Human Glial Fibrillary Acidic Protein: Complementary DNA Cloning, Chromosome Localization, and Messenger RNA Expression in Human Glioma Cell Lines of Various Phenotypes

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ABSTRACT

Gial fibrillary acidic protein (GFAP) is a constituent of intermediate filaments of gial cells of the astrocytic lineage. We cloned a human GFAP complementary DNA, deduced the amino acid sequence, and established the chromosomal location (17q21) of the GFAP gene by Southern blot hybridization of somatic cell hybrids and by in situ hybridization. The authenticity of the complementary DNA was proven by expressing it in glioma cells lacking endogenous GFAP; after microinjection of the complementary DNA, such cells became positive for staining with GFAP antibodies. The levels of fibronectin (FN) and GFAP mRNA of ten human glioblastoma cell lines, determined by Northern blot hybridization, were related to other phenotypic characteristics (cell morphology and expression of the genes encoding platelet-derived growth factor (PDGF) receptors). A high expression of GFAP mRNA was found only in cells lacking fibronectin mRNA and protein. Glioma cells with a fibroblastic phenotype (bipolar, FN+/GFAP−) were found to express both types of PDGF receptors (α and β). Relatively high levels of PDGF α-receptor mRNA, in the absence of β-receptor expression, were found in cell lines that express GFAP and lack detectable levels of fibronectin mRNA. The findings are compatible with the idea that the genes encoding PDGF receptors in glioma cells are regulated in concert with other genes, the expression of which may reflect the developmental program of normal gial cell lineages.

INTRODUCTION

Intermediate filaments are 8–10-nm fibers that form part of the cytoskeleton of virtually all cell types in higher eukaryotes (1–4). On the basis of their tissue-restricted distribution, intermediate filament proteins have been divided into five main classes: desmin; vimentin; GFAP; neurofilament proteins; and keratins. All intermediate filament proteins have the same structural organization with a central α-helical rod domain flanked by amino and carboxy termini of variable sizes.

GFAP is a constituent of gial cells of the astrocytic lineage (5) and is generally also present in primary gial brain tumors (6). However, a high proportion of cell lines established from malignant gliomas do not express GFAP (7). Immunohistochemical analyses of glioma cell lines have shown that the expression of GFAP and the extracellular matrix protein fibronectin (8) is mutually exclusive (9), even in cells derived from the same tumor (10). In order to facilitate further studies on the expression of GFAP in human gial cells of different phenotypes, we have in the present investigation cloned a full length human GFAP cDNA and determined the corresponding amino acid sequence. The chromosomal location of the GFAP gene was also established.

Recent studies have suggested that PDGF has a physiological role in the development of gial cells in the central nervous system. Thus, the bipotential O-2A progenitor cells of the optic nerve respond by proliferation to PDGF produced by type 1 astrocytes (11, 12). Given the fact that the v-sis oncogene encodes a PDGF-like growth factor (13–15), the finding that intracerebral injection of simian sarcoma virus in newborn marmosets gives rise to brain tumors of the glialblasta phenotype (16) suggests that an autocrine activation of the PDGF receptor in gial cells is a causal event in tumorigenesis. Whether a similar autocrine mechanism also operates in the genesis of human “spontaneous” glioblastoma is unclear. There are several examples of human glioma cell lines that express one or both of the PDGF chains (17–19) concomitantly with receptors for PDGF (18–20), but it is not known if activation of these genes is a cause or consequence of malignant transformation. PDGF synthesis and expression of the cognate receptor may occur in certain normal glia cells (cf., Refs. 12 and 21), and this phenotype may be retained after malignant transformation. We have reasoned that if this were the case, one should expect that expression of PDGF and its receptors be related to other phenotypic markers. This is a difficult problem to study since, particularly in the human, very few differentiation markers for gial cells are available, and the progenitor cell for gialblasta has not been identified.

In the present investigation we have used the best characterized astrocyte marker, namely GFAP, and studied its expression in relation to cell morphology and expression of PDGF α- and β-receptor and fibronectin.

