Gene Expression Profiling of Low-Grade Diffuse Astrocytomas by cDNA Arrays

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Abstract
Diffuse astrocytoma WHO grade II is a well-differentiated, slowly growing tumor that has an inherent tendency to progress to anaplastic astrocytoma (WHO grade III) and, eventually, to glioblastoma (WHO grade IV). Little is known about its molecular basis, except for p53 mutations that are found in >60% of cases. In a search for additional genetic alterations, we carried out gene expression profiling of 11 diffuse astrocytomas using cDNA expression arrays. Expression of six genes (TIMP3, c-myc, EGFR, DR-mm23, nm23-H4, and GDNF) was detected in 64–100% of diffuse astrocytomas, but not in nontumorous brain tissue. Seven genes (AAD14, SPARC, LRP, PDGFR-α, 60S ribosomal protein L5, PTN, and kRAS) were found to be up-regulated more than 2-fold in 20–60% of cases, whereas 11 genes (IFI 9-27, protein kinase CLK, TDGFI, BNI1, GABI, TYRO3, LDH-A, aducin 3, GUK1, CDC10, and KRT8) were down-regulated to less than 50% of normal levels in 64–100% of cases. Semiquantitative conventional reverse transcription-PCR was performed for 11 genes, 9 of which showed an expression profile similar to that obtained with cDNA expression arrays. Immunohistochemical staining for SPARC showed cytoplasmic immunoreactivity of neoplastic cells in all diffuse astrocytomas analyzed. These results indicate significant changes in gene expression in diffuse astrocytomas, but it remains to be shown which of these are causally related to the transformation of glial cells.

Introduction
Low-grade diffuse astrocytomas (WHO grade II) are well-differentiated tumors that typically occur in young adults, with a preferential location in the cerebral hemispheres. They grow slowly but have an inherent tendency for malignant progression to anaplastic astrocytoma (WHO grade III) and, eventually, glioblastoma (WHO grade IV). Ref. 1). This tendency of malignant progression is particularly true in the gemistocytic variant (2, 3).

The genetic profile of low-grade astrocytomas is still far from being complete. The most frequent genetic alterations are p53 mutations and LOH1 on chromosome 1p7, which are present in approximately two-thirds of the cases (4, 5). Overexpression of PDGFR-α has been observed in approximately 50% of low-grade astrocytomas, and this was often associated with LOH on chromosome 17p (6). Loss of chromosome 22q13.3 has been observed in up to 30% of astrocytomas (7), but the putative tumor suppressor gene on this chromosomal region has yet to be identified. Comparative genomic hybridization studies showed frequent loss of portions of chromosomes 1p and 19q (8), but LOH on chromosomes 1p and 19q appears to be rare in low-grade astrocytomas (9, 10).

The objective of this study was to expand the current knowledge of altered gene expression in low-grade diffuse astrocytomas as a first step in the identification of additional oncopgenes or tumor suppressor genes operative in the evolution of diffuse astrocytomas and secondary glioblastomas derived thereof.

Materials and Methods
Brain Tumor and Normal Brain Samples. Frozen tissues of 11 low-grade diffuse astrocytomas of WHO grade II (9 fibrillary and 2 gemistocytic astrocytomas) were obtained from the Department of Neurosurgery, University Hospital Zurich (Zurich, Switzerland). They were snap frozen after surgical removal and stored at −80°C until use. The mean age of patients was 31.1 ± 7.4 years (range, 20–45 years). Five patients were males, and six were females (Table 1).

As controls, we used three nontumorous brain tissue samples from two patients. A hippocampus tissue sample was obtained from an adult male with therapy-resistant epilepsy who underwent selective hippocampo-amygdalectomy. Postmortem cortex and medulla oblongata samples were from an adult female patient with antithrombin III deficiency; samples were obtained within 3 h after death and stored at −80°C until RNA isolation.

