Prognosis of Cerebellar Glioblastomas: Correlation Between Prognosis and Immunoreactivity for Epidermal Growth Factor Receptor Compared with Supratentorial Glioblastomas

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Abstract. Background: Cerebellar glioblastomas (GBM) are rare tumors whose clinicopathological characteristics are not well understood. Patients and Methods: Clinico-immunohistochemical findings were retrospectively analyzed in 43 supratentorial- and 7 cerebellar GBM. The correlation between survival and immunopositivity for p53, epidermal growth factor receptor (EGFR) and Ki-67 in these tumors was statistically analyzed and compared. Results: Of the 43 patients with supratentorial GBM, 27 (62.8%) were EGFR immunopositive; their survival was significantly shorter than that of the 16 EGFR-negative patients (p=0.0248). There was no significant correlation between survival and p53 immunopositivity (p=0.7870) and Ki-67 labeling index (p=0.7133). All 5 cerebellar GBM patients treated with radio- and chemotherapy were EGFR-immunonegative; they survived significantly longer than patients with supratentorial GBM (p=0.0296) possibly because their EGFR negativity rendered their tumors more highly radiosensitive. Conclusion: The better prognosis of patients with cerebellar, EGFR-negative tumors compared to patients with supratentorial tumors is due to the higher radiosensitivity of these tumors.

Glioblastomas (GBMs) of the cerebellum are uncommon; they comprise approximately 2.8% of all gliomas. Their specific clinical features differ from those of supratentorial GBM. However, their clinicopathological characteristics and prognosis are still not well understood (1-10) and there is no standard therapeutic approach to these tumors. In supratentorial GBM, some clinical factors have been identified as predictors of survival (11-14). Mutations and the overexpression of several oncogenes/tumor suppressor genes play important roles in tumor growth. The epidermal growth factor receptor (EGFR) and p53 are candidate molecules in the tumorigenesis and progression of gliomas and overexpression of EGFR genes or p53 mutations are involved in the poor or the good prognosis of gliomas (15-33). In the current study, we compared the expression of EGFR, p53 and the Ki-67 labeling index (LI) in cerebellar and supratentorial GBM and evaluated their role in the survival of patients with these tumors.

Patients and Methods

Clinical data and patient selection. The clinical records and immunohistochemical findings of 7 patients with cerebellar- and 43 patients with supratentorial GBM, treated at our hospital between January 1988 and March 2004, were retrospectively studied. Tumors not confirmed pathologically and those originating in the brainstem or thalamus were excluded from this study. Patients with supratentorial GBM who did not receive adjuvant radiotherapy were also excluded. The pathological diagnosis of all specimens obtained at the initial surgery was based on the histological classification promulgated by the World Health Organization (WHO). All 43 patients with supratentorial GBM underwent postoperative radiotherapy and/or chemotherapy; 5 of the 7 cerebellar GBM patients received postoperative radiotherapy and chemotherapy, while the other 2 underwent surgery only.

Tissue specimens and immunohistochemical staining. All tumor specimens were obtained by surgical resection and fixed in 10% formalin before paraffin processing. Representative slides were stained with hematoxylin and eosin for standard histological diagnosis. Histological subtypes were defined according to WHO criteria by one of the authors (K.S.). To determine alterations in p53 and the expression of EGFR and Ki-67, formalin-fixed, paraffin-embedded tumor samples were used. Mouse monoclonal antibody was employed at a 1:100 dilution for mutant p53 (DAKO JAPAN, Kyoto, Japan), a 1:40 dilution for EGFR (Novocastra Laboratories, Ltd., Newcastle, UK) and a 1:50 dilution for Ki-67 (Mib-1; Immunotech, Marseille, France). Pathological specimens (4 µm in thickness) were mounted on gelatin-coated slides and deparaffinized by 15-min xylene treatment. To block endogenous
peroxidase, the slides were immersed for 30 min in 3% hydrogen peroxidase in methanol. Each specimen was rinsed 3 times for a total of 15 min in phosphate-buffered saline (PBS), pH 7.5, with gentle stirring, incubated overnight with the primary antibodies at 4°C, and then subjected to the streptavidin-biotin method using the histofine SAB (M) kit (Nichirei Co., Tokyo, Japan). After washing in PBS, the sections were exposed to tetrahydrochloride (Wako Pure Chemical Industries, Ltd., Osaka, Japan) for 5 min in 0.05 M Tris buffer, pH 7.6, containing 0.003% hydrogen peroxide. To facilitate cytoplasmic visualization of the immunostained product, the slides were counterstained with Mayer hematoxylin. Experiments and control experiments were performed twice on different days using the same protocols and time exposures. The expression of p53 and EGFR was recorded as negative (−) or positive (+). One author (Y.K.), blinded to the pathological diagnosis and all clinical and radiological data, determined the Ki-67 LI by counting 1,000 tumor cell nuclei.

