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Platelet-derived growth factor receptor alpha in glioma: a bad seed

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Abstract

Recent collaborative, large-scale genomic profiling of the most common and aggressive brain tumor glioblastoma multiforme (GBM) has significantly advanced our understanding of this disease. The gene encoding platelet-derived growth factor receptor alpha (PDGFRα) was identified as the third of the top 11 amplified genes in clinical GBM specimens. The important roles of PDGFRα signaling during normal brain development also implicate the possible pathologic consequences of PDGFRα over-activation in glioma. Although the initial clinical trials using PDGFR kinase inhibitors have been predominantly disappointing, diagnostic and treatment modalities involving genomic profiling and personalized medicine are expected to improve the therapy targeting PDGFRα signaling. In this review, we discuss the roles of PDGFRα signaling during development of the normal central nervous system (CNS) and in pathologic conditions such as malignant glioma. We further compare various animal models of PDGF-induced gliomagenesis and their potential as a novel platform of pre-clinical drug testing. We then summarize our recent publication and how these findings will likely impact treatments for gliomas driven by PDGFRα overexpression. A better understanding of PDGFRα signaling in glioma and their microenvironment, through the use of human or mouse models, is necessary to design a more effective therapeutic strategy against gliomas harboring the aberrant PDGFRα signaling.

Keywords

Gliomas; PDGFRα signaling; glioma tumorigenesis

Malignant brain tumors are relatively rare but deadly due to their location and highly invasive capacity, rendering them inaccessible to surgical resection and resistant to current therapies[1,2]. Clinically, these tumors are classified based on the predominant cell type as determined by histological approaches[3]. Among them malignant gliomas originating from glial cells represent the most common and aggressive type of tumor in the central nervous system (CNS)[2,4]. Malignant gliomas in adults can be histologically classified into

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astrocytomas, oligodendrogliomas and mixed oligoastrocytomas. Further, within each type they can be divided into 4 grades corresponding to their malignancy and genetic alterations (WHO grade I–IV) (Figure 1). Despite intensive treatments including maximal surgical resection combined with radiotherapy and concurrent or adjuvant chemotherapy, the median survival of patients with grade IV glioblastoma multiforme (GBM) remains 13 to 16 months after diagnosis[5]. Clinical GBMs are composed of poorly differentiated glial cells with features such as uncontrolled growth, resistance to apoptosis, diffuse infiltration, cellular pleomorphism, nuclear atypia, mitotic abnormalities, microvascular proliferation and focal necrosis[2,3]. Based on genetic and clinical presentation, GBM has been classified into two subtypes. Primary GBMs, which arise *de novo* with short to no clinical history, predominantly occur in older patients, whereas secondary GBMs develop from lower-grade gliomas often occur in younger patients[2,4] (Figure 1).

Over the past decade, data have accumulated characterizing genetic alterations in human gliomas, including the activation of oncogenes and the inactivation of tumor suppressor genes[2,4,6–8]. It appears that in human gliomas, these genetic alterations occur in a pattern corresponding to distinct histologic subtypes and different grades of tumors. For example, it is believed that low-grade astrocytomas and oligodendrogliomas may develop from common glial progenitor cells that acquire features of astrocytic tumors in the presence of *TP53* mutations and oligodendrocytic tumors in the presence of 1p/19q chromosomal loss[7] (Figure 1). Additionally, platelet-derived growth factor receptor alpha (*PDGFRA*)/*PDGF-A* overexpression and isocitrate dehydrogenase 1 (*IDH1*) mutations are some of the major genetic alterations found in low-grade gliomas as well as secondary GBMs. When the low-grade tumors progress toward the high-grade secondary GBMs, additional changes such as CDK-dependent kinase inhibitor (*CDKN*) *2A*/*CDKN2B* deletion are acquired (Figure 1). In the primary GBMs, however, a distinct set of genetic changes are observed, such as epidermal growth factor receptor (*EGFR*) amplification/mutation, phosphatase and tensin homolog (*PTEN*) mutations/deletion and Mdm2 p53 binding protein homolog (*MDM2*) overexpression (Figure 1), suggesting a different cell-of-origin of these tumors is responsible for generating primary GBMs[2,4,6–8].

In this review, we briefly discuss PDGF signaling with an emphasis on PDGFRα signaling and its role in normal glial cell development. We then summarize the evidence obtained from various studies of human glioma tissues and animal models of PDGF-induced tumorigenesis. Lastly, we provide insights into how new treatments targeting PDGF signaling will benefit patients with this subset of brain tumors.

PDGFRα Signaling

PDGF was purified as a molecule released by platelets into the whole blood serum, stimulating the proliferation of various mesenchymal[9–11]and glial cells[12,13]. The mitogenic effect of this growth factor requires that target cells express the receptors for PDGF (PDGFR)[14]. The PDGF family consists of four ligands, PDGF-A, -B, -C, and -D, and two receptors, PDGFRα and PDGFRβ. The structures of PDGF-A and -B are largely similar, consisting of a PDGF/VEGF core domain with conserved cysteine residues called the cysteine knot motif and an N-terminal propeptide region that is removed intracellularly for activation prior to secretion[15]. Additionally, in PDGF-B and a membrane-bound, long alternative splice form of PDGF-A, there is a C-terminal basic “retention motif” that can interact with haparan sulfate proteoglycans of the extracellular matrix (ECM)[16]. The retention motif needs to be cleaved prior to secretion of the ligand. Expression of long- or short-form PDGF-A results from alternative splicing of exon 6 of *PDGFA* and is cell-type specific. Human glioma cells produce mainly the long form[17], whereas normal human endothelial cells express the short form of PDGF-A. Interestingly, the differential alternative