Total RNA Isolation. Total RNA was isolated from frozen brain tissues using the Atlas Pure Total RNA Labeling System (Clontech, Palo Alto, CA) according to manufacturer’s instructions. Briefly, 150–200 mg of tissue were homogenized in 3 ml of denaturing solution. After two phenol/chloroform extractions, RNA was precipitated with isopropanol, washed with 80% ethanol, and air dried. To remove genomic DNA contamination, RNA was treated with RNase-free DNase I (Clontech), and RNA was then dissolved in RNase-free H2O and stored at −80°C until analysis.

cDNA Probe Preparation. For cDNA probe synthesis, 5 μg of DNase-treated total RNA together with 1 μl of cDS primer mix (Clontech) in a total volume of 6 μl were heated to 70°C for 10 min and then cooled on ice. A mixture consisting of 4 μl of 5% first-strand cDNA buffer (Life Technologies, Inc., Gaithersburg, MD), 1 μl of 100 mM DTT, 2 μl of 10 mM dNTPs (Clontech), 1 μl of RNase block (40 units/μl; Stratagene, La Jolla, CA), and 5 μl of [α-32P]dATP (3000 Ci/mmol, 10 μCi/μl; ICN) was added into the tube and heated at 42°C for 2 min. One μl of SuperScript II RNase H reverse transcriptase (200 units/μl; Life Technologies, Inc.) was then added, and the reaction was continued at the same temperature for 50 min, followed by heating to 70°C for 15 min for enzyme inactivation. The cDNA probe was purified with a CHROMA SPIN-200DEPC-H2O column (Clontech). Incorporation of 32P into the probe was determined by counting in a liquid scintillation counter (BETAMATIC; Kontron Instruments, Montigny le Bretonneux, France). The first two fractions showing the highest counts were collected and used for hybridization with cDNA array.

Hybridization and Quantitation of cDNA Arrays. The Atlas Human Cancer 1.2 Arrays containing cDNA fragments of 1176 cancer-associated human genes/clones were purchased from Clontech. Preliminary experiments using different hybridization temperatures (64°C–68°C), washing conditions (64°C–68°C for different periods of time), and exposure time (up to 14 days) showed that up to 80% of total genes spotted onto the array could be detected using brain samples. The experimental conditions were then optimized (see below) to yield unambiguous and reproducible X-ray signals of approximately 250–300 genes after 6 days of exposure.

Array membranes were prehybridized with 5 μl of ExpressHyb solution (Clontech) at 68°C with continuous rotation in a glass hybridization roller. After prehybridization for 2 h, purified [α-32P]-labeled cDNA probes made from normal or tumor RNAs were added into different rollers, and hybridization was continued overnight at the same temperature. Arrays were subsequently

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3 The abbreviations used are: LOH, loss of heterozygosity; PDGFR, platelet-derived growth factor receptor; dNTP, deoxynucleotide triphosphate; RT, reverse transcription; T/N, tumor:normal ratio; LRP, Low-density lipoprotein receptor-related protein 1; ECM, extracellular matrix; MMP, matrix metalloproteinase.
washed twice in 200 ml of wash solution 1 (0.1 ×  SSC, 1% SDS) at 68°C for 20 min with agitation and then washed once in 200 ml of wash solution 2 (0.1 ×  SSC, 0.5% SDS) at 68°C for 20 min with agitation. After a final wash with 200 ml of 2× SSC for 5 min at room temperature, the damp membranes were sealed in plastic wrap and exposed to Kodak Biomax MS X-ray film with an intensifying screen at ~80°C for 6 days.

Array images on the X-ray film were scanned at 400 dpi by using an image scanner (X-Finity professional, Model PS4800+; FUJ Film Limited, Japan) and then analyzed using AtlasImage 1.01a software (Clontech). We first eliminated by visual inspection false positive signals due to apparent artifacts; the intensity of each spot on the array was then calculated after background subtraction. Mean values of intensity for each gene detected from multiple arrays were generated by the computer software (an averaged array). Two averaged arrays (one from 3 normal brain tissues and another from 11 tumor samples) were then compared. For generating mean intensity from multiple arrays and for subsequent comparison between two arrays, the “global” mode was used, then compared. For generating mean intensity from multiple arrays and for subsequent comparison between two arrays, the “global” mode was used, then compared.

