Differential Expression between Pilocytic and Anaplastic Astrocytomas: Identification of Apolipoprotein D as a Marker for Low-Grade, Non-Infiltrating Primary CNS Neoplasms

STEPHEN HUNTER, MD, ANDREW YOUNG, MD, PHD, JEFFREY OLSON, MD, DANIEL J. BRAT, MD, GEOFFREY BOWERS, JOSIAH N. WILCOX, PHD, DAVID JAYE, MD, PHD, SAVVAS MENDRINOS, MD, AND ANDREW NEISH, MD

Abstract. Pilocytic astrocytoma, the most common primary central nervous system neoplasm, is infiltrating, rapidly proliferating, and almost invariably fatal. This contrasts with the biologically distinct pilocytic astrocytoma, which is circumscribed, often cystic, slowly proliferating, and associated with a favorable long-term outcome. Diagnostic markers for distinguishing pilocytic astrocytomas from infiltrating anaplastic astrocytomas are currently not available. To identify genes that might either serve as markers or explain these distinct biologic behaviors, cDNA microarray analysis was used to compare the expression of 7,073 genes (nearly one quarter of the human genome) between these 2 types of astrocytoma. Messenger RNAs pooled from 3 pilocytic astrocytomas and from 4 infiltrating anaplastic astrocytomas were compared. Apolipoprotein D (apoD), which expressed 8.5-fold higher in pilocytic astrocytomas, showed the greatest level of differential expression and emerged as a potential marker for pilocytic tumors. By immunohistochemistry, 10 of 13 pilocytic astrocytomas stained positively for apoD, while none of 21 infiltrating astrocytomas showed similar staining. ApoD immunostaining was also seen in 9 of 14 of gangliogliomas, 4 of 5 subependymal giant cell astrocytomas (SEGAs), and a single pleomorphic xanthoastrocytomas (PXAs). By in situ hybridization, pilocytic astrocytomas, in contrast with infiltrating astrocytomas, showed widespread increased apoD expression. SAGE analysis using the NCBI database showed a higher level of expression of apoD RNA in pilocytic astrocytoma than in any of the other 94 neoplastic and non-neoplastic tissues in the database. ApoD is associated with decreased proliferation in some cell lines, and is the protein found in highest concentration in cyst fluid from benign cystic disease of the breast. ApoD might play a role in either decreased proliferation or cyst formation in pilocytic astrocytomas, gangliogliomas, SEGAs, and PXAs.

Key Words: Apolipoprotein D; Astrocytoma; Brain tumor; Microarray analysis; Pilocytic astrocytoma.

INTRODUCTION

No diagnostic markers are currently available to distinguish pilocytic astrocytomas—a biologically distinct and non-aggressive type of astrocytoma—from the fatal infiltrating astrocytomas. Pilocytic astrocytomas have been best characterized in the cerebellum where they are poorly infiltrative and curable by surgery alone in approximately 85% to 90% of cases (1, 2). Diffusely infiltrating astrocytomas, on the other hand, are treated with radiation or chemotherapy in addition to surgery and are almost invariably fatal. In contrast with anaplastic astrocytomas (AAs), pilocytic astrocytomas show low proliferation rates, very few molecular genetic abnormalities, and rarely progress to higher grade (1, 2). Cyst formation is common in pilocytic astrocytomas but rare in AAs. The histologic diagnosis of pilocytic astrocytomas is rarely a problem in the cerebellum. However, distinction between pilocytic and infiltrating astrocytomas may be very difficult in areas where pilocytic tumors are less common, such as the spinal cord, brainstem, or cerebral hemispheres, particularly in small specimens. In these areas accurate diagnosis is critical because of the marked differences in therapy and prognosis (3, 4).

Reasons for the marked differences in behavior between these 2 biologically distinct types of astrocytoma are not well understood. In this study, expression profiling by cDNA microarray analysis was employed to identify candidate genes expressed in pilocytic astrocytomas that distinguish these gliomas from infiltrating AAs and that might help explain their distinct biologic properties.

MATERIALS AND METHODS

Microarray Analysis

Two hundred to 300 mg of tissue from histologically typical pilocytic astrocytomas (WHO grade 1) and AA (WHO grade 3) were frozen at −80°C until RNA extraction. Anaplastic astrocytomas were chosen for comparison because they contain less incorporated non-neoplastic brain tissue than low-grade infiltrating astrocytomas (WHO grade 2). Histologic examination showed, as expected, that some non-neoplastic elements (predominately axons and oligodendrocytes) were incorporated within the infiltrating astrocytomas but not within the pilocytic astrocytomas. None of the gliomas showed significant inflammatory infiltration.

