Original Article

Methylation of O6-methylguanine DNA methyltransferase promoter is a predictive biomarker in Chinese melanoma patients treated with alkylating agents

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Background: The O6-methylguanine-DNA methyltransferase gene (*MGMT*) promoter methylation status can be used to predict the prognosis of patients with various cancers following treatment with alkylating agents. Moreover, *MGMT* promoter methylation often coexists with *TP53* gene mutation. However, *MGMT* has not been identified as a biomarker of melanoma. Therefore, this study systematically analyzed the prognostic role of *MGMT* and the correlation between *MGMT* methylation and *TP53* mutation in non-Caucasian patients with melanoma.

Methods: This study involved tumor samples and clinical data collected from 205 melanoma patients treated with alkylating agents at Peking University Cancer Hospital & Institute. The *MGMT* promoter methylation and *TP53* mutation status were analyzed respectively using methylation-specific polymerase chain reaction and polymerase chain reaction followed by Sanger sequencing. Additionally, *MGMT* protein expression in tumor samples was assessed via immunohistochemistry.

Results: *MGMT* promoter methylation was detected in 97 (47%) of the 205 tumor samples, and was significantly associated with a loss of MGMT protein expression (P=0.021). *MGMT* promoter methylation was also significantly associated with the presence of *TP53* mutation (P=0.004). Regarding prognosis, patients without *MGMT* promoter methylation exhibited worse overall survival outcomes, compared to those with methylation (hazard ratio: 1.443; 95% confidence interval: 0.731–2.342; P=0.015).

Conclusions: *MGMT* promoter methylation appears to coexist frequently with *TP53* mutation. Patients harboring *MGMT* promoter methylation within tumors may benefit from therapy with alkylating agents.

Keywords: *MGMT* promoter methylation appears to coexist frequently with *TP53* mutation. Patients harboring *MGMT* promoter methylation within tumors may benefit from therapy with alkylating agents.

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Introduction

Melanoma is a highly aggressive skin cancer with high treatment resistance. Although the U.S. Food and Drug Administration (FDA) has approved various targeted therapies for melanoma which includes BRAF and MEK mutation inhibitors, based on the improved responses observed among patients harboring such mutations, many patients acquire therapeutic resistance and therefore do not achieve persistent, long-term responses (1). Antibodies specific for immune checkpoints, such as those targeting CTLA-4 or the PD1/PDL1 interaction, have also been approved by the FDA and have become a standard of care for patients with unresectable or metastatic melanoma; however, these therapeutic antibodies provide benefits to only some patients (2).

Despite the above limitations, chemotherapy remains a useful first- or second-line treatment for advanced metastatic melanoma (3). Alkylating chemotherapeutic agents such as temozolomide (TMZ) and dacarbazine (DTIC), which are commonly used to treat melanomas, cause DNA double-strand breaks, subsequent termination of replication, and apoptosis in tumor cells (4,5). However, a previous study of TMZ monotherapy reported an extremely objective response rate (13–15%) and median overall survival (OS) (7.7–8.4 months) (6). These findings underscore the need to explore the chemorefractory mechanisms exploited by melanomas and to identify biomarkers that can predict the prognosis of patients treated with alkylating agents. Moreover, a better understanding of biomarkers might provide new combination therapy strategies.

The O6-methylguanine-DNA methyltransferase (MGMT) pathway is considered highly significant among the mechanisms of resistance to alkylating agents. MGMT is a DNA repair enzyme encoded by the *MGMT* gene at locus 10q26 (7). Largely anecdotal evidence suggests that MGMT plays a central role in preventing the transformation of a physiologic proliferation into malignancy (8). In addition to protecting normal cells from carcinogenesis, MGMT activity also protects tumor cells from the lethal effects of alkylating agents by removing methyl groups from the O6 position of guanine (9).

