrin signaling [73,74]. Macrophages are known to produce angiogenetic factors (VEGF and angiopoietins [ANG] 1/2), growth factors (EGF, TGFβ, interleukin [IL]-8, tumor necrosis factor [TNF]-α, and colony-stimulating factor [CSF]-1), and proteases (uPA, MMP-9, cathepsins, and heparinase); to organize mast cells and neutrophils; and even to initiate or promote tumorigenesis by synthesis of estrogens and mutagens (reactive oxygen and nitrogen-oxide radicals). Recent reports support causal links between chronic inflammation and tumorigenesis in several cancer types [88].

Because of low oxygen diffusion within a tissue, formation of tumor vasculature is essential for tumor development over a distance of a few hundred cell diameters and is characterized by PDGF involvement [89]. Tumor vascularization includes tumor vasculogenesis mediated by circulating endothelial progenitor cell (EPC) recruitment and neoangiogenesis from the preexisting blood vessel network [90,91]. Although the relative contribution of vasculogenesis versus angiogenesis and the exact contribution of EPCs to tumor vessels are still under debate, it is well established that vascular maturation requires interactions between endothelial cells and pericytes (structural support cells producing survival signals) and that tumor vasculogenesis is mediated by EPC homing and unfolding of a PDGF-mediated vasculogenic program [90]. Evidence of PDGF involvement in glioma vasculature formation has been documented using xenograft glioma models; although PDGFB did not result in enhanced proliferation of the U87MG glioma cell line in vitro, stable PDGFB expression significantly enhanced its in vivo tumorigenicity on orthotopic transplantation, in part, by increasing VEGF expression in tumor endothelial cells and promoting pericyte recruitment [92]. Additionally, PDGFB effects on recruitment of smooth muscle cells have been documented in genetically engineered models (GEMs) (briefly described in the previous section) [71]. Thus, targeting tumor vasculature by perturbing recruitment, activation of, and interaction between cell types necessary for vessel formation might prove a useful strategy in glioma treatment. PDGF-BB has recently been shown to be a direct lymphangiogenetic factor, overexpression of which correlated with intratumor lymphangiogenesis and lymphatic metastasis, and it provides a therapeutic target in non-CNS tumors [93]. It should also be noted that gliomas frequently exhibit breakage of the BBB and leaky blood vessels, which correlates with downregulation of the tight junction protein claudin 1/3 involved in preventing cargo traversal through the layer of epithelial cells and redistribution of the AQP4 and Kir 4.1 in astrocytic feet, ultimately resulting in $K+$ channel rectification and brain edema [76,94–96]. Glioma vasculature is also being constantly remodeled, vessel establishment followed by regression, leading to the ''wounds that never heal'' view of gliomas [97]. The reported immunocompetence of gliomas might be caused by significantly reduced leukocyte-endothelial cell interactions potentially resulting from diminished leukocyte flux and the immaturity of blood vessels lacking the necessary adhesion receptors [98]. Most of the macrophages and lymphocytes are thought to migrate into the tumor through the interstitial space rather than through transvascular emigration. Another variable involved in glioma BBB leakage is the permeability of the basal lamina surrounding the microvessel, which seems to correlate with the loss of agrin, a heparan sulfate proteoglycan constituent of the basal lamina, and overexpression of tenascin, an ECM molecule absent from mature brain tissue and present in the tumor vessel basal lamina [99].

More generally, tumor stromal cells have been shown to affect antitumor drug delivery adversely because of their effects on vascular flow and interstitial fluid pressure (IFP); they have also been proposed to provide support to tumor cells after therapy [74,100]. Drug effectiveness, which ultimately depends on uniform distribution, ability to cross the BBB and tumor ECM, and sufficient uptake by targeted cells, is severely reduced by the disorganized nature of dilated, leaky, and immature tumor vasculature. Aberrant structure of tumor vessels results in unequal and perturbed blood flow within the tumor as well as elevated IFP in the tumor core because of abnormal ECM composition, interstitial space contraction, and lymphangiogenesis [101]. In several tumor models, PDGF has been implicated in regulation of tumor IFP because of its effects on tumor stroma, a consequence somewhat alleviated by using PDGFR and PDGFR/VEGFR inhibitors [102].

