

# O<sup>6</sup>-methylguanine DNA methyltransferase status determined by promoter methylation and immunohistochemistry in gliosarcoma and their clinical implications

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**Abstract** O<sup>6</sup>-methylguanine-DNA methyltransferase (MGMT) is known as a DNA repair protein, and loss of function in MGMT is related to an increase in survival in patients with malignant gliomas treated with alkylating agents. In the present study, we determined the status of MGMT using methylation-specific polymerase chain reaction (PCR) and immunohistochemistry on paraffin-embedded specimens in 12 human gliosarcomas, and these results were then related to overall survival (OS) and response to alkylating agents. The MGMT promoter was methylated in six patients. Immunostaining of MGMT was positive in 58.3% of patients. MGMT methylation status was

correlated with immunostaining results in five patients (41.7%). The median OS and progression-free survival (PFS) of the whole population were 13.4 months [95% confidence interval (CI), 12.3–14.5 months] and 8.3 months (95% CI, 7.4–9.2 months), respectively. In patients with methylated MGMT promoter, median OS was 15.0 months, compared with 11.3 months in the unmethylated group. Median PFS of gliosarcoma patients was 10.3 months for the methylated group, whereas it was 7.3 months for the unmethylated group. On multivariate analysis, patients with methylated MGMT promoter had better prognosis than patients with unmethylated MGMT promoter with respect to OS and PFS ( $P = 0.045$  and  $0.034$ , respectively). However, there was no statistical significance between MGMT protein expression and survival. The results show that a significant fraction of gliosarcomas have MGMT promoter methylation and protein expression, and suggest that patient survival is associated with MGMT methylation status.

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## Introduction

Gliosarcomas are rare primary brain tumors characterized by a biphasic pattern with glial and mesenchymal components [1]. Known as a variant of glioblastoma multiforme (GBM) [2], gliosarcomas have often been treated in the same manner as GBM, which consists of radical resection, followed by radiotherapy and adjuvant chemotherapy [3–5].

Alkylating agents are the most effective cytotoxic agents in malignant gliomas. The cytotoxic mechanisms of

alkylating agents may be linked to alkylation of DNA bases, formation of cross-bridges, and induction of nucleotide mispair, thus leading to cell death [6, 7]. For example, 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) generates several DNA adducts, including O<sup>6</sup>-methylguanine, which preferentially pairs with thymine and leads to GC-to-AT transitions [8]. In addition, BCNU induces chloroethyl modifications at O<sup>6</sup>, which can lead to interstrand cross-linking [9]. However, alkylating drugs provide no benefit to approximately 50% of patients and do not produce long-term remission [10, 11]. A possible reason for this is the development of drug resistance, one of the major determinants of which is associated with intracellular expression of O<sup>6</sup>-methylguanine-DNA methyltransferase (MGMT), a protein that mediates repair of O<sup>6</sup>-guanine DNA adducts [12]. Several studies have shown that silencing of the gene encoding MGMT by promoter methylation leads to improved survival in patients with glioblastomas receiving alkylating agents such as BCNU and temozolomide [13–16]. In addition, lack of MGMT protein expression based on immunohistochemical assessment has been noted in malignant glioma drug responses [7, 17].

To date, a few studies have reported the relationship between MGMT promoter methylation and survival of patients with gliosarcomas [18]. Of note, these studies included patients with GBM as well as gliosarcomas, and the analysis was not conducted separately according to these two different diseases. Therefore, evidence for the impact of MGMT status on prognosis of gliosarcomas remains inconclusive. In this study, we examined the status of MGMT promoter methylation and protein expression, and assessed their prognostic role in primary gliosarcoma patients who received alkylating agent chemotherapy.