MATERIALS AND METHODS

cDNA Cloning and Nucleotide Sequence Analysis. Standard molecular biology techniques (22) were used unless otherwise indicated. The mouse GFAP cDNA clone G1 (23) was radiolabeled using [32P]deoxynucleotide triphosphate and the Klenow fragment of DNA polymerase I. The radiolabeled plasmid was used to screen a cDNA library made from the human clonal glioma cell line U-342 MGA C12:6 (24) which expresses GFAP (10). The recombinant phages were plated on Escherichia coli C600 hfl (25). Duplicate nitrocellulose filter lifts were hybridized with 32P-labeled G1 at 42°C in 20% formamide-5 × SSC (1 × SSC is 0.15 M sodium chloride-0.01 M sodium citrate)-50 mM sodium phosphate, pH 7.0-5 × Denhardt’s solution-0.1% SDS-200 μg/ml sonicated single stranded DNA and washed in 0.5 × SSC-0.1% SDS at the same temperature. Positively hybridizing clones were plaque purified and cDNA was isolated. The cDNA insert was isolated by EcoRI digestion and subcloned in PUC-13. The nucleotide sequence of the human cDNA restriction fragments was determined by the dideox-
Ylueleclone chain termination method (26) after subcloning into M13 phage derivatives (27).

Analysis of the Expression of Microinjected GFAP cDNA. To determine the expression of cloned GFAP cDNA, the clone E2C2 (see below) was inserted in sense orientation into the EcoRI site of the retroviral vector RD2 (kindly provided by Dr. L. Hellman, Department of Immunology, University of Upsala). The expression plasmid was designated pGFAP-RD2. The human U-343 MG glioma cell line, which does not express GFAP (10), was grown to subconfluence on 18- x 18-mm glass coverslips in Falcon dishes in MEM supplemented with 10% fetal calf serum (Gibco Ltd., United Kingdom) and antibiotics. pGFAP-RD2 DNA, at a concentration of 0.5 μg/ml, was microinjected into the cell nucleus using an Olympus IMT2-SYF injectoscope. The cells were then incubated at the same culture conditions for 48 h. Cells were fixed in 3% paraformaldehyde in PBS for 10 min at room temperature and in 100% acetone for 1 min at -20°C. Immunostaining of GFAP was performed using a rabbit immunoglobulin to cow glial fibrillary acidic protein (Dakopatts, Denmark) following a rhodamine-conjugated swine anti-rabbit IgG antibody (Dakopatts) at room temperature. Cells were rinsed with phosphate-buffered saline and examined by fluorescence microscopy. Cells not treated with injections of GFAP cDNA growing on the same coverslip were used as a control.

Chromosomal Localization of the Human GFAP Gene. The GFAP cDNA clone pCIA-5 described in this report (see below) was used to determine the chromosomal localization of the human GFAP gene. Cloned DNA probes for genes which have previously been regionally localized on chromosome 17 were used for comparison and are described in detail in Table 1. Probes (entire plasmid including insert and vector) were radiolabeled by nick-translation using [32P]deoxyribonucleotide triphosphates to a specific activity of 1 x 10^9 cpm/μg. 1 x 10^8 cpm were used for each Southern blot hybridization.

Isolation, propagation, and characterization of parental cell lines and somatic cell hybrids have been described (28). For regional localization of the GFAP gene on chromosome 17, a series of hybrid cell lines (Table 1), each retaining only a partial chromosome 17, were used (29, 30).

Cellular DNA was isolated as described (31), digested with an excess of restriction endonuclease HindIII or SstI, size fractionated in 0.8% agarose gels, transferred to nylon membrane using 0.4 M NaOH (32), and dried under vacuum. Prehybridization and hybridization were carried out in 1.5 x saline-sodium phosphate-EDTA (1 x saline-sodium phosphate-EDTA is 0.15 M NaCl-0.01 M NaH2PO4-1 mM disodium EDTA, pH 7.4), 1% SDS, 0.5% nonfat powdered milk, and 0.5 mg/ml carrier salmon sperm DNA at 42°C. Filters were washed with 0.1 x SSC-0.1% SDS at 68°C and exposed to Kodak XAR-5 films at -80°C with intensifying screens for 12-16 h.

