Table 2 Univariate analyses of overall survival time of patients with recurrent glioblastoma

Variable	Number of cases	MST (95% confident interval)	*p* value (log-rank)
Age			
≤50 years	12	31.2 (21.0–44.7)	0.05
>50 years	20	16.6 (12.7–19.6)	
Operation time			
Twice	24	17.1 (13.6–30.8)	0.09
≥3 times	8	33.3 (21.0–64.9)	
MIB-1 index at the first surgery			
≤30%	15	22.4 (13.2–55.5)	0.097
>30%	11	17.9 (12.3–23.7)	
MIB-1 index at the second surgery			
≤10%	13	42.9 (17.3–64.9)	0.005
>10%	19	16.9 (12.7–22.8)	
MGMT promoter status at the first surgery			
Methylated	6	30.6 (NA)	0.6
Unmethylated	13	19.6 (14.5–41.3)	
MGMT promoter status at the second surgery			
Methylated	5	16.9 (NA)	0.7
Unmethylated	18	19.6 (14.5–41.3)	

NA not applicable

Patients with three or more surgical resections tended to have longer survival times than those with two operations ($p = 0.09$). The MST of patients with three or more surgical resections was 33.3 months and of those with two operations was 17.1 months (Table 2). The MST of the patients in the total or subtotal resection (≥90% removal) group ($n = 16$; median age 60.0) and of patients in the partial (<90% removal) removal or biopsy ($n = 16$; median age 54.0) group who underwent a first surgery were 18.0 and 22.4 months, respectively ($p = 0.2$) in recurrent surgical cases. However, the overall survival time of patients in the total or subtotal resection group ($n = 10$; median age 57.0) and the partial or biopsy group ($n = 22$; median age 57.0) who underwent a second surgery were 42.9 and 18.0 months, respectively ($p = 0.03$). The MST after the second surgery for each group was 16.4 and 11.1 months, respectively ($p = 0.02$).

MIB-1 index at the second operation showed prognostic value in relapsed glioblastoma patients

The MIB-1 indexes of primary tumors were obtained from 26 patients and those of recurrent tumors from all patients. The median MIB-1 index of primary tumors was 22.5% (range 6.8–90.0%) and of recurrent tumors was 13.2% (range 0.6–85.7%). MIB-1 indexes were smaller for recurrent than for primary tumors ($p = 0.03$, Mann–Whitney U test) (Fig. 1).

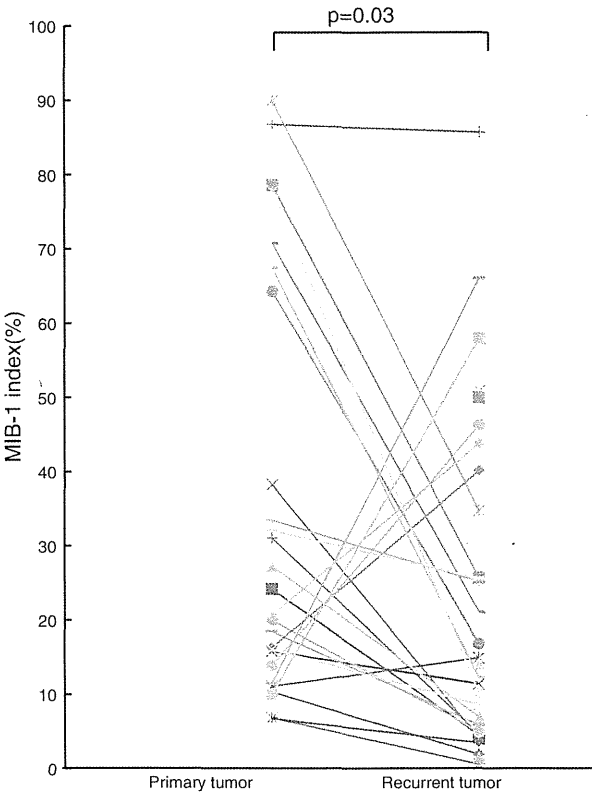

Fig. 1 Changes in MIB-1 indexes of primary and recurrent tumors. MIB-1 indexes were smaller in recurrent tumors than in primary tumors ($p = 0.03$, Mann–Whitney U test)

The MST of patients with MIB-1 indexes ≤30% was 22.4 months and of those with MIB-1 indexes >30% 17.9 months in primary tumors. Patients with MIB-1 indexes ≤30% in primary tumors tended to survive longer ($p = 0.097$) (Table 2). The MST of patients with MIB-1 indexes ≤10% was 42.9 months and of patients with indexes >10% 16.9 months in recurrent tumors. The MIB-1 indexes in recurrent tumors significantly correlated with MST ($p = 0.005$) (Table 2; Fig. 2a). The survival time of patients with MIB-1 indexes ≤10% after the second surgery was 21.6 months and of patients with indexes >10% was 6.0 months ($p = 0.0007$) (Table 3; Fig. 2b). The MIB-1 indexes in recurrent tumors significantly correlated with overall survival ($p = 0.004$), even in the multivariate analysis (Table 4). This analysis resulted in a hazard ratio (HR) of 5.252 [95% confidence interval (CI) 1.666–20.587).

Status of methylated *MGMT* promoter did not show prognostic value in relapsed glioblastoma patients

We obtained the *MGMT* promoter status in 19 cases with primary and 23 with recurrent tumors. The methylated *MGMT* promoter was found in six patients (31.6%) with primary and in five (21.7%) with recurrent tumors

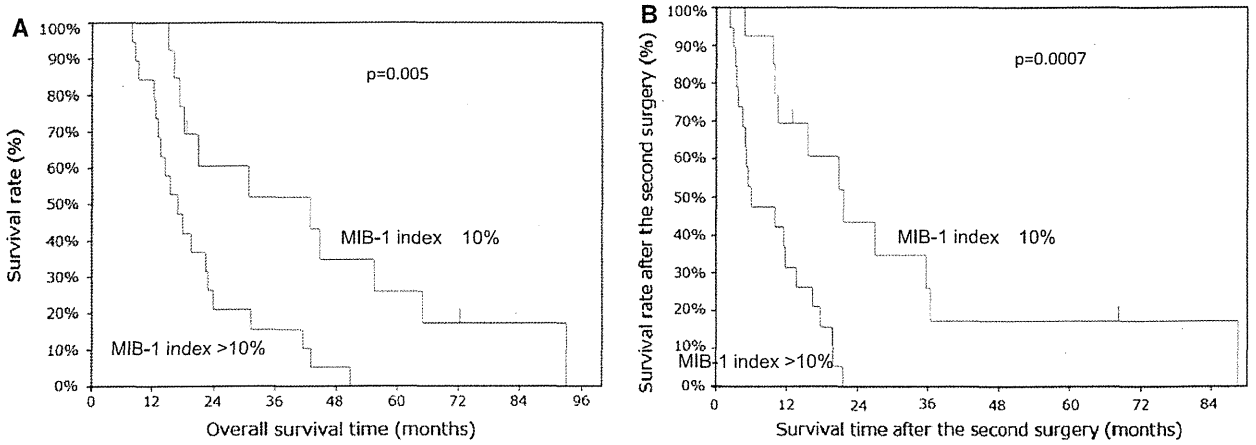

Fig. 2 **a** Kaplan–Meier survival curve comparing high and low MIB-1 indexes in recurrent tumors. The estimated overall survival rate of patients with MIB-1 indexes ≤10% was significantly higher than that of patients with indexes >10% ($p = 0.005$). **b** Kaplan–Meier survival curve after the second operation comparing high and low MIB-1 indexes in recurrent tumors. The estimated survival rate after the second operation of patients with MIB-1 indexes ≤10% was significantly higher than that of patients with indexes >10% ($p = 0.0007$)

Table 3 Univariate analyses of survival time after the second surgery of patients with recurrent glioblastoma

Variable	Number of cases	MST after second surgery (95% confident interval)	*p* value (log-rank)
MGMT promoter status at the second surgery			
Methylated	5	13.7 (NA)	0.8
Unmethylated	18	11.1 (5.0–19.9)	
MIB-1 index at the second surgery			
≤10%	13	21.6 (10.0–36.4)	0.0007
>10%	19	6.0 (3.9–13.7)	
Pseudopalisading necrosis			
Positive	8	10.9 (NA)	0.8
Negative	24	11.6 (6.0–19.7)	
Coagulation necrosis			
Positive	18	13.7 (5.4–19.7)	0.4
Negative	14	10.8 (3.7–35.7)	
Gemistocytic cell			
Positive	16	16.4 (9.7–21.6)	0.2
Negative	16	8.0 (3.9–13.7)	
Giant cell			
Positive	16	10.2 (4.7–16.4)	0.1
Negative	16	13.7 (5.0–21.6)	

NA not applicable

Table 4 Multivariate analyses of overall survival time of patients with recurrent glioblastoma

Variable	Number of cases	Hazard ratio (95% confident interval)	*p* value (Cox)
Age			
≤50 years	12	2.008 (0.811–5.506)	0.1
>50 years	20		
Operation time			
Twice	24	0.403 (0.119–1.146)	0.09
≥3 times	8		
MIB-1 index at the first surgery			
≤30%	15	1.073 (0.402–2.927)	0.9
>30%	11		
MIB-1 index at the second surgery			
≤10%	13	5.252 (1.666–20.587)	0.004
>10%	19		

(Table 1). The MST of patients with methylated or unmethylated *MGMT* promoters in primary tumors was 30.6 and 19.6 months ($p = 0.6$), respectively (Table 2). The MST of patients with methylated or unmethylated *MGMT* promoters in recurrent tumors was 16.9 and 19.6 months ($p = 0.7$), respectively (Table 2). No methylated *MGMT* promoter statuses in primary and recurrent tumors correlated with survival time (Table 2). Similarly, the methylated *MGMT* promoter in primary tumors did not correlate with the first PFS and in recurrent tumors did not correlate with the second PFS (Table 5).

Twenty patients underwent initial chemoradiotherapy with ACNU, and 12 underwent initial chemoradiotherapy with TMZ. Nine patients among 20 with initial chemoradiotherapy with ACNU were finally treated with TMZ. The MST of each of these groups was 23.3 and 14.0 months, respectively ($p = 0.02$). We then analyzed the correlation of survival time with initial chemotherapy regimen and *MGMT* promoter status in 18 patients whose *MGMT* promoter status was determined in both primary and recurrent tumors. The initial PFS of patients who received

Table 5 Univariate analyses of first and second progression-free survival (PFS) and mean survival time (MST) in 18 recurrent glioblastoma patients with *MGMT* promoter methylation status in both primary and recurrent tumors

Variable		Number of cases	First median PFS (95% confident interval)	*p* value (log-rank)	Second median PFS (95% confident interval)	*p* value (log-rank)	MST (95% confident interval)	*p* value (log-rank)
Initial treatment and the methylated *MGMT* promoter status in primary tumors								
Initial treatment	*MGMT* (primary)							
ACNU	Methylated	4	12.6 (9.1–18.3)	0.9	21.2 (2.1–NA)	0.3	49.3 (18.2–NA)	0.5
ACNU	Unmethylated	8	8.1 (3.3–26.3)		7.7 (1.6–13.6)		27.5 (15.1–64.9)	
TMZ	Methylated	1	5.2 (NA)	0.2	0.6 (NA)	0.03	9.2 (NA)	0.2
TMZ	Unmethylated	5	8.8 (2.7–22.9)		4.4 (1.0–16.4)		14.5 (7.8–41.3)	
Initial treatment and the methylated *MGMT* promoter status in recurrent tumors								
Initial treatment	*MGMT* (recurrent)							
ACNU	Methylated	3	18.1 (12.1–24.1)	0.8	13.4 (1.6–NA)	0.4	44.7 (15.5–NA)	0.6
ACNU	Unmethylated	9	9.1 (3.3–26.3)		6.6 (2.1–35.7)		31.2 (15.1–64.9)	
TMZ	Methylated	0						
TMZ	Unmethylated	6	7.6 (2.7–22.9)		3.8 (0.6–16.4)		13.4 (7.8–41.3)	
Changes of the methylated *MGMT* promoter status								
First surgery	Second surgery			0.9		0.3		0.5
Methylated	Methylated	1	3.3 (NA)		68.3 (NA)		72.2 (NA)	
Methylated	Unmethylated	4	10.9 (5.2–18.3)		4.4 (0.6–35.7)		30.6 (9.2–55.5)	
Unmethylated	Methylated	2	18.1 (12.1–24.1)		7.5 (1.6–13.4)		30.1 (15.5–44.7)	
Unmethylated	Unmethylated	11	6.4 (3.3–22.9)		5.0 (3.2–13.6)		19.6 (12.3–41.3)	