RT-Differential PCR. First-strand cDNA was synthesized as follows: 1 μg of DNase-treated total RNA together with 0.5 μg of oligo(dT)12-18 (Pharmacia, Uppsala, Sweden) in a total volume of 11 μl were heated to 70°C for 10 min and then chilled on ice. A mixture consisting of 4 μl of 5× first-strand cDNA buffer (Life Technologies, Inc.), 2 μl of 100 mM DTT, 1 μl of 10 mM dNTPs, and 1 μl of RNase block (40 units/μl; Stratagene) was added into the tube and heated at 42°C for 2 min. SuperScript II RNase H Reverse Transcriptase (200 units; Life Technologies, Inc.) was then added, and the reaction continued at 42°C for 50 min. After a 15-min inactivation step at 70°C, the cDNA was stored at −20°C until use.

RT-Differential PCR was performed by coamplification of the gene in question together with a reference gene (β-actin or GAPDH) using cDNA template generated as described above and corresponding gene-specific primer sets. The primer sequences are as follows: (a) 5’-GGCGGTTTGCTCCCAGC-3’ (sense) and 5’-GGCGGTTTGCTCCCAGC-3’ (antisense) for TIMP3; (b) 5’-CAAGAGCTTTGCTGCACTTCT-3’ (sense) and 5’-CAAGAGCTTTGCTGCACTTCT-3’ (antisense) for GAPDH; (c) 5’-GAAGCCAGAGGAGCTTCTG-3’ (sense) and 5’-GAAGCCAGAGGAGCTTCTG-3’ (antisense) for CCGTACACAGGGATCTTCA-3’ (sense) and 5’-GGCGGTTTGCTCCCAGC-3’ (antisense) for TIMP3; (b) 5’-ATCGCTATCCAAGGAGCC-3’ (sense) and 5’-ATCGCTATCCAAGGAGCC-3’ (antisense) for F11-97-R; (i) 5’-GACCTGGTGGATGCTTCT-3’ (sense) and 5’-GACCTGGTGGATGCTTCT-3’ (antisense) for ABI; (j) 5’-CTCAGCGACAGATTTCTGAG-3’ (sense) and 5’-CTCAGCGACAGATTTCTGAG-3’ (antisense) for CDC10; (k) 5’-CAAGAGCGAAGAGATCC-3’ (sense) and 5’-CAAGAGCGAAGAGATCC-3’ (antisense) for TR-YOS; (l) 5’-CACCTGATCTCCTGAGGGTTCT-3’ (sense) and 5’-CACCTGATCTCCTGAGGGTTCT-3’ (antisense) for REL; (m) 5’-ATCGCTATCCAAGGAGCC-3’ (sense) and 5’-ATCGCTATCCAAGGAGCC-3’ (antisense) for GAPDH. PCR was carried out in a total volume of 10 μl containing 0.5 μl of cDNA solution, 0.5 unit of Taq DNA polymerase (Sigma, St. Louis, MO), 1–2 mM MgCl2, 0.2 mM each dNTP, 0.1–1.0 μM sense and antisense primers, 10 mM Tris-HCl (pH 8.3), and 50 μM KCl in a Robot Thermal Cycler (Stratagene) as follows: (a) initial denaturation for 5 min at 94°C; (b) 29–35 cycles with denaturation at 94°C for 30 s, annealing at 56°C– 61°C for 1 min, and extension at 72°C for 1 min; and (c) a final extension step for 5 min at 72°C. After PCR, 7 μl of products were run on a 2% agarose gel and stained with ethidium bromide; the intensity of target and reference genes was quantified using the 1D Image Analysis Software (Kodak Digital Science). Change of gene expression was then calculated as a ratio of the intensity of tumor to control samples after normalization using a factor derived from the relative intensity of the reference gene in tumor and control samples.

