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CASE REPORT

A case of radiologically multicentric but genetically identical multiple glioblastomas

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Abstract Surgery was performed in a 65-year-old male patient with malignant gliomas at two locations in the left and right cerebral hemispheres that showed no apparent continuity in imaging studies. Slight differences in histopathological appearance were seen between the tumors, and multicentric malignant glioma was diagnosed. Detailed genetic examination showed both the left- and right-side tumors to be of the IDH-1 wild type with a p53 mutation at the same locus. Whole genome analysis by comparative genomic hybridization revealed many of the same mutations to be present in both tumors. The O^6-methylguanine-methyltransferase promoter in both cases was unmethylated, and the genetic profiles of both showed them to be homologous tumors. They were therefore inferred to be multiple gliomas from the same clone. There have been occasional reports of multicentric gliomas classified by diagnostic imaging. This report discusses the need to examine tumor origin by genomic profiling.

Keywords Multicentric glioma · Genomic profiling · IDH-1 mutation · p53 mutation · Comparative genomic hybridization · MGMT promoter methylation

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Introduction

The extent of progression of malignant gliomas, which are characterized by invasive growth, cannot be completely ascertained with even the most advanced diagnostic imaging technology. A previous autopsy investigation found that tumor cells had progressed to distant areas of the brain by the time they were diagnosed as glioblastomas by diagnostic imaging [1]. On the other hand, there is the concept of multiple gliomas. Particularly, the term "multicentric gliomas" is used for the cases in which there is no apparent continuity among individual tumors at initial diagnostic imaging studies. There have been occasional reports indicating that these tumors appeared to result from tumorigenesis at multiple sites [2–4].

We encountered a case of malignant glioma in which multiple neoplastic lesions were seen in the bilateral cerebral hemispheres at onset, with no continuity observed between the individual lesions in a detailed imaging study using high-resolution MRI. Detailed genetic analysis of the tumor tissue showed that the two tumors had almost identical gene abnormalities. This case would appear to sound a warning regarding the tumor concept referred to as multicentric glioma.

Case presentation

The patient was a 65-year-old, right-handed man being treated for hypertension. The patient's family history showed that his father and older brother had died from colon cancer. Articulation disorder that had appeared 2 weeks earlier rapidly worsened, and hemiparesis that encompassed the right side of the face was seen. A cranial CT scan showed neoplastic lesions associated with mild

surrounding cerebral edema in the right insular cortex and left corona radiata. Examination by 3-T MRI with gadolinium showed irregular enhancement of both tumors. The enhancement on the left-side tumor appeared to be continuous with the subependyma of the lateral cerebral ventricle. Although peritumoral edema that exhibited a high signal was seen on T2-weighted and FLAIR images, this was relatively localized and did not extend to commissural fibers such as the corpus callosum, and no apparent continuity was seen between the two tumors (Fig. 1a–d). Metastatic brain tumors or malignant lymphomas were suspected, and a craniotomic biopsy of the right insular cortex was first performed. The tumor was reddish-brown, soft, and hemorrhagic, and the pathological diagnosis was high-grade glioma. The neurological symptoms rapidly worsened subsequently, and the right side of the body became completely paralyzed. An imaging examination showed an increase in the size of the tumor in the left corona radiata and worsening of the surrounding cerebral edema. Consequently, complete resection of the right insular cortex tumor and biopsy of the left corona radiata tumor were performed. Because both tumors were diagnosed as high-grade gliomas, the patient received 60 Gy of local regional radiation, temozolomide 75 mg/m^2 for 42 days, and intravenous injection of 3 million units of interferon beta every other day. However, the patient's clinical symptoms and imaging findings worsened rapidly, the growth of the corona radiata tumor could not be controlled, and the patient died after a clinical course of 93 days (Fig. 1e, f). No autopsy was performed in keeping with the wishes of the patient's family.

Pathological findings

Left corona radiata tumor: The tumor cells had a slightly pale and abundant cytoplasm and highly atypical nuclei. They had grown diffusely at moderate cell density on an eosinophilic, fibrous matrix. Mitotic figures were seen sporadically, and microvascular proliferation was seen, but no apparent necrosis was observed. The MIB-1 labeling index was 30.1 %, and approximately 50 % of the cell nuclei were positive for p53 protein (clone DO7, Dako, Japan). The expression of O^6-methylguanine-methyltransferase (MGMT) protein (clone MT3.1, Neomarkers, USA) did not exceed 1.8 % (Fig. 2a–c).

