Gliosarcomas are a type of bimorphic tumor composed of glial and sarcomatous elements, and are considered to be a variant of glioblastoma, WHO grade IV. To date, only rare cases of gliosarcoma with oligodendroglial components (oligosarcoma) have been reported. We report a case of oligosarcoma consisting of gliosarcoma arising from recurrent oligodendroglioma. A 53-year-old man, who had undergone a gross total resection of oligodendroglioma (WHO grade II) 11 years earlier, presented with a local tumor recurrence. The patient underwent a second gross total resection, whereupon a histopathological examination further revealed residual features of classical oligodendroglioma, and newly-developed sarcomatous characteristics. Both the primary and recurrent tumors showed 1p/19q co-deletion and mutation of the isocitrate dehydrogenase 1 (IDH1) gene, consistent with being oligodendrogial in nature. Loss of heterozygosity (LOH) of chromosome 1p/19q and IDH1 mutation have seldom been analyzed in previous reports of oligosarcomas. We report a rare case study supported by the results of genetic analyses. Our analyses have revealed that the sarcomatous component represents a metaplastic change occurring in the oligodendroglial element.

Key words: gliosarcoma, oligodendroglioma, oligosarcoma, 1p/19q co-deletion

Gliosarcomas are an uncommon glial neoplasm of the central nervous system. According to the World Health Organization (WHO), they are considered as a glioblastoma variant, which has a bimorphic tissue pattern composed of glial and mesenchymal elements.\(^1,2\) While gliosarcomas with oligodendroglial or ependymal components have been reported in the past, most of them were published prior to the development of genetic modalities.\(^3\)\(^-\)\(^5\) In this report, we present a case of gliosarcoma coexisting with oligodendroglioma, diagnosed using a combination of immunohistochemistry and genetic analyses.

CLINICAL SUMMARY

Eleven years ago, a 53-year-old man developed generalized seizures. Subsequent examinations via computed tomography (CT) and magnetic resonance imaging (MRI) demonstrated a calcified, 3.0 cm in diameter, non-enhancing tumor in the right frontal lobe (Fig. 1a, b). Consequently, a gross total resection was performed and histopathological examination showed oligodendroglioma (WHO grade II). Loss of heterozygosity (LOH) analysis showed 1p/19q co-deletion. The patient received chemotherapy with procarbazine, nimustine (ACNU) and vincristine, and no radiotherapy was administered. On MRI performed 6 years after the initial resection, there was no evidence of tumor recurrence. However, 5 years after the last MR evaluation the patient developed generalized seizures again. CT and MRI demonstrated a 6.3 cm diameter, Gd-enhancing, recurrent tumor with focal calcification at the site of the prior resection (Fig. 1c–e). Accordingly, another gross total resection was performed on the recurrent tumor. Intraoperative findings confirmed that the tumor was intra-axial in origin at the site of the prior resection cavity in the right frontal lobe, however the tumor partly extended exophytically and had fused with the adjacent falx and dura, mimicking an extra-axial tumor. Further histopathological examinations showed residual features of oligodendroglioma and a coexisting sarcomatous finding. This indicated a diagnosis of gliosarcoma arising from oligodendroglioma or oligosarcoma, WHO grade IV. Further genetic analyses revealed that the tumor showed...
IDH1 mutation and 1p/19q co-deletion (described in detail below). The patient was treated with adjuvant radiation (54Gy) combined with chemotherapy of temozolomide (75 mg/m²). However, an allergy to temozolomide was suspected. We stopped his subsequent treatment of chemotherapy with temozolomide. On MRI performed 6 months after the second resection, there was no evidence of tumor recurrence. He consented to the submission of this case report for publication.

PATHOLOGICAL FINDINGS

Materials and methods

Tissue samples were fixed in 10% buffered formalin, embedded in paraffin, and processed for conventional histology and immunohistochemistry. Subsequently, sections of the samples (5 μm) were stained using hematoxylin and eosin (HE) for histological evaluation, and the remaining unstained serial sections were used for immunohistochemistry. Immunohistochemical studies were performed using peroxidase avidin-biotin methods (LASB kit, DakoCytomation, Carpinteria, CA, USA) on paraffin sections regarding glial fibrillary acidic protein (GFAP, prediluted; DakoCytomation, Carpinteria). At the same time, immunohistochemical studies were performed using peroxidase avidin-biotin methods (LASB kit) on paraffin sections, following heat-induced antigen retrieval. Primary antibodies were directed toward alpha-smooth muscle actin (dilution 1:400; DakoCytomation, Glostrup, Denmark), desmin (dilution 1:100; DakoCytomation, Glostrup), ZEB1 (dilution 1:200; Bethyl, Montgomery, TX, USA), IDH1-R132H (dilution 1:20; Dianova, Hamburg, Germany), alpha-thalassemia/mental retardation syndrome X-linked (ATRX) (dilution 1:100; Sigma Aldrich, Stockholm, Sweden), and Ki-67 (the MIB-1 antibody, dilution 1:100; Immunotech, Marseille, France). The Ki-67 labeling index was defined as the percentage of nuclear area stained in regions of maximum labeling. Additionally, fluorescence in situ hybridization (FISH) was performed on 4 mm-thick sections of previously prepared paraffin-embedded tissue. Cell copy numbers were
investigated by FISH using LSI 1p36/LSI 1q25 and LSI 19q13/19p13 Probe (Vysis, Downer’s Grove, IL, USA), and approximately 200 non-overlapping nuclei were scored.