## Patients and methods

### Patients and samples

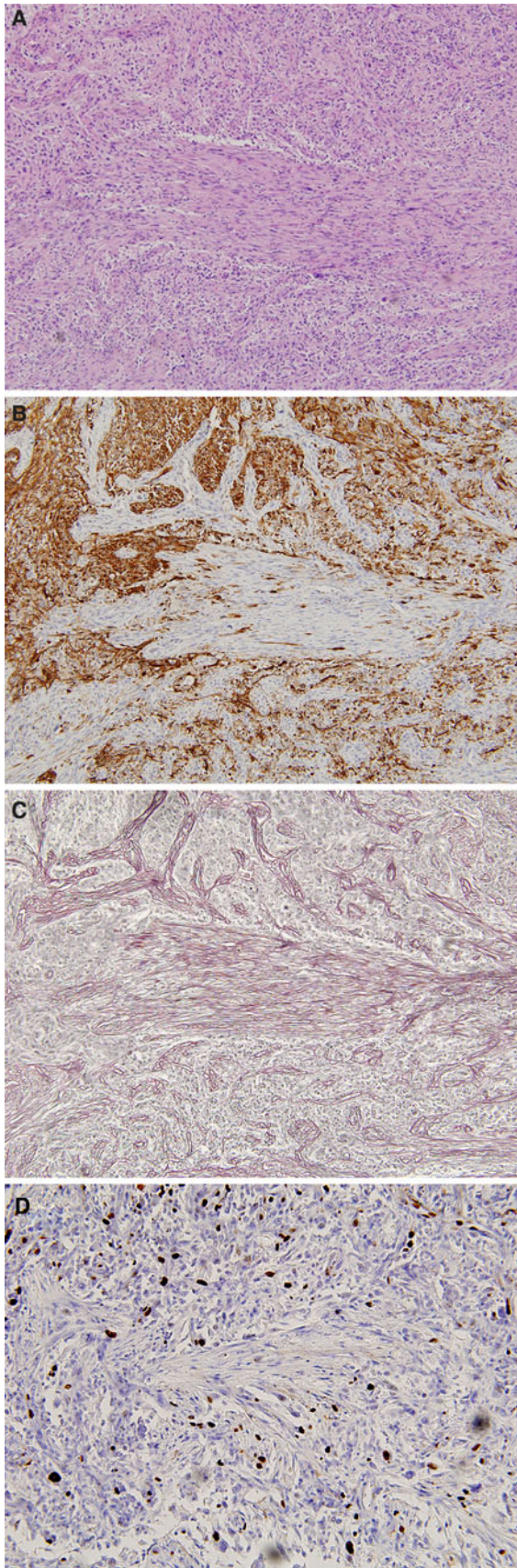
Between 1996 and 2008, a total of 18 patients with gliosarcomas were treated in the Department of Neurosurgery of Korea University Anam Hospital and Seoul National University Hospital. Among the 18 patients, 5 patients who had poorly preserved paraffin blocks and 1 patient with secondary gliosarcoma were excluded. Therefore, 12 patients were enrolled in the present study. The clinical information of the patients was collected from their medical records. All patients underwent surgical resection, the degree of which was categorized as partial, subtotal (>90% of tumor removal), or gross total resection (no distinct residual tumor) based on comparison of pre- and postoperative magnetic resonance (MR) images obtained <72 h after surgery [19]. All patients received postoperative

radiotherapy within 4 weeks of surgery. A combination protocol consisting of procarbazine, lomustine, and vincristine (PCV regimen) [20], or concomitant–adjuvant temozolomide chemotherapy in accordance with the protocol of Stupp et al. [5], was administered to the patients as first-line chemotherapy. After tumor progression, reoperation with or without additional chemotherapy, additional chemotherapy alone, or gamma knife surgery (GKS) was given to the patients.

All tumor samples were obtained from surgical resection and were fixed with formalin and embedded in paraffin. Diagnosis of gliosarcoma was confirmed upon neuropathologic re-examination in all specimens according to the histologic criteria of Meis et al. [21], i.e., the tumor was bimorphic, composed of an astrocytic malignant cell population with necrosis and secondary concomitant sarcomatous spindle cells, and confluent in at least one medium-power field (Fig. 1).

