Chromosome arm 1q gain associated with good response to chemotherapy in a malignant glioma

Case report

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✓ The authors describe the case of a patient with a glioblastoma multiforme who showed remarkably good response to chemotherapy. A genetic analysis using comparative genomic hybridization (CGH) revealed that the tumor had a gain on the q arm of chromosome 1 (1q). Using CGH for a series of genetic analyses of more than 180 patients with gliomas, six were found to have a demonstrated 1q gain. Although the tumors in all six of these cases were histopathologically diagnosed as high-grade gliomas, compared with other malignant gliomas they demonstrated a good prognosis because of their favorable chemotherapeutic sensitivity. In immunohistochemical tests, most of the tumor cells in these cases were negative for O\textsuperscript{6}-methylguanine–DNA methyltransferase, which antagonizes the effect of DNA-alkylating chemotherapeutic agents. The authors believed that a gain of 1q could be produced through the genetic events that cause loss of 1p, because these chromosomal aberrations have an imbalance of DNA copy number in common (1p < 1q). A gain of 1q is an infrequent chromosomal aberration and its clinical importance should be investigated in a larger study; however, patients with malignant gliomas demonstrating a 1q gain possibly show longer survival and good response to chemotherapy similar to patients with tumors demonstrating 1p loss. The importance of using genetic analysis for gliomas is emphasized in this report because it may help in selecting cases responsive to chemotherapy and because appropriate treatment for these patients will lead to progress in the treatment of malignant gliomas.

KEY WORDS • glioblastoma multiforme • comparative genomic hybridization • chromosome arm 1q • chemotherapy

DESPITE current therapeutic modalities including surgery, chemotherapy, and radiotherapy, only limited progress has been achieved in the treatment of GBM. In most patients, GBM leads to death within approximately 12 months,\textsuperscript{26} but authors of some reports have described the clinical features of patients with gliomas who survived for a longer duration.\textsuperscript{19,27,28}

Genetic analyses of tumors have been developed, and correlations between genetic subgrouping and clinical information for gliomas have been investigated. These investigations show that allelic loss on chromosomal arms 1p and 19q serves as a powerful predictor of both sensitivity to chemotherapy and prolonged survival in patients with oligodendroglialomas.\textsuperscript{5,26} According to recent reports, this predictor also occurs in patients with astrocytomas, although less commonly.\textsuperscript{5,12} Identification of genetic aberrations closely associated with good prognosis and response to chemotherapy appears to be important, as this information can assist in the selection of appropriate treatment for patients.

Comparative genomic hybridization is a cytogenetic analysis method that detects and maps relative DNA-sequence copy number aberrations on each chromosome.\textsuperscript{13,25} Authors of previous studies have demonstrated that CGH can be used to divide tumors of the same histopathological entity into clinically relevant subgroups, and several reports on genetic prognostic markers of gliomas using CGH have been published.\textsuperscript{2,4,15,31} More recently, genetic analysis with array-based CGH has been conducted in search of specific genetic prognostic markers of gliomas.\textsuperscript{6,11,17,21}

In this report, we validate the possibility of prolonged survival and good response to chemotherapy in patients with malignant gliomas demonstrating a q-arm copy gain of chromosome 1.

Abbreviations used in this paper: CGH = comparative genomic hybridization; DIG–dUTP = digoxigenin–11-2’-deoxyuridine 5’-triphosphate; DOP-PCR = degenerate oligonucleotide-primed polymerase chain reaction; FISH = fluorescence in situ hybridization; GBM = glioblastoma multiforme; LOH = loss of heterozygosity; MGMT = O\textsuperscript{6}-methylguanine–DNA methyltransferase; MR = magnetic resonance; PAV = procarbazine, nimustine hydrochloride, and vincristine.
Case Report