splicing of PDGF-A might determine its mitogenic capacity in these cells[17]. The two newly found PDGFs, PDGF-C and PDGF-D, contain a distinct N-terminal domain called the CUB domain. It is cleaved extracellularly after secretion of the growth factors and this cleavage is important for their activity[18]. PDGFRs share a common structure including five extracellular immunoglobulin (Ig) loops and a split intracellular tyrosine kinase (TK) domain separated by a kinase insert region[15,19]. Similar structures can also be found in other receptor tyrosine kinases (RTKs) such as vascular endothelial growth factor receptors (VEGFRs), c-Kit, c-Fms and fms-related tyrosine kinase 3 (Flt-3)[19].

PDGF ligands function as disulfide-linked homo- or hetero-dimers, PDGF-AA, PDGF-AB, PDGF-BB, PDGF-CC and PDGF-DD[18]. These dimeric isoforms are capable of binding to two structurally related RTKs with different specificities (PDGFRα and PDGFRβ)[18,20]. The binding of PDGF ligands induces dimerization of PDGFRs and juxtaposition of their intracellular tyrosine kinase domains leading to trans-autophosphorylation of multiple tyrosine residue sites[21]. The subsequent association between different SH2 domain–containing signaling molecules and phosphorylated tyrosine residues engages various downstream signaling cascades (Figure 2), gene transcription events and various cellular behaviors, such as cell proliferation, apoptosis, actin reorganization and chemotaxis[15,22]. The SH2 domain–containing signaling effectors include phosphoinositide-3-kinase (PI3K), phospholipase C (PLC)-γ, Src family kinase (SFK), protein tyrosine phosphatase SHP-2, GTPase-activating protein (GAP) for Ras, signal-transducer and activator of transcription proteins (STATs), as well as adaptor proteins such as growth factor receptor-bound protein (Grb)2, Grb7, SHC-adaptor protein (Shc), SH2/SH3 adaptor protein (Nck) and v-crk sarcoma virus CT10 oncogene homolog (Crk)[22]. PDGFRα and PDGFRβ bind to distinct but overlapping sets of these signaling molecules upon ligand stimulation. An allelic series of mutant PDGFRs have been generated and it appears that in comparison to PDGFRβ, PDGFRα relies more on the activation of specific signaling pathways to function properly in specific stages and organs during animal development[23,24]. For example, PI3K signaling is indispensable for PDGFRα during early development, whereas for PDGFRβ, disruption of PI3K alone has minimal effect on normal development[19]. Cytoplasmic domain swapping experiments further reveal that in contrast to PDGFRβ, it is not the intrinsic properties of the receptor but the ability of PDGFRα to engage specific signaling molecules that determines the activity and function of receptor signaling[25,26]. In this review, we focus on the downstream SH2 domain–containing signaling effectors of PDGFRα (Figure 2).

Members of the PI3K family consist of a regulatory p85 subunit and a catalytic p110 subunit. Upon association with the phosphorylated tyrosine residues 731 and 742 (Tyr-731/42) of the PDGFRα intracellular domain[15], PI3K is activated and produces phosphatidylinositol 3,4,5-triphosphate [PI (3,4,5)P_3 or PIP_3] in the plasma membrane. PIP_3 then recruits several downstream effectors such as Akt/PKB[15,22]. Activation of the PI3K pathway leads to several cellular effects including cell growth, survival, chemotaxis and actin reorganization[15,22] (Figure 2). PLC-γ shares the same substrate as PI3K. Upon association to Tyr-988 and -1018, it phosphorylates PIP_2 to generate inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG), which then mobilize intracellular Ca^{2+} and activates the PKC family, respectively[15,22] (Figure 2). PDGF-induced PLC-γ activation is responsible for cellular effects such as cell motility and growth. The full activation of PLC-γ, however, is dependent on PIP_3 generated by PI3K, since the recruitment of PLC-γ requires association of its pleckstrin homology (PH) domain with PIP_3 in the plasma membrane[15,22]. The SFK represents a family of tyrosine kinases including Src. Their binding sites on PDGFRα autophosphorylated tyrosine sites are Tyr-572 and -574. PDGFRα-mediated Src activation appears to be dispensable for mitogenic responses in some cell types[15,22] (Figure 2). SHP-2 is an SH2-containing protein tyrosine phosphatase that binds to phosphorylated Tyr-720 of activated PDGFRα. Its phosphatase activity can

potentially dephosphorylate the RTK and downstream effectors, leading to inactivation of these proteins. However, it has also been shown that SHP-2 is involved in the up-regulation of several effectors such as the Ras/MAPK and Src pathways, possibly through its role as an adaptor[15,22] (Figure 2). Crk is an adaptor that binds to phosphorylated Typ-702 of PDGFRα. Upon binding, it then activates the nucleotide exchange protein C3G and the subsequent activation of the JNK/SAPK pathway[15,22] (Figure 2).