### Methylation-specific PCR (MSP)

The DNA methylation status of CpG islands at the MGMT promoter was determined by chemical modification of unmethylated (but not methylated) cytosine to uracil and subsequent PCR using primers specific for either methylated or modified unmethylated DNA, as previously described [22, 23]. Briefly, genomic DNA isolated from paraffin-embedded samples was denatured by incubation with NaOH at 37°C for 20 min. The denatured DNA samples were further mixed with a bisulfate solution containing hydroquinone, and incubated at 55°C for 20 h under mineral oil. After incubation, the DNA samples were purified from the bisulfate solution using a Wizard DNA Clean-Up system (Promega, Madison, WI, USA). Modification was completed by addition of NaOH and incubation at 37°C for 20 min. Samples were concentrated by ethanol precipitation. To amplify the promoter region of the MGMT gene on the sodium-bisulfite-treated DNA sample, the following previously reported specific primer sequences were used: 5'-TTTGTGTTTTGATGTTTGTAGGTTTTTGT-3' (forward primer) and 5'-AACTCCACACTCTTCCAAAA CAAAACA-3' (reverse primer) for the unmethylated product and 5'-TTTCGACGTTTCGTAGGTTTTTCGC-3' (forward primer) and 5'-GCACTCTTCCGAAAACGAAACG-3' (reverse primer) for the methylated product [14]. PCR was performed with initial denaturation at 95°C for 3 min, followed by 40 cycles at 95°C for 30 s, annealing at 61.5°C for 1 min, extension at 72°C for 1 min, and 1 cycle of elongation at 72°C for 7 min. All reactions took place separately in two tubes under the same conditions. Normal human lymphocyte DNA was used as a negative control for methylated alleles of MGMT, and U251 glioblastoma DNA was used as a positive control. PCR products were loaded



◀ **Fig. 1** Photomicrograph showing typical features of a gliosarcoma (case 3). **a** The tumor exhibits a biphasic pattern with distinct glial and sarcomatous components [hematoxylin and eosin (H&E), original magnification,  $\times 100$ ]. **b** The gliomatous component is immunoreactive with glial fibrillary acidic protein (GFAP) (original magnification,  $\times 100$ ). **c** The sarcomatous component is positive for reticulin (original magnification,  $\times 100$ ). **d** Tumor cells show immunoreactivity against Ki-67, representing highly proliferative activity (original magnification,  $\times 200$ )

on a 12% polyacrylamide gel, stained with ethidium bromide, and examined under ultraviolet (UV) illumination.

#### Immunohistochemistry for MGMT

Formalin-fixed paraffin-embedded specimens were cut to thickness of 5  $\mu\text{m}$ . In the deparaffinized sections, the endogenous peroxidase was blocked with 3%  $\text{H}_2\text{O}_2$  in methanol. Antigen retrieval was performed in an autoclave at 120°C for 10 min in 50 mmol/l citrate buffer (pH 6.0) for MGMT immunohistochemistry in the same buffer. Blocking of nonspecific binding was accomplished with 5% skim milk. After washing in phosphate-buffered saline (PBS), the sections were incubated with anti-MGMT antibody (1:20, clone MT3.1; Neomarkers Inc., Fremont, CA, USA) overnight at 4°C, followed by staining with a streptavidin–biotin–peroxidase kit (Nichirei, Tokyo, Japan). The sections were reacted in a diaminobenzidine peroxytrichloride substrate solution, and counterstained with hematoxylin. As a negative control, the primary antibody was omitted. Human liver was used as a positive control. The immunoreactivity of MGMT protein was evaluated semiquantitatively by counting the stained cells in  $>1,000$  cells. A tumor with  $>10\%$  stained cells was considered to be MGMT positive, according to a previous study [19, 24]. Each slide was individually reviewed by a neuropathologist, and all immunohistochemical analyses were carried out in a blind manner to prevent potential bias from knowledge of the clinical data.

#### Statistical analysis

Overall survival was calculated from date of surgery to date of death, and progression-free survival (PFS) was counted from date of surgery to date of increase in tumor size  $>25\%$  or presence of a new lesion on imaging. The effect of MGMT status on survival was determined by Kaplan–Meier method. Survival curves for the groups according to MGMT status were compared using the log-rank test. Correlation between the status of MGMT promoter methylation and MGMT protein expression was analyzed by Fisher's exact test. Other prognostic factors

including age, Karnofsky performance scale (KPS), radiation dose, and extent of resection according to MGMT promoter methylation were evaluated by Mann–Whitney test and Fisher's exact test. Cox proportional-hazards regression analysis was used for hazard ratios and associated 95% confidence intervals (CI). Multivariate models were fitted by use of a Cox proportional-hazards regression model. Statistical analysis was performed using commercially available statistical software (SPSS version 11.0; SPSS Inc., Chicago, IL, USA), and probability values  $<0.05$  were considered statistically significant.