During the oncogenesis of several human cancers, *MGMT* was found to be transcriptionally silenced by promoter hypermethylation. The immediate consequence of this phenomenon is the loss of MGMT protein expression and a reduced DNA repair capacity, which results in increased sensitivity to alkylating agents and decreased tumor survival (10,11). Therefore, several studies have described the *MGMT* promoter methylation status as a predictive biomarker of drug efficacy and prognosis, especially among patients with glioblastoma (GBM) (12). For example, a phase III clinical trial conducted by the European Organization for Research and Treatment of Cancer (EORTC) and National Cancer Institute of Canada (NCIC) identified *MGMT* methylation as the strongest predictor of TMZ chemotherapy outcomes in patients with GBM; specifically, *MGMT* methylation was beneficial in terms of response, progression-free survival, and OS (12). Several additional studies have found associations of MGMT inactivation with improved outcomes in Caucasian patients with melanoma who were treated with alkylating agents (13-15). However, as Caucasian and non-Caucasian patients exhibit significant differences in terms of pathogenesis and pathologic subtypes (16,17), the predictive role of MGMT should also be determined in a large cohort of non-Caucasian patients with melanoma.

Many previous reports of ovarian, colorectal, and brain cancers, including malignant astrocytomas, have associated *MGMT* methylation with *TP53* mutation, particularly the G:C to A:T transition (5,11,18-20). By contrast, however, other studies have not identified a relationship between these factors (21,22), indicating the need for further investigation of the potential correlation between *MGMT* promoter methylation and *TP53* mutation and the corresponding effect on prognosis in patients with melanoma. Accordingly, this study aimed to investigate the role of *MGMT* promoter methylation and *TP53* mutation as prognostic biomarkers prognosis in Chinese patients with melanoma who were treated with alkylating agents.

Methods

Patients and tumor tissue samples

Archived formalin-fixed, paraffin-embedded (FFPE) melanoma samples were collected from 205 patients (including 64 with mucosal melanomas, 107 with acral melanomas, and 34 with non-acral skin melanomas) who received DTIC/TMZ and cisplatin-based chemotherapy at the Peking Cancer Hospital & Institute (Beijing, China) between 2005 and 2016 were collected for this study. The samples were analyzed by hematoxylin and eosin staining and immunohistochemistry to confirm the diagnosis of melanoma. Clinical case data were also collected, including patient age and sex, TNM (tumor-node-metastasis) stage, thickness (Breslow), ulceration, and survival outcomes

(for which patients were followed until loss to follow-up or death). This study was approved by the Medical Ethics Committee of the Beijing Cancer Hospital & Institute (2017KT21) and was conducted according to the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants.

DNA isolation and bisulfite modification

Genomic DNA was isolated using the Omega FFPE DNA Kit (Omega Bio-Tek Inc., Norcross, GA, USA). Subsequently, the isolated genomic DNA was subjected to sodium bisulfite modification using the Methylamp™ DNA Modification Kit (EpiGentek Inc., Farmingdale, NY, USA) according to the manufacturer's protocol. The bisulfite-treated DNA was stored at –80 ℃.

Detection of MGMT methylation

In each tumor sample, the methylation status of the *MGMT* promoter was determined using methylation-specific polymerase chain reaction (MS-PCR). Bisulfite-treated DNA was amplified using primers specific to either modified or unmodified DNA. The following primer sequences based on previous research (23,24) were used: 5'-TTTCGACGTTCGTAGGTTTTCGC-3' (forward) and 5'-GCACTCTTCCGAAAACGAAACG-3' (reverse) for the methylated reaction and 5'-TTTGTGTTTTGATGTTTGTAGGTTTTTGT-3' (forward) and 5'-AACTCCACACTCTTCCAAAAACAAAACA-3' (reverse) for the unmethylated modified reaction. The unmethylated reaction yielded a 91-base pair (bp) product, and the methylated reaction yielded an 81-bp product that included the PYRCpG3, PYRCpG4, and PYRCpG5 region. The PCRs were performed in total volume of 25 μL, and tumor DNA was amplified during 40 cycles at an annealing temperature of 66 ℃. The A549 cell line, which is known to exhibit *MGMT* methylation, was used as a control for methylated MGMT, and DNA from the peripheral blood leukocytes of a healthy individual was used as a control for unmethylated *MGMT*. Template-free H_2O was used as a negative PCR control. The PCR products were analyzed by 3% agarose gel electrophoresis and ethidium bromide staining.