An allelic series of *PDGFRA* tyrosine-to-phenylalanine mutations disrupting association between the RTK with different downstream effectors and signaling pathways have been generated[27]. This study demonstrated that PDGF-AA-induced DNA synthesis was strictly dependent on PI3K signaling only, whereas Src, PI3K and PLC-γ are required for PDGF-mediated chemotaxis with Src being the most important effector for cell motility[27]. Surprisingly, all PDGFRα mutants in the "add-back panel" that can only initiate one of the five downstream pathways were not able to restore the PDGF-induced chemotaxis to the level achieved by WT PDGFRα, indicating that activation of multiple effectors is required for full activity of the receptor to mediate certain cellular responses[27]. Using a similar strategy, PI3K and PLC-γ were shown to play predominant roles in preventing apoptosis of mesoderm cells during early *Xenopus* embryo development[28]. These studies suggest that distinct downstream effectors of PDGFR may be required for different cell types, species and stages of development. To further dissect the contribution of signaling pathways emanating from PDGFRα, Klinghoffer *et al.*[23] further generated knock-in mice that harbored one of the three mutants, PDGFRα-F7, PDGFRα-F731/42 or PDGFRα-F572/74. Interestingly, mice that homozygously harbored PDGFRα-F731/42, as well as PDGFRα-F7, displayed phenotypes comparable to PDGF-A- or PDGFRα-null animals, including growth retardation, skeletal and lung development abnormalities[23]. However, Src association with PDGFRα was only required for oligodendrocyte development, possibly due to the role of Src in promoting progenitor cell migration[23]. These experiments suggest that PI3K is the major effector of PDGFRα signaling during embryogenesis.

Role of PDGFRα and PDGF-A during Development of the CNS and Injury

The developmental functions of PDGFRs and PDGFs have predominantly been unveiled through genetic studies in mice[19,29]. The role of the closely related PDGF/VEGF superfamily in neural and glial development is evolutionarily conserved: in *Caenorhabditis elegans*, VERs, the VEGF family which are structurally similar to vertebrate VEGFRs, are expressed in neurons and glial cells[30]; in *Drosophila melanogaster*, the PDGFR/VEGFR homologue PDGFR/VEGFR-related receptor (PVR) and its ligands, PDGF- and VEGF-related factor (PVF), are required for ventral midline glia cell survival[31]. In the mammalian CNS, messenger RNA (mRNA) and protein expression of PDGF ligands and receptors are found throughout various brain regions[32]. In rodents, the PDGFRα-positive oligodendrocyte progenitor cells (OPCs)[33] originate at the ventricular surface near the floor plate of spinal cord in embryonic day 12.5 (E12.5) of mice (E14 for rats)[34]. Neurons and astrocytes throughout the CNS produce PDGF-A, which acts as a mitogen for OPCs[35–38]. Early studies showed PDGF-A-knockout mice develop tremor caused by severe hypomyelination on neuronal projections throughout the CNS[39,40]; PDGFRα-null mice die prenatally and the OPC isolated from the premortem animals shows defects in proliferation and differentiation[41]. Subsequent studies using PDGFRα-mutant-knock-in mice showed that downstream SFK and PI3K are important for normal myelination of the CNS[23]. Further analyses revealed that PDGF-A, but not PDGF-B, is required for proliferation, migration and normal differentiation of PDGFRα-positive OPCs[36,37,42,43], and that over-production of PDGF-A in the CNS neurons or astrocytes induces hyper-proliferation of the PDGFRα-positive O-2A progenitor cells in a paracrine manner[40,44]. The mitogenic effect of PDGF-A appears to rely on various environmental cues endowed by the ECM, which

activate various integrins[45] and chondroitin sulfate proteoglycan 4 (CSPG4)/nerve/glial antigen (NG2)[46]. The proliferation rate and the amount of PDGF-A available are proportional and the OPC population continues to grow until the concentration of PDGF-A becomes limiting; that is, the rate of PDGF-A consumption exceeds the rate of its production due to the increased number of OPCs[47]. The OPCs may also be able to sense the amount of PDGF-A by the "rheostat-like" PDGFRα function, switching downstream signaling from the PI3K pathway to PLC-γ under higher PDGF-A concentration[41]. Similar mitogenic function of PDGFRα/PDGF-A signaling has also been demonstrated during CNS injury. After experimental demyelination, the ensuing remyelination often involves increased proliferation of PDGFRα- and NG2-positive OPCs[48]. A decrease in genomic PDGFRα dosage, as shown in heterozygous PDGFRα$^{+/-}$ mice[49], resulted in impaired remyelination after myelin damage to the CNS[50].

In addition to its role as a mitogen for OPCs, PDGF-A acts as a lineage specification factor, directing the differentiation of embryonic neural progenitor cells into oligodendrocytes[51,52]. In cultured O-2A progenitor cells isolated from rat optic nerve, PDGF-A and basic fibroblast growth factor (bFGF) are also capable of maintaining proliferation and self-renewal potential, as well as preventing terminal differentiation into oligodendrocytes[53]. Interestingly, the effect of these growth factors on OPC proliferation and differentiation appear to be reserved in the adult CNS in that following demyelination, PDGF-A and bFGF levels are increased in order to promote OPC proliferation and subsequent differentiation into mature oligodendrocytes for remyelination[54]. The observation that PDGF and bFGF convert adult rat O-2A cells to their perinatal counterparts after injury to the adult brain[55] implies their capacity to reprogram OPCs to their progenitors[56].

