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REVIEW ARTICLE

Platelet-derived growth factor in glioblastoma—driver or biomarker?

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Winner of the Rudbeck Award, 2012 at the Medical Faculty of Uppsala University, for his outstanding research on growth regulation of normal and neoplastic cells.

Abstract

The platelet-derived growth factor (PDGF) family of mitogens exerts vital functions during embryonal development, e.g. in the central nervous system, where PDGF drives the proliferation of oligodendrocyte precursors. PDGF and PDGF receptors are co-expressed in human glioblastoma (GBM). Whether an aberrant activation of the PDGF receptor pathway is a driving force in glioma development has remained an open question. In experimental animals, overexpression of PDGF has convincingly been shown to induce tumors, both in wild-type animals (marmoset, rat, mouse) and in mice with targeted deletions of suppressor genes, e.g. *Tp53* or *Ink4A*. Targeting the PDGF receptor in tumor-bearing mice leads to growth inhibition and reversion of the transformed phenotype. Findings of PDGF receptor amplification or mutations in human GBM are strong indicators of a causative role of the PDGF receptor pathway. However, clinical trials using PDGF receptor antagonists have been disappointing. In conclusion, a PDGF receptor profile may be a biomarker for a subgroup of GBM originating from a PDGF receptor-responsive cell. Although compelling experimental and clinical evidence supports the notion that the PDGF receptor pathway is a driver in GBM, formal proof is still missing.

Key words: *Brain tumors, molecular biology, oncogenes, tumor biology*

Introduction

The widely cited articles by Hanahan and Weinberg on the hallmarks of cancer (1) have highlighted the importance of self-sufficiency in growth factor receptor signaling, mediated by aberrant ligand-induced activation, constitutively activated and mutated receptors, or aberrant activation of down-stream signals. The idea that cancer cells are growth-stimulated by autocrine growth factors has been around for quite a while, even before the term 'autocrine' was used to describe the phenomenon (2). For instance, Howard Temin referred to it in his pioneering work on the mechanism of RNA tumor virus-induced cell transformation (3,4). The field was tremendously boosted by the discovery that the trans-forming v-*sis* gene of simian sarcoma virus (SSV) is a retroviral version of the cellular platelet-derived

growth factor (PDGF) B-chain gene (5,6) and that the transforming activity of the gene is mediated by autocrine PDGF-BB (7).

In my own search for suitable sources for novel growth factors, I was encouraged by the early studies by Temin and others to use serum-free conditioned culture media from human established cancer cell lines. The human osteosarcoma cell line U-2 OS, established by Jan Pontén (8), turned out positive as it was found to produce growth-promoting factor (s) which could be partially purified (9). Further purification and structural and functional analyses revealed a striking resemblance to PDGF (10). Subsequently, the osteosarcoma cell-derived growth factor was shown to be identical to PDGF-AA (11). Using the recently developed PDGF receptor-binding assay, I found that one of our clonal human glioblastoma (GBM) cell lines, U-343 MGa Cl2, produced large amounts of PDGF receptor-competing activity. Through subsequent studies it became evident that the cells produce significant amounts of PDGF (12,13), mainly PDGF-AA (14). This finding was the starting-point for a new research avenue, PDGF in human and experimental brain tumors (reviewed in (15,16)).

PDGF and PDGF receptors

The platelet-derived growth factor (PDGF) family consists of covalently linked hetero- or homodimers of A-, B-, C-, and D-chains (PDGF-AA, -AB, -BB, -CC, and -DD) (for comprehensive reviews on PDGF and PDGF receptors, see (17,18)). The ligands bind
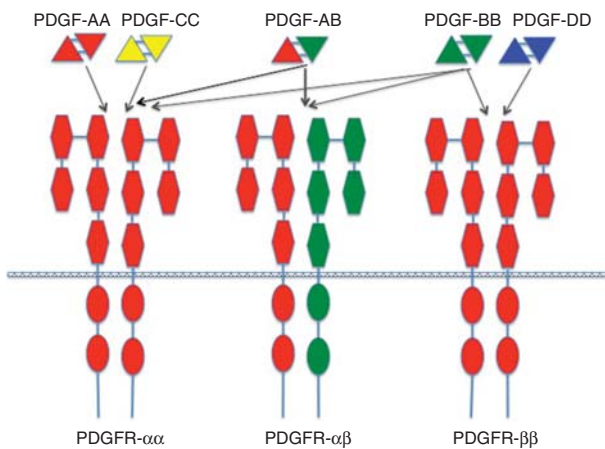