**Statistical analysis.** The correlation between each immunohistochemical result (p53-, EGFR- and Ki-67 immunopositivity) and survival in the 43 patients with supratentorial GBM was analyzed using the log-rank test. The cut-off for immunopositivity was: p53< 30% (negative), p53> 30% (positive); EGFR< 30% (negative), EGFR> 30% (positive), as previously described in our study (42). For the Ki-67 LI, the cut-off was <19.6% and >19.6% as its mean rate was 19.6%. The difference in the survival time of 5 cerebellar- and 43 supratentorial GBM patients, both groups having received postoperative radiotherapy and/or chemotherapy, was also statistically analyzed. Statistical significance was regarded as p<0.05. Survival was estimated in months from the date of the first surgery to the date of death or, in patients alive at the time of this study, the date of the last follow-up. Survival curves were plotted, and the median survival time was estimated by the Kaplan-Meier method.

**Results**

**Clinical data of supratentorial- and cerebellar GBM.** The clinical data of the 43 supratentorial- and 7 cerebellar GBM patients are summarized in Table I. Detailed clinical data on patients with cerebellar GBM are shown in Table II. The patients with supratentorial GBM included 30 males and 13 females with a mean age of 50.8 years (range 7 - 73 years). Of the cerebellar GBM patients, 4 were males and 3 females, and their mean age was 50.8 years (range 7 - 73 years). There was no significant difference in the mean age of our study population. In the group with supratentorial GBM, patients of the male gender prevailed.

Of the 43 patients with supratentorial GBM, 2 received gross total-, 8 subtotal- and 30 partial resection; 3 underwent biopsy. All received adjuvant radiation therapy (48-60 Gy in 2-Gy fractions) and 39 received nitrosourea antineoplastic agent-based chemotherapy (70 mg/m² i.v. on days 1 and 28), and 1 received ifosfamide (900 mg/m² i.v. on days 1-5), cisplatin (20 mg/m² i.v. on days 1-5) and etoposide (60 mg/m² i.v. on days 1-5). Additionally, 1 patient underwent stereotactic radiosurgery (central 36 Gy, margin 18 Gy) for residual tumor. The other 2 patients received no postoperative adjuvant therapy (Table II). The mean follow-up in these 7 patients was 17 months (range 2-64.5 months); 4 died of tumor progression and 3 are alive with residual tumor. The median survival of all 7 patients was 22.2 months, while it was 30 months for the 5 patients who received combined adjuvant therapy. In contrast, the 2 patients with no adjuvant therapy survived for only 3.25 and 4.5 months; they manifested dissemination to the contralateral side of the cerebellum and the lateral ventricle. These findings suggest that combined adjuvant therapy may prolong the postoperative survival of cerebellar GBM patients.

**Immunohistochemical analysis of supratentorial- and cerebellar GBM.** A summary of our immunohistochemical findings is shown in Tables II and III. Of the 43 supratentorial GBM, 31 (72%) were p53-immunopositive and 27 (63%) were EGFR-immunopositive. The mean Ki-67 LI was 19.6% (range 1.9 - 58.3%); 17 (39%) of the supratentorial GBMs were immunopositive at a Ki-67 LI >19.6%.

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**Table I. Clinical data for supratentorial and cerebellar glioblastomas.**

<table>
<thead>
<tr>
<th></th>
<th>Supratentorial (n=43)</th>
<th>Cerebellar (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age yrs (range)</td>
<td>50.8 (7-73)</td>
<td>49.5 (8-72)</td>
</tr>
<tr>
<td>Sex (Male/Female)</td>
<td>30 / 13</td>
<td>4 / 3</td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surgery</td>
<td>43</td>
<td>7</td>
</tr>
<tr>
<td>GTR</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>STR</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>PR</td>
<td>30</td>
<td>4</td>
</tr>
<tr>
<td>Biopsy</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Radiation therapy</td>
<td>43 (48-60 Gy)</td>
<td>5 (54-60 Gy)</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>39</td>
<td>5</td>
</tr>
<tr>
<td>Mean follow-up period months (range)</td>
<td>18.6 (2.3-112.3)</td>
<td>17 (2-64.5)</td>
</tr>
</tbody>
</table>