Examination and Operation. This 79-year-old man with a history of aortic valve regurgitation complained of numbness in his right leg, and 3 weeks later motor weakness of the right arm and mild motor aphasia developed. He was admitted to our hospital, where computed tomography scans revealed a heterogeneous mass in his left frontal lobe (Fig. 1A). His vital signs were stable, and his consciousness level was graded as 13 (E4V3M6) on the Glasgow Coma Scale. Radiography of his chest did not show a lung lesion. Magnetic resonance imaging with intravenous infusion of gadolinium showed that the mass in his left frontal lobe enhanced heterogeneously, suggesting a diagnosis of malignant glioma (Fig. 1B and C). After admission, the patient's level of consciousness progressively deteriorated, and he underwent a left frontal craniotomy to remove the tumor. The tumor was found to be soft and moderately hemorrhagic, and was not well demarcated from the surrounding brain tissue in the deep portion. The tumor mass was gross totally resected with the aid of a surgical microscope.

Histopathological Findings. Electron microscopy revealed the surgical specimen to be a hypercellular glial tumor. A prominent population of tumor cells demonstrated mitotic figures. Vascular proliferation and necrosis were also identified (Fig. 2). Subpopulations of tumor cells were positive for glial fibrillary acidic protein and S100 protein. The DNA repair protein MGMT antagonizes the effect of DNA-alkylating chemotherapeutic agents; therefore, the CGH analysis was performed according to a protocol described elsewhere. Briefly, tumor DNA was extracted from a microdissected piece of formalin-fixed and paraffin-embedded tissue section (5 μm thick) and amplified using DOP-PCR. To select the region of truly neoplastic tissue, tissue microdissection was performed after referring to the pathological findings of consecutive tissue sections that were stained with H & E or MIB-1; DNA was labeled with another DOP-PCR reaction using DIG-dUTP (Roche), and the reference DNA was amplified from 50 ng normal male DNA and labeled as described earlier except that rhodamine-dUTP (Roche) was used instead of DIG-dUTP. The probe mixture was denatured and hybridized to normal metaphase spreads (Vysis, Inc.). After unhybridized probes were washed, the metaphase spread was incubated using fluorescein isothiocyanate-conjugated anti-DIG-antibody (Roche). The preparations were washed and counterstained using 4,6-diamino-2-phenylindole in antifade solution. Red, green, and blue images were acquired and the ratios of fluorescence intensity along the chromosomes were quantitated using the CytoVision analysis system (Applied Imaging Co.).

The CGH profile is shown in Fig. 3. Chromosomal copy number aberrations (gains [+ ] and losses [− ]) that were detected using CGH analysis were as follows: +1q, +2q21-32, +3, +7, +8, +8q12.2-ter, +9p22.3-ter, +9pter-22, −2p-22, −10q22-ter, −12, −13, −14, +15q24-ter, −16q12.1-22, and +18q22-ter.

Postoperative Course. Although MR imaging performed 19 days after surgery showed a small residual tumor (Fig. 4B), another MR image obtained 3 weeks later showed remarkable reduction of the tumor (Fig. 4D). Further, the tumor disappeared almost completely 7 days after the end of the first PA V course (Fig. 4E). Two months later, however, tumor development was recognized (Fig. 4F), and the second course of PA V was started in combination with local radiotherapy (60 Gy). The tumor then disappeared again (Fig. 4G and H). The patient remained free from further tumor development and was discharged from our hospital. Eight months after the initial surgery, he died of heart failure at another hospital. His last MR image obtained 10 days before his death revealed no sign of tumor recurrence.

Summary of Additional Cases

We have analyzed the genetic profiles of more than 180

Fig. 1. Computed tomography scan (A) and MR images (B and C) obtained in a 79-year-old man at admission. A: A heterogeneous mass in the left frontal lobe is evident. B and C: Axial (B) and sagittal (C) Gd-enhanced images demonstrating a heterogeneously enhanced brain tumor with corpus callosum involvement, suggesting a diagnosis of malignant glioma.

glioma tissues using CGH (unpublished data), and six of these cases were found to have a copy gain of the q arm of chromosome 1 (1q). Clinical and genetic features of these six cases are summarized in Table 1. In general, these patients showed a tendency to have a favorable clinical course. In Case 2, the patient’s family did not allow her to undergo any adjuvant therapy because of her age. In the other five cases, chemotherapy and radiotherapy were performed. Because our CGH analysis was performed retrospectively, genetic information was obtained after the initial treatment for these patients (except for Case 6 described earlier), and did not help in determining the chemotherapeutic regimen. Immunostaining of tumor cells to detect MGMT was also performed retrospectively. All of the tumors with 1q gain contained a small number of MGMT-positive cells (MGMT scores were less than 15% in all cases), suggesting a good response to chemotherapy (Table 1).