Another question that comes to mind is how ''normal'' tumor-attracted and perpetually

stimulated normal cells actually are. In contrast to tumor cells, normal cells within the tumor are proposed to be genetically stable; yet, multiple lines of evidence from other tumor types indicate that tumor fibroblasts, macrophages, endothelial cells, and pericytes actually exhibit characteristics different from their normal tissue counterparts [73,74]. Tumor-resident fibroblasts, for example, are "activated," show high proliferation rates and markers of smooth muscle differentiation, and can enhance malignant transformation of epithelial cells. Conversely, normal tissue fibroblasts can prevent progression or even revert the phenotypes of transformed epithelial cells [103–105]. Macrophages have been shown to undergo processive maturation within the tumor, shifting from their normal antitumor functions (presenting tumor antigens to T cells or direct killing of tumor cells) to promoting tumor progression and metastasis [88]. This abnormal maturation is attributable to the immunosuppressive tumor microenvironment, which is rich in chemokines that prevent dendritic cell differentiation and actually render macrophages tumor-trophic rather than exhibiting anti tumor effects. In the case of brain tumors, glioma endothelial cells show differential MDR and MMP activity as well as increased angiogenic capabilities as compared with their normal counterparts [106,107]. Glioma-resident reactive astrocytes are induced to produce CXCL16, which is usually expressed only by dendritic and vascular cells. Normally, this chemokine is a transmembrane ligand mediating chemotaxis and adhesion of activated T cells; in the glioma environment, it is being shed into the interstitial space and potentially "quenches" CXCR6 receptors on T cells, preventing their anti-immune functions [108]. Moreover, glioma cells "exploit" astrocytes to facilitate their own invasiveness by supplying plasminogen and activating the uPA-plasmin cascade in astrocytes, which results in conversion of plasminogen to plasmin, activation of pro–MMP-2 produced by reactive astrocytes, and subsequent ECM degradation necessary for ECM invasion [109].

Neural stem cells: brain tumor tropism

It has long been thought that brain development is essentially complete at the end of embryonic life and that adult brain tissue is static and nonrenewing. Recent research has shown that adult brain undergoes continuous neurogenesis throughout life [110–113]. This essential process has been shown to occur in various organisms, ranging from birds to mammals (including human beings), and is generally mediated by NSCs and PCs present in certain areas of the adult brain, such as the subventricular zone (SVZ), dentate gyrus (DG), and olfactory bulb (OB) (Fig. 5) [113–120]. Although the SVZ and DG contain resident neural stem cells (NSCs), other areas, such as the OB, undergo neurogenesis mediated by NSC/PCs migrating into the area [117,119,121]. Organization of the adult SVZ reflects the hierarchic progression of cell differentiation from the NSCs to transit-amplifying progenitor cells (TAPs) and then to neuroblasts, which enter the rostral migratory stream (RMS) to migrate to the OB or travel into periventricular regions as a part of maintenance neurogenesis (see Fig. 5A, C). The SVZ is known to contain several GFAP-expressing type B cells that can fully reconstitute chemically ablated SVZ, and are therefore referred to as NSCs, as well as larger numbers of multipotent type C TAPs and type A neuroblasts (neuronal restricted progenitors) [117]. The subgerminal zone (SGZ) is similarly organized and contains type B cells (putative DG NSCs shown to act as NSCs in vitro), which give rise to D cells (dark cells and immature progenitors), which, in turn, divide and differentiate into mature granule neurons (see Fig. 5B, D, E) [122]. The number of NSCs decreases progressively during embryonic life and reaches low numbers comparable to the adult brain at midgestation. In the adult brain, NSCs, defined by their ability to self-renew and give rise to all neuronal and glial cell types present in the brain, amount to less than a few percent [116].

