Invited Review

Platelet-Derived Growth Factor (PDGF) in primary brain tumours of neuroglial origin

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Summary. It has become clear that disruptions in the genome of somatic cells play a causative role in tumour development. We know that the ultimate formation of a malignancy is the result of a multistep process in which the functional loss and/or the altered or increased expression of genes play important roles. One such family of genes are the oncogenes, encoding protein products with mainly growth stimulating effects. Platelet-derived growth factor (PDGF) belongs to the family of oncogenes. It is likely that PDGF plays an essential role in the development of at least a subgroup of malignant astrocytic tumours that do not contain amplification of the EGF-receptor. The expression of PDGF α-receptors is related to tumour progression in these tumours, and some of the most malignant tumours were shown to contain amplification of the PDGF α-receptor. It is also clear now from several experimental studies that PDGF can drive the transformed phenotype, and that PDGF antagonists, by blocking the PDGF autocrine pathway revert the transformed phenotype of certain tumour cells. Because of the findings that receptor protein tyrosine kinases such as the EGF- and the PDGF-receptor play a crucial role in the development of gliomas, it is possible that inhibitors of the phosphorylation of the protein tyrosine kinases will be future candidates for glioma therapy. They might be able to at least delay the development of a fully malignant glioma. The role of PDGF in other tumours of neuroglial origin in the central nervous system has not been studied as extensively as its role in gliomas. Recent data suggest that also for the primitive neuroectodermal tumours overexpression of the PDGF α-receptor is related to malignancy of the tumours. For other tumours, such as neuroblastomas, PDGF exerts a differentiating rather than a mitogenic function and is an important survival factor. Further studies are needed to elucidate the role of PDGF in these non-glial primary brain tumours. Moreover, for a complete understanding of the role of PDGF in malignancies of the CNS, it is important to explore its function in the development of the normal

CNS further.

Introduction

Despite recent advances in clinical oncology during the last decades, the possibility of a curative treatment of malignant brain tumours still seems remote. Most patients recieve intracranial operation as a first choice treatment. However, a number of complicating factors, such as tumour localization in the vicinity of vital nerve and blood vessel structures as well as the often extremely malignant behaviour of the tumour with infiltrative growth and micrometastases within the brain, may make radical resection impossible (Russel and Rubinstein, 1989). It is therefore highly warranted to look for alternative treatments. Understanding the biological behaviour of the tumour is a prerequisite for a rational non-surgical treatment.

During the last decade, huge advances have been made in the understanding of the genetics of cancer. Developments in molecular biology have pointed out the involvement of several families of genes in the development of human cancer. One such gene family is the family of oncogenes, including the family of growth factors and growth factor receptors. Oncogenes are altered versions of normal cellular genes (proto-oncogenes) that are involved in the regulation of normal cell growth, development and cell death. By several genetic alterations the proto-oncogene can be converted into an oncogene with transforming activity (Westermark et al., 1990).

One of the classic examples of the close relationship between the protein products of proto-oncogenes and the products of oncogenes was the discovery in 1983 that the Platelet-derived Growth Factor (PDGF) B-chain is the cellular counterpart of the transforming protein of simian sarcoma virus (SSV), p^{28sis} (Doolittle et al., 1983; Waterfield et al., 1983). It was then shown that SSV-transformed cells secreted a molecule with PDGF-

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like activity (Johnsson et al., 1985a). Moreover, SSV could only transform PDGF-responsive cells (Leal et al., 1985), i.e. cells bearing PDGF receptors, and SSV transformation could be blocked by antibodies to PDGF (Johnsson et al., 1985b). The cell transformation of PDGF-responsive cells by SSV *in vitro* was consistent with the generation of malignancies *in vivo* of only those cells that express PDGF receptors, such as cells derived from connective tissue and glia (Heldin et al., 1981). Thus, intracerebral injection of SSV together with its helper virus in newborn marmosets induced fibromas, fibrosarcomas and gliomas (Deinhardt, 1980). Microscopically, some of these experimental brain tumours in the newborn marmosets showed the characteristics of human glioblastoma multiforme, such as endothelial cell proliferation, vast necrosis and cellular pleomorphism. Apart from establishing the transforming activity of the PDGF B-chain, these studies provided the first clear link between oncogenes and growth factors.