Immunohistochemistry. Formalin-fixed, paraffin-embedded sections of 11 low-grade astrocytomas analyzed in the cDNA expression array were deparaffinized in xylene and rehydrated in graded ethanol. Endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide in methanol for 30 min at room temperature. The sections were microwaved in antigen unmasking solution (Vector Laboratories, Burlingame, CA) for 3 × 5 min. After incubation with 5% skimmed milk for 1 h at room temperature, the sections were incubated overnight at 4°C with the primary monoclonal antibody against SPARC (10.9 mg/ml; Hematological Technologies Inc., Essex Junction, VT; 1:15,000 dilution). The reaction was visualized using Vectastain Elite ABC kit (Vector Laboratories) and 3',3'-diaminobenzidine solution (Vector Laboratories). The sections were then counterstained with hematoxylin. Formalin-fixed, paraffin-embedded sections of human breast tumors were used as positive controls (11). Sections without primary antibody were served as negative controls.

Screening for p53 Mutations. Exons 5–8 of the p53 gene were screened for mutations in all 11 low-grade astrocytomas using single-strand conformational polymorphism followed by direct sequencing as described previously (5).

Statistical Analyses. Student’s unpaired t test was carried out to compare data in tumors with and without p53 mutations (11).

Results

Gene Expression Profile. Using the AtlasImage 1.01a software (Clontech), we initially compared gene expression levels among the three control samples and found no significant difference (<25%
difference for more than 90% of the genes detected on the arrays). Therefore, a standard array was generated from the pooled data from 11 tumors and separately with each of the individual low-grade astrocytoma arrays. We averaged array generated by pooling data from 11 tumors and separate control samples. This array was then used for comparison with an array generated by pooling data from 11 tumors and separate control samples. Therefore, a standard array was generated from the pooled data from the 11 tumors and separate control samples. This array was then used for comparison with an array generated by pooling data from the 11 tumors and separate control samples.

**Table 2. Genes detected only in low-grade astrocytomas by cDNA array**

<table>
<thead>
<tr>
<th>Code</th>
<th>Gene name</th>
<th>Intensitya</th>
<th>Frequency (%b)</th>
<th>Putative function</th>
</tr>
</thead>
<tbody>
<tr>
<td>A08g</td>
<td>Tissue inhibitor of metalloproteinase (TIMP3)</td>
<td>+++</td>
<td>11/11 (100%)</td>
<td>Plays pivotal roles in the regulation of ECM metabolism; inhibits activity of MMPs; is a bifunctional molecule that promotes growth and oncogenic transformation in some cell types. TIMP3 overexpression has been observed in breast carcinomas.</td>
</tr>
<tr>
<td>A12d</td>
<td>Epidermal growth factor receptor (EGFR)</td>
<td>++</td>
<td>11/11 (100%)</td>
<td>Involved in the control of cell proliferation</td>
</tr>
<tr>
<td>F11e</td>
<td>DR-nm23</td>
<td>++</td>
<td>7/11 (64%)</td>
<td>Binds DNA; involved in cell differentiation; implicated in pathogenesis and metastasis of tumors</td>
</tr>
<tr>
<td>A03c</td>
<td>c-myc oncogene</td>
<td>++</td>
<td>8/11 (73%)</td>
<td>Transcription factor; promotes cell proliferation and transformation</td>
</tr>
<tr>
<td>E13j</td>
<td>Gla derived neuropeptide factor (GDNPF)</td>
<td>+</td>
<td>11/11 (100%)</td>
<td>Involved in the maintenance of normal function in the nervous system; promotes survival and growth of spinal motoneurons during the period of programmed cell death and after injury</td>
</tr>
<tr>
<td>F09b</td>
<td>nm23-H4</td>
<td>+</td>
<td>10/11 (91%)</td>
<td>Regulates a diverse array of cellular events including growth and development; implicated in the pathogenesis of tumor and metastasis</td>
</tr>
</tbody>
</table>

a +++, >2000; ++, 1000–2000; +, <1000.

b Percentage of cases with detectable level of mRNA.