In addition to "maintenance" neurogenesis, the adult brain exhibits a rudimentary ability to regenerate certain cell types and structures in response to stress. The regenerative potential of the adult brain has been studied using various models of brain injury, including stab injury, total or focal brain ischemia, and tumorigenesis. Some of the recent developments in the field have indicated that SVZ- and embryonic stem cell (ESC)–derived NSCs, NSC/PCs, and ependymal cells participate in a regenerative response to perinatal and adult hypoxia or ischemia, with their migration toward injury sites potentially mediated by SCDF-1/CXCR4 [123–128]. Stress conditions, including trauma, ischemia, radiation, and epilepsy, result in increased proliferation of SVZ/sub-dentate gyrus (SDG) cells, and postischemia vascular and neuronal damage is reduced on transplantation of NSC/PCs or

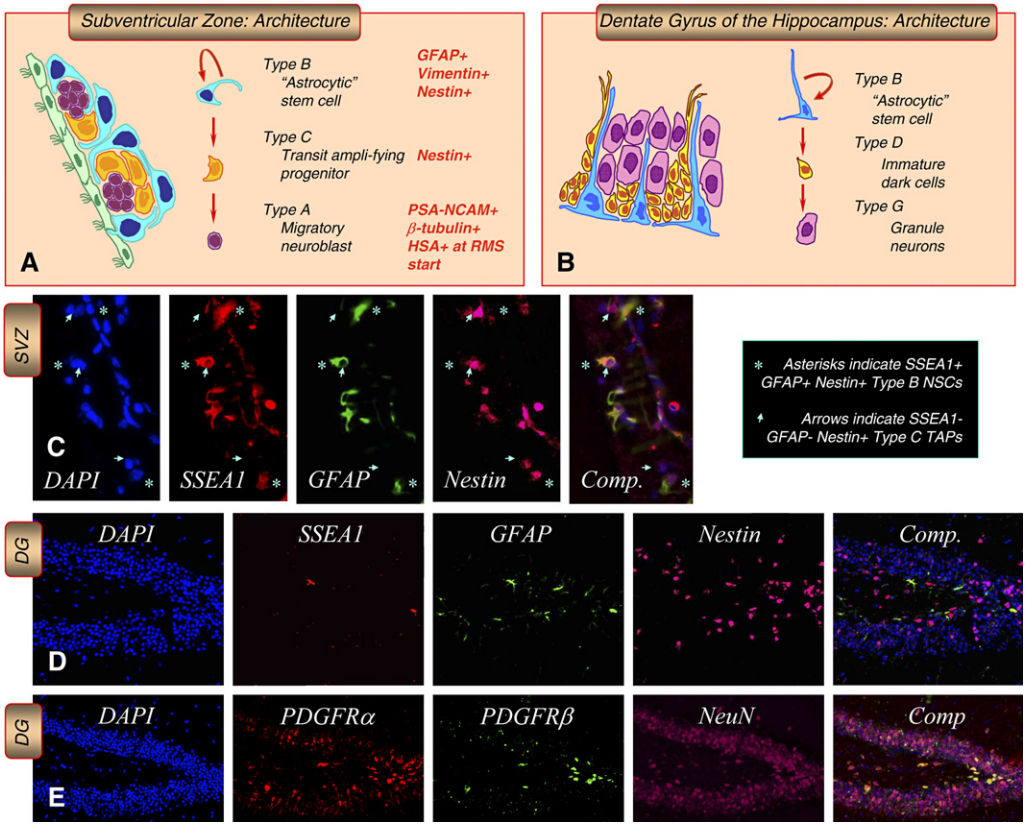

Fig. 5. Architecture and cellular constituents of the neural stem cell (NSC) niches in the adult mammalian brain. (*A*) Schematic representation of the adult SVZ composition. The SVZ is located at the side wall of the lateral ventricle and includes slowly dividing type B astrocytic NSCs that give rise to rapidly dividing (transit-amplifying) type C progenitor cells, which, in turn, produce type A neuroblasts. HSA, heat-stable antigen, mCD24a; NCAM, neural cell adhesion molecule; PSA, polysialic acid. (*B*) Schematic representation of the adult DG of the hippocampus. Dividing type B astrocytes give rise to the "dark" type D cells, which, in turn, produce new mature type G granule neurons. (*C*) Immunofluorescent staining of the adult SVZ with GFAP (*green*), nestin (*purple*), and SSEA-1 (*red*; NSC marker). Type B NSCs marked by asterisks are triple-positive for all three markers and are characterized by lower nestin expression, whereas the transit-amplifying type C cells marked by arrow do not express GFAP or SSEA-1 but stain intensely with nestin. Comp., composite. (*D*) Immunofluorescent staining of the adult DG of the hippocampus with GFAP (*green*), nestin (*purple*), and SSEA-1 (*red*). SSEA-1 is expressed in a subset of GFAP-positive type B cells; nestin labels the type D dark cells. (*E*) Immunofluorescent staining of the adult DG of the hippocampus with PDGFRs and NeuN (*purple*). Only a small subset of NeuN-expressing cells show PDGFRα (*red*) expression, a small fraction of which also stain for PDGFRβ (*green*).