## Results

### Patient characteristics

The clinical data are summarized in Table 1. There were ten male and two female patients. Patient ages ranged from 25 to 75 years (median 54 years). Ten patients (83.3%) had preoperative KPS score  $\geq 80$  (median 90, range 70–100). Tumor location was temporal in five patients (41.7%), parietal in three patients (25%), fronto-temporal in two patients (16.7%), and temporo-parietal and parieto-occipital in one patient each (8.3%). Surgical resection was performed in all patients; nine patients (75%) had gross total resection, and three patients (25%) received subtotal or partial resection. All patients underwent radiotherapy postoperatively with median dose of 5,940 cGy (95% CI, 5,739–6,141 cGy) delivered over a period of 5–8 weeks. Three patients (25%) received PCV-based chemotherapy, and the remaining nine patients (75%) were administered concomitant-adjuvant temozolomide chemotherapy. For the ten patients who suffered tumor progression, four patients underwent reoperation alone and one patient was given additional PCV chemotherapy following surgery. Three patients were treated with GKS, and one patient was administered additional chemotherapy alone, with agents including PCV and Avastin plus irinotecan. One patient declined treatment after tumor progression.

### MGMT promoter methylation and protein expression

All 12 specimens were available for determination of MGMT status by MSP and immunohistochemistry. MGMT promoter methylation was detected in six tumors (50%), whereas the remaining cases did not show a methylated band (Fig. 2). Based on immunohistochemical analysis, MGMT protein was expressed in the nucleus of tumor cells and was observed in both gliomatous and sarcomatous regions (Fig. 3). Of the six tumors with promoter methylation, two cases demonstrated lack of MGMT expression on immunohistochemistry and four samples exhibited MGMT immunopositivity. Of six tumors

with unmethylated promoter, three cases were immunostained for MGMT. There was no correlation between MGMT promoter methylation and protein expression ( $P > 0.05$ ).

### Patient survival associated with MGMT status

At time of analysis, 10 (83.3%) of the 12 patients had died, and 2 patients (16.7%) were alive in stable condition. Nine (75%) patients died from tumor progression and one male patient (8.3%) died from systemic disease unrelated to the primary gliosarcoma. The median OS and PFS of our series were 13.4 months (95% CI, 12.3–14.5 months) and 8.3 months (95% CI, 7.4–9.2 months), respectively.

In patients with MGMT promoter methylation, median OS was 15.0 months, compared with median survival of 11.3 months in the MGMT promoter unmethylated group. One-year survival rate was 80% in the MGMT methylated group, as compared with 50% in the unmethylated group. Median PFS was 10.3 months for the methylated MGMT group, compared with 7.3 months for the unmethylated MGMT group. Using a univariate Cox proportional-hazards regression model, MGMT methylation was only correlated with median OS (odds ratio; 10.11, 95% CI, 1.1–87.25,  $P = 0.035$ ) and progression-free survival in statistics (hazard ratio; 11.11, 95% CI, 1.29–95.88,  $P = 0.029$ ) (Table 2). On multivariate analysis, after adjustment for age, KPS, extent of resection, and radiation dose, MGMT methylation also significantly increased OS (hazard ratio; 7.42, 95% CI, 1.05–52.38,  $P = 0.045$ ) and progression-free survival (hazard ratio; 8.44, 95% CI, 1.17–60.99,  $P = 0.034$ ) (Fig. 4).

There was no significant difference for other prognostic factors, including age, KPS, and radiation dose in terms of MGMT methylation status (Table 3,  $P = 0.589$ , 0.310, 0.093, respectively). Extent of resection was also not correlated with MGMT methylation ( $P = 1.000$ ).

Regarding MGMT protein expression, the median OS of those patients whose tumors showed lack of MGMT expression was 8.3 months (95% CI, 5.3–11.3 months), and the median OS of those patients who had an MGMT-expressing tumor was 13.4 months (95% CI, 12.6–14.2 months). For MGMT nonexpressing tumors, median PFS was 6.1 months (95% CI, 4.0–8.2 months), and median PFS was 8.3 months (95% CI, 7.6–9.0 months) for those with MGMT expression. Differences in OS and PFS between subgroups defined by presence of MGMT protein expression did not reach statistical significance ( $P = 0.771$  and 0.381, respectively).