PDGF and PDGF receptors

PDGF was first isolated from blood platelets in 1979 as a mitogen for connective tissue cells and glial cells (Antoniades et al., 1979; Heldin et al., 1979). The molecule is a disulphide-linked dimeric protein with a molecular size of approximately 30 kDa, and consists of two subunits that are almost equal in size, the A- and B-chain. All three possible homo- and heterodimeric combinations of the PDGF A- and B-chain occur, forming PDGF-AA, PDGF-BB or PDGF-AB (reviewed by Heldin et al., 1993). PDGF is expressed by a variety of cell types in the body and is thought to play an essential role in the embryonal development and wound healing. Also, PDGF is involved in pathological disorders such as inflammation, arteriosclerosis and cancer (reviewed in Heldin and Westermark, 1990; Raines et al., 1990). Two receptors for PDGF, the α- and ß-receptors, have so far been identified (reviewed in Claesson-Welsh, 1994). These two receptors bind, with different affinities, the three PDGF isoforms, i.e. the α-receptor binds all three isoforms with high affinity, whereas the ß-receptor binds PDGF-BB with high affinity, PDGF-AB with low affinity and does not bind PDGF-AA (Claesson-Welsh, 1994). The PDGF receptors belong to a family of structurally related growth factor receptors possessing protein tyrosine kinases in their intracellular domains (Claesson-Welsh, 1994). Ligand binding induces receptor dimerization and activation of the intracellular tyrosine kinases. For a number of biological effects, the two PDGF receptors show differential signal transduction pathways, as demonstrated by several studies (Claesson-Welsh, 1994). In addition to cell growth, cell locomotion, and in particular chemotaxis, is essential in many of the biological functions of normal as well as malignant cells (reviewed in Stoker and Gherardi, 1991). A chemotactic response at least for some cell types, such as human fibroblasts, was mediated only by the PDGF ß-receptor

(Siegbahn et al., 1990). Recently, structural analysis of the PDGF receptors has linked the chemotactic responses to specific tyrosine residues in the tyrosine kinase domains (Kundra et al., 1994; Yokote et al., 1996).

The role of PDGF in the development of brain tumours, and especially those tumours that are derived from glial cells, by a possible autocrine mechanism has been the subject of extensive studies, which is the focus of this review. Since new discoveries have been made on the role of PDGF in the normal development of the central nervous system, we will first present a brief review on PDGF in normal glial and neuronal development. Apart from the PDGF receptor, several other protein tyrosine kinases have been implicated in the development of the CNS and in tumourigenesis, as is discussed elsewhere (reviewed in Weiner, 1995).

PDGF in development of the CNS

Several studies have demonstrated that the PDGF α-receptor is required for the normal development of the nervous system as well as of other organ systems. More detailed insight in the *in vivo* function of the PDGF α-receptor in the development of the nervous system has been obtained by the finding that the PDGF α-receptor gene is deleted in the Patch (Ph) mouse (Stephenson et al., 1991). In the Ph-mutant mice, defects were found in the non-neuronal derivates of the neural crests, whereas crest-derived neurons appeared normal. Consistent with these findings was the high expression of PDGF α-receptor mRNA in neural crest derivates during late embryogenesis in non-mutant mice (Orr-Urtreger et al., 1992; Schatteman et al., 1992). PDGF A- or B-chain knockout mice do not show any visible defects in the CNS (Levéen et al., 1994; Boström et al., 1996). However, since the mice die late in gestation or early in life, tissue-specific knockout targeting the nervous system remains to be done to clarify the role of PDGF in the CNS. It is also possible that double knockouts of both PDGF chains are necessary to obtain any visible morphological changes.

In a series of now classic experiments, PDGF was shown to exert a crucial role in the development of cells of the glial cell lineage in the rat optic nerve (Noble et al., 1988; Raff et al., 1988). The optic nerve serves as an excellent model for studying gliogenesis *in vitro* because of the absence of neurons and the presence of only three types of postmitotic glial cells, i.e. oligodendrocytes, type I astrocytes and type II astrocytes. It was found that PDGF is a growth promoting factor for the O-2A cell, a progenitor cell that gives rise to oligodendrocytes and type II astrocytes, and that PDGF prevents premature differentiation of the O-2A progenitor cell. Thus, PDGF was able to cause proliferation and, after a certain time, programmed differentiation of the O-2A progenitor cells (Noble et al., 1988; Raff et al., 1988). The type I astrocyte in the optic nerve was found to be the source for PDGF, and the effect was mediated by PDGF-AA via