Total cellular RNA was prepared from frozen specimens by mechanical disruption in TRizol reagent (Gibco BRL, Gaithersburg, MD) followed by chloroform extraction and alcohol precipitation according to manufacturer’s instructions. Poly-A+ RNA was isolated with Oligotex oligo-dT beads (Qiagen, Valencia, CA) according to manufacturer’s instructions and quantified with Ribogreen reagent (Molecular Probes, Eugene, OR). Three hundred nanogram samples of poly-A+ RNA from each
tumor were pooled with RNA from tumors of the same type. The pooled poly-A+ RNA specimens were shipped frozen to Incyte Genomics (Palo Alto, CA) for reverse transcription, labeling, and hybridization to the UniGem microarray, using proprietary methods (http://www.incyte.com). Ratios of balanced Cy5 and Cy3 fluorescence intensities at each target position indicate the relative expression of specific genes. Ratios greater than 1.8 and absolute values of fluorescence greater than 700 are considered likely to be significant according to Incyte internal quality control data. The coefficient of variation of Cy5/Cy3 fluorescence ratios is approximately 15%.

Immunohistochemistry

A series of 64 glial neoplasms was selected from the files of the Emory Department of Pathology. The diagnosis in all cases was confirmed by review by 2 neuropathologists (SH and DB). Five \( \mu \)m sections of formalin-fixed paraffin-embedded tissue were tested for the presence of apolipoprotein D (ApoD) using an avidin biotin-complex technique and steam heat-induced antigen retrieval. An avidin-biotinylated enzyme complex kit (DAKO, LSAB2) was used in combination with the automated DAKO AUTOSTAINER (DAKO Corp., Carpinteria, CA). Primary anti-apoD antisera (Signet, Dedham, MA) was used at a dilution of 1:160. Approximately half of the cases were also stained with anti-apoD antibody (Calbiochem, San Diego, CA) (1:160), giving essentially identical results. Positive control was axillary apocrine gland. Negative control was primary antibody replaced with buffer. Positive staining was defined as staining of greater than 5% of the cross sectional area of the tumor.

In Situ Hybridization

In situ hybridization was performed on paraffin sections with the use of human-specific \(^{35}\)S-labeled riboprobes as previously described (5). In situ hybridization results were photographed by polarized light epiluminescence microscopy (Leitz, Stuttgart, Germany) so that the silver grains appeared white. The results were evaluated by 2 individuals and graded (-, +, ++, and ++++) on the basis of the signal intensity above background per cell expressing apoD mRNA in each tissue type. As a control for both background and mRNA integrity, all tissues were hybridized with human von Willebrand’s factor (VWF) cDNA. All tissues showed VWF hybridization to endothelial cells.

RESULTS

Microarray Analysis

Genes differentially expressed at least 2-fold in anaplastic and pilocytic astrocytomas are shown in Tables 1 and 2, respectively. The greatest differential expression was seen for apoD, differentially expressed 5.5-fold and 6.5-fold on separate cDNA targets, in pilocytic astrocytomas.

Immunohistochemistry

In initial studies, 10 of 13 pilocytic astrocytomas stained positively for apoD. Positive staining was within the cytoplasm of the neoplastic cells. Granular bodies, a histologic feature found in pilocytic but not AAs, also stained positively, as did fluid within microcysts (Fig. 1). The majority of cases showed moderate staining intensity. One case with marked staining intensity showed staining of nuclei in addition to cytoplasm. Positive staining was patchy, involving approximately 15% to 75% of the tumor area in different specimens. Small pieces of tumor and peripheral regions of larger pieces of tissue tended not to stain. Two of the negative pilocytic astrocytomas were very small samples—the smallest samples in the study. The infiltrating astrocytomas included 4 low-grade astrocytomas (LGAs), 8 AAs, and 9 glioblastomas (GBMs). None of the 21 infiltrating astrocytomas stained positively for apoD within the cytoplasm of the neoplastic cells (Table 3). Twenty out of the 21 cases were completely negative, with many of these cases showing positive staining of plasma within blood vessels. A single case of anaplastic astrocytoma showed interstitial staining without staining of the neoplastic cells. In this case, positive staining was limited to a focal area surrounding a group of damaged blood vessels and also involved adjacent non-neoplastic brain tissue. This staining was interpreted as related to an exudate of plasma since it was interstitial in location, associated with hemorrhage, and the only focus in any of the cases in which either AA or non-neoplastic brain tissue stained positively.