ACNU nimustine hydrochloride, *TMZ* Temozolomide, *NA* not applicable

chemoradiotherapy with ACNU and who had methylated ($n = 4$) and unmethylated ($n = 8$) MGMT promoters in primary tumors was 12.6 and 8.1 months, respectively ($p = 0.9$; Table 5). In contrast, the initial PFS of patients receiving chemoradiotherapy with TMZ and who had methylated ($n = 1$) and unmethylated ($n = 5$) *MGMT* promoters in primary tumors was 5.2 and 8.8 months, respectively ($p = 0.2$; Table 5). Three patients who initially underwent chemoradiotherapy with ACNU and who had methylated *MGMT* promoters, and five of nine patients who had unmethylated *MGMT* promoters in recurrent tumors were finally treated with TMZ. The MST of patients who underwent initial ACNU treatment followed by TMZ and who had methylated *MGMT* promoter status in recurrent tumors was longer than that of patients who had unmethylated *MGMT* promoter status (44.7 vs. 31.2 months, Table 5); however, this finding was not significant ($p = 0.6$). None of the patients who underwent the initial TMZ treatment received other alkylating agents. There was no significant difference in the PFS and MST of primary and recurrent tumors according to *MGMT* methylation status. Six patients showed changes in the methylated *MGMT* promoter in primary and recurrent tumors. Changes in the methylated *MGMT* promoter in primary and recurrent tumors did not correlate with the first and second PFS (Table 5).

Degenerative changes in tumor cells by chemoradiotherapy

We examined the degenerative changes in tumor cells, which included pseudopalisading necrosis, coagulation necrosis, gemistocytic cells, and giant cells, in H&E-stained specimens in recurrent tumors (Table 6). The MST of patients with pseudopalisading necrosis was 19.2 months and of patients without pseudopalisading necrosis 20.2 months ($p = 0.98$). The MST of patients with coagulation necrosis was 19.6 months and of patients without coagulation necrosis 25.4 months ($p = 0.16$). The MST of patients with gemistocytic cells was 22.4 months and of patients without gemistocytic cells 17.4 months ($p = 0.27$). The MST of patients with giant cells was 16.4 months and of patients without giant cells 22.4 months ($p = 0.08$). We found no correlations between morphological changes in tumor cells and survival time (Table 6).

Table 6 Univariate analyses of overall survival time of patients with recurrent glioblastoma with regard to degenerative changes of tumor at second surgery

Variable	Number of cases	MST (95% confident interval)	p value (log-rank)
Pseudopalisading necrosis			
Positive	8	19.2 (NA)	0.98
Negative	24	20.2 (16.2–31.2)	
Coagulation necrosis			
Positive	18	19.6 (13.6–23.7)	0.16
Negative	14	25.4 (15.5–44.7)	
Gemistocytic cell			
Positive	16	22.4 (16.2–50.8)	0.27
Negative	16	17.4 (13.2–30.8)	
Giant cell			
Positive	16	16.4 (13.2–30.8)	0.08
Negative	16	22.4 (16.9–44.7)	

MST mean survival time, *NA* not applicable

Discussion

We attempted to clarify the prognostic factors in recurrent glioblastomas. Clinically, patients ≤50 years had a longer survival time than patients who were >50 years old ($p = 0.05$). Glioblastoma patients who underwent surgery twice or more ($n = 35$; MST 21.0 months) showed increased overall survival compared with those who underwent only one surgery ($n = 154$; MST 12.9 months) at our institute from 1996 to 2010 ($p = 0.008$). Patients who had three or more surgical resections tended to survive longer than those who had two operations ($p = 0.09$). Whether surgical resections of recurring glioblastomas prolong survival of glioblastoma patients is unclear, but reoperations in recurrent patients have been reported to be beneficial for selected patients [19].

The extent of surgical resection in patients with newly diagnosed glioblastoma has been a well-documented prognostic factor for survival [20, 21]. In this study, we found a significant difference in survival time between the total and subtotal resection groups and the partial and biopsy groups of 189 newly diagnosed glioblastoma patients ($p = 0.03$). The extent of surgical resection during the first surgery had no correlation with the overall survival time in patients who underwent a second surgery in recurrent surgical cases; however, there was a significant difference between survival time and the extent of surgical resection during the second surgery. These data indicate that the extent of surgical resection is important, even in recurrent cases.

MIB-1 indexes that >30% in primary tumors tended to be poor prognostic factors, which were not significant. However, MIB-1 indexes in recurrent tumors had a definite correlation with overall survival time and survival time after the second surgery. Overall, survival of patients with MIB-1 indexes ≤10% at recurrence was 42.9 months and those with indexes >10% was 16.9 months, a significant difference. Schroder et al. [22] reported that MIB-1 indexes of glioblastomas at recurrence correlated with time to

recurrence. Kunishio et al. [23] reported that MIB-1 indexes of tumors with radiation necrosis after interstitial brachytherapy were 7.6 ± 5.5%, whereas that of primary tumors was significantly higher at 17.0 ± 11.2% ($p < 0.05$). These results were similar to ours. In contrast, Ralte et al. [24] reported that the difference in the MIB-1 indexes of initial (10.33 ± 7.98) and recurrent (13.8 ± 9.40) glioblastomas were not statistically significant ($p = 0.79$). Kodera et al. [25] reported that the MIB-1 indexes of recurrent glioblastomas after stereotactic radiosurgery were significantly lower than those before. In our study, MIB-1 indexes was also smaller in recurrent (13.2%) than in primary (22.5%) tumors ($p = 0.03$, Mann–Whitney U test). It has been postulated that MIB-1 indexes were smaller in recurrent than in primary tumors when tumor cells respond to initial chemoradiotherapy and degenerative changes of the tumor cells occur. Degenerative changes, such as giant cells, gemistocytic cell formation, and coagulation necrosis, are often found in recurrent gliomas [15], but these morphological changes did not correlate with survival time in our study.

It has been reported that the methylated *MGMT* promoter was found in 44.7–48.4% of newly diagnosed glioblastoma cases [11, 26, 27]. In our study, the methylated *MGMT* promoter was found in 31.6% of primary tumors. The first PFS was 6.2 months in patients treated with chemoradiotherapy with ACNU or TMZ, and the time was shorter than a previous report that found the first PFS to be 6.9 months with radiotherapy and TMZ treatment in newly diagnosed glioblastoma patients [1]. The rate of finding the methylated *MGMT* promoter seemed to be smaller in our series than in previous reports, which may be because our series could have included unfavorable cases with regrowth that did not respond to chemoradiotherapy and therefore needed a second surgery.

Brandes et al. estimated the correlation between *MGMT* promoter methylation status at first and relapse operation and survival time in 44 TMZ-treated paired tumors. They suggested that *MGMT* methylation status determined only at the first surgery appears to be of prognostic value. The MST of patients with methylated or unmethylated *MGMT* promoters in primary tumors was 30.6 and 19.6 months, respectively [14]. In contrast to their TMZ-treated series, in our series, 12 patients were initially treated with ACNU and six with TMZ. The *MGMT* promoter methylation status in primary tumors had no correlation with survival time and PFS. Brandes et al. also showed that overall survival and survival time after second surgery were not correlated with *MGMT* methylation status of recurrent tumors, even though most patients were treated with TMZ rechallenge and nitrosourea-based chemotherapy after the second surgery. The authors suggested that this may depend in part on the low activity of second-line therapies, especially those with alkylating agents, at the time of failure after chemoradiotherapy with TMZ [14].

Twenty patients in our study had initial chemoradiotherapy with ACNU and 12 patients had TMZ. The MST of each group was 23.3 and 14.0 months ($p = 0.02$), respectively. Nine patients among 20 patients with initial chemoradiotherapy with ACNU were finally treated with TMZ. The MST of patients with methylated *MGMT* promoter status in recurrent tumors treated with initial ACNU followed by TMZ tended to be longer than that with unmethylated *MGMT* (44.7 vs. 31.2 months, $p = 0.6$, Table 5). It is possibility that patients with recurrence who maintain the methylated *MGMT* status have sensitivity to TMZ even after the failure of initial ACNU. Nagane et al. [28] reported that protein expression of MGMT by Western blotting is an important prognostic factor for recurrent glioblastoma patients treated with TMZ after failure of initial ACNU-based chemotherapy. Methylated *MGMT* promoters were found in 31.6% of primary tumors and 21.7% of recurrent tumors. Six patients showed alterations in the methylation of the *MGMT* promoter between the primary and recurrent tumor. The *MGMT* promoter status of four of the five primary tumors with methylated *MGMT* promoters (80%) changed to unmethylated and the status of two of the 13 primary tumors with unmethylated *MGMT* promoters (15.4%) changed to methylated. Brandes et al. reported that *MGMT* status changed from methylated to unmethylated in eight of 13 (61.5%) tumors and from unmethylated to methylated in six of 25 (24%). Moreover, significant changes in *MGMT* methylation status occurred more frequently in *MGMT* methylated cases than unmethylated cases [14]. There are a number of potential explanations for these changes, including regional variation within the tumor, direct influence on methylation by treatment, selection of unmethylated cell populations by treatment, and further dedifferentiation of the tumor [12, 13].

In conclusion, we performed a multivariate analysis in relapsed glioblastoma patients and showed that only MIB-1 indexes in recurrent tumors persisted as significant independent prognostic factors in cases that had second surgeries. *MGMT* promoter status was frequently observed to change from methylated to unmethylated, but the *MGMT* promoter methylation status in primary and recurrent tumors had no correlation with survival time and PFS in recurrent surgical cases.