**RT-Differential PCR.** To confirm the mRNA expression data obtained by cDNA expression array, we performed semiquantitative RT-PCR for 11 of the genes listed in Tables 2–4. Although the extent of change detected by the two methods varied, probably due to the difference in sensitivity, expression change of nine of the genes detected by RT-PCR showed the same tendency as in cDNA array experiments (Fig. 3). The expression of two genes (BIN1 and CDC10) did not differ significantly between tumors and nontumorous brain tissues by RT-PCR (data not shown). The increase versus control levels was 2.5-fold for EGFR (range, 1.1–3.3), 3.5-fold for SPARC (range, 2.1–4.6), 8.5-fold for TIMP3 (range, 2.3–17.2), 1.7-fold for GDNPF (range, 0.7–2.2), and 1.7-fold for LRP (range, 1.0–2.4). The decrease versus control levels was 18% (range, 3–75%) for IFI 9-27 and 68% (range, 57–93%) for TYRO3.

**SPARC Immunohistochemistry.** Immunohistochemical staining for SPARC showed strong cytoplasmatic staining in neoplastic cells in all diffuse astrocytomas analyzed as well as in reactive astrocytes (Fig. 4), whereas the adjacent normal brain tissue was negative.

**Discussion**

To identify novel genetic alterations in low-grade diffuse astrocytomas, we used a cDNA array technology to analyze expression patterns of >1000 cancer-associated genes in 11 low-grade diffuse astrocytomas. A total of 24 genes showed significant changes in expression: mRNA-derived cDNA of 6 genes was detectable only in tumors; 7 genes were overexpressed by more than 2-fold, and 11 genes were down-regulated to less than 50% of the control level.

**Table 3. Genes overexpressed in low-grade astrocytomas detected by cDNA array**

<table>
<thead>
<tr>
<th>Code</th>
<th>Gene name</th>
<th>Intensity ratio (mean T/mean N)a</th>
<th>Frequency (%)b</th>
<th>Putative function</th>
</tr>
</thead>
<tbody>
<tr>
<td>F12m</td>
<td>AAD14</td>
<td>4.17</td>
<td>6/11 (55%)</td>
<td>Encodes a neuronal, small conductance calcium-activated potassium channel</td>
</tr>
<tr>
<td>F04k</td>
<td>60S ribosomal protein L5 (rPL5)</td>
<td>2.95</td>
<td>7/11 (64%)</td>
<td>A eukaryotic ribosomal protein essential for normal cell growth and proliferation</td>
</tr>
<tr>
<td>D03m</td>
<td>Low density lipoprotein receptor-related protein (LRP)</td>
<td>2.87</td>
<td>6/10 (60%)</td>
<td>Multifunctional cell surface receptor; involved in lipoprotein metabolism, and cellular lipid uptake</td>
</tr>
<tr>
<td>F14a</td>
<td>Secreted protein acidic and rich in cysteine precursor (SPARC)</td>
<td>2.33</td>
<td>4/11 (36%)</td>
<td>Interacts with ECM components and regulates activation of MMPs; may be involved in tumor invasion; highly expressed in a variety of human neoplasms</td>
</tr>
<tr>
<td>B04n</td>
<td>B-cell receptor-associated protein (bBAP)</td>
<td>2.33</td>
<td>3/11 (27%)</td>
<td>A protein with sequence similarity to prohibitin; involved in lymphocyte function</td>
</tr>
<tr>
<td>E10b</td>
<td>Pleiotrophin precursor (PTN)</td>
<td>2.11</td>
<td>4/11 (36%)</td>
<td>Heparin-binding growth/differentiation factor; plays role in tumor growth and angiogenesis</td>
</tr>
<tr>
<td>A02e</td>
<td>Platelet-derived growth factor receptor alpha subunit (PDGFR-a)</td>
<td>2.07</td>
<td>2/10 (20%)</td>
<td>Binds all three PDGF isoforms; a number of different signaling pathways can be initiated leading to cell growth, actin reorganization, migration, and differentiation</td>
</tr>
</tbody>
</table>