Since apoD is associated with cyst formation and granular bodies, we expanded our immunohistochemical study to include other cystic tumors with granular bodies (i.e. gangliogliomas and pleomorphic xanthoastrocytomas [PXAs]), as well as subependymal giant cell astrocytomas (SEGAs), ependymomas, and oligodendrogliomas (Table 3). Nine of 14 gangliogliomas and the single PXA stained positively, as did 4 of 5 SEGAs. None of the 6 ependymomas showed any positive staining. One of 4 oligodendrogliomas showed a single very small focus (approximately 1% of the specimen) of definite staining within the cytoplasm of the neoplastic cells. This case was not tabulated as positive in Table 3 since staining involved less than 5% of the cross sectional area of the tumor.

In Situ Hybridization

Formalin-fixed paraffin-embedded sections from 11 pilocytic and 11 infiltrating astrocytomas (1 LGA, 8 AAs, 2 GBMs) were hybridized in situ with an apoD riboprobe. Slides were developed at 4 and 8 wk. At 8 wk, all pilocytic astrocytomas showed widespread hybridization signal above background. In contrast, the infiltrating astrocytomas showed essentially background signal levels with only small focal areas above background in several cases (Fig. 2). Seven of the pilocytic tumors were graded 3+, three as 2+, and one as 1+. Three of the infiltrating astrocytomas were considered to show focal 1+ signal, while the others were graded as 0. Similar to the immunohistochemical staining, hybridization signal in the pilocytic tumors was not uniform in distribution but did
<table>
<thead>
<tr>
<th>Differential expression</th>
<th>Gene Description</th>
<th>Gene bank accession #</th>
<th>Locus</th>
<th>Cy3 fluorescence intensity (anaplastic)</th>
<th>Balanced Cy5 fluorescence intensity (pilocytic)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.1</td>
<td>nuclear receptor subfamily 1, group I, member 3*</td>
<td>X56199</td>
<td>3p25.3–p24.1</td>
<td>2916</td>
<td>359</td>
</tr>
<tr>
<td>3.9</td>
<td>myelin-associated oligodendrocyte basic protein</td>
<td>H23137</td>
<td>Xq22</td>
<td>1087</td>
<td>277</td>
</tr>
<tr>
<td>3.5</td>
<td>proteolipid protein 1 (Pelizaeus-Merzbacher disease, spastic paraplegia 2, uncomplicated)</td>
<td>M27110</td>
<td></td>
<td>6451</td>
<td>1855</td>
</tr>
<tr>
<td>3.5</td>
<td>epidermal growth factor receptor (avian erythroblastic leukemia viral (verb-b) oncogene homolog)</td>
<td>X00588</td>
<td>7p12</td>
<td>1461</td>
<td>419</td>
</tr>
<tr>
<td>2.8</td>
<td>glial fibrillary acidic protein</td>
<td>AA059335</td>
<td>17q21</td>
<td>23,648</td>
<td>8461</td>
</tr>
<tr>
<td>2.6</td>
<td>tight junction protein 2 (zona occludens 2)</td>
<td>NM_004817</td>
<td>9q13–q21</td>
<td>3652</td>
<td>1430</td>
</tr>
<tr>
<td>2.5</td>
<td>upregulated by 1,25-dihydroxyvitamin D-3</td>
<td>S73591</td>
<td>1</td>
<td>2112</td>
<td>841</td>
</tr>
<tr>
<td>2.5</td>
<td>cathepsin H</td>
<td>X16832</td>
<td>15q24–q25</td>
<td>2072</td>
<td>835</td>
</tr>
<tr>
<td>2.3</td>
<td>phosphoprotein enriched in astrocytes 15</td>
<td>NM_003768</td>
<td>1q21.1</td>
<td>6984</td>
<td>3002</td>
</tr>
<tr>
<td>2.3</td>
<td>neurotrophic tyrosine kinase, receptor, type 2</td>
<td>NM_006180</td>
<td>9q22.1</td>
<td>3659</td>
<td>1608</td>
</tr>
<tr>
<td>2.3</td>
<td>ESTs, Moderately similar to histamine N-methyltransferase [H. sapiens]</td>
<td>AW148944</td>
<td>Unmapped</td>
<td>2526</td>
<td>1116</td>
</tr>
<tr>
<td>2.1</td>
<td>glutamate dehydrogenase 1</td>
<td>AW135046</td>
<td>8q12–q13</td>
<td>4259</td>
<td>1991</td>
</tr>
<tr>
<td>2.1</td>
<td>minichromosome maintenance deficient (S. cerevisiae) 4</td>
<td>AF004715</td>
<td>11q21</td>
<td>1569</td>
<td>730</td>
</tr>
<tr>
<td>2.0</td>
<td>jerky (mouse) homolog-like</td>
<td>NM_001098</td>
<td>22q13.2–q13.31</td>
<td>2211</td>
<td>1103</td>
</tr>
<tr>
<td>2.0</td>
<td>poly(A)-binding protein, cytoplasmic 1</td>
<td>Y00345</td>
<td>8q22.2–q23</td>
<td>1170</td>
<td>586</td>
</tr>
<tr>
<td>2.0</td>
<td>Incyte EST</td>
<td></td>
<td></td>
<td>1022</td>
<td>518</td>
</tr>
</tbody>
</table>