Figure 1. Binding specificities of PDGF ligands and receptors. The PDGF isoforms are made up as homo- or heterodimers of anti-parallel subunits covalently linked by two S-S bonds. The ligands bind to and dimerize PDGF α and β receptors with specificities depicted in the figure. The receptors have extracellular domains with five immunoglobulin domains. Each receptor has an intracellular, split tyrosine kinase domain.

to and activate heterodimeric α and β tyrosine kinase receptors with ligand specificities outlined in Figure 1. Several intracellular signaling pathways are engaged by PDGF receptor activation among which the Ras-MAPK, PI3K, and PLCγ pathways are most studied. Receptor activation culminates in cell cycle initiation, DNA synthesis, and mitosis. PDGF is not only involved in cell cycle regulation but also cell migration and chemotaxis (19-21). PDGF and PDGF receptors fulfill important functions in development, e.g. in kidney, lung, intestine, and brain tissues, mainly through paracrine receptor activation (17).

PDGF in glial development

A ground-breaking study by Richardson et al. (22) showed that cultured oligodendrocyte precursor cells (OPCs) from the developing rat optic nerve are growth-stimulated by PDGF, present in astrocyte-conditioned medium. A subsequent study showed that the OPCs express PDGFRα (23). These findings have been confirmed by *in vivo* experiments in mice, where PDGF-AA has been shown to drive the proliferation of OPCs (24-26). OPCs are likely to be stimulated *in vivo* by paracrine PDGF released from astrocytes and neurons (27). No such autocrine stimulation by normal OPCs during development has been demonstrated. Interestingly, PDGF-AA seems to be a rate-determining factor for OPC proliferation (28), but not for myelination; it is only in the total absence of PDGF that myelination becomes negatively affected (26). Continuous infusion of PDGF into the subventricular zone of the mouse brain induces precursor cell proliferation and hyperplastic, glioma-like lesions. These lesions are reversible and cannot be defined as full-blown malignancies (29). In conclusion, in normal development PDGF drives the expansion of undifferentiated OPCs, and an excess of PDGF leads to an increase in OPC number beyond the physiological need. Apparently, there is no negative feedback mechanism operating in OPCs to control their PDGF-induced proliferation. Rather OPC proliferation seems to be controlled at the level of PDGF available for receptor activation.

PDGF-induced gliomas in animal models

Experiments performed by Friedrich Deinhardt and collaborators showed that SSV is tumorigenic in marmoset monkeys (30,31). A complete record of Deinhardt's experiments was unfortunately never published, but we were lucky to obtain the original paraffin-embedded material from Deinhardt's experiments and could publish the histology of brain tumors induced by intracerebral SSV injections into newborn

animals (32). Interestingly, the virus caused lesions indistinguishable from human glioblastoma with all its hallmarks: cellular pleomorphism, necroses, and microvascular proliferations. The morphology was mixed, with oligodendroglioma-like, astrocytic, and anaplastic areas present. We now know that the transforming gene v-*sis* of SSV is a retroviral version of the PDGFB gene and that the transforming activity of v-*sis* is exerted by an autocrine PDGF receptor activation. Thus, an autocrine and uncontrolled PDGF receptor activation of target cells in primate brain in a retroviral context leads to the development of brain tumors displaying all the hallmarks of GBM. In this respect, SSV infection in marmosets has a more dramatic effect than continuous infusion in mouse brain (29).