VEGF-secreting NSCs and on administration of hematopoietic cytokines or VEGF with subsequent NSC transplantation [129–132]. Several studies of stab injury or focal ischemia point at upregulation of the basic helix-loop-helix (bHLH) transcription factor olig2 negatively correlating with neurogenesis and an astrogliogenic response in the adult SVZ mediated by Notch1 pathway activation [133,134]. Some of the signaling pathways mediating proapoptotic injury response include JNK, p53, IkappaB kinase (IKK) apoptosis-inducing factor (AIF), and PKC [135–140], whereas neuroprotective signaling is mediated by nerve growth factor (NGF) or erythropoietin (Epo) activation of Erk, Akt, and PI3K as well as NFκB, Cox2, Stat3, and p38 [141–148]. It is also worth noting that although normal cells, including NSC/PCs, migrate toward the injury sites and attempt to regenerate brain tissue, reactive astrogliosis along the infarct border directly correlates with the delayed phase of infarct expansion [149].

The first reports indicating implanted NSC tropism toward gliomas came from the xenograft mouse models. These reports demonstrated extensive ability of the labeled and implanted NSCs to migrate in juxtaposition to tumor cells and distribute themselves throughout the tumor mass on orthotopic and intravascular transplantation as well as capability for stable expression of certain genes (eg, herpes simplex virus-1 [HSV-1], TNF-related apoptosis–inducing ligand, IL-12) to provide means for antitumor therapy and development of prolonged antitumor immunity [150–153]. More recent reports indicated that implanted NSCs preferentially target intra- and extracranial tumors of neural and nonneural origin as compared with normal tissue and that tumor tropism is partially induced by VEGF and angiogenically activated tumor microvasculature [154,155]. Additional in vitro experiments indicated involvement of glioma ECM composition and specific ECM proteins expressed at the invasion front in modulating NSC behavior, accentuating the potential effects of the tumor microenvironment on tumor-trophic behavior of NSCs [156]. Furthermore, a recent study indicated that SVZ-derived nestin-positive endogenous precursor cells migrated toward and surrounded implanted brain tumor cells, starting to express markers of early committed and noncommitted precursors, and that their presence correlated with antitumorigenic effects in vivo and in vitro [157]. Another study demonstrated that a tumor-tropic NSC population was largely composed of astrocytic progenitors expressing CXCR4, blockade of which inhibited NSC migration toward the tumor [158]. Taken together, these studies indicate that because of the growth factor–rich tumor microenvironment, various populations of NSC/PCs are induced to migrate toward and into the tumor mass, a process that might occur during spontaneous gliomagenesis and involve endogenous stem cells/PCs.