In addition to the role of PDGF-A in glial-restricted progenitor cell development, PDGF-A is capable of stimulating the proliferation of PDGFRα-positive neural stem cells (NSCs) residing in the subventrical zone (SVZ) of the adult murine brain, which are capable of differentiating into neurons and oligodendrocytes[57]. The presence of this common progenitor has been long appreciated, and identified in embryonic mouse spinal cord by expression of the basic helix-loop-helix transcription factor Oligs[58]. Through observations employing *Olig2*-null animals, one of the *Olig* genes, *Olig2*, was found to be important for oligodendrocyte development[58]. *In situ* hybridization experiments further revealed that expression of *Olig2* precedes that of PDGFRα in mouse embryonic oligodendrocyte precursors[58,59], suggesting a possible link between the genes during glial development. Indeed, ectopic expression of *Olig2 in vivo* induces expression of the transcription factor Sox10, which together with Sox9, controls transcription of the gene encoding PDGFRα[60,61]. Intriguingly, in mouse embryos, *Olig* expression is regulated by Shh[59] that is also required for self-renewal of the embryonic forebrain progenitors induced by PDGF-A and bFGF[62], evincing a complex interaction of Shh, Sox, Olig and PDGF in oligodendrocyte development. Surprisingly, normal astrocytic development does not seem to be affected in *Olig2*-null animals[58]. Whether OPCs are derived from glial-restricted O-2A cells or neuron/oligodendrocyte common progenitor cells is still an open question[62]. However, these findings suggest the existence of distinct PDGF-responsive neural progenitor cells, each of which is spatiotemporally responsible for generating various neural cells during CNS development. These observations in normal CNS development not only helped to elucidate the identity of the long pursued cells-of-origin of the malignant gliomas, but also raised questions such as whether these intrinsic developmental and physiological properties and functions are preserved after cell transformation.

PDGF-B, -C, and -D during Development of the CNS

While several studies have shown that PDGF-B was able to potently induce glioma development from resident glial (progenitor) cells[63], its role in normal glial cell development is still elusive. Postmitotic CNS neurons are the major cells that express PDGF-B ligand. However, unlike PDGF-A, neuron-specific PDGF-B knockout did not impact normal CNS development or astroglial and angiogenic responses to CNS injury[64]. The role of neuron-derived PDGF-B in normal CNS development and injury is thus obscure.

To a similar extent, the roles of the two new members of the PDGF family, PDGF-C and PDGF-D, in CNS development remain relatively elusive. PDGF-C was first discovered in the spinal cord of chick embryo. Thus, it was originally named spinal cord-derived growth factor (SCDGF)[65]. In rats, PDGF-C is expressed predominantly in embryonic brain and spinal cord, while PDGF-D expression is evident in adults rather than in embryos[66]. In addition, PDGF-C was detected by immunohistochemistry (IHC) in developing brain and spinal cord in mouse embryo[67]. Interestingly, PDGF-C expression was also found in the transient external granular layer cells of the cerebellum in mouse E13 embryo[68]. The requirement of PDGF-C for normal spinal cord development was further demonstrated by studies of PDGF-C knockout mice[69]. Mice deficient in PDGF-C displayed a range of abnormalities and died perinatally due to difficulties in breathing and eating. Examination of the mouse embryos revealed the development of spina bifida occulta, an incomplete closure and deformation of the vertebrae[69]. PDGF-D knockout animals have not been reported yet and studies on its role in normal CNS development still fall behind. However, PDGF-D has been shown to stimulate blood vessel formation and wound healing processes[70], suggesting that PDGF-D might have an impact on tumor angiogenesis.

PDGFRα Signaling in Human Glioma

The first implication that PDGF autocrine signaling can contribute to cell transformation comes from the discoveries of amino acid sequence similarity between simian sarcoma virus (SSV) oncogene *v-sis* and PDGF-B chain gene[71,72]. In human glioma, a glioma cell line was shown to secrete a growth-promoting factor[73–75] that was later found to be three disulfide-linked dimers, PDGF-AA, PDGF-AB and PDGF-BB[76–78]. Each of these dimers had distinct binding affinities to receptors α and β[20]. Subsequent studies revealed that mRNA for PDGF ligands and receptors were expressed in a series of established glioma cell lines[79,80]. *In situ* hybridization and IHC analyses further demonstrated that in human glioma tissue, tumor cells express PDGF-A, -B, and PDGFRα, whereas surrounding hyperplastic endothelial cells express PDGF-B and PDGFRα, suggesting the presence of autocrine and paracrine PDGF stimulation in glioma development[81–84]. As described in the previous section during development of the CNS, the OPC number increases in response to the level of paracrine PDGF-A expression. Whereas in an autocrine situation, in which PDGF ligand is over-expressed in PDGFRα-positive progenitor cells, the rate of PDGF-A production increases together with the number of the progenitor cells, theoretically rendering these cells capable of proliferating indefinitely[29]. Thus in an autocrine situation, the growth factor sensing mechanism of OPCs might be lost, potentially leading to tumor formation.