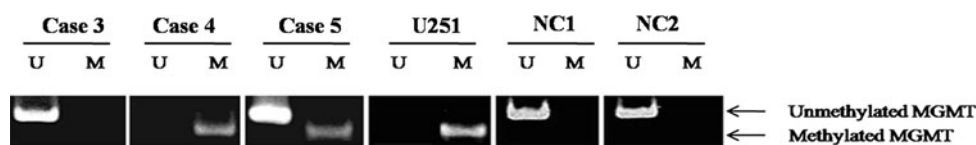
## Discussion

The extent of MGMT promoter methylation is quite variable among malignancies, ranging from 0% to 75%,

**Table 1** Clinical features and MGMT status in gliosarcoma patients

Case no.	Age (years)/sex	Symptoms/signs	Location	KPS	Extent of surgery	Adjuvant therapy	Time to progression (months)	Therapy for progression	Cause of death	Overall survival (months)	MGMT promoter methylation	MGMT protein expression
1	56/Male	Headache	Rt. T	90	Gross total	RT, concurrent TMZ	No progression	NA	Alive	16.0	Methylation	Positive
2	75/ Female	Hemiparesis	Lt. P	90	Partial	RT, concurrent TMZ	No progression	NA	Alive	6.2	Methylation	Positive
3	57/Male	Seizure	Rt. T	90	Gross total	RT, PCV	3.2	No treatment	Tumor progression	4.1	Unmethylation	Negative
4	25/Male	Headache	Rt. T-P	100	Gross total	RT, PCV	8.8	Reoperation	Tumor progression	14.0	Methylation	Positive
5	45/Male	Headache	Rt. P-O	100	Gross total	RT, PCV	6.1	Reoperation	Systemic disease	8.3	Methylation	Negative
6	65/Male	Headache	Rt. T	90	Gross total	RT, concurrent TMZ	8.3	GKS	Tumor progression	9.2	Unmethylation	Positive
7	32/ Female	Headache	Rt. P	100	Gross total	RT, concurrent TMZ	10.3	PCV, Avastin, irinotecan	Tumor progression	16.2	Unmethylation	Negative
8	53/Male	Headache	Lt. F-T	70	Subtotal	RT, concurrent TMZ	8.2	Reoperation and PCV	Tumor progression	13.4	Unmethylation	Positive
9	42/Male	Headache	Lt. T	90	Gross total	RT, concurrent TMZ	5.1	GKS	Tumor progression	6.9	Unmethylation	Negative
10	55/Male	Headache	Rt. P	90	Gross total	RT, concurrent TMZ	11.7	GKS	Tumor progression	17.1	Methylation	Positive
11	51/Male	Headache	Lt. F-T	70	Subtotal	RT, concurrent TMZ	6.3	Reoperation	Tumor progression	13.3	Unmethylation	Positive
12	68/Male	Hemiparesis	Rt. T	90	Gross total	RT, concurrent TMZ	14.8	Reoperation	Tumor progression	21.7	Methylation	Negative

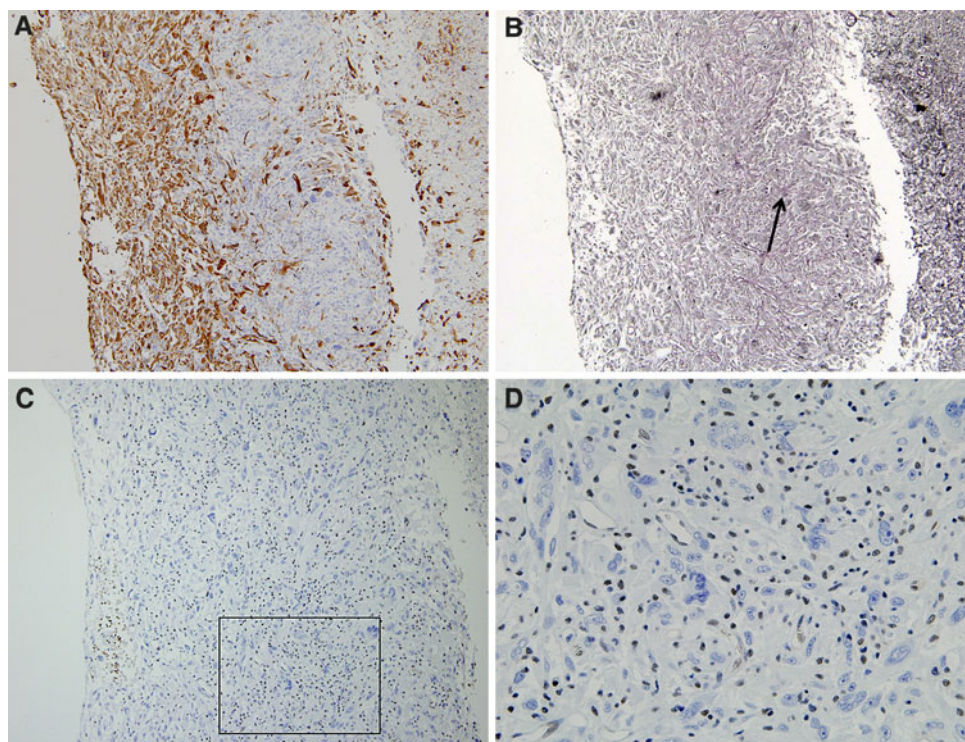
MGMT O<sup>6</sup>-methylguanine-DNA methyltransferase, GKS gamma knife surgery, KPS Karnofsky performance scale, PCV regimen consisting of procarbazine, lomustine, and vincristine, TMZ temozolomide, RT radiation therapy, NA not available