MGMT protein immunohistochemistry

FFPE tissue sections were examined by IHC with the monoclonal mouse anti-human MGMT antibody clone MT3.1 (Abcam, Cambridge, UK). The primary antibody was omitted from negative controls. Inflammatory cells and reactive stromal cells served as internal positive controls. Prior to staining, the tissue sections were deparaffinized with xylene for 30 min and rehydrated in decreasing concentrations of ethanol. Endogenous peroxidases were then blocked with 30% H_2O_2 for 20 min in phosphate-buffered saline (PBS). For antigen retrieval, the slides were heated in 0.01 M citrate buffer (pH 6.0) in a pressure cooker for 4 min and subsequently cooled to room temperature in the same buffer. After washing, the slides were incubated overnight with the primary antibody at 4 ℃ (dilution 1:100). The tumor sections were then stained using the Envision™ Detection kit (Gene Tech, Shanghai, PR China) according to the manufacturer's instructions and counterstained with hematoxylin. MGMT status was determined based on staining intensity {negative (0), low positive [1], strongly positive [2]} and percentage of positive tumor cells {0% (0), 1–50% [1], 51–100% [2]}. Only nuclear staining was regarded as positive staining.

TP53 mutation detection

Exons 5–8 of *TP53* were amplified by a two-step PCR. The following primer sequences were used for step I amplification: 5'-GTTTCTTTGCTGCCGTCTTC-3' (forward) and 5'-CCTTCCACTCGGATAAGATG-3' (reverse) for exon 5, 5'-AGCACATGACGGAGGTTGTG-3' (forward) and 5'-TCTCATGGGGTTATAGGGAG-3' (reverse) for exon 6, 5'-GCCTCCCCTGCTTGCCACAG-3' (forward) and 5'-GAGAGGTGGATGGGTAGTAG-3' (reverse) for exon 7, and 5'-TACCTGGAGCTGGAGCTTAG-3' (forward) and 5'-GAAAGAGGCAAGGAAAGGTG-3' (reverse) for exon 8. The following primer sequences were used for step II amplification: 5'-CTTTATCTGTTCACTTGTGC-3' (forward) and 5'-CAATCAGTGAGGAATCAGAG-3' (reverse) for exon 5, 5'-CATGAGCGCTGCTCAGATAG-3' (forward) and 5'-TAGGGAGGTCAAATAAGCAG-3' (reverse) for exon 6, 5'-CATCTTGGGCCTGTGTTATC-3' (forward) and 5'-GAAGAAATCGGTAAGAGGTG-3' (reverse) for exon 7, and 5'-GACAGGTAGGACCTGATTTC-3' (forward) and 5'-AAGTGAATCTGAGGCATAAC-3' (reverse) for exon 8.

Clinical data analysis and statistical methods

OS was defined as the time from diagnosis to the death of

Table 1 Correlation of *MGMT* promoter methylation with clinicopathological features

Characteristic	Methylation [%]	Unmethylation [%]	P value
Total [205]	97 [47]	108 [53]	
Gender			0.735
Male [109]	49 [45]	60 [55]	
Female [96]	47 [49]	49 [51]	
Age			0.647
≤50 [123]	55 [45]	68 [55]	
>50 [82]	39 [48]	43 [52]	
Stage			0.017
I [8]	2 [25]	6 [75]	
II [26]	6 [23]	20 [77]	
III [43]	13 [30]	30 [70]	
IV [128]	74 [58]	54 [42]	
Primary site			0.875
Mucosal [64]	30 [47]	34 [53]	
Acral [107]	53 [50]	54 [50]	
Skin [34]	15 [44]	19 [56]	

MGMT, O6-methylguanine-DNA methyltransferase.