Theories of gliomagenesis

Heterogeneous expression of stage-specific differentiation markers, often compared with cell heterogeneity characteristic of normal CNS development, and the presence of signaling abnormalities in genes or pathways implicated in stem cell proliferation and self-renewal (Notch, Shh, PTEN, EGF, and Myc) frequently found in human brain tumors make one wonder whether gliomas arise from NSCs [159]. The field of solid tumorigenesis is currently dominated by the cancer stem cell (CSC) theory, which argues clonal development of a phenotypically diverse cancer cell population differentiating unidirectionally from a single cell—the CSC (Fig. 6A). CSCs reportedly characterized by the stem cell properties of indefinite proliferation, self-renewal (which allows the accumulation of mutations), and tumorigenicity as well as by an ability to re-establish a tumor phenotypically similar to the original on transplantation were proposed to arise from a stem cell with dysregulated stem cell properties or a differentiated cell that acquired mutations allowing for its dedifferentiation to a stem cell–like state [160]. CSCs isolated from various types of glial and nonglial CNS tumors, including glioblastoma multiforme (GBM) and medulloblastoma, have been extensively characterized in regard to their differentiation properties and tumorigenicity; CSCs were also proposed to be resistant to radiation and chemotherapy and responsible for tumor recurrence [160–165]. More recent publications suggest additional possibilities for the origins and mechanisms of CSC derivation [75]. Some of these alternatives include tissue-specific stem cells, bone marrow stem cells, or transdifferentiating somatic cells giving rise to the CSCs in a process potentially mediated by cell fusion of cytoplasmic or nuclear material (resulting in the formation of syncytium, heterokaryons, or synkaryons that might undergo reductional division), horizontal gene transfer (including that of apoptotic bodies), or aneuploidy (an initiating effect as a result of carcinogenesis, as a result of fusion with an aneuploid cell, or as a result of abnormal reductional division).

Genetic analysis of human glioma samples shows them to be more or less clonal. Further, studies of human tumors and animal models of gliomagenesis indeed provide highly compelling but only correlative evidence that NSCs are likely to be the origin of gliomas [159]. For example, frequent tumor formation in the vicinity of the SVZ as well as a general likelihood of prototypic tumor-initiating lesions arising in neurogenic regions as compared with other areas of the brain on exposure to chemical carcinogens or viral oncogenes is taken as direct evidence for an NSC origin of the CSCs [159,166–169]. Yet, careful lineage tracing studies using faithful glioma models have not been published. In fact, mouse modeling experiments show that depending on the combination of mutations, gliomas can arise from NSC/PCs and fully differentiated cells, arguing against the previous hypothesis. For example, although combined activation of Ras and Akt is