the transient expression of PDGF α-receptors on the O-2A progenitor cells (Pringle et al., 1989). The expression of PDGF α-receptors in the rat CNS is tightly regulated during development, and restricted to cells of the glial cell lineage (Pringle et al., 1992). Further studies revealed that PDGF is a survival factor for O-2A progenitor cells and for newly formed oligodendrocytes (Barres et al., 1992; Raff et al., 1993). Thus, PDGF is both a mitogen and a survival factor for O-2A progenitor cells. The growth factor appears to promote survival by suppressing an active death programme in the cell. It is suggested that the requirements for survival factors is a more general phenomenon than previously thought, that occurs also in non-neuronal cells (Raff, 1992). Interestingly, PDGF has also been recognized as a trigger for apoptosis in growth-arrested murine fibroblasts (Kim et al., 1995). Thus, depending on the state of the cell, PDGF can both initiate cell growth and cell death. Recently, the role of PDGF and other growth factors in remyelination in the CNS has been reviewed (Woodruff and Franklin, 1997).

It has become clear that the function of PDGF in the CNS is not restricted to cells of the glial lineage. The PDGF A-chain was found to be expressed by neurons of embryonic and adult mice (Yeh et al., 1991), and the PDGF B-chain was demonstrated in neurons throughout the brain of a nonhuman primate (Sasahara et al., 1991). In a transgenic mouse model in which an enzymatic marker gene was placed under the transcriptional control of the PDGF B-chain promotor, the transgene was mainly expressed within neuronal cells of the mouse brain (Sasahara et al., 1991). In the developing rodent mouse retina, the PDGF A-chain is accumulated in retinal ganglion neurons during target innervation, whereas the PDGF α-receptor is expressed in retinal astrocytes, suggesting paracrine interactions between neighbouring layers of cells (Mudhar et al., 1993). Furthermore, functional PDGF ß-receptors were found on developing neurons of the rat brain, and a neurotrophic function for PDGF was suggested (Smits et al., 1991). The PDGF ß-receptors on these immature neuronal cells could also transduce a chemotactic response (AS, KF, A. Siegbahn, unpublished data). Since then, several studies have appeared that aim to identify the PDGF responsive neurons within the CNS, and to elucidate the exact role of PDGF for the nerve cell. Thus, PDGF-BB exerts trophic effects on developing GABAergic neurons of the rat cerebellum (Smits et al., 1993). Other cell culture studies revealed dopaminergic neurons of the ventral mesencephalon as targets for the neurotrophic actions of PDGF (Nikkhah et al., 1993; Othberg et al., 1995). It is likely that the PDGF receptor uses distinct signaling pathways for such different phenotypic responses as mitogenesis and neuronal differentiation (Vetter and Bishop, 1995).

Recently, a novel modulary function of PDGF-BB in regulation of both inhibitory and excitatory synaptic transmission in the brain has been demonstrated. Thus, an inhibition of native type A GABA receptors in rat hippocampal CA1 pyramidal neurons after brief activation of the PDGF ß-receptor on the cells has been reported (Valenzuela et al., 1995). Also, PDGF exerted a long-term inhibition of NMDA receptors on cultured hippocampal neurons (Valenzuela et al., 1996). The last study, in which PDGF was found to exert long-term inhibition of excitatory synaptic neurotransmission, supports recent findings that PDGF can exert neuroprotective action on neuronal populations in the CNS, either by direct or indirect patways (Cheng and Mattson, 1995; Pietz et al., 1996). A putative neuroprotective role for PDGF in the brain is of clinical interest for those non-neoplastic pathological conditions such as trauma, injury and focal ischaemia, in which elevated levels of PDGF and PDGF receptors have been found (Hermanson et al., 1995; Funa et al., 1996; Iihara et al., 1996).