Of the 7 patients with cerebellar GBM, 5 received postoperative combined radiotherapy (54-60 Gy in 2-Gy fractions) and chemotherapy; 4 were given nitrosourea antineoplastic agent-based chemotherapy (70 mg/m² i.v. on days 1 and 28), and 1 received ifosfamide (900 mg/m² i.v. on days 1-5), cisplatin (20 mg/m² i.v. on days 1-5) and etoposide (60 mg/m² i.v. on days 1-5). Additionally, 1 patient underwent stereotactic radiosurgery (central 36 Gy, margin 18 Gy) for residual tumor. The other 2 patients received no postoperative adjuvant therapy (Table II). The mean follow-up in these 7 patients was 17 months (range 2-64.5 months); 4 died of tumor progression and 3 are alive with residual tumor. The median survival of all 7 patients was 22.2 months, while it was 30 months for the 5 patients who received combined adjuvant therapy. In contrast, the 2 patients with no adjuvant therapy survived for only 3.25 and 4.5 months; they manifested dissemination to the contralateral side of the cerebellum and the lateral ventricle. These findings suggest that combined adjuvant therapy may prolong the postoperative survival of cerebellar GBM patients.
Of the 5 cerebellar GBM patients treated with combined adjuvant therapy, 4 manifested p53 immunopositivity, all 5 were negative for EGFR immunostaining. Ki-67 analysis showed that 1 tumor had a Ki-67 LI of 19.6%. Representative pathological and immunohistochemical findings are given in Figure 1.

**Table II. Clinical and immunohistochemical findings for the 7 patients with cerebellar glioblastoma.**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age (yrs)</th>
<th>Sex</th>
<th>Resection</th>
<th>Radiation</th>
<th>Chemo-therapy</th>
<th>Dissemination</th>
<th>Survival (months)</th>
<th>p53</th>
<th>EGFR</th>
<th>Ki-67 LI(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>32</td>
<td>M</td>
<td>STR</td>
<td>WB 60 Gy</td>
<td>+(IA)</td>
<td>none</td>
<td>Dead (17)</td>
<td>–</td>
<td>–</td>
<td>2.1</td>
</tr>
<tr>
<td>2</td>
<td>72</td>
<td>M</td>
<td>PR</td>
<td>none</td>
<td>none</td>
<td>+†</td>
<td>Dead (2)</td>
<td>+</td>
<td>–</td>
<td>5.7</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>F</td>
<td>STR</td>
<td>WB 60 Gy EL 14 Gy</td>
<td>+(ICE)</td>
<td>none</td>
<td>Alive (64.5)</td>
<td>+</td>
<td>–</td>
<td>3.6</td>
</tr>
<tr>
<td>4</td>
<td>56</td>
<td>M</td>
<td>PR</td>
<td>EL 54 Gy</td>
<td>+(IA)</td>
<td>none</td>
<td>Dead (34)</td>
<td>+</td>
<td>–</td>
<td>31.6</td>
</tr>
<tr>
<td>5</td>
<td>41</td>
<td>M</td>
<td>PR</td>
<td>EL 60 Gy</td>
<td>+(AV)</td>
<td>none</td>
<td>Alive (41.5)</td>
<td>+</td>
<td>–</td>
<td>11.0</td>
</tr>
<tr>
<td>6</td>
<td>70</td>
<td>F</td>
<td>PR</td>
<td>none</td>
<td>none</td>
<td>+§</td>
<td>Dead (4.5)</td>
<td>+</td>
<td>–</td>
<td>29.1</td>
</tr>
<tr>
<td>7</td>
<td>68</td>
<td>F</td>
<td>STR</td>
<td>EL 60 Gy γ (central 36 Gy, marginal 18 Gy)</td>
<td>+(AV)</td>
<td>none</td>
<td>Alive (15)</td>
<td>+</td>
<td>–</td>
<td>18.5</td>
</tr>
</tbody>
</table>


Of the 5 cerebellar GBM patients treated with combined adjuvant therapy, 4 manifested p53 immunopositivity, all 5 were negative for EGFR immunostaining. Ki-67 analysis showed that 1 tumor had a Ki-67 LI of 19.6%. Representative pathological and immunohistochemical findings are given in Figure 1.