* Expressed in females only (by chance, all 3 pilocytic tumors were from males).
**TABLE 2**

<table>
<thead>
<tr>
<th>Differential expression</th>
<th>Gene bank accession #</th>
<th>Gene locus</th>
<th>Cy3 fluorescence intensity (anaplastic)</th>
<th>Cy5 fluorescence intensity (pilocytic)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Balanced</td>
<td></td>
<td></td>
<td>8.5</td>
<td>6.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.0</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.5</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.2</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.1</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.0</td>
<td>2.0</td>
</tr>
</tbody>
</table>

**Genes Overexpressed in Pilocytic Astrocytoma Compared to Anaplastic Astrocytoma**

- Apolipoprotein D
- Sparc/osteonectin, cwcv and kazal-like domains proteoglycan (testican)
- Cell adhesion molecule with homology to L1CAM (close homologue of L1)
- ESTs
- KIAA0193 gene product
- Protease, serine, 11 (IGF binding)
- Putative transmembrane protein with homology to L1CAM (close homologue of L1)
- KIAA0193 gene product
- Low density lipoprotein-related protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane-protein
- Low density lipoprotein-related protein 3
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
- Putative transmembrane protein 1 (alpha-2-macroglobulin receptor)
Apolipoprotein D in Pilocytic and Anaplastic Astrocytomas

Fig. 1. ApoD immunohistochemistry. A: Pilocytic astrocytoma showing diffuse labeling of neoplastic cells. Blood vessel in lower left-hand corner does not label but contains positively labeling plasma. B: Infiltrating anaplastic astrocytoma does not label. Again, note blood vessel containing labeled plasma. C: Ganglioglioma with labeled neoplastic cells and granular bodies. D: Microcyst within pilocytic astrocytoma contains labeled fluid.

TABLE 3

ApoD Immunohistochemistry of Primary Brain Tumors

<table>
<thead>
<tr>
<th></th>
<th># positive</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Infiltrating:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pilocytic Astrocytoma</td>
<td>10</td>
<td>13</td>
</tr>
<tr>
<td>Ganglioglioma</td>
<td>9</td>
<td>14</td>
</tr>
<tr>
<td>SEGA</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>PXA</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Ependymoma</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Infiltrating:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oligodendroglioma</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Astrocytoma</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Anaplastic Astrocytoma</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Glioblastoma</td>
<td>0</td>
<td>9</td>
</tr>
</tbody>
</table>

Pilocytic astrocytomas frequent cystic change and a predilection to form numerous granular bodies. Thus apoD is not a marker specifically for pilocytic astrocytomas, but rather for a group of low proliferative, non-infiltrating and potentially curable primary brain tumors.