References

1. Stupp R, Mason WP, van den Bent MJ et al (2005) Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 352:987–996

2. Birol Sarica F, Tufan K, Cekinmez M et al (2010) Effectiveness of temozolomide treatment used at the same time with radiotherapy and adjuvant temozolomide; concomitant therapy of glioblastoma multiforme: multivariate analysis and other prognostic factors. J Neurosurg Sci 54:7–19
3. Gorlia T, van den Bent MJ, Hegi ME et al (2008) Nomograms for predicting survival of patients with newly diagnosed glioblastoma: prognostic factor analysis of EORTC and NCIC trial 26981–22981/CE.3. Lancet Oncol 9:29–38
4. Li SW, Qiu XG, Chen BS et al (2009) Prognostic factors influencing clinical outcomes of glioblastoma multiforme. Chin Med J (Engl) 122:1245–1249
5. Mineo JF, Bordron A, Baroncini M et al (2007) Prognosis factors of survival time in patients with glioblastoma multiforme: a multivariate analysis of 340 patients. Acta Neurochir (Wien) 149:245–252 discussion 252-243
6. Stupp R, Hegi ME, Mason WP et al (2009) Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. Lancet Oncol 10:459–466
7. Carson KA, Grossman SA, Fisher JD et al (2007) Prognostic factors for survival in adult patients with recurrent glioma enrolled onto the new approaches to brain tumor therapy CNS consortium phase I and II clinical trials. J Clin Oncol 25:2601–2606
8. Cunningham JM, Kimmel DW, Scheithauer BW et al (1997) Analysis of proliferation markers and p53 expression in gliomas of astrocytic origin: relationships and prognostic value. J Neurosurg 86:121–130
9. Inoue T, Kumabe T, Kanamori et al (2010) Prognostic factors for patients with gliomatosis cerebri: retrospective analysis of 17 consecutive cases. Neurosurg Rev 34:197–208
10. Moskowitz SI, Jin T, Prayson RA (2006) Role of MIB1 in predicting survival in patients with glioblastomas. J Neurooncol 76:193–200
11. Hegi ME, Diserens AC, Gorlia T et al (2005) MGMT gene silencing and benefit from temozolomide in glioblastoma. N Engl J Med 352:997–1003
12. Jung TY, Jung S, Moon KS et al (2010) Changes of the O6-methylguanine-DNA methyltransferase promoter methylation and MGMT protein expression after adjuvant treatment in glioblastoma. Oncol Rep 23:1269–1276
13. Parkinson JF, Wheeler HR, Clarkson A et al (2008) Variation of O(6)-methylguanine-DNA methyltransferase (MGMT) promoter methylation in serial samples in glioblastoma. J Neurooncol 87:71–78
14. Brandes AA, Franceschi E, Tosoni A et al (2010) O(6)-methylguanine DNA-methyltransferase methylation status can change between first surgery for newly diagnosed glioblastoma and second surgery for recurrence: clinical implications. Neuro Oncol 12:283–288
15. Ogashiwa M, Nakadai M, Asoh Y et al (1987) Morphological analysis of recurrent gliomas. Giant cell and gemistocytic cell formation. Neurol Med Chir (Tokyo) 27:276–282
16. Ogashiwa M, Nakadai M, Asoh Y et al (1985) Morphological analysis of recurrence of glioma. Neurol Med Chir (Tokyo) 25:1010–1018
17. Willson N, Duffy PE (1974) Morphologic changes associated with combined BCNU and radiation therapy in glioblastoma multiforme. Neurology 24:465–471
18. Palmisano WA, Divine KK, Saccomanno G et al (2000) Predicting lung cancer by detecting aberrant promoter methylation in sputum. Cancer Res 60:5954–5958
19. Soults CB, Canute GS, Ryken TC (1998) Evidence-based review of the role of reoperation in the management of malignant glioma. Neurosurg Focus 4:e11
20. Ammirati M, Vick N, Liao YL et al (1987) Effect of the extent of surgical resection on survival and quality of life in patients with supratentorial glioblastomas and anaplastic astrocytomas. Neurosurgery 21:201–206
21. Lacroix M, Abi-Said D, Fourney DR et al (2001) A multivariate analysis of 416 patients with glioblastoma multiforme: prognosis, extent of resection, and survival. J Neurosurg 95:190–198
22. Schroder R, Feisel KD, Ernestus RI (2002) Ki-67 labeling is correlated with the time to recurrence in primary glioblastoma. J Neurooncol 56:127–132
23. Kunishio K, Matsumoto K, Higashi H et al (1999) Proliferative potential of malignant glioma cells before and after interstitial brachytherapy. Neurol Med Chir (Tokyo) 39:341–349
24. Ralte AM, Sharma MC, Karak AK et al (2001) Clinicopathological features, MIB-1 labeling index and apoptotic index in recurrent astrocytic tumors. Pathol Oncol Res 7:267–278
25. Kodera T, Kubota T, Kabuto M et al (2000) Analysis of the proliferative potential of tumor cells after stereotactic radiosurgery for recurrent astrocytic tumors. Neurol Res 22:802–808
26. Costa BM, Caeiro C, Guimaraes I et al (2010) Prognostic value of MGMT promoter methylation in glioblastoma patients treated with temozolomide-based chemoradiation: a Portuguese multicentre study. Oncol Rep 23:1655–1662
27. Preusser M, Charles Janzer R, Felsberg J et al (2008) Anti-O6-methylguanine-methyltransferase (MGMT) immunohistochemistry in glioblastoma multiforme: observer variability and lack of association with patient survival impede its use as clinical biomarker. Brain Pathol 18:520–532
28. Nagane M, Kobayashi K, Ohnishi A et al (2007) Prognostic significance of O6-methylguanine-DNA methyltransferase protein expression in patients with recurrent glioblastoma treated with temozolomide. Jpn J Clin Oncol 37:897–906

Neurol Med Chir (Tokyo) 52, 553~557, 2012

The 70th Annual Meeting Special Topics — Part II: Multidisciplinary Treatment for High Grade Gliomas

Usefulness of Multimodal Examination and Intraoperative Magnetic Resonance Imaging System in Glioma Surgery

Kaori SAKURADA,[1] Kenichiro MATSUDA,[1] Hayato FUNIU,[1] Atsushi KUGE,[1] Sunao TAKEMURA,[1] Shinya SATO,[1] and Takamasa KAYAMA[1]

[1]*Department of Neurosurgery, Yamagata University Faculty of Medicine, Yamagata, Yamagata*

Abstract

Extensive surgical removal of tumor tissue can contribute to longer survival for patients with gliomas. Intraoperative magnetic resonance (iMR) imaging is important for safe and maximal resection of brain tumors. A new operating room equipped with a 1.5-T MR imaging system and neuronavigation opened at Yamagata University Hospital in 2008. Using this new suite, we have safely treated over 200 cases. Use of iMR imaging improved glioma resection rates in 25 (34%) of 73 cases, and gross total resection was achieved in 48 patients (66%). Motor evoked potential (MEP) monitoring was performed in combination with iMR imaging for 32 gliomas. MEP monitoring was successful in 30 cases (94%). Transient decreases in MEP amplitude were seen in two patients. One patient showed transient motor weakness and another showed improvement of motor function. The iMR imaging system provides useful information for tumor resection that allows intraoperative modification of surgical strategies. Combining MEP monitoring with iMR imaging appears to offer the most effective method for safe glioma surgery near eloquent areas.

Key words: intraoperative magnetic resonance imaging, glioma, intraoperative monitoring

Introduction

Intraoperative magnetic resonance (iMR) imaging and neuronavigation have substantially changed the principles of surgery for brain tumors. iMR imaging can provide updated information on anatomical structures and unanticipated brain events, thereby allowing safer and more accurate surgery.[1-4] Our institution installed a fully integrated neurosurgical suite including neuronavigation and an intraoperative 1.5-T high-field MR imaging system (Surgical Suite®) in 2008. This system provides high-quality images with short scan times. The Surgical Suite® has separate components in the operating room and the MR imaging room, so can be used not only for intraoperative scanning, but also for pre- and postoperative scans and brain checks for inpatients at Yamagata University Hospital. Using this new suite, we have safely treated over 200 cases of brain tumors, including gliomas, metastatic brain tumors, meningiomas, and pituitary adenomas. This study reviews our initial experiences, to evaluate the advantages and limitations of this suite in glioma surgery.

Received April 18, 2012; Accepted June 6, 2012

Material and Methods

Figure 1 shows the appearance of the operating system, Surgical Suite® (GE Healthcare, Milwaukee, Wisconsin, USA). The high-field (1.5-T) MR imaging system (Signa HDx; GE Healthcare) is located in a separate room, which allows us to use standard surgical instruments irrespective of their magnetic properties. Intraoperative MR imaging includes T_1-weighted, T_2-weighted, T_2^*-weighted, fluid-attenuated inversion recovery, diffusion-weighted, and T_1-weighted with gadolinium imaging. If necessary, diffusion tensor imaging can be performed. About 1 hour is needed for iMR imaging, including patient transfer time.

A total of 202 consecutive patients were treated in the Surgical Suite® at Yamagata University Hospital between July 2008 and September 2011. The histological diagnoses were glioma (n = 73, 36.1%), pituitary adenoma (n = 35, 17.3%), meningioma (n = 18, 8.9%), metastatic tumor (n = 19, 9.4%), and others (n = 57, 28.2%).

Surgical planning was based on multiple sequences of MR imaging performed 1 or 2 days before surgery. Using a navigation planning workstation (iPLAN 2.6; Brainlab AG, Feldkirchen, Germany), target lesions and important anatomical structures were coded as colored objects. Diffusion tensor imaging and functional MR imaging were performed to visualize important tract and functional areas (pyramidal tract, motor cortex, and speech area). If the tumor was located in or around the pyramidal tract and or motor cortex, electrophysiological monitoring such as motor evoked potential (MEP) monitoring was performed by transcortical stimulation using reduction to <50% amplitude compared to before tumor resection as the warning level. If the surgeon considered that the goals of surgery had been met or the risk of injury to a functional area or tract was high, iMR imaging was performed. If iMR imaging indicated incomplete resection, the surgical planning was revised according to the newly obtained images, and the surgical procedure was resumed based on the updated navigation information.

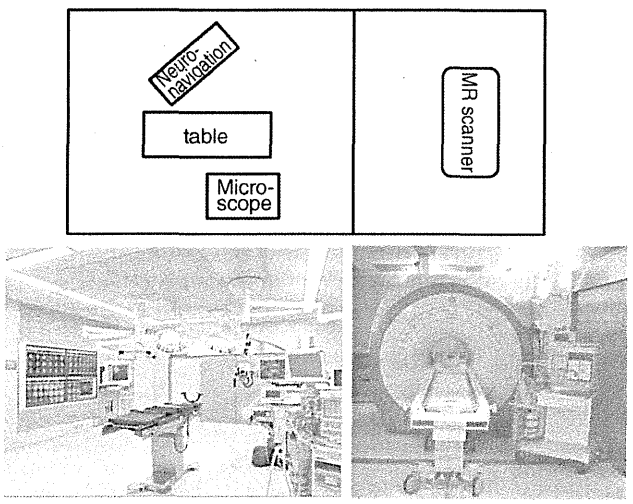

Fig. 1 Layout and photographs of the Surgical Suite®. A magnetically shielded sliding door separates the magnetic resonance (MR) imaging room (*right*) from the operation room (*left*).

The extent of resection (EOR) was determined by comparing the MR images obtained before surgery with those obtained within 72 hours after surgery. EOR was calculated based on manual segmentation of the tumor outline in the planning software. Glioma volume was defined as the volume of increased intensity on T_1-weighted imaging with gadolinium. Tumor volume for non-enhanced tumors was defined as the area of increased intensity on T_2-weighted imaging. Subtotal or greater resection was defined as a postoperative finding of a >95% reduction in tumor volume.

Results

iMR imaging was performed 225 times during the 202 procedures. The period of interruption for each intraoperative MR imaging session was about 1 hour. The results of iMR imaging affected the surgical strategy in 33 of these 202 cases, including strategies for 25 of 73 gliomas (34%), 3 of 35 pituitary adenomas (8.6%), and 5 of 57 others. Additional removal after iMR imaging was performed in over 50% of cases of non-enhanced tumors and recurrent lesions. Gross total resection was achieved in 66% of 73 glioma cases (n = 48).

Volumetric analysis of primary supratentorial gliomas (n = 40) found that mean initial tumor volumes were 54.9 cm^3 (range 1.2–160.0 cm^3) in

Table 1 Summary of volumetric analysis in primary supratentorial gliomas

	LGG	HGG	All	HGG by Sanai et al.[9]
Number of patients	7	33	40	500
Gd enhancement (+/−)	2/5	29/4	31/9	—
Tumor volume (cm^3)*	18.3 (1.0–49.0)	54.9 (1.2–160.0)	48.4 (1.0–160.0)	65.8 (0.3–476.1)
Additional removal (+)	2 (33%)	9 (27%)	11 (32%)	—
Additional removal volume (cm^3)*	3.9 (0.4–7.3)	2.4 (0.3–8.0)	2.6 (0.3–8.0)	—
Residual tumor volume (cm^3)*	4.6 (6.5–12.7)	2.3 (0.8–25.5)	2.6 (0.8–25.5)	2.3 (0–80)
Intraoperative resection rate*	80.5% (40.7–100)	93.5% (34.5–100)	91.7% (34.5–100)	—
Resection rate*	83.2% (40.7–100)	95.1% (34.5–100)	93.5% (34.5–100)	96% (10–100)

*Values are mean (range). Gd: gadolinium, HGG: high-grade glioma, LGG: low-grade glioma.

high-grade glioma (HGG) and 18.3 cm^3 (range 1.0–49.0 cm^3) in low-grade glioma (LGG). The volume of additional removal after iMR imaging was 2.4 cm^3 (range 0.3–8.0 cm^3) in HGG and 3.9 cm^3 (range 0.4–7.3 cm^3) in LGG. Residual tumor volume was 2.3 cm^3 (range 0.8–25.5 cm^3) in HGG and 4.6 cm^3 (range 6.5–12.7 cm^3) in LGG. The EOR was 95.1% in HGG and 83.2% in LGG (Table 1). In addition, iMR imaging revealed an unexpected brain event in 1 patient (acute subdural hematoma). To evaluate the impact of iMR imaging, we analyzed the relationship between supratentorial glioma surgical staging (Table 2) and the incidence of additional removal after iMR imaging. This surgical staging was proposed by Nomura and Kayama and the Japanese Brain Tumor group in 2004.[5] We analyzed 44 cases of primary supratentorial glioma (4 cases were added following the volumetric analysis): stage 1, n = 0; stage 2, n = 5; stage 3, n = 21; stage 4, n = 12; stage 5, n = 6. No additional removal was performed for cases in stages 1, 2, or 5. Additional removal after iMR imaging achieved subtotal resection in 3 cases (14%) in stage 3 and 3 cases (25%) in stage 4 (Fig. 2).