**Correlation between immunohistochemical findings and survival time.** Kaplan-Meier survival curves for patients with p53-, EGFR- and Ki-67 immunopositive supratentorial GBM are presented in Figure 2. The survival of patients with EGFR-negative tumors was significantly longer than that of patients with EGFR-positive tumors (p=0.0248). On the other hand, there was no significant correlation between survival and p53 immunopositivity (p=0.7870) and the Ki-67 LI (Ki-67 LI<19.6% vs. LI>19.6%, p=0.7133).

The median survival was 30 months in the 5 patients with EGFR-negative cerebellar GBMs who received adjuvant therapy, being significantly longer than in the 43 supratentorial GBM patients (p=0.0296, Figure 3). These observations suggest that the longer survival of patients with cerebellar GBM is attributable to the greater radiosensitivity of their EGFR-negative tumors.

**Table III. Immunopositivity of supratentorial- and cerebellar glioblastomas.**

<table>
<thead>
<tr>
<th>Immunopositivity</th>
<th>p53</th>
<th>EGFR</th>
<th>Ki-67</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supratentorial (n=43)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>positive (&gt;30%)</td>
<td>31 (72%)</td>
<td>27 (63%)</td>
<td>17 (39%)†</td>
</tr>
<tr>
<td>negative (&lt;30%)</td>
<td>12 (28%)</td>
<td>16 (37%)</td>
<td>26 (61%)§</td>
</tr>
<tr>
<td>Cerebellar (n=5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>positive (&gt;30%)</td>
<td>4 (80%)</td>
<td>0 (0%)</td>
<td>1 (20%)†</td>
</tr>
<tr>
<td>negative (&lt;30%)</td>
<td>1 (20%)</td>
<td>5 (100%)</td>
<td>4 (80%)§</td>
</tr>
</tbody>
</table>

†: Ki-67 labeling index >19.6%, §: Ki-67 labeling index<19.6%.

**Discussion**

**Immunohistochemical factors and survival.** Ours is the first report detailing the immunohistochemical features of cerebellar GBM. Our results suggest that the longer survival of patients with cerebellar and supratentorial GBM who received adjuvant radiation therapy is correlated with the EGFR-immunonegativity of their tumors.
Figure 1. Case 4. A: Photomicrograph showing nuclear atypia, endothelial proliferation and necrosis (H&E. original magnification x 100) B, C: Tumor tissue is negative for EGFR (B) and positive for p53 staining (original magnification x 400) D: The Ki-67 labeling index is 31.6% (original magnification x 400). Case 6. E: Photomicrograph showing endothelial proliferation, necrosis and pseudopalisading (H&E. original magnification x 100) F, G: Tumor tissue is negative for EGFR- (F) and positive for p53 staining (G) (original magnification x 400) H: The Ki-67 labeling index is 29.1% (original magnification x 400). Case 7. I: Photomicrograph showing nuclear atypia, endothelial proliferation and necrosis. (H&E. original magnification x 100) J, K: Tumor tissue is negative for EGFR- (J) and positive for p53 staining (K) (original magnification x 400) L: The Ki-67 labeling index is 18.5% (original magnification x 400).

Figure 2. Kaplan-Meier survival curves for patients with EGFR (A) and p53 (B) immunopositivity and a Ki-67 LI (C) of supratentorial glioblastomas. EGFR: epidermal growth factor receptor, LI: labeling index.
Glioblastomas are thought to develop either through a progression from low-grade or anaplastic astrocytoma to secondary glioblastoma or de novo as primary glioblastoma. They follow distinct genetic pathways: primary glioblastomas typically overexpress EGFR genes but rarely have p53 mutations, while secondary glioblastomas frequently manifest p53 mutations but rarely overexpress EGFR (39). The most common alterations in high-grade astrocytomas include p53 mutations and EGFR overexpression (40). Peraud et al. (41) reported that 11% of their primary glioblastomas manifested p53 mutations and that 32% overexpressed EGFR; among secondary glioblastomas, these rates were 67% and 0%, respectively. At present, there is no consensus regarding the prognostic value of most of these commonly altered genes in supratentorial glioblastoma. In some studies, p53 gene mutation or p53 immunopositivity as a marker of anomaly in the p53 pathway was predictive of a shorter survival in glioma patients (15-17), whereas in others there was no significant relationship (18-25). Conversely, there are reports that p53 mutations predicted longer survival in patients with glioblastoma (26, 27). There is also no consensus regarding the prognostic value of EGFR amplification/overexpression in glioblastoma. Some investigators found no correlation with survival (18, 19, 22, 28), while others contend that this aberration is predictive of a poor prognosis (15, 16, 20, 21, 29-32).