**Fig. 2** Representative figure of methylation-specific polymerase chain reaction analysis for O<sup>6</sup>-methylguanine DNA methyltransferase (MGMT) in human gliosarcomas. Methylated [81 base pairs (bp)] and unmethylated (93 bp) polymerase chain reaction products of each

sample were run in a separate lane. Case 5 shows both methylated and unmethylated bands, which is regarded as MGMT promoter methylation. U251: methylated control from glioblastoma cell line, NC1 and 2: unmethylated normal control from peripheral blood lymphocytes

**Fig. 3** Photomicrographs demonstrating MGMT expression in a gliosarcoma (case 4). **a** The gliomatous region is positively immunostained for GFAP protein (brown color; original magnification,  $\times 100$ ). **b** Reticulin fibers are mostly seen in the sarcomatous compartment (arrows; original magnification,  $\times 100$ ). **c** MGMT-positive cells are shown in both gliomatous and sarcomatous components (original magnification,  $\times 100$ ). **d** Higher magnification of outlined area in **c** shows nuclear staining of MGMT in tumor cells (dark-brown color; original magnification,  $\times 400$ )



**Table 2** Results of univariate Cox proportional-hazard regression analysis

Variables	Overall				Progression-free survival			
	Hazard ratio	95% CI		P-value	Hazard ratio	95% CI		P-value
		LL	UL			LL	UL	
Age (years)	0.98	0.93	1.03	0.457	0.98	0.94	1.03	0.476
KPS	0.97	0.90	1.04	0.417	0.95	0.88	1.03	0.246
Extent of resection <sup>a</sup>	0.44	0.07	2.70	0.378	0.29	0.04	2.11	0.221
Radiation dose (cGy) <sup>b</sup>	1.00	1.00	1.00	0.565	1.00	1.00	1.00	0.349
MGMT methylation status	10.11	1.17	87.25	0.035*	11.11	1.29	95.88	0.029*

KPS Karnofsky performance scale, CI confidence interval, LL lower limit, UL upper limit