Upregulation of apoD in pilocytic astrocytomas was also confirmed by serial analysis of gene expression (SAGE), using a public database (http://www.ncbi.nlm.nih.gov/SAGE). According to this SAGE database, which is maintained by the National Center for Biotechnology Information, the probability is 100% that apoD is overexpressed in pilocytic astrocytoma compared with all grades of infiltrating astrocytoma, i.e. LGA, AA and GBM. ApoD expression in pilocytic astrocytoma (2,853 tags per million base pairs [tmbp]) was at least 4 times higher than in any of the other 94 tissues available in this database, with the single exception of mesothelial cells (2,200 tmbp). Other tissues of interest in this database included normal white matter (497 tmbp), normal cerebellum (135 tmbp), thalamus (164 tmbp), pooled GBMs (210 tmbp), AAs (95 tmbp), and LGAs (399 tmbps). Increased expression relative to both non-neoplastic brain tissue and low-grade infiltrating astrocytoma suggests that increased expression of apoD in pilocytic astrocytomas does not simply reflect decreased proliferation in these tumors. In addition to apoD, serine protease 11 and alpha-1-antichymotrypsin (overexpressed 1.9-fold by microarray) were also found to be differentially expressed in pilocytic astrocytomas by both SAGE and microarray.
Fig. 2. ApoD in situ hybridization at 8 wk: positive signal by epiluminescence appears as white granules. A: Non-neoplastic cerebellum (lower right) showing minimal to absent signal with adjacent pilocytic astrocytoma (upper left). B: Microcystic area in pilocytic astrocytoma showing strong signal. C: Pilocytic astrocytoma showing strong signal in a subpopulation of neoplastic cells. D: Anaplastic astrocytoma showing absent to minimal signal. Positive signal in lower right corner is within the leptomeninges, a site known to express apoD RNA normally.

analysis. No other genes were found to be differentially expressed by both microarray and SAGE analysis.

The clinical aggressiveness and associated poor prognosis of astrocytomas is directly related to their infiltrative or invasive behavior (1, 2). Individual neoplastic cells may infiltrate through the brain tissue and be found long distances from primary tumor. Consequently, infiltrating glial neoplasms cannot be successfully removed by surgery and will almost invariably recur, eventually causing death. In contrast, non-infiltrating gliomas, such as pilocytic astrocytomas, are cured by surgery in a high percentage of cases. The expression of myelin-associated and proteolipid proteins in AAs (Table 1) most likely reflects the incorporation of non-neoplastic elements within the infiltrating astrocytomas compared with the solid, poorly infiltrative pilocytic astrocytomas.

Several genes that may be related to the invasive behavior of gliomas (i.e. proteases, adhesion molecules, and extracellular matrix proteins) were differentially expressed between pilocytic astrocytomas and AAs in this study. Cathepsin H was overexpressed 2.5-fold in AAs. This is consistent with a previous study demonstrating the importance of Cathepsin H in the progression and invasive behavior of astrocytomas (16). Another potential candidate for a role in the invasive behavior of astrocytomas is zona occludens protein 2 (17). This tight junction protein, which is known to be expressed by astrocytes, was overexpressed 2.6-fold in AAs. The function of this protein in astrocytomas is not clear. Interestingly, several extracellular matrix molecules were overexpressed in pilocytic astrocytomas compared with AAs, including perlecan (×2.9) and testican (×3.0). These members of the sparc/osteonectin family of extracellular matrix molecules are involved in tumor invasion and growth. Perlecan for example is an angiogenic modulator and both binds to and acts as a major storage site for...
basic fibroblast growth factor (18). Production of these matrix molecules or members of their families have been correlated with angiogenesis or malignant behavior of several tumors, including melanoma (19) and colon carcinoma (20). Conversely, perlecan suppresses growth and invasion in fibrosarcoma cells (21).

In conclusion, this study identified apoD as a potentially useful marker for low-grade non-infiltrating primary brain tumors, including pilocytic astrocytomas, gangliogliomas, SEGAs, and PXAs. Additional work needs to be done to determine whether apoD will be more useful as a marker at the protein or RNA level, and to what extent it will be useful as a marker in frozen or fixed tissue, cerebrospinal fluid, or cyst fluid. The exact role that this gene, as well as other matrix and adhesion genes, might play in the unique behavior of pilocytic astrocytomas remains to be elucidated. Based on what is currently known about this protein, apoD may play a role in either cyst formation or the low proliferative rate of these tumors.

ACKNOWLEDGMENT

We thank Dr Erwin G. Van Meir for his invaluable assistance with this investigation.

REFERENCES

1. Kleihues P, Cavenee WK. Pathology and genetics of tumors of the nervous system. WHO classification of tumors 2000;45–51

Received August 31, 2001
Revision received October 25, 2001 and November 12, 2001
Accepted November 19, 2001