MEP monitoring was combined with iMR imaging in 32 gliomas. MEP monitoring was successful in 30 cases (94%), and was only unsuccessful in 2 patients with preoperative severe motor weakness. Table 3 shows the results of MEP monitoring and postoperative motor function. Preoperative motor deficits were improved in 4 patients and remained unchanged in 19 patients. Permanent motor deficits were identified in 5 patients, 2 patients with decreased amplitude of MEP and 3 patients with no changes in MEP amplitude. Transient decreases in MEP amplitude were seen in 2 patients. One of these two patients had left temporal glioblastoma and showed transient decreases in MEP amplitude caused by middle cerebral artery compression (Fig. 3). iMR imaging showed no ischemic changes, but diffusion-weighted imaging after surgery clearly showed an ischemic lesion around the left internal capsule.

Table 2 Surgical staging for glioma

Stage	Definition
1	tumor size ≤1 cm or within one gyrus
2	Stage 1 (+1) or tumor size >1 cm to <3 cm
3	Stage 2 (+1) or tumor size >3 cm
4	Stage 3 (+1) or stage 2 (+1+1)
5	Stage 3 (+1+1) or stage 2 (+1+1+1) or multiple lesions, disseminated lesions, extra-CNS lesions

+1: Eloquent area (motor, speech, visual); thalamus, basal ganglia, bilateral lesions; sylvian fissure (insular cortex). CNS: central nervous system. Reproduced with permission from Kayama et al.: [A proposed staging system for glioma surgery]. *No Shinkei Geka Journal* 13: 448–453, 2004 (Japanese),[5] ©2004, The Japanese Congress of Neurological Surgeons.

Discussion

The present study suggests that the best use for iMR imaging and integrated navigation seems to be for glioma surgery. In our series, 34% of glioma cases

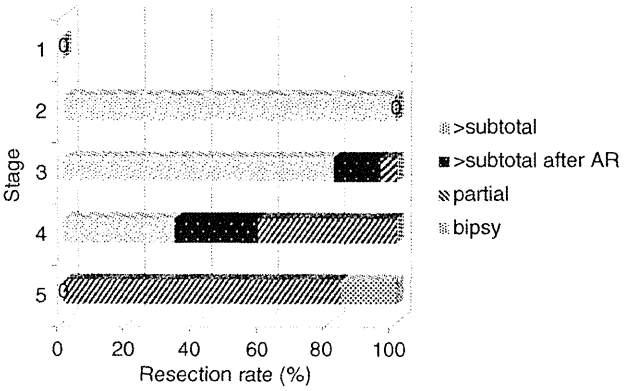

Fig. 2 Surgical staging and resection rate. Tumors in stages 1, 2, and 5 underwent no additional removal (AR) after intraoperative magnetic resonance imaging. AR was performed for 14% of stage 3 cases and 25% of stage 4 cases.

Table 3 Results of intraoperative motor evoked potential (MEP) monitoring and postoperative motor function

MEP	Postoperative motor function				Total
	Improve	No change	Transient aggravation	Permanent aggravation	
No change	3	19	1	3	26
Transient decrease in amplitude	1	0	1	0	2
Permanent decrease in amplitude (<50%)	0	0	0	2	2
Total	4	19	2	5	30

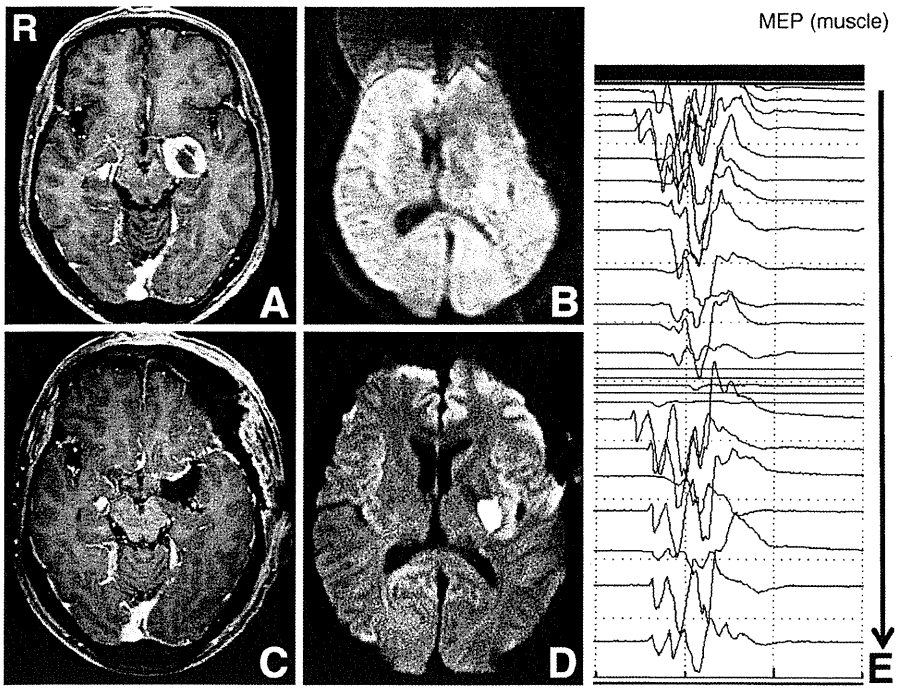

Fig. 3 Representative case of left temporal glioblastoma showing transient decrease in motor evoked potential (MEP) amplitude. A: Preoperative T_1-weighted magnetic resonance (MR) image with gadolinium. B: Intraoperative diffusion-weighted MR image. C: Postoperative T_1-weighted MR image with gadolinium. D: Postoperative diffusion-weighted MR image. E: Temporal changes in response of the thenar muscles. Transient decrease in amplitude occurred during middle cerebral artery compression, but recovered after releasing the compression.

were improved and the EOR was increased following iMR imaging. This modality offers particularly important contributions to modification of surgery in non-enhanced and recurrent tumors. In addition, iMR imaging is a good method to overcome technical problems encountered during surgery, such as unclear margins of microscopic and pathological findings and brain shift. In patients with newly diagnosed glioblastomas, increased EOR parallels improvements in overall survival, even at the highest levels of resection, and subtotal resections as low as 78% confer survival benefits.[9] Our results are similar to these findings (Table 1). In our series, the resection rate of LGG was lower than that of HGG. Three of 7 LGGs were located in eloquent areas, so we intentionally performed partial resection. The resection rates of these cases ranged from 40.7% to 71.9%. Safer resection could be performed for LGG than for HGG considering the long natural history of LGG. Randomized controlled trials of iMR imaging-guided glioma surgery did not demonstrate survival benefits.[10] However, iMR imaging guidance in glioma surgery did help surgeons achieve the optimum EOR.

This study analyzed surgical staging and additional (modified) removal after iMR imaging. For tumors classified in stages 1, 2, or 5, no modification was required after iMR imaging. Stage 5 tumors are not resectable to ensure preservation of brain function. In contrast, tumors in stages 3 and 4 underwent the modification after iMR imaging in 15–25% of cases. Moreover, over 50% of recurrent or non-enhanced tumors underwent modification after iMR imaging. iMR imaging is mainly useful for safety management in patients with stage 1, 2, or 5 lesions. Safety management is one of the important purposes of iMR imaging. In our series, we were able to detect left acute subdural hemorrhage during right temporal glioblastoma removal,[7] and could remove the hematoma immediately after tumor resection. The postoperative course was uneventful and the patient remained alive as of 26 months postoperatively.

Review of iMR imaging-guided resection of glioblastoma pointed out the limitations in the available literature,[6] and also suggested cost as an important outcome parameter. Our Surgical Suite® has separate components in the operating room and MR imaging room. Consequently, the system can be used not only for intraoperative imaging, but also for pre- and postoperative imaging and brain checks for inpatients. We have also tried to use iMR imaging in various neurosurgical operations. T_1-weight-

ed imaging with gadolinium clearly shows residual tumor in the pituitary region, and intraoperative time-resolved contrast-kinetics imaging can reveal complete resection of arteriovenous malformation without the need for conventional catheter angiography.[8]

The present study examined the associations between MEP monitoring and postoperative motor function in 32 patients. MEP monitoring was successful in 30 patients (94%), excluding 2 patients with severe preoperative motor weakness. Preoperative motor deficits recovered in 4 patients, whereas 19 patients showed no changes in motor function. Permanent deficits occurred in 5 patients. We were able to detect decreases in amplitude for 2 patients, but the remaining 3 patients did not show any change in MEP amplitude during surgery. One patient with precentral gyrus glioblastoma suffered motor weakness of the upper limb, and one patient had frontal gliosarcoma. We temporarily clipped the feeding arteries, and checked MEP responses within 20–30 minutes during gliosarcoma removal. We confirmed that MEP responses were unchanged, and then cut the feeder vessels. Postoperative MR imaging revealed an ischemic lesion, including the pyramidal tract. We speculate that the blood supply to the pyramidal tract during surgery was sufficient, but was altered by leptomeningeal anastomosis after surgery. Figure 3 shows the usefulness of MEP monitoring. Compression of the middle cerebral artery caused decrease in MEP amplitude. We were able to prevent permanent deficits based on the warnings provided by MEP monitoring. MEP monitoring is an essential tool for preserving motor function in patients with glioma near the motor cortex or pyramidal tract. We have safely performed 6 awake craniotomies with the use of this iMR imaging system. Combining MEP monitoring with awake craniotomy and iMR imaging appears to offer the most effective method for safe glioma surgery near eloquent areas. Further study of the impact of an iMR imaging system on surgical success and patient survival within the context of a large, prospective, population-based project is needed to confirm the present findings.

The present study shows that iMR imaging provides useful information that allows intraoperative modification of the surgical strategy, and MEP monitoring provides useful information for preserving motor function in patients with gliomas near the primary motor cortex and pyramidal tract. Combined use of iMR imaging, neuronavigation, and MEP monitoring offers the optimal tool for treating brain tumors around the motor cortex or pyramidal tract. This approach could be very helpful for maximizing resection and minimizing morbidity.