For the genetic analyses, tumor DNAs were isolated from frozen samples using a QIAamp DNA Mini Kit (Qiagen, Tokyo, Japan), and corresponding non-tumor DNA was isolated from blood samples using the QIAamp DNA Blood Mini Kit (Qiagen). We used frozen samples of the recurrent tumor which was diagnosed as the sarcomatous component. LOH was detected by PCR-based LOH assay using 24 micro-satellite markers, previously described.6 We used the capillary electrophoresis data of five representative loci on chromosome 1p22 (D1S206, D1S435, D1S2766) and 19q13.3 (D19S219, D19S420).

Histopathology and immunohistochemistry

Histopathologically, the primary tumor showed classical oligodendroglial features, such as tumor cells with rounded, homogeneous nuclei and perinuclear halos (Fig. 2a). The tumor cells also exhibited intense staining for the mutant form of IDH1 (Fig. 3a). In addition, the Ki-67 labeling index was less than 5%. The histopathological and immunohistochemical findings were compatible with the diagnosis of oligodendroglioma, WHO grade II.

The recurrent tumor showed a bimorphic pattern, classical oligodendroglioma and sarcomatous components, with the latter showing densely packed, long bundles of spindle cells associated with focal necrosis (Fig. 2b–d). Moreover, the sarcomatous component showed high mitotic activity and nuclear atypia (Fig. 2e). The Ki-67 labeling index was 8% and 40% in the glial and sarcomatous components. While GFAP immunoreactivity was limited to the glial component, alpha-smooth muscle actin (SMA) and desmin were expressed in the sarcomatous component (Fig. 3d, e). Both components in the recurrent tumor exhibited positive staining for IDH1-R132H (Fig. 3b, c), ZEB1 (Fig. 3f, g) and ATRX (Fig. 3h, i). In contrast, p53 was negative in both components. These suggested a monoclonal tumor origin from the prior oligodendroglioma.

Genetic study

We performed PCR-based LOH analysis (Fig. 4). The capillary electrophoresis data of five representative loci on chromosome 1p22 (D1S206, D1S435, D1S2766) and 19q13.3 (D19S219, D19S420) showed allelic losses (arrows) in the primary tumor, as opposed to the corresponding reference genotype of blood leukocytes of the patient. This showed evidence of 1p/19q co-deletion in the primary tumor. In the recurrent tumor, allelic losses were partially recognized on D1S206, D1S435 and D19S420. Contrary to the finding of the primary tumor, PCR-based LOH analysis showed evidence of partial loss of chromosomes 1p and 19q in the recurrent tumor. FISH analysis revealed evidence
of losses of chromosomes 1p and 19q in both the glial and sarcomatous components (Fig. 5a–d).

**DISCUSSION**

Here we have presented a rare case of gliosarcoma arising from oligodendroglia. According to the 2016 *WHO Classification of Tumours of the Central Nervous System*, gliosarcoma is considered to be a glioblastoma variant characterized by a bimorphic tissue pattern with alternating areas displaying glial and mesenchymal differentiation.\(^1\,^2\) *IDH1* and 1p/19q analyses were performed on both the primary and recurrent tumors. Both of the tumor cells demonstrated the *IDH1* mutant protein expression. This suggests the tumor evolved from low grade glioma, as the *IDH1* mutation rarely occurs in primary glioblastomas and gliosarcomas.\(^7\) Mainly observed in classical oligodendrogliomas, 1p/19q co-deletion is considered to be a significant predictor of outcome for patients with tumors of oligodendroglial and oligoastrocytic histology.\(^8\,^9\) In this case, 1p/19q co-deletion was found in the glial and sarcomatous components of the recurrent tumor.