A number of studies have shown that intracerebral delivery of PDGF-encoding retroviral vectors in rodents leads to the development of brain tumors at high frequency. A recombinant Moloney murine leukemia virus encoding PDGFB (MoMuLV/ PDGFB) injected together with replication-competent helper virus (MoMuLV) was found to induce glioma-like lesions in wild-type mice (33). The tumors mostly resembled human glioblastoma with extensive necrotic areas. Some tumors displayed characteristics of primitive neuroectodermal tumors, and some were phenocopies of human low-grade oligodendrogliomas. Only a minority of the cells, identified as infiltrating astrocytes, were glial fibrillary acidic protein (GFAP)-positive, whereas a high proportion were nestin and PDGFRα-positive. These characteristics suggested that the tumors were derived from a PDGFRα-positive neural progenitor transformed by a persistent autocrine activation of the PDGF receptor signaling pathway. Eric Holland and collaborators have made important discoveries and significantly contributed to our understanding of PDGF-induced brain tumors in the mouse. A useful mouse model was developed, where PDGF is encoded by a recombinant avian RCAS virus and delivered intracerebrally in transgenic mice with a targeted expression of the virus receptor tv-a under the control of GFAP (GTv-a mice) or nestin (NTv-a mice) promoters (34). RCAS-PDGFB generated brain tumors in both types of transgenic mice: oligodendrogliomas in NTv-a mice, and oligodendrogliomas or oligoastrocytomas in GTv-a mice. Tumor latency was shortened, and malignancy was enhanced in mice with homozygous deletion of the Ink4a-Arf locus, which is a common aberration in human GBM. Using the 2′,3′-cyclic nucleotide 3′-phospho-diesterase (CNPase) promoter to drive the expression of tv-a in transgenic mice (CTv-a mice), Lene Uhrbom's research group was able to target PDGF expression to cells of the oligodendrocyte lineage. In the absence of other genetic aberrations, RCAS-PDGFB generated low-grade oligodendrogliomas (35), whereas high-grade oligodendrogliomas as well as astrocytomas were induced in *Cdkn2a null* (p16^{Ink4a}-/-, p19^{Arf}-/-) mice (36). Collectively, these data show that a single oncogene, i.e. *Pdgfb*, can cause tumors with different cellular origin (GFAP-positive astrocytes, nestin-positive neural stem cells, and CNPase-positive oligodendrocyte progenitors). The impact of these findings on the identification of the cell of origin of human glioblastoma has been discussed (37). PDGFB has also been shown to induce GBM-like tumors when expressed as a transgene under the control of the *Gfap* promoter in Tp53 *null*, but not wild-type, mice (38).

High expression of PDGFB, generated by deleting the translation-inhibiting sequence in the 5′ end of PDGFB cDNA inserted into the RCAS vector, was shown to give rise to high-grade tumors in wild-type mice, whereas the full-length sequence mostly induced low-grade oligodendrogliomas (39). Tumor grade is thus dose-dependent. Low expression of PDGF causes low-grade tumors, and high expression yields high-grade tumors. In this context it is interesting to note that the v-*sis* gene lacks this particular sequence (40), which may explain why SSV causes high-grade tumors in marmosets. The finding that an oncogene coding for a structurally and functionally normal growth factor can cause high-grade tumors is difficult to understand given the widely accepted concept that malignant tumors are caused by multiple genetic events and display a diversity of phenotypic aberrations (1). This question was addressed decades ago (41) but has not yet been fully answered. There are three main alternatives.

(1) Mitogenic stimulation and unrestricted cell proliferation induced by excessive amounts of PDGF is accompanied by secondary genetic aberrations causing tumor progression. This idea was adopted in a recent review (42), where replicative stress was supposed to be the driving force behind the mutagenic events. Direct evidence for this hypothesis, e.g. whole-genome sequence data for PDGF-induced tumors, is, however, lacking.

(2) PDGF synthesis driven by retroviral vectors synergizes with insertional mutagenesis of complementary oncogenes or suppressor genes. Retroviral long terminal repeats are strong promoters and enhancers, which could activate cellular genes at a distance from the insertion sites. Further, integrated proviral DNA could theoretically interrupt genes and give rise to