Overexpression and gene amplification of PDGFRα occurred mostly in lower-grade gliomas as well as secondary GBMs[2,4,85–87], representing a distinct subtype of GBMs from those with EGFR overexpression (Figure 1)[88]. In a clinical study of IHC staining on 103 grade II astrocytomas, PDGFR expression was found to be an independent prognostic factor for these patients with low-grade gliomas[89]. Overexpression of PDGF ligands, however, varies among gliomas of different grades, with PDGF-A being expressed in all grades and PDGF-

B only in high-grade GBMs[90], suggesting PDGF-B may be involved in the conversion of low- to high-grade gliomas. In fact, it has been shown that in human oligodendroglioma tissue[91] and in a mouse glioma model[92], the level of PDGF signaling may predict grade and malignancy.

High-throughput approaches such as comparative genomic hybridization (CGH) and microarray have been established, enabling glioma profiling in a whole-genome scale[93–96]. The most comprehensive and reliable analysis of genomic alteration in primary GBM tissue has been conducted by The Cancer Genome Atlas Research Network (TCGA)[97], a collaborative project sponsored by the National Cancer Institute (NCI), National Institutes of Health in USA[97]. This multi-institutional effort provides invaluable resources for gene expression and mutation, DNA copy number alterations (CNA), microRNA expression and DNA methylation data for GBMs. Most of the previously appreciated genetic aberrations such as alterations in the RB, TP53 and RTK pathways were re-captured in the initial TCGA studies. However, an unexpected higher frequency (~13%) of focal amplifications of the 4q12 locus harboring *PDGFRA* was reported for primary GBMs in the TCGA collection than that published previously[97,98]. Further classification of these GBMs by gene expression signatures revealed that *PDGFRA* overexpression occurs together with *TP53* and *IDH1* mutations in the Proneural subtype of GBMs, which also express oligodendrocyte lineage genes such as *OLIG2* and *SOX*[99]. Interestingly, within this subtype of GBMs, *PDGFRA* amplifications and *PIK3CA/PIK3R1* mutations (leading to constitutively active PI3K subunits p110 and p85, respectively) mostly occur in a mutually exclusive manner[99], signifying an overlapping functionality between these two alterations in gliomagenesis. Indeed, analyses in different cohorts of gliomas of different grades using other methods such as single nucleotide polymorphism array (SNP-Chip)[100] or a Western blot–based proteomic analysis[101] confirmed the importance of PI3K/AKT/mTOR pathway activation in *PDGFRA*-amplified gliomas.

The Proneural subtype of GBMs harboring *PDGFRA* amplification from the TCGA dataset share similar gene expression profiles with the previously reported pro-neural (*PN*) subclass[94]. Nearly all WHO grade III tumors including astrocytomas, oligodendrogliomas and mixed oligoastrocytomas have been classified as a *PN* subclass, as well as secondary GBMs[94,99]. Patients with tumors are younger and survive longer. Analyses of signature gene expression in *PN* tumors also revealed that these tumors resemble neuroblast/glial progenitor cells in the fetal and adult brain indicating a possible cell of origin of this subclass of glioma[94]. Indeed, putative NSCs residing in the adult mouse SVZ are PDGFRα-positive and respond to PDGF-A stimulation by forming a glioma-like lesion[57], suggesting susceptibility of adult NSCs to oncogenic transformation by *PDGFRA* alteration.

PDGF-C and PDGF-D in Human Glioma

The two novel PDGF ligands, PDGF-C and PDGF-D, were first implicated in the development of glioma by a study that examined the expression of PDGF ligands and receptors in glioma cell lines and primary glioma tissues[102,103]. Lokker and colleagues found that PDGF-C and -D were expressed at high levels in tumor tissues as compared with those in the normal brain. Additionally, autocrine loops involving PDGF-C/PDGFRα and PDGF-D/PDGFRβ exist in all tumors examined. However, the pathologic consequences of PDGF-C and -D overexpression and whether they compensate PDGF-A and -B functions or merely initiate redundant signaling cascades remains elusive. Interestingly, in a subcutaneous tumor model, PDGF-C was associated with tumor resistance to anti-VEGF therapies. The resistant tumors tend to up-regulate the level of PDGF-C after anti-VEGF treatment, possibly compensating the function of the inhibited VEGF in stimulating angiogenesis[104]. Indeed, in patients with recurrent GBMs after anti-VEGF therapy, PDGF-

C together with c-Met was expressed at high levels, especially in the center of the tumor mass[105]. The mechanism of resistance to anti-VEGF therapy may involve the capacity of PDGF-C to alter the structure of blood vessels by recruiting perivascular cells[106], thus stabilizing the vessels and rendering them insensitive to anti-VEGF treatment. The function of PDGF-D/PDGFRα autocrine signaling remains unknown despite its presence in several GBM cell lines and primary tissues[102,103]. However, it has been shown that PDGF-D was responsible for inducing chemotactic tropism of PDGFR-positive stem cells toward glioma cells in the mouse brain[107]. This observation is likely important for the development of therapeutic strategies using stem cells as a drug carrier.

