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COMMUNICATION

Genetic Analysis of a Case of Glioblastoma with Oligodendroglial Component Arising During the Progression of Diffuse Astrocytoma

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Abstract The most recent definition of glioblastoma with oligodendroglioma component (GBMO) assigned clinical significance to the observation of oligodendroglial foci within glioblastomas. However, the pathological mechanism of its histogenesis has not yet been determined. We report the genetic analysis of a GBMO case that evolved from an astrocyte lineage. A 37-year-old male underwent a third craniotomy for the removal of recurrent lesions of a secondary glioblastoma originating from a previous diffuse astrocytoma. The lesion in the right frontal lobe contained oligodendroglial foci within a glioblastoma background, while the remaining lesions showed only classic glioblastoma histology. Genetic analyses revealed distal 10q loss of heterozygosity (LOH) occurring de novo in the oligodendroglial tissue, as well as 10p, 17p LOH, and isocitrate dehydrogenase-1 gene (*IDH1*) mutations inherited from the previous lesions. The final recurrent glioblastoma underwent LOH on almost the entire of chromosome 10. Based on these results, the importance of an oligodendroglial component in glioblastomas may be limited.

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Introduction

Until recently, glioblastoma (GBM) was the only term used to designate astrocytic tumors. However, in 2007, the WHO Classification of Tumors of the Central Nervous System introduced glioblastoma with oligodendroglioma component (GBMO) to describe those tumors with better prognoses than classic GBM [1]. This suggested that some GBMs represent the ultimate level of malignancy in the oligodendroglial lineage. However, heterogeneous morphology is an intrinsic histological characteristic of GBMs, and it is therefore possible that they include oligodendroglial components as a phenotypic variation, regardless of their origin.

Because it remains controversial whether oligodendroglial components derive from an oligodendroglial lineage, it is thought that genetic studies may prove useful in clarifying this. In the present report, we performed genetic analyses in a case of secondary GBM derived from diffuse astrocytoma manifesting with oligodendroglial components in a recurrent lesion.

Clinical Summary

A 37-year-old patient was first admitted to our hospital in August 2004, when he was 30 years old, for the removal of a non-enhanced tumor in the right frontal lobe and insular cortex (Fig. 1a). The histopathological diagnosis was diffuse astrocytoma, grade II (Fig. 2a). After partial removal of the lesion, the patient was followed up without adjuvant therapy, following his preference, with short-term magnetic resonance

imaging (MRI) surveys. As seen on an MRI scan in April 2009, the residual tumor developed an enhanced lesion followed by gradual enlargement (Fig. 1b).

A second operation in December 2009 revealed that the lesion was malignant (Fig. 2b). An MRI scan demonstrated no residual enhanced lesion after postoperative concurrent temozolomide and radiation therapy (60 Gy), so follow-up was again instituted while the patient received maintenance temozolomide. Follow-up MRI scans revealed two novel isolated recurrent lesions in the sylvian fissure and frontal lobe in January and April 2011, respectively (Fig. 1c). Additionally, a small enhanced lesion was present in the contralateral frontal lobe. In May 2011, the lesions in the right hemisphere were completely removed surgically, followed by stereotactic biopsy of the contralateral lesion.

Following cyber-knife therapy to the contralateral lesion, the patient was discharged uneventfully and was followed up for 5 months, receiving maintenance temozolomide and concurrent interferon-β therapy. In July 2011, a recurrent lesion was detected in the right frontal lobe, which led to the patient's death in December 2011, 7 months after the third surgery. .