truncation or inactivation of the gene product. In order to probe this idea and search for genes that synergize with PDGF in gliomagenesis, Uhrbom et al. (33) constructed a recombinant MoMuLV coding for a full-length PDGFB cDNA. The recombinant virus was combined with a replication-competent helper virus to increase the frequency of proviral insertions in order to render the genetic screen more effective. The experiment generated 108 brain tumors from which 647 insertion sites were obtained with as many as 66 common insertion sites (43), reviewed in (44). A number of the tagged genes are known to be involved in oncogenesis, e.g. *Trp53*, *Eef1a1*, *Gli*, *Fos*, and *Ccnd*, providing proof of concept for the genetic screen. The vast majority of tagged genes, however, had not previously been implicated in oncogenesis or PDGF signaling. One of these, *Sox5*, was subsequently found to be a suppressor of PDGF-induced brain tumors and cause cellular senescence (45). Five insertions in the *Nfix* locus were found, the functional implication of which is still unknown and worth pursuing. NFIX has been shown to be a critical factor in development and to induce quiescence in neural stem cells (46,47). Insertion into the *Nfix* locus may interfere with the differentiation program and thereby synergize with PDGF in tumor development. Whether insertional mutagenesis also operates in the RCAS-PDGF-induced brain tumors is an open question. The oncogenic potential of retroviruses was dramatically illustrated by the clinical trials on gene therapy in X-linked severe combined immunodeficiency (XSCID) patients a few years ago. Three out of 11 patients developed T cell tumors, all of which caused by proviral insertions in the *LMO2* locus (48,49).

(3) The generation of high-grade gliomas by PDGF overexpression is caused by an autocrine activation of transformed cells as well as recruitment of non-transformed cells, which are attracted to the tumor site by paracrine activation. In fact, PDGF virus-induced gliomas in the rat contain a large number of untransformed cells, which apparently have migrated and populated the tumor (50). According to this model, PDGF is sufficient to drive tumor formation in the absence of other genetic aberrations. This model may seem unlikely and less attractive than those described above. The model presumes that an excessive, PDGF-induced, cell proliferation is not controlled by any feedback regulation. Interestingly, this may indeed be the case. In PDGF-transgenic mice, the number of PDGF-
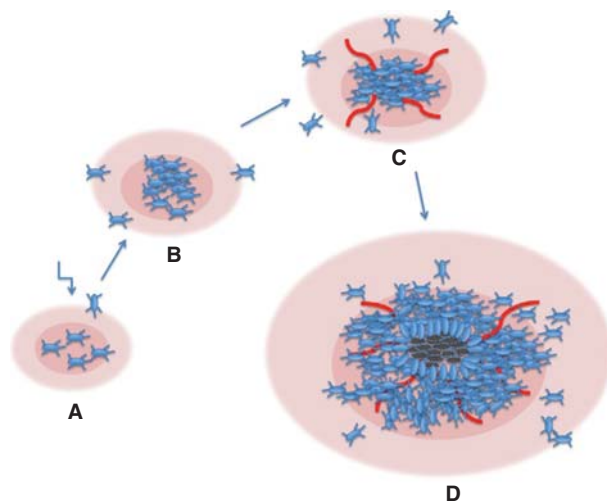

Figure 2. Schematic model for the development of high-grade gliomas in experimental animals following infection with PDGF-encoding retroviruses. **A.** Cells are infected, produce PDGF, and start to proliferate in an uncontrolled fashion. **B.** The PDGF-transformed population expands, and neighboring cells are attracted by paracrine stimulation. **C.** Cell density has increased, and local hypoxia elicits an angiogenic response. **D.** The expanding population has grown beyond the supply of nutrients, and necroses appear. Pink areas denote PDGF released by the tumor cells.

responsive oligodendrocyte precursors was proportional to the supply of PDGF and seemingly unsaturable (51). In the case of a local production of autocrine/paracrine PDGF, the pool of stimulated cells may increase progressively to the point where hypoxia drives angiogenesis and where necroses may occur in severely hypoxic areas. Such a process may then yield tumors, which closely resemble glioblastoma multiforme (see Figure 2 for a schematic representation of this model).

PDGF and PDGF receptors in human glioma

Our observation that human glioma cells in culture produce PDGF became an incentive for studies of the expression of PDGF and PDGF receptors in human glioma. A rather coherent picture has emerged from these studies, pointing to a causative role for PDGF in gliomagenesis. PDGFA and PDGFα receptors are co-expressed in glioblastoma (52) and oligodendroglioma (53). The PDGFRα gene is amplified, mutated, or rearranged in a fraction of glioblastoma tumors (54-60). These findings strongly suggest that genetic aberrations and overactivity of the PDGFα receptor signaling pathway are important events in the development of a subset of glioblastomas. This view is strengthened by transcriptome analysis of glioblastoma, which has indicated that the proneural subgroup of GBM is characterized by aberrations in the

PDGF/PDGFR pathway (60). Indeed, overactivity of the PDGFα receptor pathway may be an initiating event in human glioblastoma as a result of an increased *PDGFA* gene dosage generated by chromosomal non-disjunction by the occurrence of multiple copies of chromosome 7, which harbors the PDGF A-chain gene (61).