PDGF-induced Brain Tumors in Animal Models

Despite intensive research, the prognosis for patients diagnosed with high-grade glioma remains dismal. A major hurdle to progress is the lack of animal models accurately recapitulating the behavior and neuropathological features of human tumors[108]. Several genetically engineered mouse (GEM) models of spontaneous glioma formation have been developed by generating mice with astrocyte-specific transgenic expression of *v-src*[109] or *H-ras*[110] and with the concomitant loss of the neurofibromatosis 1 (*NF1*) and tumor *p53* suppressors[111,112]. Recently, transgenic mice that harbor astrocyte-specific expression of PDGF-B (hGFAPpPDGFB mice) were generated and assessed for spontaneous glioma formation[113] (Table 1). The loss of p53 or the overexpression of PDGF-B alone was unable to induce brain tumor growth. However, when Hede and colleagues crossed hGFAPpPDGFB mice with p53-null mice, the resultant hGFAPpPDGFB/p53-null mice developed an aggressive GBM-like tumor with characteristics frequently found in human GBMs[113]. The researchers also observed the increased expression of PDGFRα in tumor cells, while PDGFRβ was expressed in the tumor vasculature, and that the tumor expressed a series of lineage markers indicating the presence of various cell types including neurons, astrocytes and oligodendrocytes, especially in large tumors.

Spontaneous gliomas have been found to develop in transgenic mice overexpressing the long isoform of PDGF-A, PDGF-A_L, under the control of a GFAP promoter[114] (Table 1). Unlike hGFAPpPDGFB mice, hGFAPpPDGFA_L mice did not require additional genetic aberrations such as *p53* deletion in order to develop spontaneous tumors. These mice were also found to harbor neoplastic cells positive for PDGFRα, Olig2 and NG2 in the SVZ, corpus callosum, hippocampus and cerebellum[114], suggesting that the resident OPCs in these areas of the brain were likely the target of transformation. Alternatively, GFAP-positive NSCs could also be the cell of origin for PDGF-induced gliomas in the hGFAPpPDGFA_L mice, since PDGF-A has been reported to be capable of biasing the fate of stem cells residing in the SVZ toward OPC-like cells[57]. Histologically, spontaneous gliomas formed in hGFAPpPDGFAL mice showed characteristics of both astrocytes and oligodendrocytes and were determined to be WHO grade III oligoastrocytomas (Table 1). Although it is difficult to assess the tumor-promoting potential of PDGF-B, short- and long-form PDGF-A by comparing the transgenic mice generated by overexpression of these genes, due to the differences in promoter strength and transgenic copy numbers among these mice, these studies have suggested that glioma-specific long-form PDGF-A may exert tumorigenic effects through distinct signaling pathways than those stimulated by PDGF-B and short-form PDGF-A[114].

Other GEM models of PDGF-induced glioma development have also been described, most of which involve a single intracranial injection of retroviruses expressing PDGF-B ligand[115–119] (Table 1) targeted nonspecifically, or to astrocytes (GFAP-positive) or neural progenitor cells (nestin-positive) using cell type-specific promoters. In these studies, PDGF-B overexpression induces glioma growth from various cell types including glial progenitor

cells, astrocytes and neural progenitor cells, and the resulting tumors frequently exhibited characteristics of oligodendrogliomas or mixed oligoastrocytomas (Table 1). These two models, termed germ-line transgenic or retrovirus induction, differ in many ways such as the incidence of tumorigenesis and histological features. Discrepancy may be due to different times of stimulation during animal development, different cells being targeted or retroviral-mediated insertional mutagenesis in genes that cooperate with PDGF to induce tumorigenesis[120]. Additionally, the local injection of various agents including cells or viruses into the brain of mice may induce the proliferation of VEGF-expressing Olig2-positive glial cells and a local increase in angiogenesis[121,122]. These factors may contribute to the differential tumor induction potentials observed in these models where PDGF-B alone was targeted to GFAP-positive cells in the brain. Despite the differences between the transgenic and the virus-mediated models, an increase in tumor malignancy by concomitantly introducing a loss of p53 function or the *Ink4a/Arf* locus was evident in all studies[113,116,119,123] (Table 1), demonstrating a cooperative effect of PDGF overexpression and the disruption of a p53 or RB pathway in glioma tumorigenesis. These observations in the animal models thus provide an experimental basis for TCGA analyses, demonstrating that the three core signaling pathways (RTK, RB, and P53) are important for GBM viability.

PDGFRα-positive progenitor cells that respond to PDGF stimulation can be found in various parts of the adult brain[57,113,117]. Exogenous PDGF-A infusion into the adult SVZ induces PDGFRα-positive neural progenitor cells to generate glioma-like lesions in the mouse brain[57]. To investigate how PDGF-A–mediated PDGFRα activation contributes to glioma formation, we utilized an orthotopic transplantation model, in which we implanted various PDGF receptor or ligand overexpressing human or mouse cells into the brains of mice to assess their tumorigenic potentials[124]. In order to determine which signaling pathway(s) downstream of PDGFRα are necessary for its oncogenic capacity, we chose PDGF-A instead of PDGF-B since PDGF-A only stimulates PDGFRα but not PDGFRβ. When the tyrosine phosphorylation sites for PI3K binding were mutated to phenylalanine, PDGFRα lost the capacity to transform mouse astrocytes, both *in vitro* in soft agar and *in vivo* in the brain of mice. Unexpectedly, the association between PDGFRα and a downstream effecter (SHP-2) is required for maximal tumorigenic potential observed in cells overexpressing the wild-type receptor. This is significant, since a recent automated signaling network-based analysis on the TCGA genomic data identified SHP-2/*PTPN11* as one of six linker genes that are not altered in GBMs but are statistically enriched for interactions with the commonly altered genes in GBMs[125], including *EGFR, PDGFRA, PIK3R1, KIT* and *KDR*. Our observation thus provides functional validation of the role of SHP-2/*PTPN11* in mediating PDGFRα signaling in gliomagenesis.