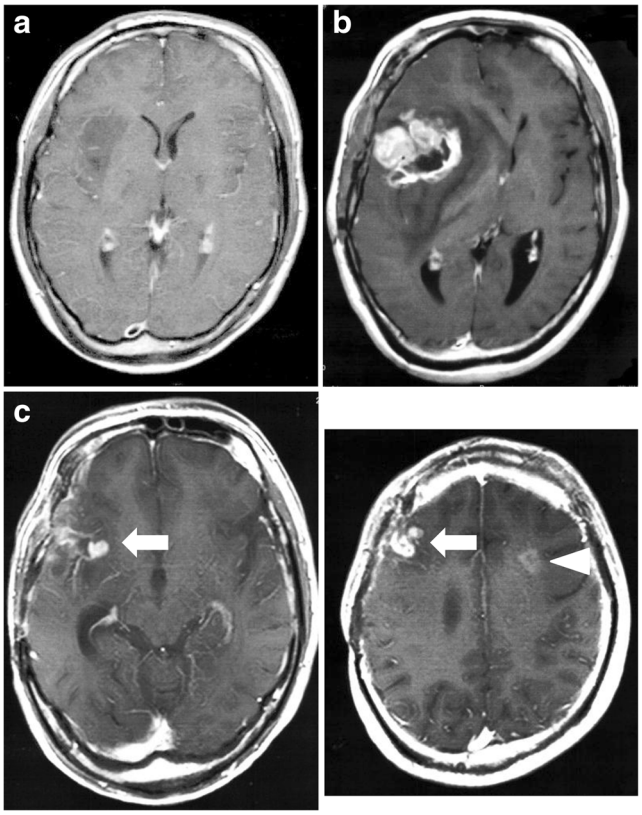

Fig. 1 T1-weighted gadolinium-enhanced MRI images. **a** The primary tumor showed a non-enhanced lesion in the right insula. **b** The first recurrent tumor with its well-enhanced, enlarging mass. **c** The second recurrence appeared as a well-enhanced mass in the right sylvian fissure and right frontal lobe (*arrow*). Slight lesion enhancement was also detected on the contralateral side (*arrowhead*)

Pathological Findings

The primary tumor showed a diffuse proliferation of glioma cells with mild nuclear atypia and modest cellularity in a fibrillary background (Fig. 2a). Tumor cells diffusely infiltrated the surrounding cerebral cortex. Mitosis, necrosis, and microvascular proliferation were not observed. Immunohistochemistry revealed nuclear accumulation of p53 and a low MIB-1 staining index in tumor cells. The lesion was thus diagnosed as diffuse astrocytoma, WHO grade II.

The first recurrence specimens obtained from the second surgery showed that cellularity and the degree of nuclear atypia / polymorphism were markedly increased compared with the primary tumor (Fig. 2b). Brisk mitotic activity and microvascular proliferation were also noted, indicating malignant progression of the tumor. The second recurrence specimens obtained from the third surgery showed both oligodendrocytic and astrocytic tumor cells focally intermingling with each other (Fig. 3a, b), both of which were immunopositive for IDH1 R132H (Fig. 3b) and p53 (Fig. 3c). This suggested that both components had a common genetic background. Another area of the tumor showed a high level of tumor cell proliferation with hyperchromatic, atypical nuclei and numerous mitotic figures associated with pseudopalisading necrosis (Fig. 3d). The MIB-1 staining index had increased to 31.3 % (data not shown). The tumor cells in this area again showed immunoreactivity for both IDH1 R132H and p53, suggesting a common origin of this component. Taken together, this suggested that the lesion of the second recurrence was high-grade oligoastrocytoma with necrosis, meeting the criteria of GBMO.

Genetic Findings

Genomic DNA extraction from peripheral blood leukocytes and snap-frozen tumor tissue was performed using the QIAamp DNA mini Kit (Qiagen Science, Germantown, MD) according to the manufacturer's protocol. Loss of heterozygosity (LOH) was evaluated by allelic imbalance detection using 14 microsatellite markers on chromosomes 1p, 10, 17p (TP53 locus), and 19q [2]. The *IDH1* mutation was evaluated by direct sequencing according to the method of Hartmann et al. with slight modifications [3]. This study was approved by the Ethics Committee of Kyushu University.

The genetic analysis results are summarized in Table 1. In brief, the *IDH1* mutation and LOH at 10p and 17p occurred in the primary tumor, while the recurrent lesion with GBMO histology harbored a mutation at 10q LOH distal to the PTEN (10q23) locus. The final contralateral lesion underwent LOH on almost the entire of chromosome 10.

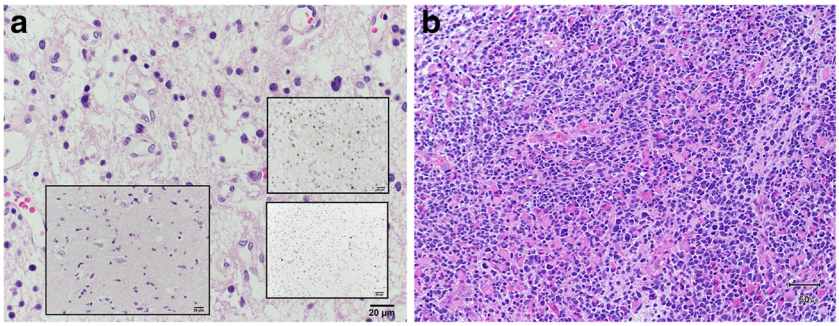

Fig. 2 The primary lesion showing diffuse proliferation of glioma cells with mild nuclear atypia in a fibrillary background. **a** Hematoxylin and eosin (H&E) staining. Neither mitosis nor necrosis was present. Tumor cells contained rod-shaped, naked nuclei diffusely infiltrating the surrounding cerebral cortex (*left inset*), and were immunopositive for p53 (*right upper inset*). The MIB-1 staining index was low (*right lower inset*). **b** The lesion on the first recurrence showed a high level of anaplastic glioma cell proliferation with brisk mitotic activity (H&E staining)