Right insular cortex tumor: The tumor cells had eosinophilic cytoplasm, processes, and highly atypical small round nuclei with a perinuclear halo. They had grown diffusely at moderate cell density on an eosinophilic, fibrous matrix. Mitotic figures and microvascular proliferation were seen in abundance, and necrosis was observed. The MIB-1 labeling index was 32.6 %, and approximately 70 % of the tumor cell nuclei were positive for p53 protein. MGMT protein expression did not exceed 1.9 % (Fig. 2d–f).

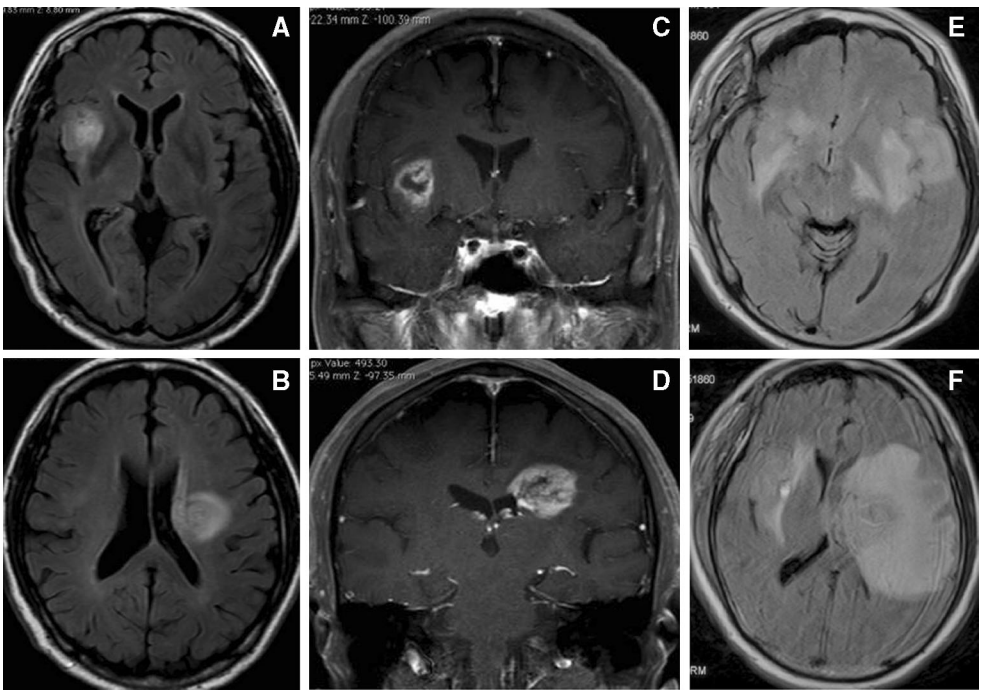


Fig. 1 MR images. **a, b** Initial axial FLAIR image demonstrated no apparent continuity between two tumors. **c**, **d** Initial gadolinium-enhanced coronal T1-weighted image demonstrated two distant heterogeneously enhanced tumors. **e**, **f** Axial FLAIR image following radiochemotherapy demonstrated massive growth of the left hemispheric tumor

Genetic analysis

Examination of IDH-1 and p53 by direct sequencing showed both the left- and right-side tumors to be of the IDH-1 wild type (Fig. 3a, b), while p53 showed the same mutation at codon 175 in exon 5 from CGC (Arg) to CAC (His) in both tumors (Fig. 3c, d). Methylation analysis of MGMT by methylation-specific PCR detected a distinct unmethylated band in both tumors (Fig. 3e). Moreover, comparative genomic hybridization (CGH) analysis of the two tumors confirmed the presence of identical changes in the number of chromosome copies: +7, −9p21, and −10q for the left-side tumor and +7, +8, −9pter-13, −10, −14q24-ter, −15qcen-22.2, −18q21-ter, and −19q for the right-side tumor (Fig. 3f, g).