Rodriguez *et al.*\(^5\) performed 1p and 19q FISH analysis on seven gliosarcomas in which several patterns of 1p/19q loss were detected. However, *IDH1* analysis was not performed. Hiniker *et al.*\(^10\) reported a case of gliosarcoma, and performed both *IDH1* analysis and 1p/19q FISH analysis. The *IDH1* mutation was found in both the primary and recurrent tumors. In addition, 1p/19q co-deletion was found in the primary tumor, but it was not identified in the recurrent tumor. In their report, retention of alleles of chromosomes 1p and 19q was shown by SNP array analysis. It was postulated that the absence of 1p/19q co-deletion in gliosarcoma was affected by the duplication of the remaining intact chromosomes 1 and 19 in gliosarcoma associated with the loss of the second derivative chromosome containing 1q and 19p. Additionally, they described that there was a slight possibility that the two tumors arose entirely independently (“collision tumor”). Shoji *et al.*\(^11\) reported a case of a sarcomatous tumor occurring at the site of resected
oligodendroglioma, and found IDH1 mutation and 1p/19q co-deletion in the recurrent sarcomatous tumor. The case had no oligodendroglial component in the recurrent tumor. Vajtai et al. reported a case of gliosarcoma, which showed IDH1 mutation in the primary and secondary tumors. However, they performed 1p and 19q analysis using only PCR-based LOH analysis, which did not show 1p/19q co-deletion in each of the oligodendrogial and sarcomatous components of the recurrent tumor.

Figure 4 Loss of heterozygosity (LOH) analyses with micro-satellite markers. The capillary electrophoresis data of five representative loci on chromosome 1p22 (D1S206, D1S435, D1S2766) and 19q13.3 (D19S219, D19S420) showed allelic losses (arrows) in the primary tumor, as opposed to the corresponding reference genotype of blood leukocytes of the patient. In the recurrent tumor, allelic losses were partially recognized on D1S206, D1S435 and D19S420.

Figure 5 Fluorescence in situ hybridization analysis showed chromosome 1p/19q co-deletion in the (a, b) oligodendrogial and (c, d) sarcomatous components in the recurrent tumor: 1 orange signal corresponding to (a, c) 1p36 and (b, d) 19q13.
sarcomatous components in the recurrent tumor. Yasuda et al.\textsuperscript{13} reported a case of gliosarcoma arising from oligodendroglioma, which exhibited 1p/19q co-deletion and \textit{IDH1} mutation in both the oligodendrogial and sarcomatous components in the recurrent tumor. Their case received chemotherapy with procarbazine, ACNU and vincristine, and radiotherapy was administered. They noted that glial or epithelial to mesenchymal transition (EMT) after irradiation treatment for glioblastoma had been reported,\textsuperscript{14} thus it is possible that radiation therapy may induce EMT in oligodendroglioma. In addition to these findings, the secondary gliosarcomas arising from astrocytoma after radiation therapy have been reported by Cheong et al.\textsuperscript{15} In a separate study, Codispoti et al.\textsuperscript{16} reported sarcomatous transformation in astrocytoma in the absence of radiation therapy. Similarly in our case, the patient received no radiotherapy, therefore, sarcomatous transformation in oligodendroglioma occurred without any association with radiotherapy. On the other hand, both components in the recurrent tumor in our case exhibited ZEB1 positivity associated with reduced expression of E-cadherin. We cannot rule out the possibility that chemotherapy administered to our patient induced EMT because chemotherapy has also been reported in the literature to induce EMT.\textsuperscript{17}

Although, changes in chromosomal aberration of recurrent oligodendrogial tumors have been reported previously, the tumors with 1p/19q co-deletion also demonstrated the same aberrations in the recurrent tumor.\textsuperscript{18–20} In our case, the presence of 1p/19q co-deletion and \textit{IDH1} mutation in both of the glial and sarcomatous components was found in the recurrent tumor as well as the primary tumor. These findings support the notion that the sarcomatous component evolved from the oligodendrogial element. In addition, the majority of the recurrent tumor was composed of spindle cells with nuclear atypia that showed positivity for alpha-SMA, \textit{IDH1-R132H} and ZEB1. These findings suggest that spindle cells of the recurrent tumor is neoplastic rather than reactive, which is consistent with the report by Vajtai et al.\textsuperscript{12}

In conclusion, we presented a rare case of gliosarcoma in which 1p/19q co-deletion and \textit{IDH1} mutation has been found in both the oligodendrogial and sarcomatous components in the recurrent tumor. To determine the nature of sarcomatous recurrence and genetic events, an immunohistochemistry examination and genetic analysis by FISH or PCR-based LOH analysis must be performed.

ACKNOWLEDGMENT

The authors would like to thank F. Doi and T. Akiyama for technical assistance.

DISCLOSURE STATEMENT

None declared.

REFERENCES