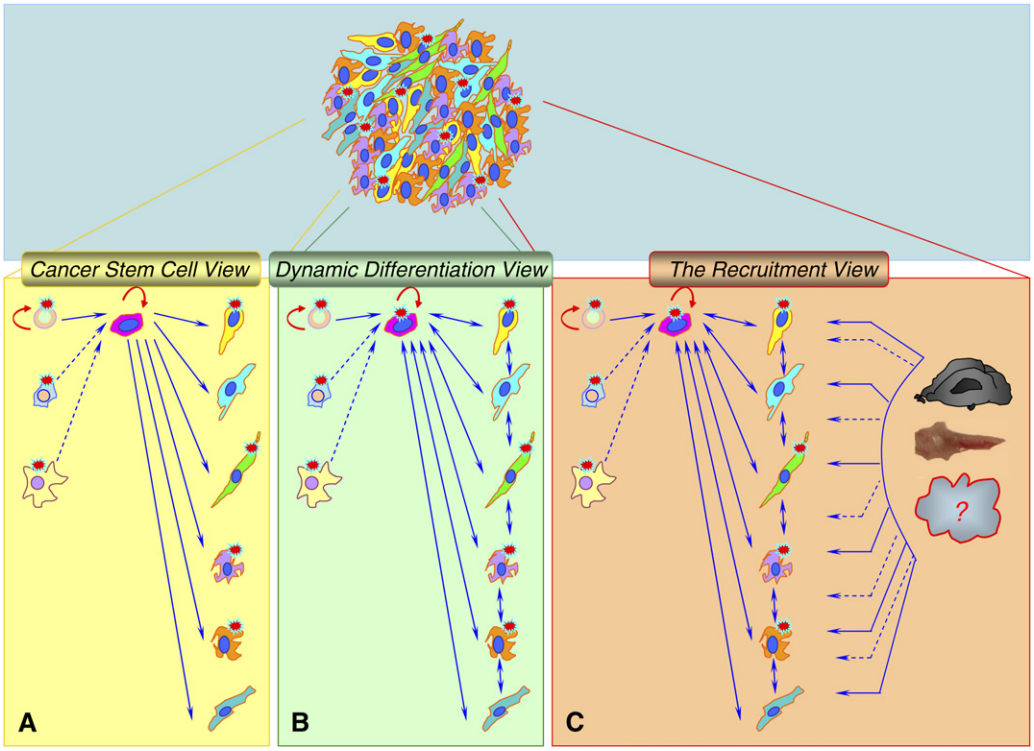

Fig. 6. Views of tumorigenesis. (*A*) CSC view proposing unidirectional development of a clonal tumor cell population. All cells within the tumor are derived from the originating CSC arising from a mutated normal stem cell or a differentiated cell, and thereby contain all mutations found in this cell. (*B*) Dynamic differentiation view proposing multidirectional development of a clonal tumor cell population. As in the CSC view, all progeny of the cell of origin contain mutations found in the originating cell. This model adds the possibility of a changing lineage and differentiation state in tumor cells depending on acquisition of mutations, and allows the stem-like characteristic to be a result of tumor progression rather than a necessary characteristic of its initiation. (*C*) Recruitment view proposing multidirectional development of a nonclonal tumor cell population. This view proposes that brain tumors contain the progeny of the cell of origin and the recruited ''normal'' cells that are paracrine stimulated by the progeny cells. All progeny cells contain mutations of the originating cell and are characterized by the dynamic lineage and differentiation state. A part of the tumor is contributed by recruited cells, which may be recruited at various stages of the normal differentiation lineage and may contain mutations different from the progeny cells.

sufficient to generate gliomas from nestin-positive PCs, they can also be derived from GFAP-positive astrocytes on additional overexpression of the *Myc* oncogene or loss of the *ink4a*/*arf* tumor suppressor locus, both of which are sufficient to induce or maintain undifferentiated characteristics in vitro [170,171]. The caveat of the system is that although infection of nestin-expressing cells exclusively targets an NSC/PC subset of brain cells (including type B NSCs and type C TAPs), the GFAP expression pattern causes targeting of fully differentiated astrocytes and the bona fide type B NSCs. Nevertheless, it should be noted that NSCs are mostly nondividing quiescent cells that are much lower in number and that because retroviral-mediated gene transfer in this system requires cell division, gliomagenesis is much more likely to occur in GFAP-expressing, frequently dividing, and fully differentiated astrocytes [116,117]. These data also indicate that the presence of more mutations is necessary to induce glioma formation from more differentiated cells. In addition, if CSCs arose directly from NSCs, which, in principle, need a single (or a few) mutation(s) in genes or proteins involved in regulation of stem cell proliferation or self-renewal to become cancerous, why would early gliomas exhibit a variety of genetic mutations and chromosomal abnormalities? Chromosomal analysis of brain tumor CSCs derived from gliomas and

