Recurrent anaplastic ganglioglioma: pathological characterization of tumor cells

Case report

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A total resection of a left frontal lobe tumor in a 26-year-old man revealed differentiated ganglioglioma with small foci of atypical glial cells exhibiting mild atypia. Six and one-half years later, a large, well-demarcated tumor recurred; at that time, histological analysis revealed both typical ganglioglioma and highly cellular anaplastic areas, the latter predominating. Although the patient subsequently underwent total and subtotal resections, radiation therapy, and chemotherapy, tumors continued to recur at progressively shorter intervals and he died at the age of 35 years. Biopsies of tissue obtained at the last three resections and the autopsy revealed only anaplastic tumor cells. Routine histological examinations indicated that these tumors were uniformly composed of undifferentiated cells. However, pathological studies using immunohistochemical analysis, electron microscopy, and immunoblot analysis demonstrated that a small number of recurrent anaplastic cells had astrocytic features. Results of Ki-67/MIB-1 labeling and silver nucleolar organizer region counts for those cells were high for glial tumors. A retrospective study of the initial tumor showed slightly high MIB-1 labeling for atypical glial cells. This case is characterized by pathological findings of recurrent tumors that correspond to an unusual form of malignant glioma exhibiting slight astrocytic differentiation. The present case suggests that a longer follow-up period (> 5 years) is necessary in cases of ganglioglioma with mild atypia and that careful examinations, including proliferating potential analysis of initial tumor cells, could be important for the diagnosis and treatment of ganglioglioma.

KEY WORDS • ganglioglioma • anaplastic ganglioglioma • histopathology • ultrastructural study • Ki-67 labeling • silver nucleolar organizer region

Gangliogliomas of the central nervous system are rather uncommon lesions comprising approximately 1% of all brain tumors.\(^5,7,11\) Surgical resection is the treatment of choice for gangliogliomas, and favorable clinical outcomes have been reported in several relatively large series.\(^6,7,11,15,22\) However, the role of adjuvant therapy and the effect of histological grade on survival remain unclear. Anaplastic gangliogliomas are extremely rare, and anaplastic changes are found mostly in the glial elements of the tumor.\(^2,2,4,8,11,20\) Patients with anaplastic ganglioglioma often develop tumor recurrences.\(^21\) To date, extensive pathological studies using data on differentiation and proliferative activity in primary and recurrent tumors have not been described in the literature. Here we report a case of ganglioglioma in the cerebral hemisphere of a young adult who underwent a second operation 6.5 years after an initial, total tumor resection. The outcome after the second operation included repeated recurrences and eventually death. This report examines the pathological features of the primary and recurrent tumors, using immunohistochemical and histochemical analyses, electron microscopy, and immunoblotting.

Case Report

This man began to have seizure episodes at the age of 24 years. His family medical history was unremarkable. Seizures occurred once a year for the following 2 years.

First Examination and Operation. In April 1985, a computerized tomography (CT) scan revealed a left frontal tumor (Fig. 1 left) and the patient, then 26 years old, underwent surgery for total removal of a 4 × 4-cm–solid pinkish tumor.

Postoperative Course. No postoperative radiotherapy was administered. On recovery, the patient returned to his carpentry job.

Tumor Recurrence and Treatment. Six and a one-half years after the initial operation, the patient developed loss of memory and aphasia. Computerized tomography and
magnetic resonance (MR) imaging disclosed a large, iso-
dense high-density tumor of the left frontal lobe, appar-
ently a recurrent tumor (Fig. 1 right). In December 1991, 
the 9 × 8-cm tumor was totally resected and the cut sur-
face appeared to be partly hemorrhagic. Postoperative 
treatment for the malignant tumor was planned at that 
time, but the patient declined radiotherapy. Nine months 
after the surgery, the tumor recurred again. In November 
1992, the tumor was subtotally resected. The patient 
received radiotherapy of 60 Gy to the tumor in a 5-week 
course beginning 45 days postoperatively. However, his 
aphasia progressed gradually. Computerized tomography 
and MR imaging revealed an increase in the size of the 
tumor. In January 1993, the tumor was again subtotally 
resected, followed by chemotherapy (methotrexate 5 mg 
administered twice locally). Two months after a final 
operation, performed in May 1993, the patient died at 35 
years of age. An autopsy was performed but was limited 
to the brain.