\*  $P < 0.05$ , statistically significant

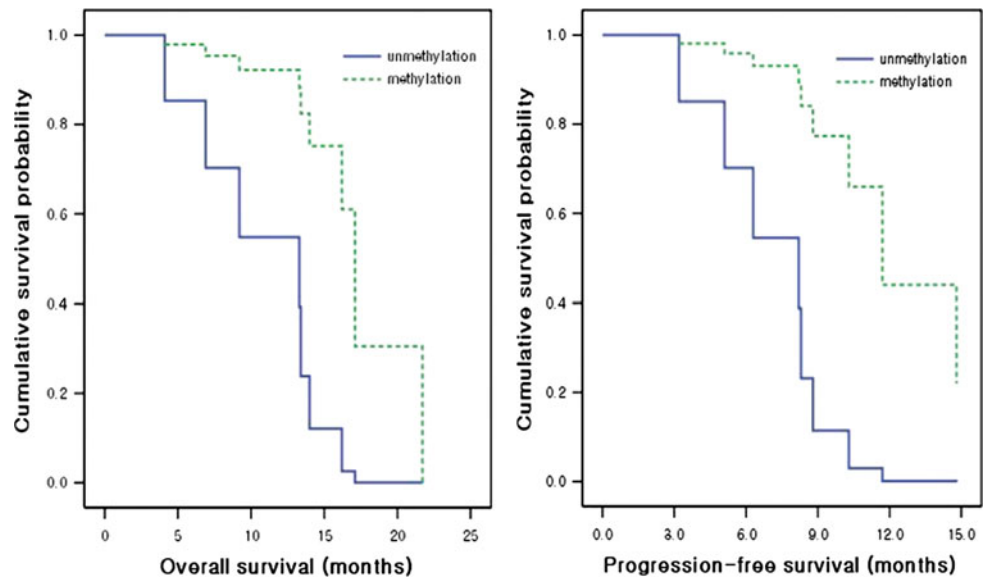
<sup>a</sup> At time of primary surgery

<sup>b</sup> Not including delivered by gamma knife surgery

depending on the specific type of tumor [22, 25]. However, the status of MGMT including promoter methylation and protein expression in gliosarcomas has not been thoroughly

studied. In the present study, we showed that a significant fraction (50%) of gliosarcomas had MGMT promoter methylation. Considering that soft tissue sarcomas

**Fig. 4** Overall (a) and progression-free (b) survival curves by multivariate Cox regression in gliosarcoma patients, depending on methylation status of MGMT promoter. There were significant statistical differences in both overall survival and progression-free survival between the two groups ( $P = 0.011$  and  $0.008$ , respectively; log-rank test)



**Table 3** Baseline characteristics of variables by MGMT promoter methylation status

Variables	MGMT methylation ( $n = 6$ )		MGMT unmethylation ( $n = 6$ )	
	Mean $\pm$ SD	(Min, Max)	Mean $\pm$ SD	(Min, Max)
Age (years)	54.0 $\pm$ 17.7	(40.0, 69.8)	50.0 $\pm$ 11.6	(39.5, 59.0)
KPS	93.3 $\pm$ 5.2	(90.0, 100.0)	85.0 $\pm$ 12.2	(70.0, 92.5)
Radiation dose <sup>a</sup> (cGy)	6266.7 $\pm$ 432.6	(5940.0, 6570.0)	5910.0 $\pm$ 177.0	(5850.0, 5985.0)
PFS (months)	10.3 $\pm$ 4.3 <sup>b</sup>	(6.2, 15.1)	7.3 $\pm$ 2.5 <sup>b</sup>	(4.6, 8.8)
OS (months)	15.0 $\pm$ 5.8 <sup>b</sup>	(7.8, 18.3)	11.3 $\pm$ 4.6 <sup>b</sup>	(6.2, 14.1)

MGMT O<sup>6</sup>-methylguanine-DNA methyltransferase, SD standard deviation, KPS Karnofsky performance scale, PFS progression-free survival, OS overall survival,  $n$  patient number, Min minimum, Max maximum

<sup>a</sup> Not including delivered by gamma knife surgery

<sup>b</sup> Median  $\pm$  standard deviation (SD)

represent 15% of the methylated MGMT [26], gliosarcomas may have a different DNA repair activity from other types of sarcomas. The different MGMT methylation status between gliosarcomas and soft tissue sarcomas may be related to the genetic profile of gliosarcomas. Previous studies have suggested that the sarcomatous component of gliosarcomas originated from aberrant mesenchymal differentiation of highly malignant glial cells, demonstrating that each subpopulation of the tumor shares the same genetic aberration, including *p53* mutations, *p16* deletions, and *PTEN* mutations [27, 28]. Therefore, gliosarcomas have been regarded as a variant of glioblastomas and treated like malignant gliomas, i.e., maximal tumor resection followed by radiotherapy and chemotherapy [1, 29].