There are high levels of Akt phosphorylation in our model of PDGFRα/PDGF-A–overexpressing tumors[124]. However, using a RCAS/*tv-a* system, Dai and colleagues have shown that combined activation of Akt and K-Ras generated astrocytomas while PDGF-B overexpression led mostly to oligodendrogliomas[126]. They further showed that the blockade target of rapamycin (mTOR) converted PDGF-B–induced astrocytomas to oligodendroglioma-like tumors[127]. Thus it appears that in the RCAS/*tv-a* system, PDGF-B and Ras/Akt/mTOR signaling pathways promote the formation of distinct histological types of animal brain tumors. However, in human glioma, as shown by a proteomic study, the activation of mTOR and Ras/Erk pathways frequently co-occurred in the PDGF-B–enriched subgroup[101] and two of four oligodendrogliomas in this study were of the PDGF-B/mTOR/Ras activation subtype, suggesting a more complex connection between PDGF signaling and glioma cell lineage specification may exist in humans than found in mice. Additionally, since there was no concomitant overexpression of PDGFRα in tumors generated from RCAS/*tv-a*–mediated PDGF-B expression, it will be interesting to determine whether

targeted co-overexpression of PDGFRα and PDGF-B in the mouse brain will generate gliomas with similar histopathological characteristics as does PDGF-B alone.

Prospects for PDGF-targeted Therapy in Glioma

Although PDGF targeted therapies using various tyrosine kinase inhibitors have been extensively studied over the past decade, the results were generally disappointing and have yet to shift the standard clinical treatment of GBM, which encompasses maximal surgical resection, followed by TMZ and radiation[128]. In order to design therapeutic strategies that can benefit patients with glioma driven and maintained by PDGF signaling, more thorough pre-clinical studies are warranted. Various animal models that are candidates for this purpose have been proposed[129]. Additionally, the identification of new potential molecular targets in tumors induced by PDGF signaling is also important. The application of systems biology to the genomic data obtained from patient samples is expected to aid in this process[130]. To generate a network of genes that share a regulatory program, the commonly altered genes in GBM were grouped into several functional “modules”. The interactions between each cancer-altered gene and other non-altered “linkers” can be identified within and among different modules[101]. SHP2/*PTPN11* was identified in this network approach as one of the six linkers and was also shown to be important in the PDGF-driven gliomagenesis in our model[124]. Molecules such as SHP-2/*PTPN11*, which are not commonly overexpressed or mutated in GBM, are often understudied. However, several signaling pathways may converge on these molecules to exert downstream function. Besides its involvement in regulating the PDGFRα/PI3K/Akt pathway in gliomas[124], SHP-2 has also been known to mediate the EGFR/PI3K/Akt[131] and the Ras/MAPK signaling pathways[132] (Figure 2). Thus, it represents a molecule whose loss may lead to the disruption of multiple signaling cascades involved in tumorigenesis. As tumors are considered to be “network-addicted” rather than “oncogene-addicted”[133] strategies targeting such a molecule in monotherapy or in combination with other cytotoxic drugs may prove more efficient than targeting multiple upstream RTKs. Recent advances in large-scale whole-genome profiling of GBMs should yield to the discovery of more promising targets similar to these. However, preclinical studies of targeted drugs require a more stringent selection of animal models that accurately emulate human tumors. In addition, personalized medicine should be administered to each individual, employing the data gained from genomic profiling and systems biology.

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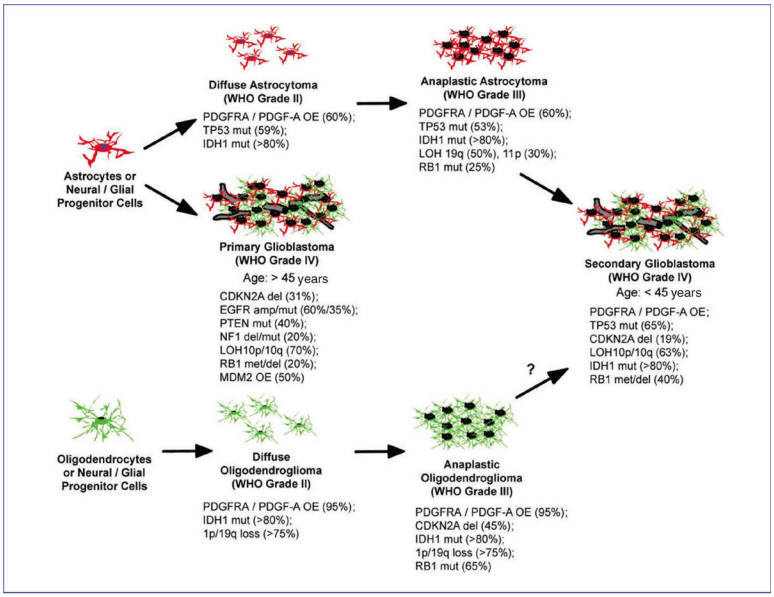

Figure 1. Progression the genetic alterations in human malignant gliomas

Astrocytoma may arise from astrocytes or progenitor cells that accumulate genetic alterations and become transformed. *IDH1* and *TP53* mutations are believed the first alterations occurring in these tumors. Oligodendrogliomas can develop from the same progenitor cells or mature oligodendrocytes that acquire a distinct set of genetic changes. The earliest changes that occur include *IDH1* mutation and a 1p/19p loss. Representative genetic changes are shown with the estimated frequency of occurrence within each type of tumor. OE, overexpression; mut, mutation; del, deletion; met, promoter methylation; amp, amplification.