medulloblastomas indicate that they are aneuploid and show different karyotypes, and it has been argued that these CSCs might be derived from a mutated NSC that fused with an aneuploid cell or acquired aneuploidy as a result of cell fusion and reductional division after it [75]. Yet, most researchers believe that oncogenic mutations are rare events and that a normal cell requires four to seven of these events to become cancerous [172]. Although the CSC theory has been supported to a great extent in hematologic malignancies, the existence of CSCs in gliomas is mostly substantiated by a retrospective analysis of tumor cells addressing their tumorigenicity, gene expression, and clonogenic capacity, for example [161–165]. Nevertheless, it should be noted that tumor cells are known to express a variety of genes (likely including the stem cell markers) aberrantly, and therefore cannot be categorized as any cell type present during normal development. Likewise, it is not clear whether extensive in vitro transdifferentiation abilities of normal and tumor cells are necessarily applicable to gliomagenesis [173–176]. In addition, it has been proposed that the neurosphere assay commonly used to identify and isolate NSCs as well as CSCs might, in fact, select for highly proliferative cells more similar to the TAPs of the nervous system rather than the actual stem cells [177].

An additional implication of the CSC theory is that because CSCs always represent the most undifferentiated cell type present in the tumor, the tumor cell population is derived by unidirectional differentiation from the CSCs (see Fig. 6A). Nevertheless, it is well established that gliomas tend to acquire more undifferentiated characteristics as they progress toward malignancy rather than being characterized by extremely undifferentiated characteristics as they initiate. Mouse modeling experiments help to identify undifferentiated characteristics of gliomas and expression of early genes as a consequence of tumorigenesis, indicating that changes in signaling directly affect the apparent lineage and differentiation state of brain tumor cells. For example, PDGF-stimulated oligodendroglioma cells shift toward astrocytic morphology on activation of Akt, whereas astrocytoma cells shift to oligodendroglial morphology on treatment with mTOR inhibitors in vitro and in vivo [178]. Therefore, heterogeneous expression of stage-specific differentiation markers might merely reflect inherent regional instability in signaling and gene expression patterns typical for cancer cells rather than multipotential tumor origins (Fig. 6B).

Unfortunately, analysis of human gliomas cannot inherently demonstrate tumor cell(s) of origin or provide a definitive answer as to the extent of their involvement in tumor development. Interestingly, careful study of gene expression profiles of recurrent human tumors that arose after surgical resection and therapy revealed that some of the chromosomal deletions found in the original tumor resection were absent from the recurrent tumor [179]. These results would argue that the recurrent tumor did not arise from the same CSC progeny cell(s) that gave rise to the original tumor but, instead, were derived from some other cells recruited into the tumor and potentially acquired additional mutations while being a part of the tumor environment (Fig. 6C). The CSC model cannot account for this phenomenon, because tumor clonality is one of its cornerstones, which translates into the inevitable presence of all mutations found in the CSC-derived original tumor in its recurrent counterpart. Detailed analysis of PDGF expression in modeled oligodendrogliomas supports nonclonal complexity characteristic of gliomagenesis [71]. PDGF expression in the tumors is not uniform and generally correlates with higher grade tumor structures, such as microvasculature and pseudopalisading necrosis; certain cells show intense PDGF expression, whereas others exhibit PDGF amounts less than the level of detection and may be paracrine stimulated by the PDGF-producing cells (Fig. 7) [71]. Differential vulnerability to mutation acquisition could be present in the autocrine-stimulated progeny cells versus the paracrine-stimulated recruited cells; yet, it might be possible for the recruited cells to acquire additional mutations during tumor progression, implicating them in tumor recurrence. In this system, the appearance of ''recurrent tumor CSCs'' or, more generally, a cell population showing characteristics of CSCs might thus be a direct consequence of the acquisition of dedifferentiating mutations in the recruited population rather than the actual cause of original tumorigenesis.