Results

Histopathological Findings

Examined using light microscopy, the tumor extracted 
at the initial resection was primarily composed of an ast-
trogial and ganglion cell component. Large polygonal

TABLE 1
Proliferation potential of neoplastic cells in primary and 
recurrent tumors in a patient with ganglioglioma*

<table>
<thead>
<tr>
<th>Histological Findings</th>
<th>Primary Tumor</th>
<th>Recurrences of Tumor</th>
</tr>
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<tbody>
<tr>
<td>mitotic figures/10 hpfs</td>
<td>0.8</td>
<td>4.3 4.4 0.8 19</td>
</tr>
<tr>
<td>Ki-67 score (%)</td>
<td>NE</td>
<td>NE 24.6 9.7 23.2</td>
</tr>
<tr>
<td>MIB-1 score (%)</td>
<td>7.0</td>
<td>7.8 28.9 8.3 NE</td>
</tr>
<tr>
<td>AgNORs/nucleus</td>
<td>NE</td>
<td>2.95 3.55 1.69 3.08</td>
</tr>
</tbody>
</table>

* Abbreviations: AgNORs = silver nuclear organizer regions; hpfs = high-power fields; NE = not examined.

ganglion cells were irregularly arranged in groups and 
binucleated ganglion cells were present (Fig. 2A). The 
ganglion cells were immunoreactive for neurofilament 
protein (NFP), synaptophysin (SY) (Fig. 2B), and neuron-
specific enolase (NSE). Astroglial components (includ-
ing gemistocytes) stained positive for both glial fibrillar 
acidic protein (GFAP) (Fig. 2C) and S-100 protein. In 
other areas, small immature cells were found that dis-
played considerably high cellularity, rare rosette for-
matin, and small numbers of mitotic figures (Fig. 2D). 
Immunohistochemically, a large number of these cells re-
acted positively to vimentin and some to GFAP.

In the tissue removed at the second resection, most 
tumors were uniformly composed of small cells with 
round-to-oval nuclei and scanty cytoplasm (Fig. 2E), 
although differentiated ganglioglioma were present on 
the surfaces of the tumors. In areas of small neoplastic 
cells, mitoses were often present. Areas of hemorrhage 
and necrosis were seen, but there was no pseudopalisad-
ing around necrotic areas, cellular pleomorphism, or en-
dotheilial proliferation. Reticulin fibers were sparse and 
surrounded small neoplastic cells. The small neoplastic 
cells displayed positive immunoreactivity to antivimentin 
but not to anti-NFP, -SY, -desmin, or anti–smooth muscle 
actin. The GFAP immunoreactivity was equivocal on rou-
tine immunostaining, but definite on protease-treated par-
afin sections (Fig. 2F).

In the specimens obtained at the third, fourth, and fifth 
resections, there were no areas of differentiated gangli-
oglioma. The histology and immunophenotype of the 
tumor cells of those resections were similar to those of 
the small neoplastic cells observed at the second resection. In 
tissue from the third resection, spindle-shaped cells with 
fascicular patterns were noted (Fig. 2G). Some tumor cells 
had eccentric, pale areas in their cytoplasm. In areas of small neoplastic 
cells observed at the second resection. In 
the tissue from the fourth resection, mitoses were 
numerous (19 in 10 high-power fields).

The brain autopsy disclosed swelling, herniation, and 
recurrent tumors with meningeal dissemination of the 
small neoplastic cells.

No tumor cells displayed any detectable immunoreac-
tivity for p53 protein.

Proliferative Markers

The results of proliferating activity analyses for immu-
ture tumor cells are summarized in Table 1. Nuclear label-
ing with Ki-67 or MIB-1 antibodies was observed in 
immature neoplastic cells, but not in differentiated gan-
glion cells.

Electron Microscopy Findings

Electron microscopy evaluation of tissue samples ob-
tained at the second and third resections revealed small 
tumor cells embedded in considerably abundant, fuzzy 
matrices, without surrounding basal lamina or attachment 
devices (Fig. 3A). The tumor cells contained mitochon-
dria and numerous rough endoplasmic reticula with 
marked dilation and frequent concentric lamination in 
their cytoplasm. They had short cell processes which 
included scattered intermediate filaments. Cilia were rare-
ly observed in the tumor cells (Fig. 3B). No neurosecreto-
ry granules were identified.
Western Blot Analysis

Frozen tumor tissue specimens obtained from the fourth operation were prepared for immunoblotting with anti-GFAP antibody. The analysis revealed a weak band at approximately 50 kD in the fractions obtained from cytoskeletal preparations from the fourth resection (Fig. 4).

Discussion

Histologically, gangliogliomas are defined as neoplasms composed of an intimate admixture of neoplastic astrocytes and atypical ganglion cells. Immunohistochemical analysis using neuronal (SY, NFP, NSE) and glial (GFAP) markers has been shown to be useful in the
Anaplastic changes and appeared to be composed of small undifferentiated cells; at the level of light microscopy, these anaplastic lesions could be diagnosed as recurrent anaplastic ganglioglioma. Clinical and radiological data, as well as pathological findings, suggest that the recurrent tumor could have arisen from a preexisting ganglioglioma and was designated as Grade I with small areas corresponding to Grade III. In previous studies of large series of gangliogliomas, the frequencies of Grades I, II, and III were approximately 85%, 10%, and 5%, respectively. Thus, the primary tumor described in the present case was partly hypercellular and contained a few mitotic figures. Therefore, it was designated as Grade I with small areas corresponding to Grade III. In previous studies of large series of gangliogliomas, the frequencies of Grades I, II, and III were approximately 85%, 10%, and 5%, respectively. Thus, the primary tumor described in the present case was a very rare type of lesion.