The above described studies establish the neurotrophic action of PDGF on several neuronal subpopulations in cell culture, by promoting cell survival and the outgrowth of neurites and dendrites on the cells. That PDGF may have a neurotrophic effect in the developing mammalian brain also *in vivo*, was shown in an intraocular transplantation model (Giacobini et al., 1992, 1993). Different areas of fetal rat brain were transplanted into the anterior eye chamber of a host rat, and the effects of PDGF-AA and PDGF-BB on the development of the grafts was monitored. Thus, PDGF-AA and PDGF-BB had differential effects on intraocular-transplanted pieces of fetal parietal cortex and hippocampus (Giacobini et al., 1992). Also, PDGF-AA promoted the outgrowth and maintenance of dopaminergic fibers from developing substantia nigra neurons, whereas PDGF-BB promoted the survival of the tyroxine hydroxylase-positive cells in this area (Giacobini et al., 1993). These experiments show that the PDGF receptors on the cells of the developing brain are functional, and provide the first direct evidence that PDGF can influence the development of cells of the CNS *in vivo*. Moreover, a possible neurotrophic effect of PDGF on mesencephalic dopaminergic neurons is of great clinical interest for neurodegenerative disorders such as Parkinson's disease. Although most studies so far impute the neurotrophic effects of PDGF to the PDGF ß-receptor, there are also reports that describe PDGF α-receptors on various neuronal populations in the adult CNS, such as fetal dopaminergic neurons and motoneurons of the spinal cord (Ballagi et al., 1993; Vignais et al., 1995).

Although a large number of questions still remain unanswered concerning the role of PDGF in the CNS, it is clear that there is a distinct and differential expression of the two PDGF receptors in the mammalian brain, which seems to be developmentally regulated. This differential expression of the receptors together with the widespread distribution of the PDGF ligands suggests important functions for PDGF in both gliogenesis and neurogenesis. To gain full insight into the role that PDGF plays in the complex process of development of

the CNS, it will be of great interest to identify both the stimulating and inhibitory factors that regulate the transcription of the genes for PDGF α- and PDGF ß-receptors at the molecular level. The recent isolation and molecular cloning of the promotor genes for both the PDGF α-receptor (Wang and Stiles, 1994; Afink et al., 1995) and the PDGF ß-receptor (Ballagi et al., 1995; Shinbrot et al., 1997) will facilitate these studies.

PDGF in primary tumours of neuroglial origin

Gliomas

Glioma is the most common malignant primary brain tumour. The tumours are derived from neuro-epithelial cells and include a heterogeneous group of tumours. They are classified into different grades of malignancy, according to histopathological findings such as cellular pleomorphism, necrosis and vascular proliferations (Russel and Rubinstein, 1989). According to the WHO criteria, the astrocytic tumours can be classified in three subgroups: low grade astrocytoma, anaplastic astrocytoma and the most malignant form, glioblastoma multiforme (GBM) (Kleihues et al., 1993). Apart from the astrocytic tumours, the gliomas include ependymomas, oligodendrogliomas and mixed gliomas (oligoastrocytomas).

The finding that intracerebral injection of SSV, a retroviral form of the PDGF B-chain, causes gliomas in newborn marmosets suggested that PDGF has a role in the pathogenesis of gliomas (Deinhardt, 1980). Human glioma cells in culture often express the genes encoding the two PDGF chains (Eva et al., 1982; Nistér et al., 1988a) and secretion of PDGF protein in the cell culture medium has been shown (Nistér et al., 1988b). Also, the expression of PDGF receptors on human malignant glioma cell lines has been demonstrated (Nistér et al., 1992). In this last study, a large number of glioma cell lines were tested for the presence of PDGF receptors, and all three phenotypes, i.e. expressing both PDGF receptors or exclusively α- or ß-receptors, were found (Nistér et al., 1992). Interestingly, when the data on the expression of both PDGF chains, both PDGF receptors as well as the pattern of receptor binding specificity for these glioma cell lines were all taken into account, the majority of cell lines showed a phenotype that makes autocrine growth stimulation by PDGF possible (Nistér et al., 1992). All these findings support the autocrine hypothesis (Sporn and Roberts, 1985), indicating that malignant cells exhibit a relative growth factor autonomy and a relative independence of exogeneous growth factors for their survival and multiplication compared to their normal counterparts, by endogenous production of growth factors that act with functional receptors on the same cell. Recently, more direct proof for the importance of PDGF for autocrine growth stimulation and the malignant phenotype of glioma cells has come from several experimental studies. It was reported that a dominant-negative mutant of PDGF was able to revert the transformed phenotype of two human astrocytoma cell lines (Shamah et al., 1993). The authors used two earlier described dominant-negative PDGF constructs, consisting of mutations within a PDGF-A cDNA clone that form stable PDGF-A homodimers lacking mitogenic activity and are able to form dimers with wild-type PDGF-A and PDGF-B (Mercola et al., 1990). Other strategies to obtain PDGF antagonistic effects on glioma cells have been used successfully, such as by PDGF neutralizing antibodies (Vassbotn et al., 1994) and a truncated receptor (Strawn et al., 1994). In the latter study, the truncated PDGF receptor was also shown to impair the growth of glioma cells transplanted as a xenograft in nude mice (Strawn et al., 1994).