Driver or biomarker?

In the discussion on the causative role of PDGF and PDGFR in the development of human brain tumors, we may be guided by an analogous discussion over a century ago regarding micro-organisms as etiologic agents in human disease. This subject was brought up by Henle and Koch and resulted in what became known as Koch's postulates (reviewed in (62)). Although Koch's postulates cannot be directly translated into the etiology or pathogenesis of a non-viral cancer, such as glioblastoma multiforme, they can be adjusted as follows:

(1) PDGF receptor signaling must be aberrantly activated in brain tumor cells. As has been summarized above, PDGF and PDGFR are expressed at high levels in most GBM. Since the cell of origin of human GBM is yet unknown, we cannot make any direct comparison between the levels of activation in the normal cell(s) of origin versus GBM cells. However, the PDGFRα gene is amplified in a subset of GBM, which strongly indicates that it confers a selective growth advantage and thus has a mechanistic role in the pathogenesis of the tumor. The recurrent findings of receptor mutations and rearrangements, albeit rare, are strong indicators of a mechanistic role, although we cannot formally rule out the possibility that these changes are passenger mutations and not drivers.

(2) Aberrant activation of PDGF receptors in the cell of origin must cause GBM *in vivo*. As described above, studies in marmosets, rats, and mice have provided convincing evidence that overexpression of PDGF, mainly PDGFB but also PDGFA (61), causes GBM in experimental animals. It needs to be said, however, that these findings in no way prove that similar mechanisms operate in human GBM.

(3) Blocking the PDGFR signal transduction pathway in GBM should retard the growth of glioma cells *in vivo*. There are a few examples where PDGF antagonists have been shown to retard cell growth in GBM cultures (63,64). However, clinical trials on the use of PDGF receptor kinase inhibitors have been disappointing (65,66).

These results are in striking contrast to studies on PDGFB-induced gliomas in mice, where low-molecular-weight PDGF receptor kinase inhibitors are efficacious in reverting the transformed phenotype and cause tumor growth retardation *in vivo* (67). It is also notable that imatinib, a low-molecular-weight PDGFR inhibitor, is an effective drug in the treatment of other PDGF-driven tumors such as gastrointestinal stroma tumors with *PDGFRα* mutations and dermatofibrosarcoma tuberans with an activating *PDGFB* translocation (reviewed in (68,69)). In the absence of in-depth pharmacokinetic and pharmacodynamic studies in GBM, we cannot draw firm conclusions regarding the potential of PDGF receptor antagonists in the treatment of GBM. However, the possibility remains that aberrant activation of the PDGFR signaling pathway is an initial event in the proneural and mesenchymal subgroups of GBM (61) but is rendered redundant during tumor progression. This would point to a hit-and-run mechanism in contrast to the clinically more favorable situation of oncogene addiction (70,71) where oncogene inactivation leads to the death of tumor cells but spares normal cells. A third possibility could be that PDGFR activation is a driver in a subset GBM in all stages but intervention of the pathway needs to be combined with an interference with other aberrations, e.g. in the PTEN, TP53, INK4A/ARF pathways.

In conclusion, a PDGF receptor profile, such as has been demonstrated in the proneural subgroup of GBM, is likely to be a marker of the cell of origin. Such tumors are probably derived from PDGFRα-positive cells. There is also considerable evidence that a constitutively active PDGFR pathway is a driver in a subset of GBM, although formal proof is still lacking.

Targeting the PDGFR, or other signaling pathways, in GBM has so far not been clinically successful. Maybe an unprejudiced search for GBM antagonists by high-throughput screening will be more fruitful than the 'intelligent design' of targeted drugs. A promising substance has recently been identified (72). There is currently a vibrant activity in basic, translational, and clinical research on GBM as well as in cancer research in general. It is my personal belief that these research activities will bear fruit and translate into a better treatment of GBM, which until now has remained a tumor with a dismal prognosis.

Acknowledgements

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