The first recurrent tumor had a large area of small, poorly differentiated cells as well as a small area of typical ganglioglioma. Clinical and radiological data, as well as pathological findings, suggest that the recurrent tumor could have arisen from a preexisting ganglioglioma and could be diagnosed as recurrent anaplastic ganglioglioma. At the level of light microscopy, these anaplastic lesions appeared to be composed of small undifferentiated cells; these cells became the single neoplastic components in subsequent recurrent tumors. Anaplastic changes and malignant transformations of gangliogliomas are exceptional and for the most part are restricted to the glial component in reported cases. Thus, anaplastic lesions of gangliogliomas correspond to glioblastoma or anaplastic glioma. However, our patient’s tumor did not show features typically seen in glioblastoma. On conventionally stained histological slides, the recurrent tumors showed no evidence of glial differentiation and might be difficult to distinguish from primitive neuroectodermal tumor or unclassified sarcoma. Immunohistochemically, the tumor cells revealed a definite immunoreactivity for GFAP only when the formalin-fixed, paraffin-embedded tissue sections were pretreated with protease. Western blot analysis performed on a sample from the fourth resection detected weak expression of GFAP molecules in the tumor. These findings suggest that recurrent anaplastic cells might contain small quantities of GFAP. Under the electron microscope, most of the recurrent anaplastic tumor cells showed undifferentiated features, but some displayed glial features with intermediate filaments, cilia, and concentric laminations of the endoplasmic reticulum. Combining these analyses, glial (astrocytic) differentiation was partly identified in the recurrent anaplastic tumors.

In our patient’s recurrent tumors, a high level of proliferative activity was signaled by frequent mitotic figures on routine histological examinations. We used MIB-1 and Ki-67 labeling and the silver nucleolar organizer region (AgNOR) method to analyze proliferative capacity quantitatively. The monoclonal antibody MIB-1 recognizes the same or a very similar epitope as Ki-67, although the immunohistochemical technique for labeling each of these antigens is different and the MIB-1 proliferating cell indices (PCIs) sometimes correlate poorly with the Ki-67 PCIs. The Ki-67 PCIs ranged from 0.6% to 3.2% (mean 1.7%) and from 1.3% to 12.4% (mean 6.2%) for astrocytomas and glioblastomas, respectively. Thus, the MIB-1 and Ki-67 PCIs of our recurrent tumor were consistent with those of glioblastoma. In addition, the AgNOR impregnation showed moderate-to-high AgNOR counts compared to those in the literature. A high proliferative capacity of the recurrent tumor appears to be consistent with the progression of the present case. It is noteworthy that the Ki-67 and MIB-1 PCIs and the AgNOR counts all decreased in the third recurrent tumor. This might be due to the effect of radiation therapy, which was used after the operation on the second recurrence. However, intratumoral differences in indices of tumor cell proliferation should be considered, because regional differences in the expression of the Ki-67 protein and AgNOR counts in glioblastoma have been suggested by Onda et al.

The proliferative potential of the initial tumor in this case was measured using MIB-1 monoclonal antibody because only paraffin sections were available. Wolf and colleagues showed that the labeling indices for Ki-67 were less than 10% in all gangliogliomas (61 cases), except for a single case of a Grade II ganglioglioma, and that 74% of gangliogliomas had a Ki-67 PCI of less than 1%. In our patient’s initial tumor, MIB-1 was observed in the nuclei of less differentiated glial cells with moderate cellularity and rare mitotic figures; the MIB-1 PCI was 7%. Thus the proliferative capacity of the initial tumor in the present case was considered high for gangliogliomas. The high MIB-1 PCI might be related to the development of tumor.
Recurrent anaplastic ganglioglioma

recurrence. A previous study\textsuperscript{21} showed a significant correlation between WHO grading of gangliogliomas and Ki-67 indices and that four of six patients with WHO Grade II tumors developed tumor recurrences or tumor progression. Therefore, the present case supports the use of Ki-67 or MIB-1 in specifying the grade and evaluating the prognosis.

Currently, postoperative radiation therapy is recommended only if there is tumor progression or histological evidence of anaplastic ganglioglioma. The present case indicates that postoperative radiation might be necessary for the treatment of Grade III lesions and that careful follow up over a longer period (> 5 years) could be recommended.

Conclusions

A patient developed ganglioglioma with mild atypia in the cerebral hemisphere and underwent total tumor resection. Six and one-half years later, recurrences began and eventually led to a fatal outcome. The recurrent tumors had an unusual histology: they were composed of undifferentiated cells. After extensive pathological study, these tumors were diagnosed as malignant glioma with slightly astrocytic differentiation. This case suggests that ganglioglioma with mild atypia and high MIB-1 labeling can recur more than 5 years after total resection and that careful histological examinations with proliferative markers for the primary tumor should be applied to the management of ganglioglioma.

Acknowledgments

The authors thank Dr. S. R. VandenBerg for his comments on the pathological findings and Masako Kohno and Kohji Isoda for their expert technical assistance.

References


Manuscript received September 18, 1995. Accepted in final form January 15, 1996.

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