Summary

The roles of various members of the PDGF family during development are mirrored by a variety of functions of PDGFs during gliomagenesis. Elevated transcription and increased protein levels of these growth factors have been correlated with several types of cancer, including gliomas.

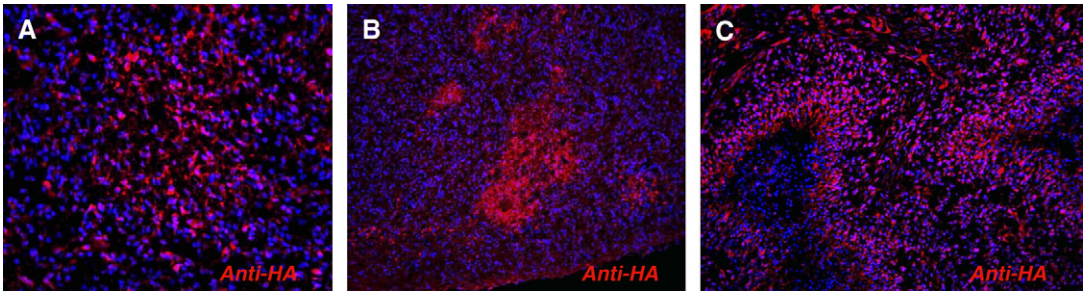

Fig. 7. Nonuniform PDGF expression in the PDGF-induced oligodendrogliomas of RCAS-tv-a mouse model using somatic cell gene transfer–mediated gliomagenesis. (*A*) Region of the low-grade oligodendroglioma is characterized by the nonuniform expression of PDGF, as assessed by the anti-hemagglutinin (HA) staining detecting the tag present on the PDGF sequence used to initiate the tumor. Some tumor cells show intense staining, and thus produce high levels of PDGF, whereas others show PDGF levels lower than the level of detection. (*B*) Region of the high-grade PDGF-induced tumor area shows the correlation between the increased amounts of PDGF (as assessed by the anti-HA staining) and microvascular proliferation. (*C*) Region of the high-grade tumor area contains pseudopalisading necrosis and vascular-associated cells showing nonuniform PDGF expression, which is somewhat higher in cells composing the rim of the pseudopalisading and contributing to the perivascular areas.

PDGF overexpression has been shown to be causal in glioma formation in GEMs, regionally variable, and quantitatively correlated with higher grade tumor structures. To some degree, these effects may be attributable to the ability of PDGFs to cause phenotypic dedifferentiation or prevent glial cell differentiation. Activation of signaling pathways downstream of the PDGFRs has been shown to be causal in glioma initiation, whereas activation of other signaling pathways correlated with glioma progression.

Similar to normal development, the action of PDGFs during gliomagenesis is not only autocrine but paracrine in nature. PDGF production by glioma cells can stimulate its expressors and recruit other cell types into the tumor. PDGF has been implicated in regulating tumor vasculogenesis mediated by the recruitment of EPCs, pericytes, and vascular smooth muscle cells and in tumor IFP by its effects on tumor stroma. Additionally, brain tumors are known to contain implanted as well as endogenous NSC/PCs attracted to the growth factor–rich glioma environment, migrating in juxtaposition and homing to glioma cells.

The realization of the importance of interaction between the brain tumor microenvironment and tumor cells and active recruitment of nonstromal cell types leads to a more complex view of brain tumor formation. Although the popular CSC theory proposes the existence of a clonal brain tumor, the ability of various mutations and PDGF signaling to alter the apparent lineage and differentiation state and extensive paracrine modes of action of PDGF suggest additional nonclonal complexity during gliomagenesis. Although the existence of a normal stromal component may certainly be present in the tumors, the recruitment view of brain tumorigenesis proposes the possibility of nonclonal tumor development mediated by perpetual growth factor stimulation. The recruitment of nontumor cell types and the possibility of mutations arising in the recruited cell population allow for its expansion and alteration of the cellular composition of the tumors.

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