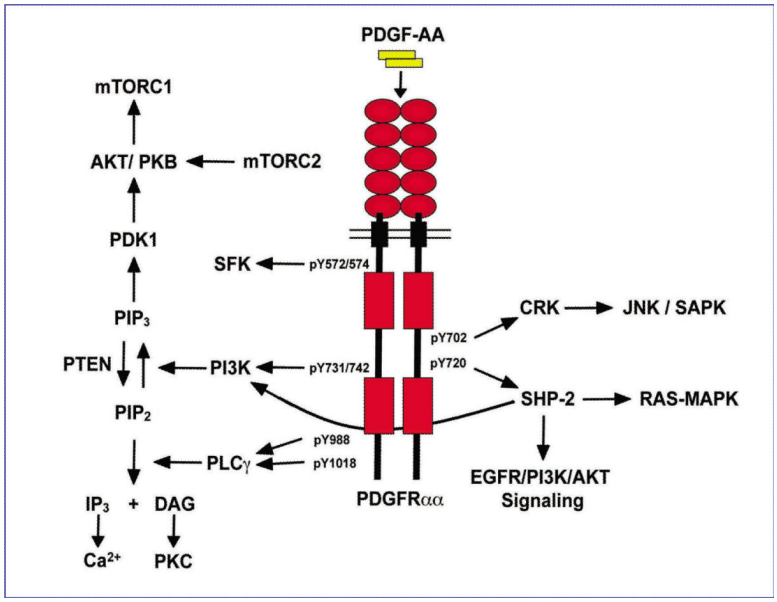

Figure 2. PDGFRα/PDGF-A signaling

PDGF-AA homodimers bind and induce the dimerization and autophosphorylation of the PDGFRαα homodimer receptor. Subsequently, SH2 domain-containing signaling effectors are recruited to the receptor by binding to specific phosphorylated tyrosine residues, initiating downstream signaling cascades.

Table 1

Summary of animal models of PDGF-induced spontaneous brain tumor formation.

Method	Vector	PDGF	Strain	Age	Target Cell	Histology	Reference(s)
Virus Sup I.C. Inj	MoMuLV	PDGF-B	C57BL/6	Neonatal	Non-specific	GBM / PNET	[115]
Virus Sup I.C. Inj	MoMuLV	PDGF-B	$p53^{-/-}$	Neonatal	Non-specific	GBM / PNET	[123]
Virus Sup I.C. Inj	MoMuLV	PDGF-B	$Ink4a/Arf^{-/-}$	Neonatal	Non-specific	GBM / PNET	[123]
Packaging Cell I.C. Inj	RCAS	PDGF-B	Ntv-a	Neonatal	$Nestin^{+}$ cells	Low-grade Oligo	[116]
Packaging Cell I.C. Inj	RCAS	PDGF-B	Ntv-a, $Ink4a/Arf^{-/-}$	Neonatal	$Nestin^{+}$ cells	Anaplastic Oligo	[116]
Packaging Cell I.C. Inj	RCAS	PDGF-B	Gtv-a	Neonatal	$GFAP^{+}$ cells	Low-grade Oligo/ Oligoastro	[116]
Packaging Cell I.C. Inj	RCAS	PDGF-B	Gtv-a, $Ink4a/Arf^{-/-}$	Neonatal	$GFAP^{+}$ cells	Anaplastic Oligo/ Oligoastro	[116]
Packaging Cell I.C. Inj	RCAS	PDGF-B	Gtv-a, $p53^{-/-}$	Neonatal	$GFAP^{+}$ cells	Low-grade Oligo/ Oligoastro	[116]
Packaging Cell I.C. Inj	RCAS	ACC-PDGF B-HA	Ntv-a	Neonatal	$Nestin^{+}$ cells	High-grade Oligo	[92]
Virus Sup C.C. Inj	pQ(Retroviral)	PDGF-B	Sprague Dawley Rat	Adult	Non-specific	GBM	[117]
Virus Sup L.V, Inj	pQ(Retroviral)	PDGF-B	Sprague Dawley	Neonatal	SVZ Cells	GBM	[117]
Packaging Cell L.V. Inj	pCEG (Retroviral)	PDGF-B	Rat C57BL/6	E14	SVZ Cells	High-grade Oligo	[52, 118]
Protein L.V.Infusion	NA	PDGF-A	CD-1	Adult	SVZ Cells	Low-grade Glioma	[57]
Zygote Pronuclear Inj	IRESβGEO	PDGF-B	Transgenic hGFAPpPDGFB, $p53^{-/-}$	EO	$GFAP^{+}$ cells	GBM / High-grade Oligo	[113]
Zygote Pronuclear Inj	IRESβpGEO	Long-form PDGF-A	Transgenic hGFAPpPDGFA	EO	$GFAP^{+}$ cells	Grade III Oligoastro	[114]