References

1) Black PM, Alexander E 3rd, Martin C, Moriarty T, Nabavi A, Wong TZ, Schwartz RB, Jolesz F: Craniotomy for tumor treatment in an intraoperative magnetic resonance imaging unit. *Neurosurgery* 45: 423–430, 1999
2) Black PM, Moriarty T, Alexander E 3rd, Stieg P, Woodard EJ, Gleason PL, Martin CH, Kikinis R, Schwarz RB, Jolesz F: Development and implementation of intraoperative magnetic resonance imaging and its neurosurgical applications. *Neurosurgery* 41: 831–845, 1997
3) Claus EB, Horlacher A, Hsu L, Schwartz RB, Dello-lacono D, Talos F, Jolesz F, Black PM: Survival rates in patients with low-grade glioma after intraoperative magnetic resonance image guide. *Cancer* 103: 1227–1233, 2005
4) Fahlbusch R, Ganslandt O, Buchfelder M, Schott W, Nimsky C: Intraoperative magnetic resonance imaging during transsphenoidal surgery. *J Neurosurg* 95: 381–390, 2001
5) Kayama T, Sonoda Y, Sato S, Fujimaki T, Shibui S, Nomura K: [A proposed staging system for glioma surgery]. *No Shinkei Geka Journal* 13: 448–453, 2004 (Japanese)
6) Kubben PL, ter Meulen KJ, Schijins OE, ter Laak-Poort MP, Van Overbeekr JJ, van Santbrik H: Intraoperative MRI-guided resection of glioblastoma multiforme: a systematic review. *Lancet Oncol* 12: 1062–1070, 2011
7) Sakurada K, Kikuchi Z, Kuge A, Takemura S, Kokubo Y, Sato S, Kayama T: [Detection of acute subdural hemorrhage using intraoperative MR imaging during glioma surgery: case report]. *No Shinkei Geka* 38: 1115–1120, 2010 (Japanese)
8) Sakurada K, Kuge A, Takemura S, Huniu H, Kokubo Y, Kondo R, Sato S, Kayama T: Intraoperative magnetic resonance imaging in the successful surgical treatment of an arteriovenous malformation: case report. *Neurol Med Chir (Tokyo)* 51: 512–514, 2011
9) Sanai N, Polley MY, McDermott MW, Parsa AT, Berger MS: An extent of resection threshold for newly diagnosed glioblastoma. *J Neurosurg* 115: 3–8, 2011
10) Senft C, Bink A, Vatter H, Gasser T, Seifert V: Intraoperative MRI-guidance and extent of resection in glioma surgery: a randomized, controlled trial. *Lancet Oncol* 12: 997–1003, 2011

Address reprint requests to: Kaori Sakurada, MD, PhD, Department of Neurosurgery, Yamagata University Faculty of Medicine, 2-2-2 Iidanishi, Yamagata, Yamagata 990-9585, Japan.

Brain Tumor Pathol
DOI 10.1007/s10014-012-0119-8

ORIGINAL ARTICLE

Clinical and histological characteristics of recurrent oligodendroglial tumors: comparison between primary and recurrent tumors in 18 cases

Masayuki Kanamori · Toshihiro Kumabe · Ichiyo Shibahara · Ryuta Saito · Yoji Yamashita · Yukihiko Sonoda · Hiroyoshi Suzuki · Mika Watanabe · Teiji Tominaga

Received: 29 May 2012 / Accepted: 17 September 2012

Abstract Changes in histological and genetic characteristics were investigated in 18 paired primary and recurrent oligodendroglial tumors, using sequencing analysis for isocitrate dehydrogenase (IDH) 1 and 2 gene mutation, Ki-67 and p53 immunohistochemistry, and fluorescent in situ hybridization for loss of heterozygosity of chromosomes 1p and 19q (1p/19q co-deletion). Malignant transformation occurred in 5 of 8 cases with World Health Organization (WHO) grade II tumors, but in 0 of 10 cases with WHO grade III tumors progressing to glioblastoma. Thirteen of the 18 cases carried IDH1 gene mutation. Tumors with IDH1 mutation tended to survive for longer, even after recurrence, but newly developed microvascular proliferation, tumor necrosis, and elevated Ki-67 labeling index were common. Eleven of the 13 IDH1-mutation tumors had either 1p/19q co-deletion or nuclear expression of p53, but all 5 IDH1/2 wild-type tumors had neither. All cases had the same profile for 1p/19q status at recurrence, but nuclear expression of p53 changed from negative to positive in 2 of 6 cases with IDH1 mutation and 1p/19q co-deletion. WHO grade II oligodendroglial tumors show a high rate of malignant transformation, possibly involving p53 in tumors with IDH1 mutation and 1p/19q co-deletion. Tumors with IDH1 mutation had a more aggressive histological phenotype despite their better prognosis.

Keywords Recurrent oligodendroglial tumor · IDH1/2 gene · p53 · 1p/19q co-deletion · Ki-67 labeling index

M. Kanamori · T. Kumabe (✉) · I. Shibahara · R. Saito · Y. Yamashita · Y. Sonoda · T. Tominaga
Department of Neurosurgery, Tohoku University Graduate School of Medicine, 1-1 Seiryo-machi, Aoba-ku, Sendai 980-8574, Japan
e-mail: kuma@nsg.med.tohoku.ac.jp

H. Suzuki
Department of Pathology and Laboratory Medicine, Sendai Medical Center, Sendai, Japan

M. Watanabe
Department of Pathology, Tohoku University Hospital, Sendai, Japan

Introduction

Oligodendroglial tumors, including oligodendroglioma (OD), oligoastrocytoma (OA), anaplastic oligodendroglioma (AOD), and anaplastic oligoastrocytoma (AOA), account for 6.8 % of all cases of glial tumors in Japan [1]. These entities have better prognoses than astrocytic tumors, but most patients ultimately experience disease progression. The changes in histological diagnosis for the 95 cases treated prospectively in the North Central Cancer Treatment Group study were recently reported [2]. The World Health Organization (WHO) grade increased after recurrence in 51 % of all cases, in 57 % of WHO grade II tumors, and in 13 % of WHO grade III tumors. Additionally, significant changes occurred in the diagnosis of recurrent tumor to pure astrocytic, pure oligodendroglial, and mixed tumor [2]. Therefore, dynamic changes could occur in the histological character of recurrent oligodendroglial tumors.

Changes in genetic aberrations at progression have also been demonstrated. For example, p16 deletions, gain of chromosome 7, and loss of chromosome 10 are considered to be associated with progression [2–4]. Recently, somatic mutations in the isocitrate dehydrogenase 1 (IDH1) gene or IDH2 gene have been found in adult astrocytomas or ODs [5–7]. Oligodendroglial tumors with IDH1 or IDH2

Published online: 02 October 2012

mutations have better prognosis [8–10], and express mutually exclusive loss of heterozygosity of chromosomes 1p and 19q (1p/19q co-deletion) or p53 mutations [11]. In contrast, IDH1/2 wild-type tumors have poor prognosis and show no 1p/19q co-deletion. The mechanisms of these histological changes—or how these representative genetic aberrations are associated with tumor progression in IDH1 or IDH2 mutated and wild-type recurrent oligodendroglial tumors—remain unclear.

The present study retrospectively reviewed the clinical and histological findings of recurrent oligodendroglial tumors to clarify the dynamic changes in all recurrent oligodendroglial tumors, and analyzed the histological and genetic characteristics of recurrent tumors with IDH1 or IDH2 mutations, those with IDH1/2 wild-type genes, and those with 1p/19q co-deleted or retained.

Materials and methods

Patients

Our facility treated 123 patients with histologically verified oligodendroglial tumors between April 1978 and July 2011. The histological diagnosis was established by two neuropathologists (H.S. and M.W.) using current WHO criteria [12]. However, the definitive criteria for glioblastoma with oligodendroglial component (GBMO) remains to be established at this point. In this series, the criteria for GBMO were as follows: the tumor was composed of typical glioblastoma, including marked atypia, high cellularity, pseudopalisading or large necrosis, microvascular proliferation, and an oligodendroglial component. AOA with a small area of tumor necrosis was excluded from GBMO. During this period, 36 patients had recurrent disease, 18 of whom underwent salvage surgery. We reviewed the histological findings of the primary and recurrent tumors. This study was approved by the institutional ethics committee of Tohoku University Graduate School of Medicine, in accordance with the Declaration of Helsinki. Informed consent for molecular and histological analyses of the specimens was obtained from all patients involved in this study.

Analysis of IDH1 and IDH2 gene mutations

Analysis of IDH1 and IDH2 gene mutations was performed as described previously [7]. Genomic deoxyribonucleic acid (DNA) was extracted from snap-frozen tissues, and the DNA fragments including codon 132 of the IDH1 gene and codon 172 of the IDH2 gene were amplified. Direct sequencing analysis of the purified polymerase chain reaction product was performed in an automated DNA analyzer (CEQ 8000; Beckman Coulter, Brea, CA, USA).

Immunohistochemical analysis and fluorescent in situ hybridization (FISH)

Immunohistochemical and FISH analysis was performed as described previously [13]. For immunohistochemical analysis, formalin-fixed, paraffin-embedded tissues were cut into 2 μm thick paraffin sections. The primary antibodies were mouse monoclonal antibody for p53 (Nichirei, Tokyo, Japan; not diluted), and Ki-67 (DAKO, Glostrup, Denmark; 1:300). The cut-off value of the labeling index was 10 % for p53 staining. The Ki-67 labeling index was quantified by counting the number of positive cells among 1000 cells in the regions with the most immunoreactivity. For the FISH analysis of 1p/19q co-deletion, 2 μm thick paraffin-embedded sections were deparaffinized and hybridized with locus-specific probes for 19q13 (SpectrumOrange; Abbott Molecular, Des Plaines, IL, USA), 19p13 (SpectrumGreen; Abbott Molecular), 1p36 (SpectrumOrange), and 1q25 (SpectrumGreen). Nuclei were counterstained with 4′,6-diamino-2-phenylindole and mounted in antifade solution.

Statistical analysis

Continuous variables were compared with Student's *t* test, and categorical variables were compared with the chi-squared test or Fisher's exact test. Kaplan–Meier curves were generated for the progression-free survival rate after initial surgery and the overall survival rate after recurrence. We compared curves for the two groups with the log-rank test. A probability value of less than 0.05 was considered statistically significant. All statistical analyses were performed with Dr. SPSS (SPSS, Chicago, IL, USA).

Results

Demographics of the cases with recurrent oligodendroglial tumors

The 13 male and 5 female patients were aged 29–78 (median 50.5) years at initial treatment. The intervals between surgeries for primary and recurrent tumors ranged from 1 to 234 (median 28) months. For histological diagnosis, debulking surgery was performed in 15 cases and biopsy in 3. The histological diagnosis of primary tumors was OD in 6, OA in 2, AOD in 6, and AOA in 4 (Fig. 1). The strategies for postoperative treatment were described previously [13]. Postoperatively, only radiation therapy was performed in 5 patients, only chemotherapy in 1, a combination of radiation and chemotherapy in 9, and no additional treatment in 3. Radiation therapy consisted of 1.2 Gy twice daily for a total of 72 Gy in 60 fractions to the tumor bed for AOD and AOA, and 2.0 Gy once daily

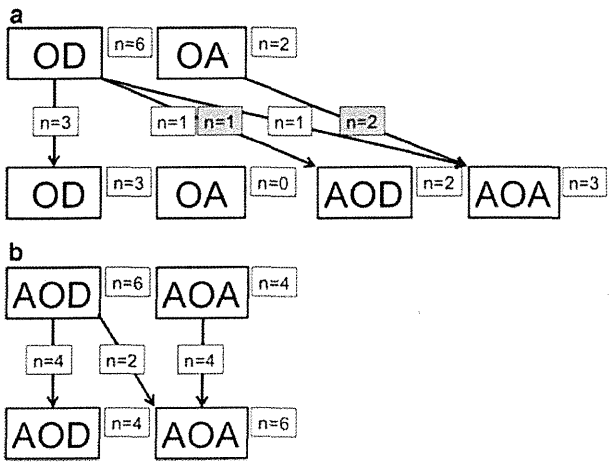

Fig. 1 Changes in the histological diagnoses of primary (*upper row*) and recurrent (*lower row*) oligodendroglial tumors among WHO grade II tumors (**a**) and WHO grade III tumors (**b**). The cases who did not receive any adjuvant therapy are indicated in *gray*. *OD* oligodendroglioma, *OA* oligoastrocytoma, *AOD* anaplastic oligodendroglioma, *AOA* anaplastic oligoastrocytoma

for a total of 60 Gy in 30 fractions for OD and OA. Chemotherapy consisted of nimustine hydrochloride (ACNU) in 7 patients, combination chemotherapy of procarbazine, ACNU, and vincristine in 2, and temozolomide in 1.