Discussion

The concept of multicentric gliomas was first proposed 50 years ago [2–4]. However, multicentric gliomas are almost never referred to as a pathological concept, notably in WHO classifications. Numerous autopsy studies have shown that by the time a glioma is detected by imaging, tumor cells are already present in areas distant from the tumor body [1]. For a pathologist, the concept of the multicentric glioma itself is like a wastebasket diagnosis when discussing the clinical condition referred to as glioma [5].

Clinically, however, multicentric gliomas have been encountered in between 2.4 and 4.9 % of glioma patients [3, 4, 6]; thus, they necessarily attract interest as a rare condition. Salvati et al. [3] reported that among 25 patients with multicentric gliomas (2 % of glioma patients they encountered), 21 had synchronous multicentric glioblastoma. Autopsy was performed in 11 of these 21 patients, and all 11 cases showed no continuity between the multicentric glioblastomas [4]. In a study conducted during the MRI era, Hefti et al. [7] reported that of the 247 patients they encountered, 24 (9.7 %) had a total of 67 lesions that were multicentric high-grade gliomas. Through detailed MRI evaluations, they confirmed a distribution suggestive of diffuse progression of the white matter and progression in the cerebral ventricles or subependyma of the cerebral ventricles, even in the absence of apparent continuity as observed in imaging studies. They noted the limitations of diagnostic imaging and expressed a negative view regarding multicentric tumorigenesis. In recent years, evaluation by methods such as high magnetic field MRI, PET, and MRS [8] has permitted the identification of tumor