Autocrine growth stimulation via PDGF α-receptors is a possible mechanism for the growth of human glioma cells even *in vivo* (Hermanson et al., 1992). In this study, PDGF A- and B-chain were consistently found in tumour cells of high malignancy grades, which together with the finding of PDGF α-receptor on tumour cells of all histopathological grades, makes an autocrine loop possible (Hermanson et al., 1988, 1992) (Fig. 1). These studies also revealed a putative role for PDGF in neovascularization of the tumour because of the presence of high levels of PDGF ß-receptors on hyperplastic endothelium in the high grade gliomas (Hermanson et al., 1988). A paracrine activation of these endothelial cells via PDGF produced by the tumours cells, or an autocrine activation via PDGF produced by the endothelial cells themselves (DiCorleto et al., 1983), are possible pathogenic mechanisms (Fig. 1). Neo-vascularization in tumour tissue in turn supports further growth of the tumour. In fact, an angiogenic activity of PDGF *in vivo* has later been established (Risau et al., 1992).

Support for the concept that glioblastoma multi-forme is a heterogeneous group of tumours with different molecular phenotypes comes from several studies (von Deimling et al., 1993). Several genetic alterations such as chromosomal loss and gene amplifi-cation may contribute to the pathogenesis of the tumour (reviewed in Westermark and Nistér, 1995). Amplifi-cation and/or overexpression of the genes for various growth factors and growth factor receptors in glial tumours has been reported, such as amplification of the EGF receptor gene (Libermann et al., 1985). Excessive EGF signalling may lead to loss of growth control of the tumour cells. Amplification of the EGF receptor was found to be associated with loss of genetic material on chromosome 10 in glioblastoma multiforme (von Deimling et al., 1992). Amplification of the PDGF α-recepor was found in only a few cases of glioblastoma multiforme (Fleming et al., 1992; Kumabe et al., 1992). Interestingly, amplification of the genes for the PDGF α-receptor and the EGF receptor occurs in different subtypes of glioblastomas (Fleming et al., 1992). Recently, the other subtype of malignant glioma with overexpression and/or amplification of the PDGF α-receptor gene was shown to be associated with loss of

heterozygosity (LOH) on chromosome 17p (Hermanson et al., 1996). Because of this finding, the p53 tumour suppressor gene (reviewed in Greenblatt et al., 1994) was examined for mutations by using SSCP analysis and direct sequencing. In contrast to the association between LOH on chromosome 17p and increased PDGF α-receptor expression, no significant statistical correlation was found between p53 mutations and increased PDGF α-receptor mRNA levels (Hermanson et al., 1996). Because elevated levels of PDGF α-receptor mRNA