The histological diagnosis of the recurrent tumors was OD in 3 cases, AOD in 6, and AOA in 9 (Fig. 1). As radiation may induce cellular atypia, astrocytoma-like morphology in oligodendroglioma, and endothelial cell atypia mimicking microvascular proliferation [20], we diagnosed a malignant transformation when newly developed glomeruloid or epithelioid microvascular proliferation or brisk mitosis (>6 mitosis/10 high power fields) was found. Malignant transformation occurred in 5 of 8 cases (63 %) with WHO grade II tumors (Fig. 1a). These 5 cases had newly developed definitive microvascular proliferation. All of the cases had brisk mitosis. The changes in the histological diagnosis of primary and recurrent tumors were OD to AOD in 2 cases, OD to AOA in 1, and OA to AOA in 2 (Fig. 1a). Initial treatment after surgery was no treatment in 3 cases (Fig. 2) and only radiation therapy in 2

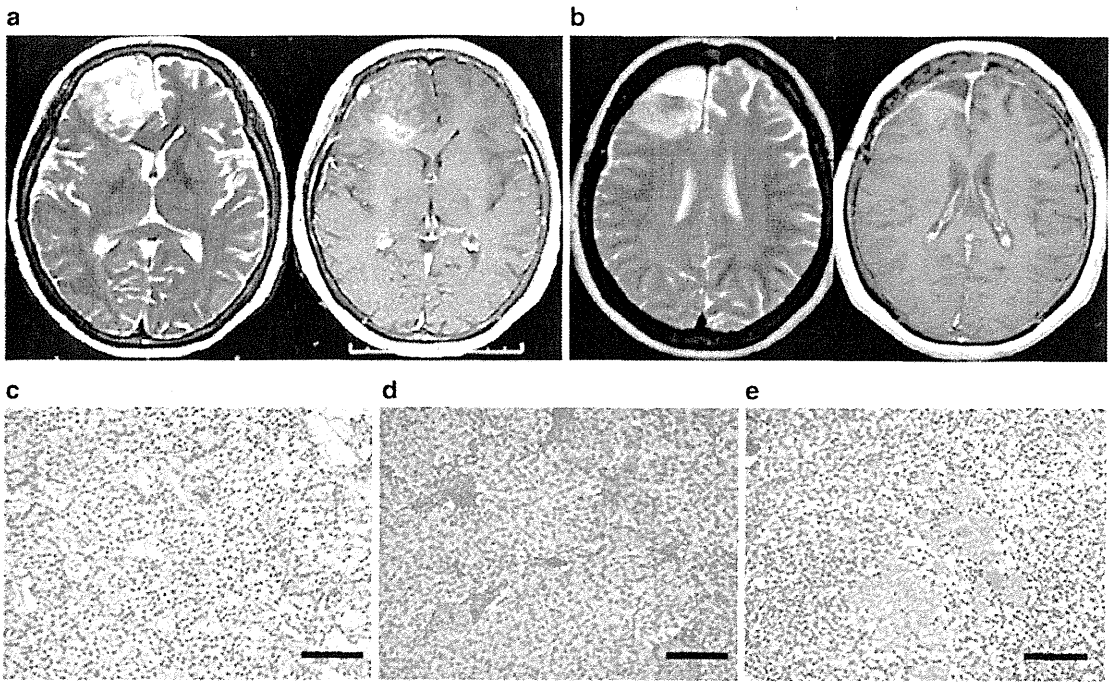

Fig. 2 Representative case with malignant transformation in a 41-year-old woman with right oligodendroglioma in the right frontal lobe. T2-weighted (*left*) and T1-weighted with gadolinium (*right*) magnetic resonance (MR) images at first presentation (**a**), demonstrating that the tumor had a distinct border and slight enhancement. Primary tumor tissues obtained from resection of the tumor consisted of round to oval neoplastic cells with a perinuclear halo but without microvascular proliferation or necrosis, and the diagnosis was oligodendroglioma (**c**). Analysis for IDH1 mutation revealed the tumor had the IDH1 R132H mutation. She did not receive any adjuvant treatments. T2-weighted (*left*) and T1-weighted with gadolinium (*right*) MR images obtained 45 months after the first surgery (**b**), demonstrating that local recurrence developed with slight enhancement. She underwent salvage surgery. Histological examination of recurrent tumor tissues obtained from resection of the tumor revealed that nuclear atypia and cellularity had not increased at recurrence, but new microvascular proliferation (**d**) and tumor necrosis (**e**) had developed. The recurrent tumor was diagnosed as anaplastic oligodendroglioma. ×200. *Bar* = 100 μm

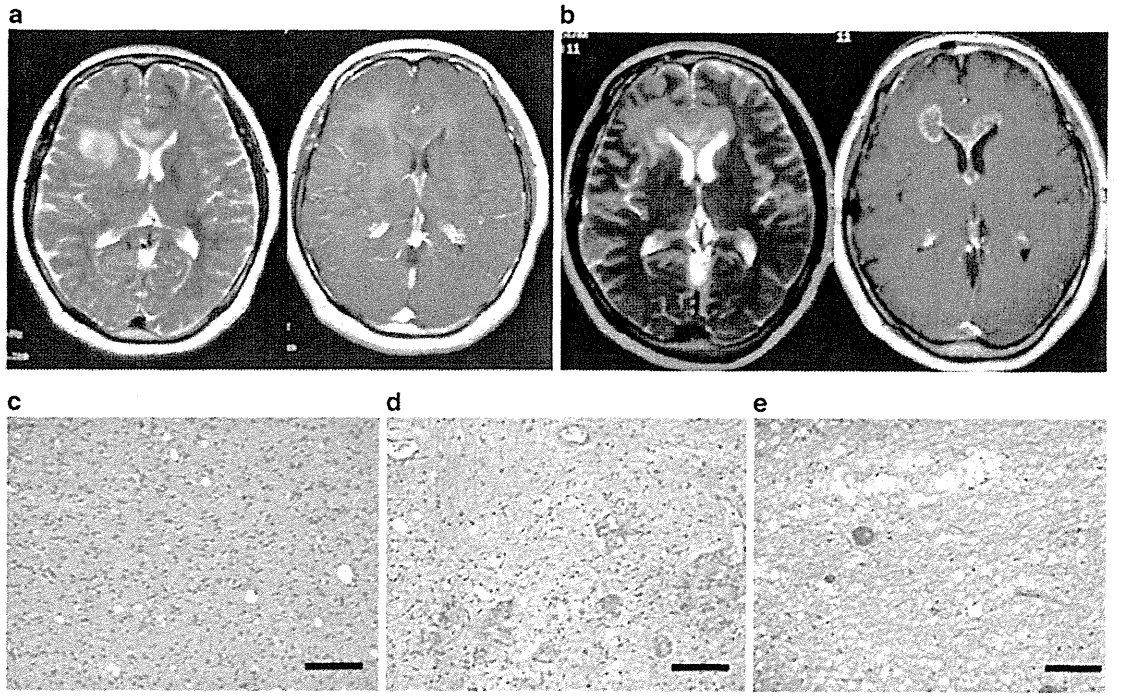

Fig. 3 Representative case with malignant transformation in a 53-year-old woman with oligodendroglioma in the right frontal lobe and corpus callosum. T2-weighted (*left*) and T1-weighted with gadolinium (*right*) magnetic resonance (MR) images at first presentation (**a**), demonstrating that the tumor had an ill-defined tumor border extending to the corpus callosum without enhancement. Primary tumor obtained from open biopsy consisted of round cells with a perinuclear halo but without microvascular proliferation or necrosis, and the diagnosis was oligodendroglioma (**c**). Analysis of IDH1 mutation revealed that the tumor had wild-type IDH1 and IDH2 genes. She received 60 Gy of radiation therapy to the tumor bed. T2-weighted (*left*) and T1-weighted with gadolinium (*right*) MR images obtained 14 months after the first surgery (**b**), demonstrating a strongly enhanced lesion in the right frontal lobe and corpus callosum. Histological examination of recurrent tumor tissues revealed new microvascular proliferations (**d**) without significant changes in cellularity and nuclear atypia. The recurrent tumor was diagnosed as anaplastic oligodendroglioma. Treatment-related coagulation necroses were also observed (**e**). ×200. *Bar* = 100 μm

(Fig. 3). Time to progression was 14, 26, 45, 129, and 236 months, respectively. Newly developed findings of tumor necrosis were present in 4 cases and microvascular proliferation in 5 (Figs. 2, 3). Similar to WHO grade III tumors, it is difficult to diagnose the recurrent tumors after adjuvant therapy as glioblastoma. Our criteria for glioblastoma at recurrence include the existence of astrocytic tumor cells with marked atypia, typical pseudopalisading necrosis. In contrast to the high incidence of malignant transformation in WHO grade II to grade III oligodendroglial tumors, no tumors progressed to glioblastoma or GBMO among the WHO grade III tumors (Fig. 1b). Eight of the 10 WHO grade III tumors had the same histological diagnosis, except for 2 cases with emergence of an astrocytic component (Fig. 1b).

Clinical characteristics of recurrent tumor with or without IDH1 mutation

Analysis for mutation of the IDH1 and IDH2 genes revealed that 13 of the 18 tumors carried mutation in the IDH1 gene. The clinical characteristics are shown in Table 1. The age of onset was younger in the cases with IDH1 mutation ($p = 0.062$). Kaplan–Meier analysis revealed that time to progression was statistically longer in

Table 1 Clinical characteristics of 18 recurrent oligodendroglial tumors with or without IDH1/2 mutation

	IDH1 mutation tumors ($n = 13$)	IDH1/2 wild-type tumors ($n = 5$)	p value
Age at initial presentation (median)	29–70 (41)	52–78 (53)	0.062
Sex (male:female)	9:4	4:1	0.67
Salvage therapy after recurrence			0.99
Radiation therapy or GKS	1	0	
Chemotherapy	9	4	
Both	3	1	
Second recurrence			0.575
Local	5	2	
Dissemination	3	2	

GKS gamma knife radiosurgery

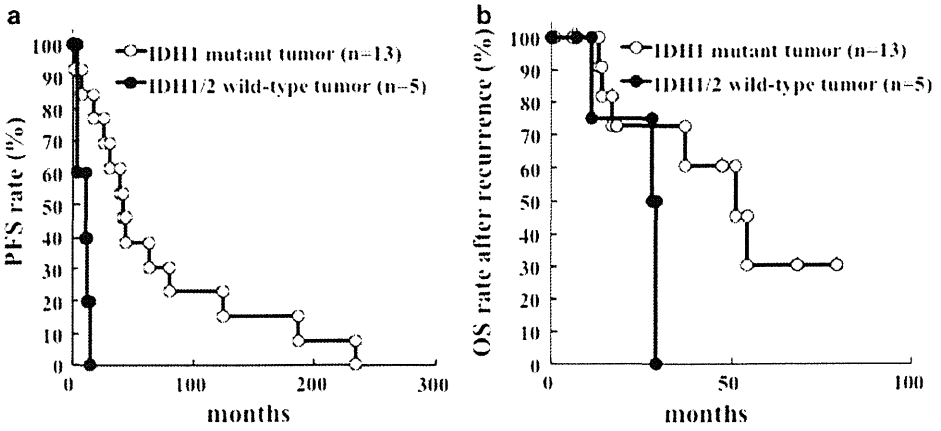

Fig. 4 Kaplan–Meier analysis demonstrating the progression-free survival (PFS) rate after initial surgery (**a**) and the overall survival (OS) rate after first recurrence (**b**) in patients with IDH1-mutation and IDH1/2 wild-type tumors

Table 2 Changes in histological findings for recurrent oligodendroglial tumors with or without IDH1/2 mutation

	IDH1 mutation tumor (n = 13)		IDH1/2 wild-type tumor (n = 5)	
	Primary	Recurrent	Primary	Recurrent
Histological diagnosis				
OD	3	1	3	2
OA	2	0	0	0
AOD	5	4	1	2
AOA	3	8	1	1
Necrosis				
Tumor necrosis	3	7	0	1
Coagulation necrosis	0	2	0	3
Absent	10	4	5	1
Microvascular proliferation				
Present	6	11	2	2
Absent	7	2	3	3
Ki-67 labeling index (mean) (%)	1.1–42.5 (17.5)[a]	3.3–62.6 (36.3)[a,b]	4.5–13.6 (13.4)	1.3–34.8 (9.2)[b]

OD oligodendroglioma, *OA* oligoastrocytoma, *AOD* anaplastic oligodendroglioma, *AOA* anaplastic oligoastrocytoma

[a,b] $p < 0.05$

IDH1-mutation tumors than in wild-type tumors (median 41 vs. 14 months, $p < 0.005$) (Fig. 4a). After recurrence, all patients received salvage surgery and salvage chemotherapy and/or radiation therapy (Table 1). The patterns of second failure were similar for the IDH1-mutation and IDH1/2 wild-type tumors (Table 1). However, the IDH1-mutation tumor still had the tendency for better prognosis (median 51 vs. 28 months, $p = 0.16$) (Fig. 4b). Six of 13 patients with IDH1-mutation tumor survived for more than 3 years after recurrence, as compared to none with IDH1/2 wild-type tumor (Fig. 4b).