For in situ chromosomal hybridization, pCIA-5 was nick-translated with [3H]dCTP to a specific activity of 2 x 10^7 cpm/μg. Normal human metaphase chromosomes from one male and two females were used for hybridization. The methods for in situ hybridization and autoradiography were as described (33, 34). Hybridization was carried out at a concentration of 100 ng labeled probe/ml hybridization solution, and with a 1000-fold excess of carrier herring sperm DNA. Washes after hybridization were at 40°C. Slides were exposed for 8-16 days at 4°C and subsequently developed and G-banded using 0.25% Wright's stain in 0.6 M phosphate buffer (pH 6.8) for 5-15 min. The chromosomal positions of grains located on chromosomes or touching chromosomes were plotted onto ideograms of G-banded human chromosomes as described by banding technique (35).

Cell Lines and Culture Conditions. The establishment of cell lines from human malignant gliomas has been described (36). The cell lines used in the present investigation originated from tumors that were diagnosed as glioblastoma multiforme. All cell lines were grown as monolayer cultures in Eagle's MEM supplemented with 10% newborn calf serum (GIBCO) and antibiotics (100 units of penicillin and 50 μg of streptomycin per ml). Cultures were maintained at 37°C in humidified air containing 5% CO2. By using phase contrast microscopy we assigned the cell lines to either of five morphology groups (19): epithelioid; polygonal; pleomorphic/astrocystoid; fascicular; or bipolar/fibroblastic.

Northern Blot Analysis of Human Glioma Cell Lines. Cells were grown to confluency in routine medium on 100 mm plastic Petri dishes or 850-cm2 Falcon roller bottles. Total cellular RNA was isolated using the LICI/urea method. mRNA was enriched by poly(A) selection on oligodeoxynucleotide cellulose (Pharmacia). Ten μg of poly(A)^+ RNA were denatured for 5 min at 65°C in 50% formamide, 2.2 M formaldehyde, 20 mM morpholinepropanesulfonic acid (pH 7.0), 5 mM sodium acetate, 1 mM EDTA, and 10% glycerol; run on 0.8% agarose-formaldehyde gels; transferred to nitrocellulose filters; and baked at 80°C for 4 h. cDNA probes were 32P-labeled by random priming. Hybridization (10°C/ml) was performed in 50% formamide-5 x SSC-5 mM EDTA-0.5% SDS, 1 x Denhardt's, 100 μg/ml salmon sperm DNA at 42°C for 12 h and washing in 0.5% SDS-0.1 x SSC at 50°C for 2 h. Kodak XAR-5 film was used for exposure at -70°C.

The following human cDNA probes were used for Northern blot hybridization analyses: (a) the GFAP cDNA probe pCIA-5 as described in this communication; (b) the PDGF β-receptor cDNA probe PDGFR-2A3 (37); (c) the PDGF α-receptor cDNA probe PDGFR A1 (38); (d) the fibronectin probe pHFN1 consisting of a 380-base pair PstI insert in pBR322 and encoding the active cell attachment domain of fibronectin (39).

Indirect Immunofluorescence Staining. Glioma cells were suspended in Eagle's MEM-10% fetal calf serum, seeded sparsely on coverslips in 35-mm Petri dishes, and allowed to grow for 2-3 days. Before the first incubation, as well as between each subsequent incubation, cells were washed 3 times in PBS. Incubations were made at room temperature in a moist chamber. Rabbit anti-fibronectin (1:50) (kindly provided by Dr. Antti Vaheri) was allowed to react for 30 min, and bound antibodies were visualized using rhodamine-conjugated goat anti-rabbit immunoglobulin (1:50, 30 min) (Dakopatts). Cells were fixed with 3% paraformaldehyde in PBS for 20 min at room temperature and with 100% acetone for 1 min at -20°C and then incubated with the monoclonal GFAP-3 antibody (1:100) (kindly provided by Dr. Peter Collins) for 30 min. The antibody was visualized by a fluorescein isothiocyanate-conjugated anti mouse immunoglobulin (1:20, 30 min) (Dakopatts).