a T, tumor; N, nontumorous brain tissue.
b Percentage of cases with >2-fold increase in mRNA expression.
Tables 2–4). The genes identified have putative functions in a variety of cellular processes. They can be classified into three major categories: (a) cell growth and transformation, cell cycle control, and apoptosis (c-myc, EGFR, PDGFR-α, TGFβ1, PTN, BNI, GAB1, and GDNF); (b) cytokine, protein kinase, signal transduction and cell surface receptors, and associated proteins (IFN-9-27, AAD14, CLK, LDH-A, LRP, GUK1, CDC10, DR-nm23, and nm-23-H4); (c) cell adhesion and basement membrane and ECM proteins (SPARC, TIMP3, adducin 3, TYRO3, and KRT8) (Tables 2–4). It is notable that this study has identified several genes, such as PDGFR-α, c-myc, EGFR, LRP, and SPARC, for which overexpression has been previously reported in astrocytic brain tumors, demonstrating the suitability and power of cDNA array technology in the identification of transformation-associated genes.

PDGFR-α overexpression in low-grade astrocytomas was first detected by in situ hybridization (12, 13). In the present study, PDGFR-α overexpression was seen in 2 of 10 low-grade astrocytomas (Table 3; Fig. 2B). Hermanson et al. (6) reported that a high level of PDGFR-α expression was significantly correlated with LOH on chromosome 17p in astrocytic brain tumors. This is corroborated by our observation that the two low-grade astrocytomas with more than 2-fold overexpression of PDGFR-α contained a p53 mutation (Fig. 2).

Overexpression of the c-myc oncogene has previously been detected by immunohistochemistry in 5% of low-grade, 33% of intermediate-grade, and 76% of high-grade astrocytic gliomas (14). In the present study, we show that in normal brain tissue, the c-myc mRNA level was below the detection limit by cDNA array hybridization and RT-PCR (Fig. 3), whereas 73% of the low-grade astrocytomas showed c-myc expression (Figs. 2A and 3), supporting the view that c-myc overexpression may be involved in the pathogenesis of low-grade astrocytomas.

EGFR amplification and overexpression are genetic hallmarks of primary (de novo) glioblastomas (15, 16). In contrast, EGFR amplification (analyzed by Southern blot or differential PCR) and EGFR overexpression (analyzed by immunohistochemistry; Ref. 16) are rarely detected in low-grade astrocytomas (17, 18) and the secondary glioblastomas derived thereof (5, 16). In the present study, we found that in normal brain tissue, EGFR mRNA was not detectable by cDNA expression array and was only detectable at low levels by RT-PCR. A small up-regulation of EGFR expression (1.1–3.3-fold) was observed in all low-grade astrocytomas analyzed (Fig. 3). This level of EGFR overexpression is significantly lower than that observed in primary glioblastomas, and it remains to be shown whether it plays a significant role in the development of low-grade astrocytomas.

LRP is a multifunctional cell surface receptor involved in lipoprotein metabolism and cellular lipid uptake. It can bind specifically to α₂-macroglobulin-proteinase complexes and regulates proteinase activity, which is necessary for cellular migration and invasive processes (19, 20). It may also function in cell growth and repair (21). A recent study using RT-PCR and immunohistochemistry showed that LRP was overexpressed in 4 of 25 high-grade gliomas and that this often occurred in association with EGFR amplification (21). In the present study, LRP up-regulation was observed in 60% of low-grade astrocytomas (Table 3), indicating that this gene is already involved in early stages of gliomagenesis.

SPARC and TIMP3 identified in this study are involved in cell adhesion and cell-ECM interactions. SPARC is a 43,000 secreted glycoprotein that interacts with the ECM components. In endothelial cells and fibroblasts, SPARC acts as a negative mediator of spreading and is associated with pathophysiological events requiring tissue remodeling and de novo formation of basement membranes (22). SPARC also regulates and coordinates endothelial cell proliferation and migration during wound healing and angiogenesis (23, 24). Immunohistochemistry showed that SPARC is expressed in normal steroiogenic cells, chondrocytes, placental trophoblasts, vascular smooth muscle cells, and endothelial cells (11). Strong reactivity was also found in fibrocytes and endothelial cells involved in tissue repair (11). SPARC is highly expressed in a variety of human neoplasms, including colorectal cancer (22), ovarian cancer (25), melanomas (23, 26), meningiomas (27), and gliomas (28). Using subtractive hybridization, immunoblotting, and immunohistochemistry, Rempel et al. (28) recently showed that SPARC is overexpressed in the majority of low-grade astrocytomas, anaplastic astrocytomas, and glioblastomas.