To investigate the better prognosis for recurrent tumors with IDH1 mutation, the histological characteristics and immunohistochemistry for Ki-67 were examined. Contrary to expectations, malignant transformation occurred in 4 of 5 WHO grade II tumors with IDH1 mutations, and in 1 of 3 WHO grade II tumors with wild-type IDH1/2 (Table 2). Newly developed microvascular proliferation and/or tumor necrosis were found in 5 recurrent tumors with IDH1 mutation (Fig. 2; Table 2). Coagulation necrosis, caused by radiation therapy and/or chemotherapy, was predominantly found in 3 of 5 recurrent tumors with wild-type IDH1/2 (Fig. 3; Table 2). Immunohistochemical analysis showed that the Ki-67 labeling index was significantly higher in recurrent tumors with IDH1 mutation than IDH1-mutated primary tumor ($p = 0.012$) and IDH1/2 wild-type recurrent tumors ($p = 0.016$) (Table 2).

Clinical characteristics of recurrent tumor with or without 1p/19q co-deletion

Alternatively, we analyzed the correlations between 1p/19q co-deletions and prognosis in all of the tumors examined and in tumors with IDH1 mutation. Kaplan–Meier analysis revealed that time to progression was longer in 1p/19q co-deleted tumors than in 1p/19q retained tumors (median 63 vs. 12 months, $p < 0.05$, all tumor; median 63 vs. 30 months, $p = 0.077$, tumor with IDH1 mutation). However, when the prognostic value of 1p/19q co-deletions was analyzed in relation to overall survival after recurrence, there was no significant difference between 1p/19q co-deleted and 1p/19q retained tumors (median 51 vs. 28 months, $p = 0.43$, all tumor; median 51 months vs. not reached, $p = 0.90$, tumor with IDH1 mutation). In contrast to the mutation status of IDH1, the rate of malignant transformation and changes in Ki-67 labeling index were not different between 1p/19q co-deleted and 1p/19q retained tumors in all tumors and in tumors with IDH1 mutation.

Changes in nuclear expression of p53 and 1p/19q co-deletions in IDH1-mutation and IDH1/2 wild-type tumors

To explore the genetic changes leading to tumor progression, nuclear expression of p53 and 1p/19q co-deletion was examined in primary and recurrent oligodendroglial tumors. Nuclear expression of p53 and 1p/19q co-deletion were found only in primary tumors with IDH1 mutation in a mutually exclusive fashion, whereas tumors without IDH1 mutation did not have either of them (Fig. 5). Changes of negative to positive nuclear expression of p53 in recurrent tumors occurred in 2 of 6 IDH1-mutation tumors with 1p/19q co-deletions and without nuclear expression of p53 in the primary tumors (Figs. 5, 6, 7). One case had a newly developed astrocytic component at recurrence (Fig. 6), and the other had predominant growth of the oligodendroglial component (Fig. 7). 1p/19q co-deletion was found in 6 of the 18 primary tumors. All cases had the same profile for 1p/19q status (Figs. 5, 6, 7).

Discussion

We reported previously that a significant number of patients with oligodendroglial tumors developed disseminated recurrent disease [13]. Salvage debulking surgery could be performed in 18 of the 36 patients with recurrent oligodendroglial tumors, whereas 14 patients developed disseminated disease at first recurrence. In this study, we focused on the cases of resectable local recurrent disease and reviewed the changes in the histological and genetic findings for recurrent oligodendroglial tumors.

Malignant transformation occurred in 63 % of WHO grade II tumors. Similar incidences of malignant transformation have been reported in WHO grade II tumors [2]. The prospective European Organisation for Research and Treatment of Cancer 22845 study compared the efficacy and safety of postoperative early radiation therapy against WHO grade II tumors to those of postoperative policies of "wait and see" [14]. In that cohort, malignant transformation occurred in 66 % of the wait and see group and in 72 % of the early irradiated group (no significant difference between the groups), and no apparent associations were found between early radiation therapy and malignant transformation. Similarly, 3 of the 5 of our patients with malignant transformation at recurrence did not receive radiation therapy at initial treatment.

Malignant transformation to glioblastoma has been controversial. In contrast to our findings, 18.1 % of AOA and 22.5 % of OA, but none of AOD or OD, were reported to transform to glioblastoma at recurrence [2]. Such differences could be attributed to the diagnostic criteria of AOA and glioblastoma. A new entity of GBMO has been proposed [12, 15, 16], defined as glioblastoma with foci that resemble OD [12]. Based on observations suggesting that the presence of necrosis is associated with worse prognosis in AOA [17], AOA with necrosis was classified as GBMO in the WHO classification [12]. GBMO has better prognosis than "classic" glioblastoma and worse prognosis than AOD, anaplastic astrocytoma, and AOA without necrosis [17]. Although AOA with necrosis could be an entity independent of other tumors, no definite criteria have been established to differentiate GBMO, AOA with or without necrosis, and glioblastoma [18] in primary tumors. Furthermore, the differential diagnosis of this

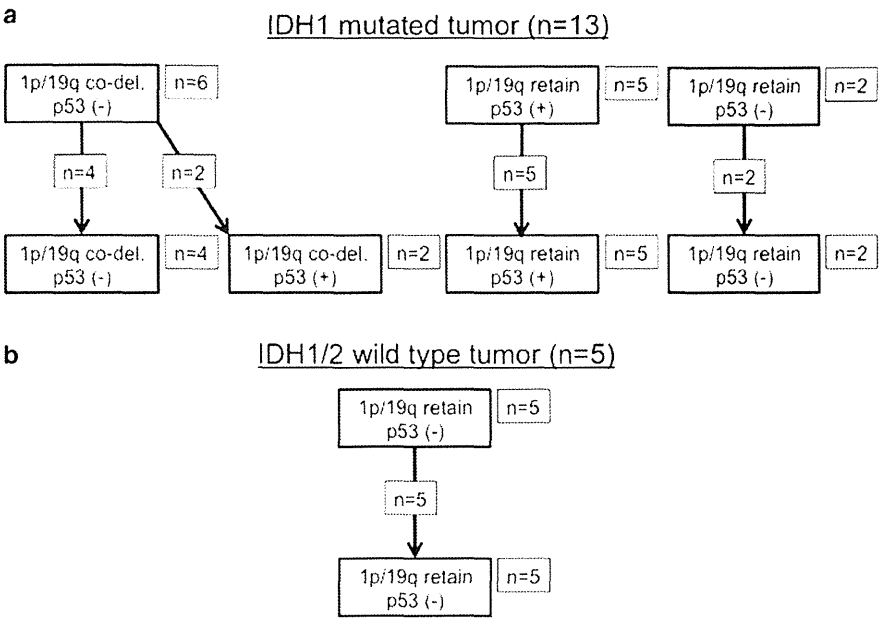

Fig. 5 Changes in the genetic mutations of primary and recurrent oligodendroglial tumors with (**a**) or without (**b**) IDH1 mutation. *1p/19 co-del.* loss of heterozygosity of chromosomes 1p and 19q, *1p/19q retain* retention of heterozygosity of chromosomes 1p and 19q, *p53* (+) positive for nuclear expression of p53, *p53* (−) negative for nuclear expression of p53

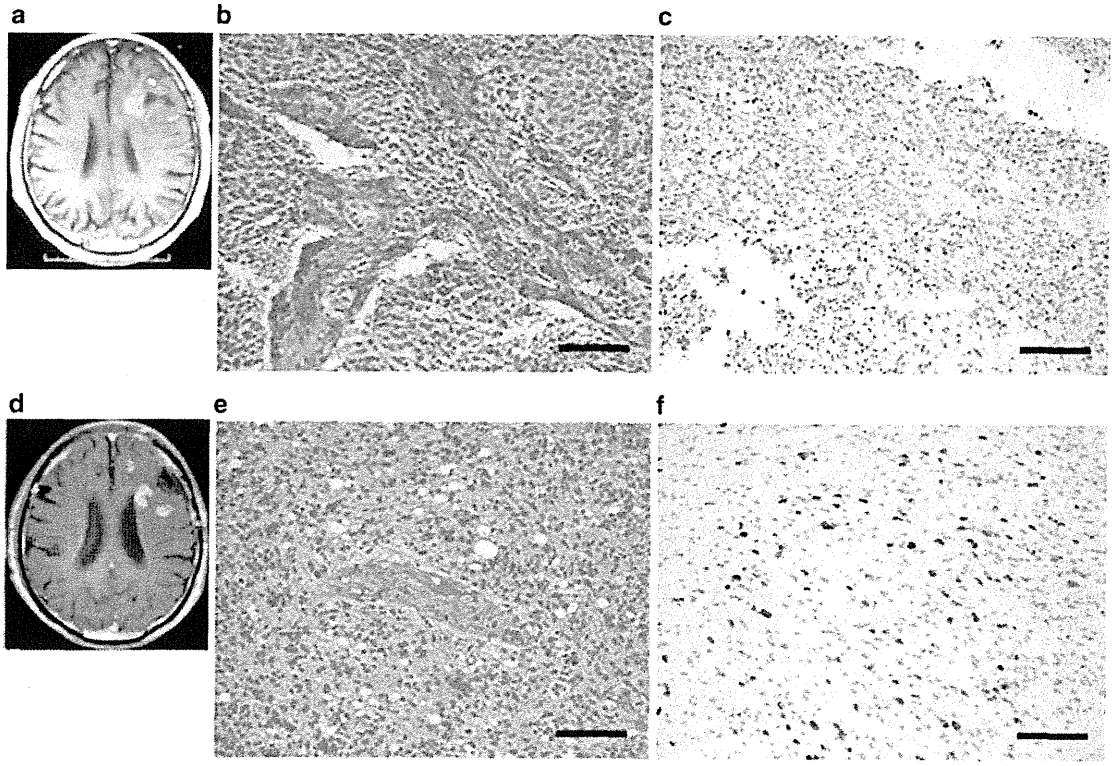

Fig. 6 Representative case of nuclear expression of p53 changing from negative to positive at recurrence in a 70-year-old man with anaplastic oligodendroglioma in the left frontal lobe. T1-weighted magnetic resonance (MR) image with gadolinium at first presentation (**a**), demonstrating heterogeneous enhancement of the tumor. Primary tumor tissues obtained from resection of the tumor consisted of round to oval neoplastic cells with a perinuclear halo, significant nuclear atypia, and increased cellularity. Microvascular proliferations were also found (**b**). Immunohistochemistry for p53 revealed that tumor cells were negative for p53 (**c**). Analysis for IDH1 mutation and fluorescent in situ hybridization for chromosomes 1p and 19q revealed that the tumor had both IDH1 mutation and 1p/19q co-deletion. The diagnosis was anaplastic oligodendroglioma. He received 72 Gy of hyperfractionated radiation therapy to the tumor bed. T1-weighted MR image with gadolinium obtained 45 months after the first surgery (**d**), demonstrating that local recurrence developed with slight enhancement. He underwent salvage surgery. Emergence of an astrocytic component, as well as higher cellularity and the development of tumor necrosis, was found (**e**). Immunohistochemistry revealed that nuclear expression of p53 had changed from negative to positive at recurrence (**f**), whereas the recurrent tumor had 1p/19q co-deletion. The diagnosis was anaplastic oligoastrocytoma. ×200. *Bar* = 100 μm

entity was more confusing in recurrent tumors because prior treatment could cause coagulation necrosis due to ischemia through chemotherapy and radiation therapy associated vascular damage. We agree that it is sometimes difficult to distinguish the ischemic coagulation necrosis associated with vascular damage from the so-called "tumor necrosis" that occurs spontaneously due to the relative hypoxia caused by high proliferative potential. In this study, we considered "tumor necrosis" to consist of "dirty-looking" necrobiotic neoplastic cells surrounded by the tumor cells with high viability, and "coagulation necrosis" to consist of homogeneously necrotic ghost cells that do not appear to be surrounded by viable tumor cells. Based on this classification, 5 cases had recurrent disease of AOA with newly developed tumor necrosis. Two of them did not receive any adjuvant therapy, and the remaining 3 underwent radiochemotherapy. The primary tumors were OD in 1, OA in 2, AOD in 1, and AOA without necrosis in 1. Whether AOA with newly developed tumor necrosis should be regarded as a malignant transformation or not is an important consideration when assessing the effects of prior treatments and scheduling salvage treatments in such cases. On the contrary, we found coagulation necrosis in 5 recurrent tumors, and all of them received radiation therapy before recurrence. Initial diagnosis was OD in 2 and AOD in 3. One case with OD developed glomeruloid microvascular proliferation with coagulation necrosis at recurrence, and was diagnosed as a malignant transformation from WHO grade II to III tumor—not to glioblastoma, because we did not find pseudopalisading or tumor necrosis, or an astrocytic component. Similarly, the remaining 4 cases did not have these features either, so these cases received the same histological diagnosis as the primary tumors: not glioblastoma or GBMO.