Discussion

The 2007 WHO introduction of GBMO as a condition with an improved prognosis to classic GBM [1] suggested that the detection of oligodendroglial foci in GBMs should provide prognostic value. A previous report of GBMO with better prognosis found that affected cases had more frequent 1p/19q co-deletions than usual oligodendroglial tumors [4]. This genetic alteration, seen in most oligodendroglial tumors, is well known as a marker of good prognosis [5]. These results suggest that GBMOs arise from typical oligodendroglial genetic pathways and explains the better prognosis of this tumor entity. On the other hand, some recent reports showed that most GBMO cases lack 1p/19q co-deletions [6, 7]. These studies proposed the hypothesis that some GBM cells differentiate into oligodendroglial components, suggesting that the possibility that the astrocytic lineage also develops GBMOs cannot as yet be excluded. It therefore remains unclear whether the presence of an oligodendroglial component can be recognized as an intrinsic prognostic factor, or whether good

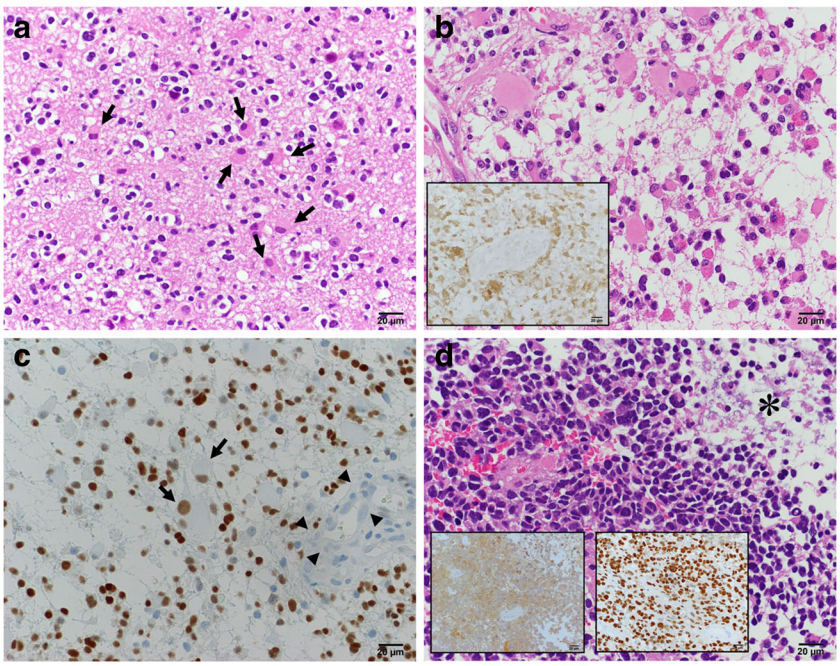

Fig. 3 Microscopic examination of the second recurrence. **a** The lesion in the right frontal lobe showed diffuse proliferation of tumor cells with roundish nuclei and a perinuclear halo on hematoxylin and eosin (H&E) staining. Minigemistocytes with eosinophilic cytoplasm and eccentric nuclei were occasionally noted (*arrows*). **b** Another area showed a mixture of astrocytic tumor cells with plump eosinophilic cytoplasms and large nuclei, and oligodendrocytic tumor cells with roundish, small nuclei including minigemistocytes and refractile eosinophilic granular cells (H&E staining). Immunohistochemistry for IDH1 R132H revealed that both astrocytic and oligodendrocytic tumor cells expressed mutant IDH1 (inset). Note the lack of staining in the blood vessel cells (*center*). **c** Immunohistochemistry for p53 revealed that both astrocytic (*arrows*) and oligodendrocytic tumor cells showed nuclear accumulation of p53. Note the lack of staining in the blood vessel cells (*arrowhead*). **d** By contrast, the lesion in the right sylvian fissure showed a highly anaplastic area with a dense proliferation of hyperchromatic tumor cells, brisk mitotic activity, and necrosis (*asterisk*) (H&E staining). Tumor cells were diffusely immunopositive for IDH1 R132H (*left inset*) and p53 (*right inset*)