were found in all malignancy grades with the highest levels in the most malignant tumours, overexpression of the PDGF α-receptor is probably an early event in the development of glial tumours and related to tumour progression (Westermark et al., 1995; Hermanson et al., 1996). In agreement with this notion, amplification of the PDGFα-receptor gene has so far only been demonstrated in glioblastoma multiforme (GBM), the most malignant tumour (Fleming et al., 1992; Kumabe et al., 1992).
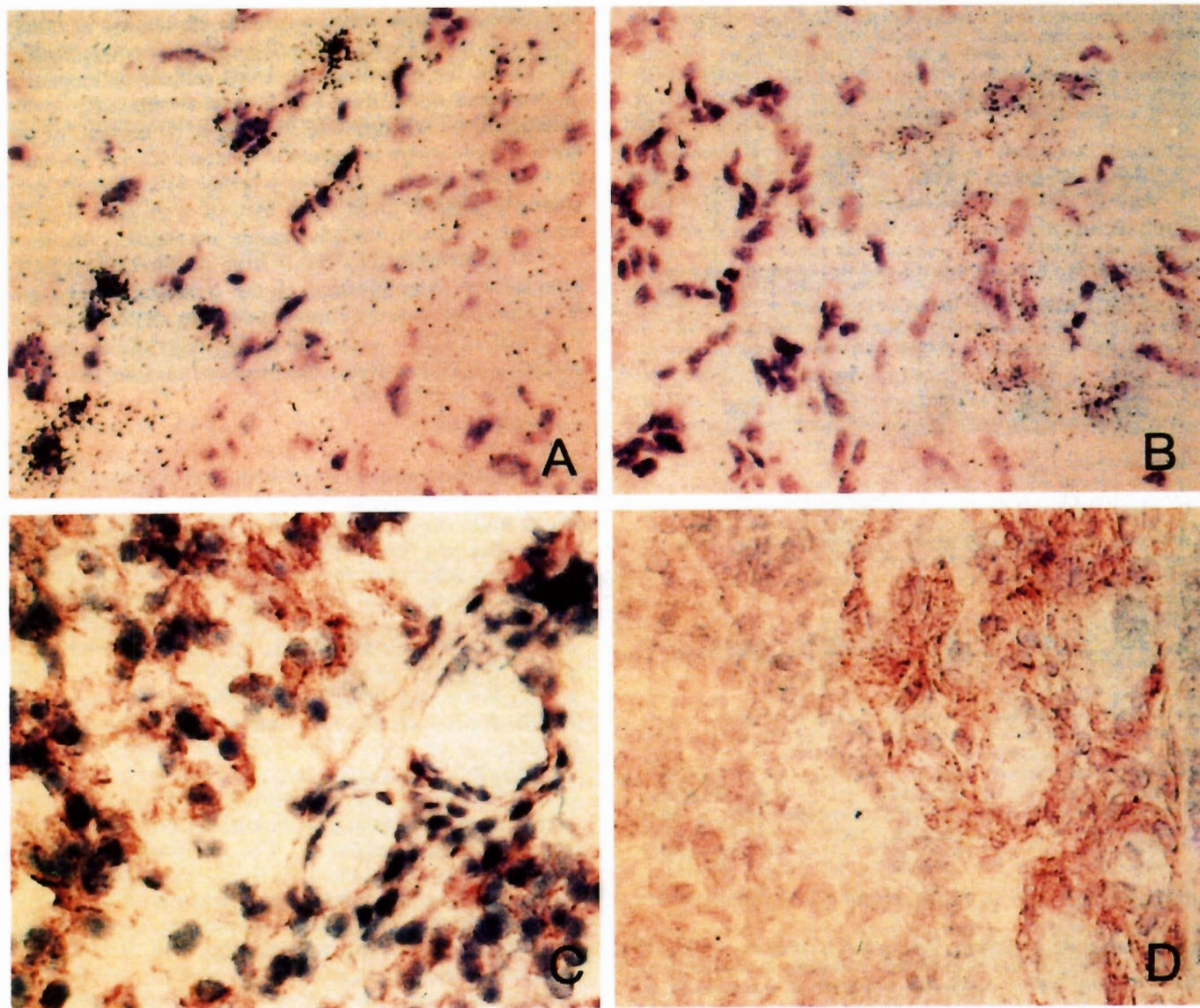

Fig. 1. Photomicrographs showing the border between glioma cells proper and hyperplastic capillaries in human glioblastoma tissue. In situ hybridization and immunohistochemistry to detect PDGF receptor (PDGFR) mRNA and protein respectively were performed. **A.** Strong in situ hybridization signals over tumour cells when probed with PDGFR-α cRNA. **B.** The same area as in A, showing strong PDGF-ß signals confined to the proliferative vessels; only background signals are seen over tumour cells. **C.** Strong immunostaining of tumour cells and almost no staining of hyperplastic capillaries with the anti-PDGFR-α antibodies. **D.** Area corresponding to that in C stained with the monoclonal anti-PDGFR-ß antibody. A strong staining is seen on hyperplastic capillaries, whereas no staining is recorded on tumour cells. (Adapted from Hermanson et al., 1992; with permission from the publisher).

Clinically, patients with GBM that show amplification of the EGF receptor are usually older than those patients with neither EGF receptor gene amplification nor LOH of 17p (von Deimling et al., 1993). Also, the tumours most often arise de novo, i.e. without a history of previous lower-grade tumour, and show an extremely malignant behaviour at clinical presentation. It is interesting to speculate that the subset of GBM with gene amplification of the EGF receptor is derived from an immature uncommitted EGF-responsive precursor cell that has recently been demonstrated in the adult mammalian CNS (Reynolds and Weiss, 1992). By one or more molecular defects the precursor cells have become transformed and have abolished their normal differentiation pathway. The other subset of GBM with LOH of 17p and overexpression and/or amplification of the PDGF α-receptor gene could then be derived from another precursor cell, that is PDGF α-receptor responsive and committed to the O-2A cell lineage (Fig. 2). Such O-2A precursor cells have also been found to be present in the mature mammalian brain (Wolswijk and Noble, 1989).