We found that the median survival of patients with EGFR-negative supratentorial GBM was significantly longer than that of EGFR-positive patients (p=0.0248). However, there was no significant correlation between their p53 immunopositivity and prognosis (p=0.7871). Barker et al. (21), who studied a series of 170 glioblastomas to examine the correlation between individual molecular pathogenetic events and treatment responses by determining their positivity for EGFR- and p53 immunostaining, their p53 mutation status and response to external beam radiation, reported that EGFR immunopositivity predicted a poor radiographically-assessed radiation response (p=0.046). They found no significant relationship between p53 immunoreactivity or mutation and radiation responses and concluded that the observed relative radioresistance of some glioblastomas is attributable to the overexpression of EGFR. Zhu et al. (32) also reported that EGFR positivity was a significant, independent indicator of a poor prognosis in 71 radiation-treated patients with astrocytic gliomas. These reports are consistent with the results of our study, suggesting that the longer survival (median 18.9 months) of our patients with EGFR-negative supratentorial GBM was attributable to the good radiosensitivity of their tumors.

Of our 7 cerebellar GBM patients, the 5 who had EGFR-negative tumors and received adjuvant radiotherapy had a median survival of 30 months, significantly longer than that of the 43 supratentorial GBM patients (p=0.0296). Although our sample size of cerebellar GBM patients is small, we suggest that cerebellar GBM may have a lower incidence of EGFR immunopositivity and that the greater radiosensitivity of EGFR-negative tumors may contribute to the better prognosis of patients with these tumors compared to patients with supratentorial GBM.

Clinical factors and prognosis. The therapeutic modalities to address cerebellar- and supratentorial GBM are surgical resection, external irradiation and chemotherapy. Because of the rarity of cerebellar GBM and the proximity of these tumors to the brain stem, there is currently no standard therapeutic approach, and the prognosis of patients with these tumors remains controversial. In our study, the median survival of 5 EGFR-negative cerebellar GBM patients, who received postoperative adjuvant radiotherapy and chemotherapy, was 30 months. In contrast, the 2 patients who underwent surgery alone survived for only 3.25 and 4.5 months, respectively, and both manifested dissemination to the contralateral cerebellum and lateral ventricle after surgery. Djalilian et al. (6) reviewed 71 malignant cerebellar gliomas reported in the literature between 1975 and 1994 and 7 cases treated at their institution and reported that, according to multivariate analysis, surgical resection (vs. biopsy) and radiation therapy were associated with extended survival in patients with grade IV tumors. Chamberlain and Silver (4) found that in 18 patients with poorly-differentiated cerebellar gliomas, distant metastasis, seen in 28% of their series, was associated with inadequate radiation doses to the posterior fossa and concurrent treatment failure at the primary cerebellar site. They suggested that high-dose limited-field irradiation directed at the primary tumor, the
current strategy for supratentorial gliomas, should be the radiotherapy of choice. We concur and suggest that patients with cerebellar GBM, especially those with EGFR-immunonegative tumors, may benefit from postoperative radiotherapy.

As our findings suggest that EGFR-negative cerebellar GBM may be highly chemosensitive and radiosensitive, we recommend aggressive multimodality therapy (surgery, radiotherapy and chemotherapy) in patients with these tumors. As our sample size was small, we are in the process of collecting additional cases with cerebellar GBM in an effort to identify predictive factors and develop a standard therapeutic approach for these rare tumors.

Conclusion

Cerebellar GBMs are rare. Our study suggested that EGFR immunonegativity was correlated with longer survival in patients with cerebellar- and supratentorial GBM who received adjuvant radiation therapy. Although our sample of cerebellar GBM was small, we postulate that the incidence of EGFR-positivity is lower in these than supratentorial GBM and that the longer survival of patients with EGFR-negative cerebellar GBM is attributable to the greater radiosensitivity of their tumors. We suggest that patients with cerebellar GBM receive aggressive multimodality therapy.

References