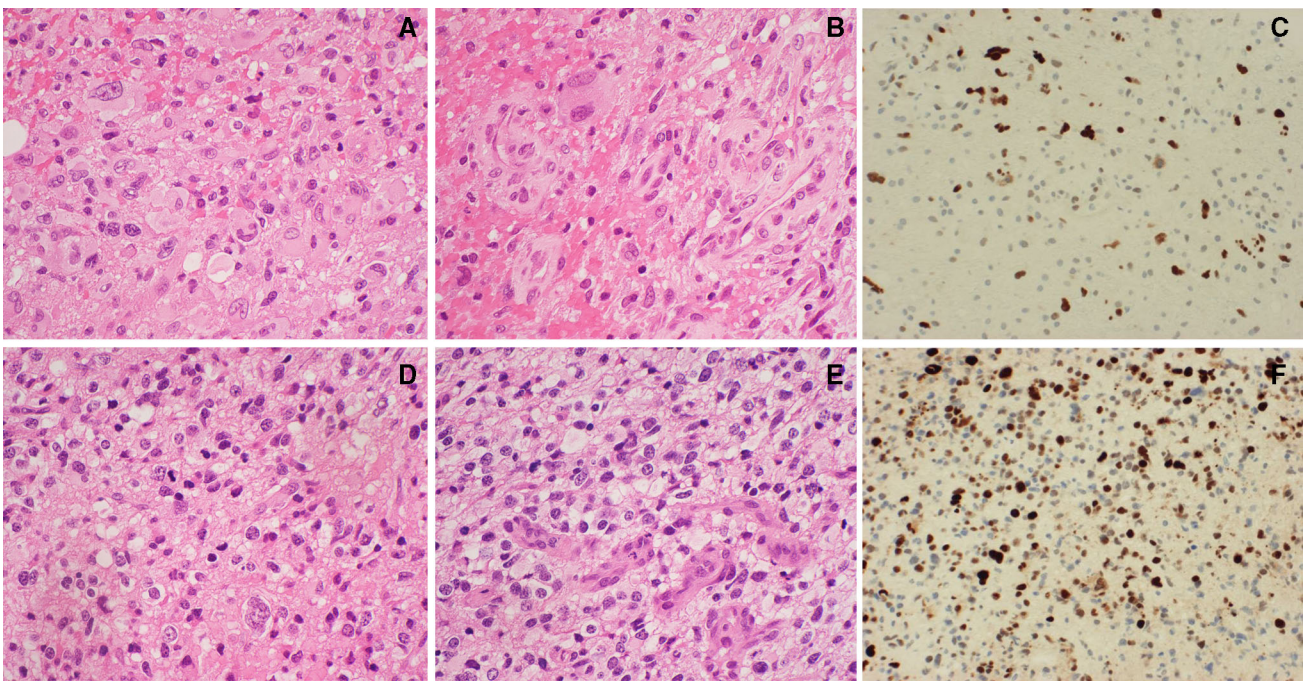
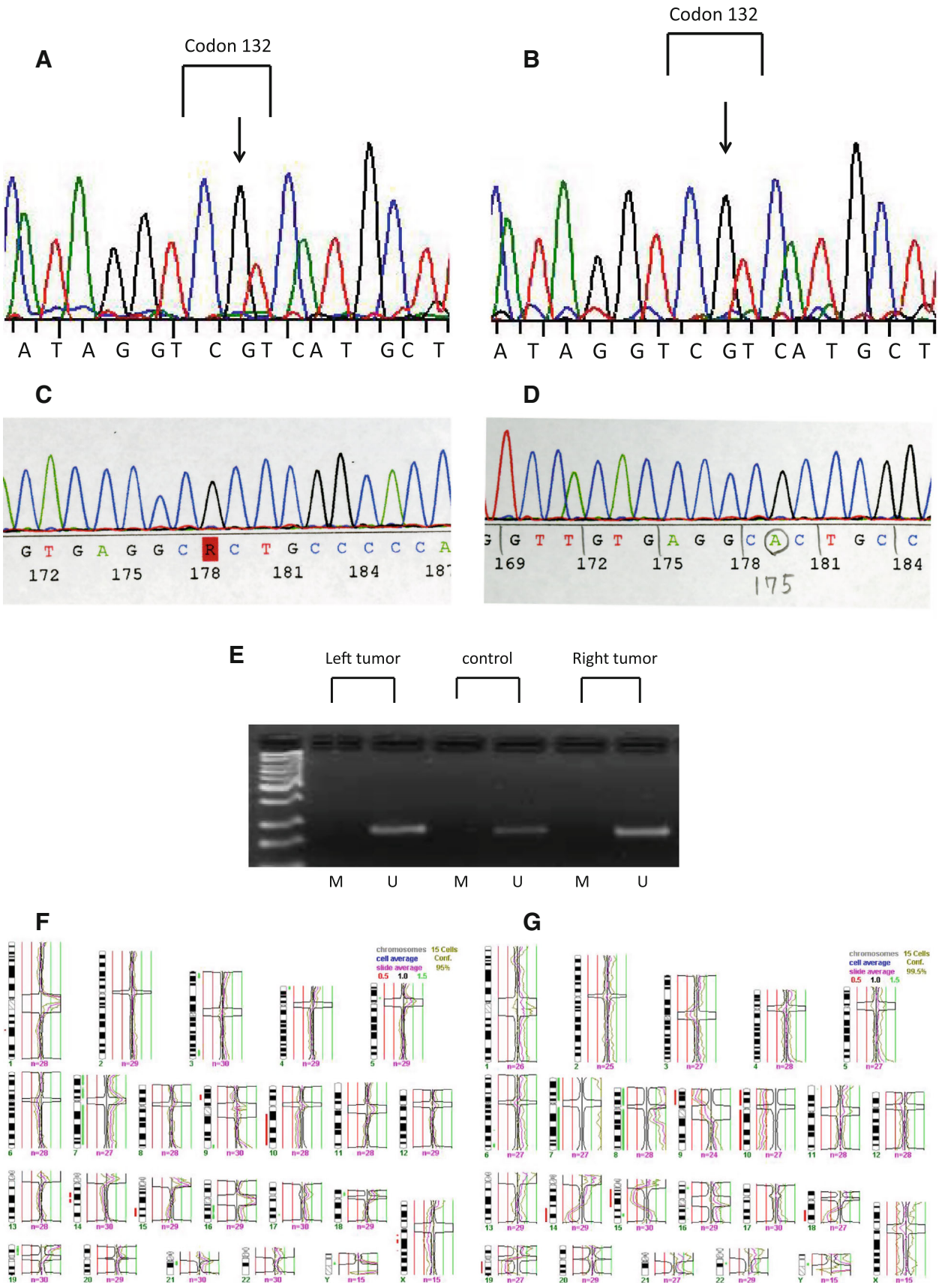