Case 1. This 53-year-old woman underwent a left frontal partial lobectomy, and a GBM tumor was diagnosed. Adjuvant chemotherapy using ranimustine and vincristine was administered as well as 60-Gy whole-brain radiotherapy. She did not undergo additional chemotherapy, and the tumor recurred within 8 months after surgery. She died of the disease 10 months after surgery.

Case 2. This 77-year-old woman had a history of surgery for removal of a right frontal tumor, which was diagnosed as an oligodendroglioma. She developed a tumor again after remaining disease free for 7 years and underwent a second craniotomy. This time the tumor was diagnosed as an anaplastic oligoastrocytoma, and we performed a CGH analysis on the tumor sample. We could not obtain a CGH profile from the first tumor because of the age of the sample. Instead, FISH was performed using 1p36 and 1q25 probes according to the method described previously.29 The FISH analysis confirmed 1p loss in 61.5% of the cells of the primary tumor, and this tumor was genetically diagnosed as a tumor with 1p loss. Magnetic resonance images obtained 3 months after the second surgery showed no sign of tumor recurrence, but another MR image obtained 1 year after surgery showed remarkable tumor regrowth. Her family decided that, due to her age, she should not undergo any adjuvant therapy. She died of the disease 17 months postoperatively.

Case 3. This 14-year-old boy underwent a right frontal partial lobectomy for a relatively well-circumscribed tumor that was demonstrated using MR imaging with gadolinium enhancement. The tumor was histologically diagnosed as a GBM with nuclear atypism, mitosis, and vascular proliferation. Adjuvant therapy included chemotherapy with ranimustine and 60-Gy whole-brain radiotherapy. After 6 years, he remains free from recurrence. He did not undergo any additional chemotherapy after the initial cycle. In this case, the tumor was relatively well circumscribed, and total resection of the tumor might have led to a good outcome.

Case 4. This 47-year-old woman underwent radiotherapy alone as adjuvant therapy, based on the patient’s own preference. The tumor was diagnosed as anaplastic astrocytoma, and reduction of the residual tumor after radiotherapy was confirmed using MR imaging. Tumor regrowth was not recognized on neuroimages for 27 months until MR imaging revealed recurrence. After recognition of tumor recurrence, the patient refused to undergo any adjuvant chemotherapy.

Case 5. The tumor in this 66-year-old man was diagnosed as a GBM, and was treated with radiotherapy and chemotherapy (ranimustine). Magnetic resonance imaging
revealed necrosis within the residual tumor; however, the patient died of interstitial pneumonia 8 months after the surgery without signs of residual tumor.

In all cases except Case 6 (the present case), patients received only one cycle of chemotherapy (if any).

**Discussion**

Cairncross and colleagues\(^5\) reported longer survival and better response to chemotherapy in patients with oligodendrogliaoma demonstrating chromosomal 1p/19q loss, which has drawn attention to identifying genetic subgroups of malignant brain tumors that show sensitivity to chemotherapy and longer survival. Ino and coworkers\(^12\) reported longer survival times and better therapeutic responses in patients with malignant astrocytic gliomas demonstrating 1p loss. The importance of categorizing malignant gliomas into genetic subgroups for the purpose of predicting their prognosis and chemotherapeutic sensitivity has received increasing attention. Subgrouping malignant gliomas according to genetic aberration could be utilized to classify GBMs into clinically relevant subgroups, and furthermore could help in determining the adjuvant chemotherapeutic regimen.