Oligodendroglial tumors carrying IDH1 or IDH2 mutations have longer progression-free survival and overall

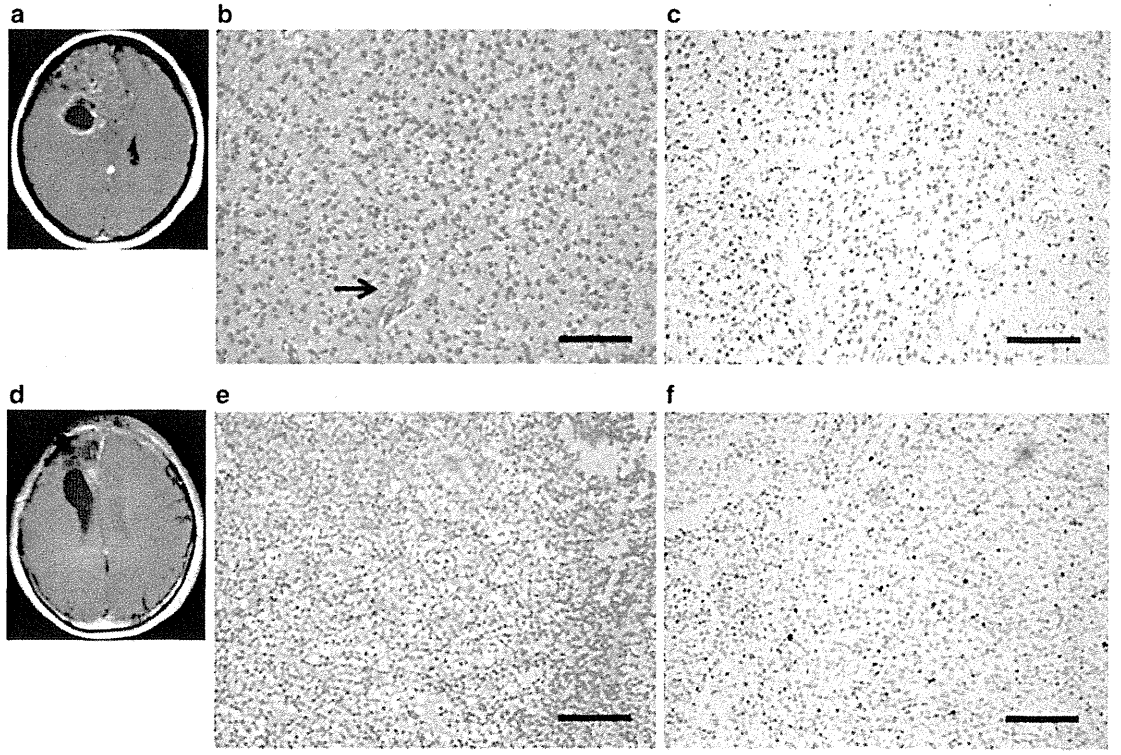

Fig. 7 Representative case of nuclear expression of p53 changing from negative to positive at recurrence in a 40-year-old woman with anaplastic oligoastrocytoma in the right frontal lobe. T1-weighted magnetic resonance (MR) image with gadolinium at first presentation (**a**), demonstrating heterogeneous enhancement of the tumor with cyst formation. Primary tumor tissues obtained from tumor resection consisted of round cells with a perinuclear halo and gemistocytic neoplastic astrocytes. Microvascular proliferation was also found (**b** *arrow*). Immunohistochemistry for p53 revealed that the tumor cells were negative for p53 (**c**). Analysis for IDH1 mutation and fluorescent in situ hybridization for chromosomes 1p and 19q revealed that the tumor had both IDH1 mutation and 1p/19q co-deletion. The diagnosis was anaplastic oligoastrocytoma. She received 72 Gy of hyperfractionated radiation therapy to the tumor bed and administration of nimustine hydrochloride. T1-weighted MR image with gadolinium obtained 95 months after the first surgery (**d**), demonstrating that local recurrence had developed with enhancement. Increase in the oligodendroglial component was found (**e**). Immunohistochemistry revealed that nuclear expression of p53 changed from negative to positive at recurrence (**f**), whereas the recurrent tumor had 1p/19q co-deletion. The diagnosis was anaplastic oligoastrocytoma. ×200. *Bar* = 100 μm

survival rates [8–10]. We found recurrent tumors with IDH1 mutation to have a long latency to progression, a higher rate of malignant transformation, and elevated proliferation activities. Despite such histological features, recurrent tumors with IDH1 mutation tended to have better outcome. In contrast, IDH1/2 wild-type tumors frequently showed mixtures of coagulation necrosis and degenerated tumor or normal cell tumor, and low proliferation activities, possibly because IDH1/2 wild-type recurrent tumors progressed early after initial treatment, before the effect disappeared. Considering the absence of apparent differences in salvage therapies and patterns of failure, tumors with IDH1 mutation could remain less aggressive or more sensitive to treatment, even at recurrence.

We explored the changes in the representative genetic aberrations, including nuclear expression of p53 and 1p/19q co-deletions, to clarify the molecular mechanisms leading to tumor progression in IDH1-mutation or IDH1/2 wild-type tumors. Previously, changes in chromosomal aberrations have been reported [3, 4]. Tumor with 1p/19q co-deletion demonstrated the same aberrations in recurrent tumor, whereas recurrent tumor frequently showed increased polysomies, rather than any specific chromosomal changes, and deletions of p16 loci [3]. In our series, no changes in 1p/19q status were found at recurrence. However, nuclear expression of p53 changed from negative to positive in 2 IDH1-mutation tumors. WHO grade II gliomas carrying IDH mutation had either 1p/19q or aberrant nuclear expression of p53, and these aberrations were mutually exclusive. We found that 11 of 13 primary tumors with IDH1 mutation carried one of these aberrations exclusively. Interestingly, 2 tumors with both IDH1 mutation and 1p/19q co-deletion also showed aberration of nuclear expression of p53 at recurrence. This finding suggests that de novo p53 mutation or the proliferation of a small subset of cells with nuclear expression of p53 could lead to tumor progression in some IDH1-mutation oligodendroglial tumors. The biphasic type of AOA consists of

distinct areas with 1p/19q co-deletion and nuclear expression of p53 [19]. We found that a small subset of tumors contain cells with nuclear expression of p53, as shown in Figs. 5 and 6. Therefore, we presume that small numbers of the cells with aberrant function of p53 proliferated in the process of IDH1-mutation tumor recurrence.

In conclusion, this study found that a high rate of malignant transformation occurred in WHO grade II oligodendroglial tumors at recurrence, which may involve p53 in tumors with IDH1 mutation and 1p/19q co-deletion. Recurrent tumors with IDH1 mutation had a more aggressive histological phenotype despite their better prognosis.

References

1. Committee of Brain Tumor Registry of Japan (2009) Report of Brain Tumor Registry of Japan (1984–2000), 12th edition. Neurol Med Chir (Tokyo) 49 Suppl:i–vii, 1–101
2. Jaeckle KA, Decker PA, Ballman KV et al (2011) Transformation of low grade glioma and correlation with outcome: an NCCTG database analysis. J Neurooncol 104:253–259
3. Fallon KB, Palmer CA, Roth KA et al (2004) Prognostic value of 1p, 19q, 9p, 10q, and EGFR-FISH analyses in recurrent oligodendrogliomas. J Neuropathol Exp Neurol 63:314–322
4. Campbell BA, Horsman DE, Maguire J et al (2008) Chromosomal alterations in oligodendroglial tumours over multiple surgeries: is tumour progression associated with change in 1p/19q status? J Neurooncol 89:37–45
5. Balss J, Meyer J, Mueller W, Korshunov A, Hartmann C, von Deimling A (2008) Analysis of the IDH1 codon 132 mutation in brain tumors. Acta Neuropathol 116:597–602
6. Parsons DW, Jones S, Zhang X et al (2008) An integrated genomic analysis of human glioblastoma multiforme. Science 321:1807–1812
7. Sonoda Y, Kumabe T, Nakamura T et al (2009) Analysis of IDH1 and IDH2 mutations in Japanese glioma patients. Cancer Sci 100:1996–1998
8. Metellus P, Coulibaly B, Colin C et al (2010) Absence of IDH mutation identifies a novel radiologic and molecular subtype of WHO grade II gliomas with dismal prognosis. Acta Neuropathol 120:719–729
9. Li S, Yan C, Huang L, Qiu X, Wang Z, Jiang T (2012) Molecular prognostic factors of anaplastic oligodendroglial tumors and its relationship: a single institutional review of 77 patients from China. Neuro Oncol 14:109–116
10. van den Bent MJ, Dubbink HJ, Marie Y et al (2010) IDH1 and IDH2 mutations are prognostic but not predictive for outcome in anaplastic oligodendroglial tumors: a report of the European Organization for Research and Treatment of Cancer Brain Tumor Group. Clin Cancer Res 16:1597–1604
11. Ichimura K, Pearson DM, Kocialkowski S et al (2009) IDH1 mutations are present in the majority of common adult gliomas but rare in primary glioblastomas. Neuro Oncol 11:341–347
12. Louis DN, Ohgaki H, Wiestler OD, Cavenee WK (2007) WHO classification of tumours of the central nervous system. IARC, Lyon
13. Kanamori M, Kumabe T, Sonoda Y, Nishino Y, Watanabe M, Tominaga T (2009) Predictive factors for overall and progression-free survival, and dissemination in oligodendroglial tumors. J Neurooncol 93:219–228
14. van den Bent MJ, Afra D, de Witte O et al (2005) EORTC Radiotherapy and Brain Tumor Groups and the UK Medical Research Council. Long-term efficacy of early versus delayed radiotherapy for low-grade astrocytoma and oligodendroglioma in adults: the EORTC 22845 randomised trial. Lancet 366: 985–990
15. Kraus JA, Lamszus K, Glesmann N et al (2001) Molecular genetic alterations in glioblastomas with oligodendroglial component. Acta Neuropathol 101:311–320
16. He J, Mokhtari K, Sanson M et al (2001) Glioblastomas with an oligodendroglial component: a pathological and molecular study. J Neuropathol Exp Neurol 60:863–871
17. Miller CR, Dunham CP, Scheithauer BW, Perry A (2006) Significance of necrosis in grading of oligodendroglial neoplasms: a clinicopathologic and genetic study of newly diagnosed high-grade gliomas. J Clin Oncol 24:5419–5426
18. Marucci G (2011) The effect of WHO reclassification of necrotic anaplastic oligoastrocytomas on incidence and survival in glioblastoma. J Neurooncol 104:621–622
19. Qu M, Olofsson T, Sigurdardottir S et al (2007) Genetically distinct astrocytic and oligodendroglial components in oligoastrocytomas. Acta Neuropathol 113:129–136
20. Perry A, Brat DJ (2010) Practical surgical neuropathology. Elsevier, Philadelphia, p 421