Table 1 Regional localization of the GFAP gene on chromosome 17

<table>
<thead>
<tr>
<th>Cell line</th>
<th>p53 (17q13)</th>
<th>Her-2/new (17q12-q21)</th>
<th>NGFR (17q12-q21)</th>
<th>Hox-2 (17q21)</th>
<th>GFAP (17q21)</th>
<th>PKC-α (17q22-q24)</th>
<th>Chromosome 17 region retained</th>
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<tr>
<td>SKBR 3</td>
<td>+</td>
<td>+ + + +</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>17pter-17qter</td>
<td></td>
</tr>
<tr>
<td>c131</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>17pter-17qter</td>
<td></td>
</tr>
<tr>
<td>N9</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>17q12-17q21</td>
<td></td>
</tr>
<tr>
<td>c19</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>17q11-12, 17q21-pter</td>
<td></td>
</tr>
<tr>
<td>275S</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>17q21-17qter</td>
<td></td>
</tr>
</tbody>
</table>

* K. Huebner, unpublished results.
Isolation of Human GFAP cDNA. A human glioma cell line (U-343 MGA C1 2.6) cDNA library was screened with the mouse GFAP cDNA probe G1. Several positive clones were found. One clone, CIA-5, containing a 1.1-kilobase insert was chosen for further characterization. Since this cDNA apparently was truncated in the 5' end, we rescreened the cDNA library with a 5' 120-base pair Sstl-Acc fragment from pCIA-5. The clone E2C2 was isolated and found to have a 1.4-kilobase insert, the downstream nucleotides.

Fig. 1. Nuclcotidc sequence of the human GFAP cDNA clone E2C2 and the HUMAN GLIAL FIBRILLARY ACID PROTEIN (GFP) translation initiation site (ATG) and the predicted open reading frame (ORF). The ORF is indicated by the shaded box and the nucleotide sequence is shown in the lower panel. The start codon is indicated by an asterisk and the stop codon is indicated by a vertical line. The coding sequence is partial and is flanked by 5' untranslated sequence (120 bases) and 3' untranslated sequence (25 bases).

RESULTS

Isolation of Human GFAP cDNA. A human glioma cell line (U-343 MGA C1 2.6) cDNA library was screened with the mouse GFAP cDNA probe G1. Several positive clones were found. One clone, CIA-5, containing a 1.1-kilobase insert was chosen for further characterization. Since this cDNA apparently was truncated in the 5' end, we rescreened the cDNA library with a 5' 120-base pair Sstl-Acc fragment from pCIA-5. The clone E2C2 was isolated and found to have a 1.4-kilobase insert, the downstream nucleotides. The authenticity of the human GFAP cDNA was further examined by subcloning into the expression vector RD2 and the authenticity of the human GFAP cDNA was further examined by subcloning into the expression vector RD2.
introduction by microinjection into U-343 MG cells, which lack endogenous GFAP expression (see below). Indirect immunofluorescence staining with GFAP antibodies revealed a typical network of GFAP fibers in a fraction of the microinjected cells (Fig. 3); cells in adjacent areas served as controls and were devoid of immunostainable fibers.