The existence of other subsets of GBM, representing the transformed phenotypes of other yet unidentified precursor cells, is possible (von Deimling et al., 1993). Most likely there are several genetic events of crucial importance for the growth of glioma cells, including the overexpression of positive growth regulators as well as the loss of growth inhibitors (reviewed in Westermark

Fig. 2. Hypothetical model for the origin of a subgroup of malignant astrocytic tumours in which the PDGF α-receptor is involved in the pathogenesis. PDGF α-receptors on the cell membranes are schematically drawn (ʃ). **A.** Under normal conditions the O-2A progenitor cells give rise to the oligodendrocyte and the type II astrocyte cell lineages. During the differentiation process the progenitor cells gradually loose their PDGF α-receptors. **B.** In the case of cell transformation, the O-2A progenitor cells might give rise to astrocytoma cells of low malignancy grade that fail to loose their receptors and finally turn into high grade astrocytomas with overexpression of PDGF α-receptors. An autocrine growth stimulation by PDGF produced by the tumour cells can now take place.

and Nistér, 1995). It has been assumed from the SSV-induced tumours in newborn marmosets that autocrine growth stimulation via the PDGF receptor is an early event in the multistep process of the pathogenesis of gliomas (Deinhardt, 1980). In this respect, the recent finding of metastatic tumour formation in athymic mice by injection of PDGF-B/v-sis transduced non-tumourigenic human glioma cell line expressing PDGF ß-receptors and low amounts of endogenous PDGF-B, is interesting (Patopova et al., 1996). The authors speculate that the induction of the fully malignant phenotype by sufficient stimulation of the PDGF ß-receptors may depend on preexisting lesions, such as loss of p53 function. These preexisting lesions, not sufficient themselves to give transformation of the cells with metastatic spread, could be related to the autocrine production of endogenous PDGF-B (Patopova et al., 1996).

Other neuroepithelial tumours

In contrast to the large number of studies on the role of growth factors like PDGF and EGF in the pathogenesis of glial tumours, little is known about these growth factors in other primary brain tumours of neuroglial origin. Here we will highlight some data of interest on a few non-glial neuroepithelial tumours.

Neuroblastoma

Neuroblastoma is an embryonal tumour that is composed of neural crest-derived sympathetic neuroblasts with varying degree of differentiation (Evans, 1980). Among the solid tumours that occur in early childhood, neuroblastoma is one of the most common tumours. Several cell lines of human neuroblastomas have been established that provide useful tools for studying neuronal differentiation by agents such as retinoic acids and phorbol-esters. For one such cell line it was shown that PDGF could potentiate the neuronal differentiation of the cells induced by phorbol-ester (Påhlman et al., 1992). The neuronal differentiation of the neuroblastoma cells induced increased levels of neuronal specific peptides/proteins such as NPY and GAP-43, and morphological changes of the cells such as increased neurite outgrowth. The undifferentiated cells contained both types of PDGF receptors. After differentiation of these neuroblastoma cells into a neuronal phenotype, a downregulation of the PDGF α-receptors was observed whereas the level of PDGF ß-receptors was unchanged (Påhlman et al., 1992). In a more recent study, both types of PDGF receptors were found on a number of human neuroblastoma cell lines (Matsui et al., 1993). PDGF induced neuronal differentiation of the cells, and a chemotactic as well as a small mitogenic activity was induced by both PDGF-AA and PDGF-BB (Matsui et al., 1993). Thus, these two studies show that PDGF is a differentiation factor rather than a mitogen for neuroblastoma cells in culture, and
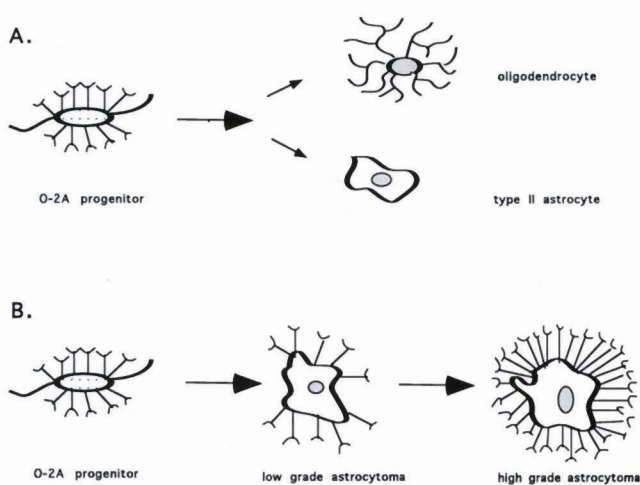
suggest that PDGF is involved in the development of sympathetic neurons. Studies in our own laboratory on a mouse neuroblastoma cell line, NB41, have demonstrated the importance of PDGF as a survival factor (Funa and Åhgren, 1998). By using an anti-sense strategy to block PDGF ß-receptor expression, it was shown that deprivation of PDGF ß-receptors converted the cells to morphologically immature neuroblasts and induced their apoptosis. An intriguing question is whether PDGF in cooperation with another mitogen has enhanced mitogenic activity on the immature neuroblastoma cells, as has been shown for PDGF in combination with bFGF on O-2A progenitor cells (Bögler et al., 1990). It will also be interesting to explore a possible correlation between the expression of PDGF receptors in neuroblastoma tumours and clinical prognosis. Certain genetic abnormalities in neuroblastomas, like amplification of N-myc, are strongly correlated to poor prognosis (reviewed in Brodeur, 1989).