Table 4 Genes repressed in low-grade astrocytomas detected by cDNA array

<table>
<thead>
<tr>
<th>Code</th>
<th>Gene name</th>
<th>Intensity ratio (mean T/mean N)</th>
<th>Frequency (%)</th>
<th>Putative function</th>
</tr>
</thead>
<tbody>
<tr>
<td>A01h</td>
<td>Interferon-inducible protein 9–27 (IFI-9–27)</td>
<td>0.04</td>
<td>11/11 (100%)</td>
<td>Regulates B-cell development and activation; generates signal transduction complex mediating antiproliferative activity of IFNs; may be implicated in cell growth control</td>
</tr>
<tr>
<td>A14j</td>
<td>Protein kinase CLK</td>
<td>0.10</td>
<td>10/10 (91%)</td>
<td>Homologue to yeast cdc2/cdc28 protein kinase that regulates cell cycle. EGFR-related protein; activates intracellular components in the ras/raf/MEK/MAPK pathway</td>
</tr>
<tr>
<td>E05c</td>
<td>Teratocarcinoma-derived growth factor (TGFβ1)</td>
<td>0.13</td>
<td>10/10 (91%)</td>
<td>Plays role in cellular growth response, transformation, and apoptosis; involved in the signaling pathway of different tyrosine kinases, including EGFR</td>
</tr>
<tr>
<td>B13m</td>
<td>GRB2-associated binder-1 (GAB1)</td>
<td>0.17</td>
<td>11/11 (100%)</td>
<td>Plays role in cellular growth response, transformation, and apoptosis; involved in the signaling pathway of different tyrosine kinases, including EGFR</td>
</tr>
<tr>
<td>A11h</td>
<td>Box-dependent myc-interacting protein 1 (BIN1)</td>
<td>0.17</td>
<td>10/10 (91%)</td>
<td>Interacts with c-myc and inhibits cell proliferation and malignant cell transformation by MYC</td>
</tr>
<tr>
<td>A04e</td>
<td>Tyrosine protein kinase SKY (TYRO3)</td>
<td>0.25</td>
<td>10/10 (91%)</td>
<td>Member of a subfamily of related receptor tyrosine kinases; may function as an adhesion molecule mediating cell-cell and cell-matrix interactions, expressed predominantly in brain; receptor of the GASP gene (growth arrest specific gene 6)</td>
</tr>
<tr>
<td>F05d</td>
<td>Lactate-dehydrogenase-A (LDH-A)</td>
<td>0.30</td>
<td>9/9 (82%)</td>
<td>Subunit of L-lactate dehydrogenase; interacts with protein kinases A, C, and other transcription factors through its multiple cis-acting promoter elements</td>
</tr>
<tr>
<td>F14l</td>
<td>Adducin 3</td>
<td>0.42</td>
<td>7/7 (64%)</td>
<td>Binds calmodulin; substrate of PKC and other kinases; likely to play an important role in skeletal organization of the cell membrane</td>
</tr>
<tr>
<td>B12m</td>
<td>Guanylate kinase (GUK1)</td>
<td>0.43</td>
<td>10/10 (91%)</td>
<td>Catalyses the conversion of GMP to GTP in cGMP cycle.</td>
</tr>
<tr>
<td>F03g</td>
<td>Keratin type II cytoskeletal 8 (KRT8)</td>
<td>0.46</td>
<td>7/7 (64%)</td>
<td>Intermediate filament protein; contains a p53-binding domain.</td>
</tr>
<tr>
<td>A10m</td>
<td>CDC10 protein homologue (CDC10)</td>
<td>0.49</td>
<td>8/9 (73%)</td>
<td>Belongs to the septin family, involved in cytokinesis; expressed ubiquitously in normal tissue</td>
</tr>
</tbody>
</table>

* T, tumor; N, nontumorous brain tissue.

* Percentage of cases with less than 50% of mRNA level compared to that of control.