In malignant gliomas, alkylating agent-based chemotherapy has been shown to increase response rates and patient survival when used as an adjuvant to surgery and radiation. However, malignant gliomas are often resistant to alkylating agents [12], and the action of MGMT is

known to be a major determinant of chemotherapy resistance [12, 17]. In gliosarcomas, we also found that unmethylated MGMT was detected in six patients, resulting in poor prognosis. This suggests that MGMT methylation status may be associated with tumor response to alkylating agents. MGMT is a DNA-repair enzyme which is ubiquitously expressed in normal human tissues. MGMT protects cells against the potentially deleterious effects of alkylating agents, which include mutations, sister chromatid exchanges, recombination, and chromosomal aberrations [22, 30]. MGMT removes mutagenic and cytotoxic adducts from the O<sup>6</sup>-position of guanine in DNA. However, methylation of the MGMT promoter induces epigenetic silencing of the gene, and O<sup>6</sup>-alkyl guanine is allowed to mispair with thymine during DNA replication, resulting in a G-to-A transition [8, 31–34]. Consequently, gliomas with methylated MGMT promoters are sensitive to alkylating agents, whereas unmethylated MGMT promoters maintain DNA repair activity [14]. Given the important role of MGMT in

tumor resistance to alkylating agents, O<sup>6</sup>-benzylguanine or a poly(ADP-ribose) polymerase inhibitor can be helpful in combination treatment for malignant gliomas [35–37].

In the present study, MGMT promoter methylation was observed in 50% of the gliosarcomas, and lack of MGMT expression was detected in 41.7% of the cases. However, there was not a consistent correlation between promoter methylation and MGMT protein expression. Several studies have suggested that methylation status may not be correlated with MGMT protein expression [38, 39]. MGMT expression can be detected from nonneoplastic components of the glioma [22, 40]. Alternatively, MGMT promoter methylation occurs in only one allele, and an unmethylated allele can produce MGMT expression in a subset of the tumor [19]. Furthermore, MSP is a highly sensitive technique in which a methylated band may be observed even if tumor cells carry MGMT promoter methylation in a minor portion. In the case of epigenetic silencing, dimethylation of histone H3 lysine 9 and methyl-CpG binding proteins can be determining factors for MGMT expression, as well as DNA hypermethylation [41]. Recent studies have documented that methylation status is homogeneous in the same tumor, but MGMT expression can be heterogeneous regionally [42]. Taken together, MGMT protein can be expressed in gliosarcomas even though the MGMT promoter is methylated.

Although a number of studies have documented that MGMT promoter methylation is related with survival in malignant gliomas treated with alkylating agents [13, 32, 43], only one report, to the best of our knowledge, has discussed the association between MGMT status and prognosis, in which MGMT promoter methylation had a positive effect on survival for gliosarcomas [18]. However, this study included both gliosarcomas and GBM, and the survival analysis was not performed separately according to these two different entities. This led us to investigate MGMT status associated with prognosis in a group of patients with gliosarcomas who received chemotherapy based on alkylating agents. In our study, there was longer OS in gliosarcoma patients with methylated MGMT (median OS, 15.0 months) compared with unmethylated MGMT tumors (median OS, 11.3 months). With respect to PFS, there was a similar pattern between methylated and unmethylated MGMT in human gliosarcomas (median PFS, 10.3 months versus 7.3 months). However, the differences in OS and PFS between subgroups defined by presence of MGMT protein expression was not statistically significant. Recent work has shown that MGMT protein expression is not a reliable biomarker for diagnosis and patient outcome, although it examined various cutoff values of MGMT expression, inter- or intraobserver agreement, two anti-MGMT antibodies, and endothelial or hematogenous cells showing MGMT immunoreactivity. It

is suggested that novel anti-MGMT antibodies should be directed against other epitopes for better clinical markers [39]. In addition, we may consider that irrelevant mAb of the same isotype is more appropriate for negative control in MGMT immunostaining studies. Therefore, MGMT immunostaining should be investigated further for clinical correlation in malignant gliomas, although immunostaining is a more convenient procedure for clinical application. Together with these findings, we suggest that MGMT promoter methylation may serve as a good prognostic factor in gliosarcoma patients treated with alkylating agent chemotherapy.

Our current study has limitations, including the retrospective method of analysis as well as the heterogeneity of treatment modalities. In addition, various prognostic factors should also be considered in detail, although most cases had KPS >70 and gross total resection in this study. Therefore, large prospective studies will be necessary to determine the impact of MGMT status on gliosarcoma prognosis, despite the rarity of these tumors.

## Conclusions

We examined MGMT status in gliosarcomas and found that a significant fraction of the tumors showed MGMT promoter methylation and protein expression. Our findings suggest that MGMT promoter methylation is associated with good prognosis in patients with gliosarcomas.

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