Table 1 The results of LOH analysis

Marker	Location	LOH				
		1st tumor: DA	2nd tumor: GBM	3rd Tumor: GBM	3rd Tumor: GBMO	Contralateral GBM
D1S2644	1p36					
D1S2766	1p22					
D1S435	1p22					
D10S1649	10p15	■		■	■	■
D10S213	10p11	■				
D10S1652	10q21					■
D10S1765	10q23					■
D10S587	10q26				■	■
D17S1791	17p13	■	■	■	■	■
D17S831	17p13		■	■	■	■
D19S219	19q13					
D19S418	19q13					
D19S420	19q13					
D19S921	19q13					

■ LOH □ No LOH

prognoses are achieved only in cases showing genetic commonality to oligodendroglial tumors.

Our case is essentially that of secondary GBM, which was excluded in previous representative GBMO studies [4–8]. Additionally, the *IDH1* mutation detected in our case is known to be less common in GBMOs [9]. These findings suggest that our case is another distinct entity separate from 'standard' GBMO. Nonetheless, the fact that oligodendroglial foci can appear during recurrence of a GBM with common histology suggests that glioblastoma cells can also appear oligodendroglioma-like. Therefore, genetic analysis is essential to distinguish the bioactivity of GBMs with an oligodendroglial component to accurately predict prognosis.

The central pathology review of the EORTC 26981 trial revealed that, of the GBMO cases in this trial, 15 % of all pathologically confirmed GBMs contained at least two genetically distinct subgroups characterized by mutually-exclusive *IDH1* mutations and epidermal growth factor receptor gene (*EGFR*) amplifications [7]. The within-trial gene expression-based subclassification also supported a heterogeneous signature, representing a comparable frequency of proneural and classical subgroups. However, the controversy following the redefinition of the GBMO concept in 2007 WHO classification [10] suggests that this definition should be revisited based on recent insights.

Genetic analyses revealed that the present case already harbored 10p, 17p (TP53 locus) LOH and *IDH1* mutations at the primary tumor site, which were then followed by a mutation at distal 10q LOH on the GBMO foci at the second recurrence. Contrary to expectations, the 1p/19q co-deletion, typically seen in the oligodendroglial lineage, did not appear in this case. A recent consensus agreed that the hallmark of divergence to an astrocytic or oligodendroglial lineage was the occurrence of an *IDH1* mutation as the initial event, followed by a TP53 mutation or 1p/19q co-deletion, respectively [11]. Accordingly, although the mutation status of TP53 is unknown, the present case appears to have genetically arisen along the astrocytic lineage.

Another genetic point of information in the present case is the accumulation of partial chromosome 10 LOH during tumor progression. The primary lesion already expressed 10p LOH, and distal 10q LOH arose when oligodendroglial foci developed at the second recurrence. LOH on chromosome 10 is known as the most common genetic event in GBMs. Moreover, the progression of astrocytoma is associated with an increased loss of 10p and 10q sequences, probably reflecting the increased involvement of tumor suppressor genes [12]. Our previous study revealed that, while 10q LOH is implicated in the malignant progression of gliomas, 10p LOH is often seen even in grade II diffuse astrocytomas.

This indicates that this genetic alteration has a less malignant effect which is why it is seen in the early stages of astrocytoma formation or progression [13]. Accordingly, the 10p LOH detected in the primary lesion of the present case also supports the fact that it evolved from an astrocytic origin.

Our current results indicate a common genetic background for astrocytic tumors. These findings also suggest that oligodendroglial components can appear during the progression of genetically astrocytic tumors, and that at least some GBMO cases originate from non-oligodendroglial tumors. In the present case, the distal 10q (10q25–26) LOH was associated with the development of the oligodendroglial component. The relevance of distal 10q (distal to the PTEN locus) LOH in malignant gliomas remains controversial [12–14], so more genetic studies, including sporadic reports like ours, would contribute to the understanding of the mechanism leading to GBMO development.

In summary, we experienced a rare case of secondary GBMO that evolved along an astrocytic lineage with the accumulation of partial chromosome 10 LOH. Further studies are needed to confirm the importance of the detection of oligodendroglial components in glioblastomas, especially in secondary cases such as ours.

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