Chromosomal Localization of the Human GFAP Gene. A panel of cellular DNAs derived from 20 well-characterized rodent-human cell hybrids retaining overlapping subsets of human chromosome regions (see Fig. 4) was tested for retention of the GFAP gene by Southern blot analysis. An example of such a Southern blot, after HindIII digestion of control and hybrid DNAs, is shown in Fig. 5. Lane 1 contains mouse DNA and shows only one obvious band (approximately 7.2 kbp). HindIII cleaved human DNA (Fig. 5, Lane 2) shows one major GFAP specific fragment of 20 kbp and numerous (at least 10 are clear on longer exposure) more weakly hybridizing bands of various lower molecular weights; these bands most likely represent cross-hybridizing members of a gene family, while the strongly hybridizing band at 20 kbp is the cognate gene. Lanes 3–6 (Fig. 5) contain DNA from hybrids which contain the GFAP cognate gene at 20 kbp, while hybrids in Lanes 7–9 do not retain the cognate gene. The cross-hybridizing bands seen in human DNA do not appear in any of the hybrid DNAs in Lanes 3–9 (or in hybrid c131, which retains an entire chromosome 17; not shown) and thus do not segregate with the cognate gene. Since the hybrid in Lane 3 (Fig. 5) retains human chromosome 17 as its sole human chromosome and other positive hybrids (Fig. 5, Lanes 4–6) also retain chromosome 17 or a region of 17 while hybrids in Lanes 7–9 do not retain chromosome 17, the GFAP cognate gene must be linked to chromosome 17 (cf. Fig. 4). Similar analysis of the entire chromosome panel confirmed linkage of GFAP to chromosome 17 (cf. Fig. 4). Hybrid and control DNAs were similarly analyzed after cleavage with restriction enzyme SstI with similar results. GFAP-specific SstI fragments of 2.8, 1.2, and <0.5 kbp, representing the cognate GFAP gene, segregated concordantly (and with a specific region of human chromosome 17) in the hybrid panel, while at least six cross-hybridizing SstI fragments were not linked to chromosome 17.

Analysis of the somatic cell hybrids retaining specific regions of chromosome 17 (see legend to Fig. 4) was also the basis for the regional localization of the cognate GFAP gene on chromosome 17, as summarized in Table 1. Since the cognate locus segregates with the Hox-2 locus in these hybrids (see Table 1), the GFAP locus is distal to the NGFR locus and proximal to the PKC locus and located at 17q21.

The regional localization of the human GFAP locus was also determined by in situ hybridization. A total of 105 metaphase cells were scored of which 35 (33%) had grains on chromosome 17. Specific labeling was observed over the midregion of the long arm with a distinct peak at band 17q21. Forty-six% of...
total grains on chromosome 17 clustered at this band. A representation of the grain distribution on chromosome 17 is shown in Fig. 6. In conclusion, the in situ hybridization data assign the GFAP locus to band 17q21 and thus confirm the data obtained by Southern blot analysis of cell hybrids.

Expression of Fibronectin and GFAP in Human Glioma Cell Lines. The levels of fibronectin and GFAP mRNA were studied by Northern blot analysis of poly(A)+ RNA isolated from ten established human malignant glioma cell lines (Fig. 7; Table 2). This panel of cell lines includes three sublines derived from the U-343 glioma (U-343 MG, U-343 MGa Cl 2:6, U-343 MGa 31L) (18, 42). The fibronectin probe hybridized to a 6-kilobase transcript whereas the GFAP cDNA revealed a 2.7-kilobase mRNA. Fibronectin mRNA was found in four of the cell lines (U-251 MG sp, U-1242 MG, U-343 MG, U-178 MG). GFAP mRNA was found in five of the ten lines analyzed (U-343 MGa 31L, U-343 MGa Cl 2:6, U-251 MG sp, U-373 MG, U-1231 MG). (The 2.7-kilobase band seen on the blot in U-343 MGa 31L hybridized with fibronectin cDNA represents a signal remaining from the previous hybridization with GFAP cDNA). The pattern of mRNA expression of fibronectin and GFAP in the different cell lines was found to conform with the expression of protein, as estimated by indirect immunofluorescence staining (Table 2).

PDGF α- and β-Receptor mRNA in Human Glioma Cells: Relation to Cell Morphology and Levels of Fibronectin and GFAP mRNA. Northern blots of poly(A)+ RNA from the ten glioma cell lines were hybridized with PDGF α- and β-receptor cDNA probes (Fig. 7). The strongest signal of the 6.5-kilobase PDGF α-receptor transcript was found in U-343 MGa 31L; detectable levels were also found in U-373 MG, U-178 MG, U-343 MG, and U-1242 MG after longer exposure times. Abundant levels of the 5.4-kilobase PDGF β-receptor mRNA were found in U-178 MG, U-343 MG, U-1242 MG, and U-1231 MG; a weak signal was found in U-1240 MG. The weak signal seen with the PDGF β-receptor probe on Northern blot of U-343 MGa 31L cells represents cross-hybridizing to α-receptor mRNA (cf. Ref. 38).