Medulloblastoma and other PNETs

Among the primitive neuroectodermal tumours (PNETs) medulloblastoma is the prototype that is localized in the cerebellum, predominantly in the vermis (Russel and Rubinstein, 1989). The tumours are characterized by immature tumour cells that have a multipotent capacity for differentiation into different phenotypes, such as neuronal and glial, or less common muscle, melanotic, etc. (Provias and Becker, 1996). Medulloblastoma is the most common pediatric CNS tumour, and comprises about 20% of all malignant brain tumours in childhood. The tumour probably originates from one or more stem cells that by genetic defects and/or environmental factors have abolished the differentiation pathway at different stages. According to this theory, each tumour cell represents a different stage of maturation along the neuronal or glial cell lineage (Trojanowski et al., 1992, 1994). There has been a search for growth factors and growth factor receptors as putative regulators of cell division and differentiation of the "medulloblast" (reviewed in Goumnerova, 1996). Interestingly, the presence of TrkC, the tyrosine kinase receptor for the neurotrophin NT-3, was found at high levels in medulloblastomas *in vivo*, and was associated with a better clinical prognosis (Segal et al., 1994). Recently, it was also shown that both PDGF α- and ß-receptors are expressed on medulloblastomas and PNETs, and that the expression of both PDGF receptors was associated with an immature neuronal phenotype of the cells (Smits et al., 1996). This "aberrant" expression of the PDGF α-receptor on tumour cells with neuronal differentiation was a consistent finding in this study, and the authors speculate that it might be a feature of the malignant phenotype of the tumour cells. Further studies are needed to find out whether the expression of the PDGF α-receptor is associated with tumour progression and with a poor clinical outcome for the patients.

Clinical applications

There is still no established therapeutic strategy available that is based on the inhibition of growth factors like PDGF for patients suffering from intracranial tumours. However, several molecules with PDGF antagonistic effect have been used successfully in experimental systems.

Monoclonal antibodies to PDGF were shown to inhibit the smooth muscle proliferation in the rat intima that occurs after angioplasty, and are of potential interest for the treatment of diseases such as arteriosclerosis (Ferns et al., 1991). Recently, neutralizing antibodies to PDGF were able to reverse the transformed phenotype of glioma cells, demonstrating that specific antagonist acting at the cell surface can block the autocrine pathway (Vassbotn et al., 1994). Other PDGF antagonists that have been successfully used experimentally are soluble receptors (Duan et al., 1991), dominant-negative mutants (Shamah et al., 1993; Vassbotn et al., 1993) and tyrosine kinase inhibitors (Kovalenko et al., 1994). Although these PDGF antagonists were used for functional studies of PDGF and to reverse PDGF-dependent autocrine transformation, some of them are potentially interesting for clinical trials. One such type of compounds are the tyrphostins, synthetic low-molecular weight compounds with broad specific protein kinase inhitory effects (Kovalenko et al., 1994). Because of their broad specificity and their high efficiency at low doses, the tyrphostins are attractive candidates for therapy of tumours in which growth stimulation by protein tyrosine kinases such as the PDGF- and EGF-receptors play a crucial role. It was recently shown that relatively low amounts of tyrphostins inhibit EGF- and PDGF-responsive glioma cell growth by blocking the protein tyrosine kinase activity of the receptors (Oude Weernink et al., 1996).

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