Investigators in previous studies reported on genetic subgrouping of malignant astrocytic gliomas in an effort to establish clinically relevant classifications.\(^2,4,15,31\) In those studies, malignant glioma with a 1q gain is relatively rare; Kunwar et al.\(^15\) found 1q gain in only approximately 8.5% of patients with an anaplastic astrocytoma. The incidence of 1p loss is approximately 10 to 12% in malignant gliomas,\(^2,12\) and longer survival times and better chemotherapeutic sensitivity in patients with gliomas demonstrating 1p loss have been thoroughly investigated. To our knowledge, however, no study has focused on 1q gain in terms of longer survival and better chemotherapeutic sensitivity.

The patient in the present case demonstrated a 1q copy gain. We believed that this 1q gain was a similar type of genetic aberration as a 1p loss, because the DNA copy number on 1p was decreased in comparison to that on 1q. We therefore believed that the tumor might show a good response to a chemotherapeutic regimen of PAV (similar to cases with 1p loss). An additional reason we used PAV treatment was because the tumor did not express a high level of MGMT during immunohistochemical tests, which was true with all of the other tumors with a 1q gain. Only one of our patients with a 1q gain (Case 1) did not show a good response to adjuvant therapy even with low MGMT expression; however, this patient underwent chemotherapy with ranimustine alone, and additional chemotherapy might have
been effective, as was seen in the present case (Case 6). Multiple cycles of adjuvant chemotherapy might be needed to control the development of tumors with a 1q copy gain.

Investigators in several studies reported a low expression of MGMT in oligodendrogliomas with a 1p loss, and our analysis suggests that tumors with a 1q gain share this common epigenetic feature of blunting MGMT expression. Our data suggest that malignant gliomas with a 1q gain contain only a small proportion of cells with high MGMT expression and show good chemotherapeutic sensitivity. In our study, no case of a supratentorial malignant glioma with a 1q gain showed a high level of MGMT expression in histopathological tests. Further investigation of this finding is needed, however.

Because tumors with a 1q gain and those with a 1p loss share common clinical and genetic features, we hypothesized that these aberrations developed, at least in part, through a common genetic pathway (Fig. 5). In the development of a 1q gain and 1p loss, there must be a chromosomal breakpoint at (or close to) the centromere of chromosome 1, and a gain of the entire chromosome 1 is associated with promotion of a 1q gain while maintaining the imbalance between 1p and 1q. It is noteworthy that in our data, the histopathological diagnosis for all tumors with a 1q gain was malignant glioma, which is expected to have genomic instability compared with low-grade tumors. In such tumors with genetic instability, chromosomal aneuploidy develops frequently, and gain of the entire chromosome 1 can occur relatively easily. Interestingly, in one of our patients with a 1q gain (Case 2) anaplastic oligoastrocytoma was diagnosed, which typically shows a 1p loss; the primary tumor in this patient was a low-grade oligodendroglioma with a 1p loss confirmed using FISH. In this case, it is possible that the 1p loss occurred first, and then a gain of the entire chromosome 1 developed. However, our FISH analysis also showed that approximately 17% of the primary tumor cells harbored a 1q gain.