The data on mRNA expression of fibronectin, GFAP, and PDGF α- and β-receptor were compiled in Table 2 and related to cell morphology as described (19). A striking feature is the essentially inverse relation between the expression of GFAP and fibronectin genes, as has previously been found with regard to the corresponding proteins (9, 10). One exception was found (U-251 MG sp), in which a fibronectin mRNA signal of medium strength was found together with GFAP mRNA and protein expression. Table 2 also makes it clear that glioma cell lines expressing both types of PDGF receptor mRNA, have a fibroblastic phenotype, i.e., bipolar, fibroblast-like morphology and high levels of fibronectin mRNA. Relatively high levels of GFAP α-receptor mRNA, in the absence of β-receptor mRNA expression, were found in two cell lines (U-343 MGa 31L, U-373 MG); these belong to the group of cell lines that express GFAP and lack detectable levels of fibronectin mRNA.

DISCUSSION

We have cloned human GFAP cDNA and deduced the primary structure of the protein. The translation product appears to be a 432-residue protein with 88 and 93% sequence similarity, respectively, with the two stretches of porcine GFAP that
were previously subjected to amino acid sequence analysis (40). The authenticity of the cDNA was confirmed by its expression in microinjected cells. An immunostainable, fibriillary network was apparent in transfected U-343 MG cells that lack endogenous GFAP expression.

During the progress of the present work, Reeves et al. (43) reported on the cloning of human GFAP cDNA; our present data confirm their coding sequence.

An alignment with the assumed coding sequence of the mouse gene showed that the predicted initiating ATG (Met) in the mouse sequence corresponds to ACG at positions 41-43 in the human nucleotide sequence (Fig. 1). Inspection of the assumed 5'-untranslated sequence of the mouse gene (41) shows the presence of a short open reading frame, in which the first 13 codons are almost identical to the 5' end of the human coding sequence. Since the NH2-terminus of mouse GFAP has not been established by amino acid sequence analysis, we do not know at present if the assumed initiating ATG of the mouse gene is the authentic one. If this is indeed the case, the conservation of a sequence, that is untranslated in the mouse and that is conserved in the human, is remarkable.

Southern blot analysis of human/mouse hybrid cell lines and in situ hybridization were used to assign the human GFAP gene to chromosome 17, band q21. Interestingly, there are several other genes of interest in relation to GFAP that are located on this region of chromosome 17. These include the erbB2 (rat neuro/glioblastoma-derived oncogene homologue) gene, the NGFR (nerve growth factor receptor) gene, and the NFI (von Recklinghausen neurofibromatosis) gene (44). Several members of the cytokeratin gene family have also been mapped to this region of chromosome 17 (44). Recent mapping studies, assigning the mouse GFAP gene to chromosome 11 (45), indicate that GFAP is part of a large syntenic gene cluster including at least 22 genes located on human chromosome 17 and mouse chromosome 11 (44, 45).

Recent studies have shown that there exist two types of PDGF receptors that differ in ligand specificity. The PDGF α-receptor binds all three isoforms of PDGF (PDGF-AA, -AB, -BB) with high affinity, whereas the β-receptor binds only PDGF-BB with high affinity (46, 47). The two receptors are structurally homologous (20, 37, 48), and both have been shown to transduce mitogenic signals in response to the proper ligand (49, 50). Available information suggests that the α- and β-receptors are differentially expressed in normal cells. Thus, the O-2A glial progenitor cell of the rat opticus nerve expresses α-receptors only (51), whereas human meningial cells (52) and rat brain capillary endothelial cells (53) express only β-receptors. Various kinds of fibroblasts in culture, however, express both types of receptors.