Fig. 2 Histopathological findings of both tumors. **a–c** *Left* Tumor in the corona radiata. **a**, **b** Hematoxylin and eosin stain (original magnification ×400) demonstrated diffusely proliferating atypical tumor cells having abundant pale cytoplasm and microvascular proliferation without prominent necrosis. **c** p53 protein immunostain (original magnification ×200) demonstrated approximately 50 % of tumor cells positive for p53 protein. **d–f** *Right* Tumor in the insular cortex. **d**, **e** Hematoxylin and eosin stain (original magnification ×400) demonstrated diffusely proliferating atypical tumor cells having tiny eosinophilic cytoplasm and small round nucleus with perinuclear halo. Microvascular proliferation and focal necrosis were observed. **f** p53 protein immunostain, ×200. Approximately 70 % of the tumor cells had immunopositivity to p53 protein


A
Codon 132
A T A G GT C GT CA T GC T
B
Codon 132
A T A G G T C GT CA T G C T
C
G T G A G G C R C T G C C C C C A
172 175 178 181 184
D
G G T T G T G A G G C A C T G C C
169 172 175 178 181 184
175
E
Left tumor
control
Right tumor
M U M U M U
F
G

◀**Fig. 3** Genomic analysis of both tumors. **a**, **b** Direct sequence analysis of codon 132 of IDH-1 gene (**a** *left side*, **b** *right side*) demonstrated both tumors to be of the wild type. **c**, **d** Direct sequence analysis of exon 5 of p53 gene (**a** *left side*, **b** *right side*) demonstrated the same mutation at codon 175 in exon 5 in both tumors. **e** Methylation-specific PCR of the promoter region of the MGMT gene. Both tumors demonstrated a distinct unmethylated band. **f**, **g** Whole genome analysis using a comparative genomic analysis method. (**f** *left side*, **g** *right side*). Both tumors demonstrated identical changes in the number of chromosome copies such as 7 gain, 9p loss, and 10q loss

continuity that could not be identified with conventional MRI. However, it is unlikely that it will be possible to visualize individual tumor cells on the order of 100 microns in size in the near future.

An easier way to establish that a tumor is a multicentric glioma may be to examine the genomic profiles of tissue from two or more tumors [9]. With recent advances in technology for the molecular analysis of gliomas, it is now considered possible to postulate on the process of individual tumor development by elucidating the stepwise mutations involved in glioma development and profiling the genospecies that are key to these mutations [10]. In the present case, the tumors appeared to be multicentric in imaging studies, and they were considered to be slightly different gliomas pathomorphologically. However, the genomic profiles of the tumors were revealed to be almost identical by the analysis of the genes that were essential to glioma development and its treatment. The analytical methods used included direct sequencing of IDH-1 and p53, whole genome analysis by CGH [11], and examining the methylation status of the MGMT promoter (a gene that confers resistance to anticancer drugs). Thus, the two tumors seen in this patient may have developed from the same clone, progressed to multiple sites and formed tumors through continuity that was not identifiable by imaging, and differed morphologically depending on the local environment. In brief, in the analyses that have been performed thus far, we were able to demonstrate pathologically that the lesions were "multifocal," that is, belonging to the same tumor, even though they would be identified as multicentric gliomas clinically, making this a valuable report.

Salvati et al. [4] reported mean survival in 25 patients with multicentric gliomas to be extremely short at 7.6 months (0.5–18 months), and survival in the case described here was also short at only 93 days. This may indicate that multicentricity observed by imaging is the final form of extensive progression in the brain of gliomas that cannot be clearly identified on images. Lim et al. [12] have reported that in many cases where tumor localization exhibited continuity with the subventricular zone, tumor cells were repeatedly supplied from glioma stem cells, resulting in multifocal progression.

The left corona radiata tumor in the present case was in contact with the lateral cerebral ventricle, and enhancement indicating subventricular extension was seen. During the course of the treatment, the left-side tumor exhibited resistance to therapy, and rapid growth was seen in that tumor. This suggests that the left-side tumor may have been the origin of the pathophysiology and the right-side tumor an invasive secondary tumor. Considering glioma pathophysiology from this perspective will likely come to play an important role clinically.

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