* EGFR, epidermal growth factor; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein/extracellular signal-regulated kinase; PKC, protein kinase C.
In this study, RT-PCR revealed that SPARC expression was up-regulated by 2.1–4.6-fold in all low-grade astrocytomas (Fig. 3), lending further evidence to the view that SPARC is an invasion-related candidate gene in low-grade astrocytomas (28).

TIMP3 is a member of the TIMP family, which is a group of multifunctional secreted proteins that play pivotal roles in the regulation of ECM metabolism by inhibiting the activity of various MMPs. Deregulation of either TIMPs or MMPs leads to unbalance of activities between these two types of enzymes, and this is considered to be related to the invasive phenotype of human neoplasms, including gliomas (29, 30). In addition to their inhibitory activity on metalloproteinases, some of the TIMP family proteins have other distinct properties such as growth factor function and promotion of oncogenic transformation (31–33). Simultaneous overexpression of TIMP2 and MMPs has been observed in recurrent gliomas more frequently than in primary tumors (34), suggesting that overexpression of these genes contributes to the invasive and more aggressive nature of the recurrent gliomas. TIMP3 overexpression has been observed in all breast carcinomas analyzed (32, 33). However, a recent study (35) showed an aberrant methylation pattern in the TIMP3 promoter region in several primary tumors, including brain tumors, suggesting a tumor suppressor role for TIMP3. In this study, we found that TIMP3 was overexpressed (2.3–17.2-fold by RT-PCR) in all low-grade astrocytomas. A recent study using serial analysis of gene expression reported a 13-fold increase in TIMP4 expression in glioblastomas (36). These results suggest that TIMP3 and TIMP4 and the interplay between TIMPs and MMPs may play a significant role in the pathogenesis of astrocytic brain tumors.

Several additional genes were found to be up-regulated. DR-nm23, nm23-H4, 60S ribosomal protein L5, and PTN have been implicated in the pathogenesis, angiogenesis, and metastasis of nonneural tumors (Tables 2 and 3). The possible role of AAD14, hBAP, and GNDPF in the multistep process of malignant transformation is currently unknown.

IFI 9-27 regulates B-cell development and activation, mediates antiproliferative activity of IFN-α and -γ, and may be implicated in cell growth control (37–39). IFI 9-27 triggers aggregation and inhibits proliferation of leukemic B cells (38). In this study, we show that IFI 9-27 is down-regulated in all low-grade astrocytomas analyzed. RT-PCR confirmed that expression of this gene was reduced to 3–50% of control levels in approximately 50% of the low-grade astrocytomas.
whereas nontumorous brain tissue showed a high level of expression (Fig. 3). It remains to be elucidated whether this down-regulation of IFI 9-27 is a causative event in the pathogenesis of low-grade astrocytomas. Several other down-regulated genes such as TDGF1, BINT, GAB1, TYRO3, LDH-A, adducin 3, and KRT8 (Table 4) have been implicated in the pathogenesis of nonneural tumors. A role for protein kinase CLK, adducin 3, GUK1, and CDC10 in tumorigenesis has not been reported previously.

It should be pointed out that the cDNA array methodology examines the mRNA level rather than protein concentrations that can be regulated not only by transcriptional but also by posttranscriptional mechanisms. Thus, some genes with small changes in mRNA level (<2-fold in this study) but with significant change in protein level may have not been identified in this study.

The results obtained in this study demonstrate the complexity of genes/pathways that may be involved in the development of low-grade astrocytomas (Tables 2–4) and point to some interesting candidate genes worth further investigation. Whether these up- or down-regulated genes are causally related to the transformation of glial cells remains to be investigated.

In summary, the establishment of gene expression profiles in low-grade astrocytomas using cDNA array technology has demonstrated significant expression changes in a number of genes implicated in various cellular pathways related to the control of cell growth, differentiation, and tumor invasion. It provides information for additional molecular studies aimed at a clarification of their role in the development of astrocytic brain tumors.

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References


GENETIC ALTERATIONS IN LOW-GRADE ASTROCYTOMAS


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