The present investigation showed a wide range of α- and β-receptor mRNA levels in human glioma cells, as determined by Northern blot hybridization analysis. A comparison of PDGF receptor expression with cell morphology and fibronectin/GFAP phenotype revealed an interesting pattern. One subgroup was formed by cells with a fibroblastic phenotype; such cells displayed the same pattern of PDGF receptor expression as bona fide fibroblasts; i.e., they expressed both types of receptor mRNA. One might argue that these cells are actually derived from a separate mesenchymal component, present in the original tumor. The high frequency of establishment of glioma cell lines with such phenotype argues against this notion. Another argument is the finding of the same unique marker chromosome in the "fibroblastic" cell line U-343 MG, having the GFAP+/FN- phenotype, and the "glial" line U-343 MGa and clones thereof, which display the GFAP+FN- phenotype. These cell lines were derived from different primary cultures of the same glioma biopsy. Since all derivatives have an inversion in chromosome 1 [inv(1)p13q43] (42, 54) they most probably have the same clonal origin. Unfortunately, this finding does not absolutely prove a monoclonal origin, since we lack data on the karyotype of the host tissue. It is also interesting that other studies have indicated that certain normal glial cells produce fibronectin (55, 56). The possible relationship between these cells and the fibronectin-positive glial cells remains to be elucidated.

A relationship between the pattern of PDGF receptor expression and glioma cell phenotype was further evidenced by the finding that cell lines expressing GFAP had no detectable, or very low, levels of PDGF β-receptor mRNA. In this group we found two cell lines (U-343 MGa 31L and U-373 MG) that expressed relatively high levels of PDGF α-receptor mRNA. Expression of α-receptors in U-343 MGa 31L has been described previously; these cells were used for initial characterization (20) and cDNA cloning (38) of the α-receptor.

Our study highlights the phenotypic heterogeneity of glioma cell populations, with regard to cells both within a certain population and between different populations. Cellular heterogeneity is not a specific trait of gliomas and is considered an important characteristic of malignant tumor cell populations in general, inasmuch as it may form the basis for tumor progression (see Ref. 57 for review). According to Nowell's original hypothesis (58), phenotypic heterogeneity may be the result of the genetic instability of tumor cells allowing for the generation of a plethora of genotypic and phenotypic variants. Another, not

Table 2. Phenotypic characteristics of human glioma cell lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Morphology</th>
<th>GFAP mRNA</th>
<th>Immunofluorescence</th>
<th>PDGF α-receptor mRNA</th>
<th>PDGF β-receptor mRNA</th>
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</thead>
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<tr>
<td>U-1231 MG</td>
<td>Epithelioid</td>
<td>++++</td>
<td>+/+/-</td>
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<td>-</td>
</tr>
<tr>
<td>U-343 Mg</td>
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<td>+++</td>
<td>+++/-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
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<td>Pleo/Astro</td>
<td>++++</td>
<td>+++</td>
<td>-</td>
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<tr>
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<td>Pleo/Astro</td>
<td>+++</td>
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<tr>
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<td>+++</td>
<td>+++/-</td>
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* Pleo/Astro, pleomorphic/astrocytoid; Bip/Fibr, bipolar, fibroblastic; n.d., not determined.
mutually exclusive hypothesis infers that neoplastic stem cells give rise to variants through more or less normal differentiation processes that do not involve any structural changes in the genome. Our findings that PDGF receptor expression is related to cell morphology and synthesis of GFAP and fibroactin are compatible with the second hypothesis for explaining the differential PDGF receptor expression in glioma cells. Previous studies on the expression of mRNA encoding the subunits chains of PDGF have shown A chain transcripts in most cell lines whereas B chain mRNA was present mainly in clones or cell lines composed of small cells or cells with an epithelioid or pleomorphic/astrocystic morphology (18, 19). As is also shown in Table 1, only such cells were found to express GFAP. These findings, in conjunction with the present report, are compatible with the idea that the expression of the genes encoding PDGF and its receptors in glioma cells is regulated in concert with other gene families which may or may not be developmentally regulated in normal glial cell lineages.

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REFERENCES


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Human Glial Fibrillary Acidic Protein: Complementary DNA Cloning, Chromosome Localization, and Messenger RNA Expression in Human Glioma Cell Lines of Various Phenotypes

Erik Bongcam-Rudloff, Monica Nistér, Christer Betsholtz, et al.


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