**TABLE 1**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age (yrs)</th>
<th>Sex</th>
<th>FU (mos)</th>
<th>Outcome</th>
<th>Op</th>
<th>Radiation Therapy</th>
<th>Chemo</th>
<th>Histopathological Finding</th>
<th>MGMT Score (%)</th>
<th>Chromosomal Copy Number Aberrations</th>
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<tr>
<td>1</td>
<td>53</td>
<td>F</td>
<td>10</td>
<td>died</td>
<td>PR</td>
<td>yes ranimustine</td>
<td>GBM</td>
<td>9.4</td>
<td>+1q, -5q32-ter, +7, +9p, -10, -12q15-21, -13, +14q21-23, -16q, -19q, +20, -22q</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>77</td>
<td>F</td>
<td>17</td>
<td>died</td>
<td>biop</td>
<td>no</td>
<td>none</td>
<td>AOA</td>
<td>3.3</td>
<td>+1q, +3q, -4, +7, -9, -11, +12, -14q, -15q, +16, -18, -19q, +20</td>
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<tr>
<td>3</td>
<td>14</td>
<td>M</td>
<td>72</td>
<td>remission</td>
<td>TR</td>
<td>yes ranimustine</td>
<td>GBM</td>
<td>12.9</td>
<td>+1q, +2p21-11, -2q11.2-22, +5p, +7p, +9q13-33, -11p, -19q</td>
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<tr>
<td>4</td>
<td>47</td>
<td>F</td>
<td>27</td>
<td>remission</td>
<td>STR</td>
<td>yes</td>
<td>none</td>
<td>AA</td>
<td>3.4</td>
<td>+1q, +2, -4, +5, +7, +8, +9q13-33, -10, +14, +21, -X</td>
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<tr>
<td>5</td>
<td>66</td>
<td>M</td>
<td>12</td>
<td>died</td>
<td>PR</td>
<td>yes ranimustine</td>
<td>GBM</td>
<td>3.4</td>
<td>+1q, +7, -10, -16p, +17q, -19q, -22</td>
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</tr>
<tr>
<td>6†</td>
<td>79</td>
<td>M</td>
<td>8</td>
<td>died</td>
<td>STR</td>
<td>yes</td>
<td>PAV</td>
<td>GBM</td>
<td>1.7</td>
<td>(see Case Report)</td>
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* AA = anaplastic astrocytoma; AOA = anaplastic oligoastrocytoma; biop = biopsy procedure; chemo = chemotherapy; FU = follow up; PR = partial removal; STR = subtotal removal; TR = total removal.
† Present case.

Fig. 4. Axial Gd-enhanced MR images obtained in the patient in the present case during chemotherapy. A: Image obtained before craniotomy (same as Fig. 1B). B: Image obtained on postoperative Day 19 showing a small residual tumor. C: Image obtained on postoperative Day 40 before PAV treatment showing remarkable progression of the tumor. D and E: Images obtained on Days 25 (D) and 53 (E) of PAV treatment demonstrating remarkable reduction of the tumor. F: Image obtained on postoperative Day 125 before the second course of PAV treatment showing tumor development. G and H: Images obtained 1 (G) and 2 (H) months after the last PAV treatment showing the tumor has disappeared again.
Chromosome arm 1q gain and chemotherapy response in a GBM

Fig. 5. Diagram representing the hypothetical pathway of a 1q copy gain. In the development of a 1q gain and 1p loss, a chromosomal breakpoint arises at (or close to) the centromere of chromosome 1, and a gain of the entire chromosome 1 (ch1) is associated with promotion of a 1q gain while maintaining an imbalance between 1p and 1q (1p < 1q).

We report on a patient with a GBM demonstrating chromosome 1q-arm copy gain in CGH analysis. This patient showed a good response to a chemotherapy regimen of PAV and a good prognosis. We also report five other cases that demonstrated the same genetic aberration. All of the tumors in these cases expressed low-level MGMT. Although further investigation is needed, there is a possibility that patients with gliomas showing a chromosome 1q gain also demonstrate good response to chemotherapy, similar to patients with oligodendrogliomas demonstrating chromosome 1p/19q loss, and thus achieve longer survival durations. We believe genetic analysis of gliomas using CGH is useful because it helps select patients with good chemotherapeutic sensitivity and avoids omitting appropriate treatment for those patients. These efforts will lead to progress in the treatment of patients with malignant gliomas.

Conclusions

We report on a patient with a GBM demonstrating chromosome 1q-arm copy gain in CGH analysis. This patient showed a good response to a chemotherapy regimen of PAV and a good prognosis. We also report five other cases that demonstrated the same genetic aberration. All of the tumors in these cases expressed low-level MGMT. Although further investigation is needed, there is a possibility that patients with gliomas showing a chromosome 1q gain also demonstrate good response to chemotherapy, similar to patients with oligodendrogliomas demonstrating chromosome 1p/19q loss, and thus achieve longer survival durations. We believe genetic analysis of gliomas using CGH is useful because it helps select patients with good chemotherapeutic sensitivity and avoids omitting appropriate treatment for those patients. These efforts will lead to progress in the treatment of patients with malignant gliomas.

References


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