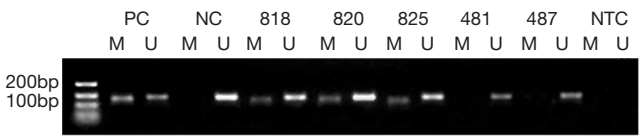

Figure 1 MS-PCR analysis of *MGMT* promoter methylation status in DNA from melanoma patients. PC, positive control; NC, normal control; NTC, no template control; M, methylated; U, unmethylated.

the patient. For the patients who were still alive at the time of the study, OS was defined as the time from diagnosis to the current date. The patients' clinicopathological characteristics were correlated with the *MGMT* promoter methylation status and MGMT protein expression, and the significance of these associations was determined using Pearson's chi-square test, the continuity correction test, Fisher's exact test, and the linear-by-linear association test as appropriate. Survival data were used to generate Kaplan-Meier curves, which were compared according to *MGMT* promoter methylation status using the log-rank test. A Cox regression model was used for both univariate and multivariate analyses. Age, sex, primary site, stage, *MGMT* promoter methylation, MGMT protein expression, *TP53* mutation status, and the combination of *MGMT* promoter methylation and *TP53* mutation status were included in the multivariate model, which used a stepwise method for variable selection. The level of statistical significance was set at $P<0.05$, and all tests were two-sided. The statistical analysis was performed using IBM SPSS statistical software (version 20.0; IBM, Inc., Armonk, NY, USA).

Results

Correlation of MGMT promoter methylation with clinicopathological features

Using MS-PCR, we found that the *MGMT* promoter was methylated in 97 (47%) of 205 tested melanomas (*Table 1* and *Figure 1*). We next used the Pearson method to evaluate the associations of *MGMT* promoter methylation with different clinicopathological features (*Table 1*), and observed a significant association with tumor stage (P=0.017), but not with sex (P=0.735), age (P=0.647), or the primary tumor site (P=0.875).

Association of MGMT protein expression with MGMT promoter methylation

We next used IHC to detect positive nuclear staining for the MGMT protein in 15 of 97 cases with *MGMT* promoter methylation and 42 of 108 cases without methylation (*Table 2*). As described in the Materials and Methods, IHC staining was scored from 0 to 2 on the basis of the staining intensity and percentage of positive tumor cells (*Figure 2*). Strongly positive nuclear MGMT protein staining (IHC score =2) was detected in 8 of 97 patients with methylation and 25 of 108 patients without methylation, whereas low positive nuclear staining (IHC score =1) was detected in 25 of 97 cases with methylation and 17 of 108 cases without methylation.

Using Pearson's chi-square test, we identified a significant association between MGMT expression and *MGMT* promoter methylation in patients with melanoma (P=0.021). Notably, both strongly positive and weakly positive nuclear MGMT protein expression were significantly associated with the *MGMT* promoter methylation status in our melanoma samples (P=0.001 and 0.027, respectively). However, we observed no significant associations of MGMT protein

Table 2 Correlation of MGMT protein expression with clinicopathological features

Characteristic	Positive [%]	Negative [%]	P value
Total [205]	66 [32]	139 [68]	
Gender			0.278
Male [109]	23 [21]	86 [79]	
Female [96]	31 [32]	65 [68]	
Age			0.332
≤50 [123]	27 [22]	96 [78]	
>50 [82]	27 [33]	55 [67]	
Stage			0.149
I [8]	1 [1]	7 [99]	
II [26]	12 [46]	14 [54]	
III [43]	9 [21]	34 [79]	
IV [128]	34 [27]	94 [73]	
Primary site			0.247
Mucosal [64]	13 [20]	51 [80]	
Acral [107]	28 [26]	79 [74]	
Skin [34]	14 [42]	20 [58]	
Methylation status			0.021
Methylation [97]	15 [15]	82 [85]	
Unmethylation [108]	42 [39]	66 [61]	

MGMT, O6-methylguanine-DNA methyltransferase; positive, positive nuclear staining; negative, negative nuclear staining.

expression with sex (P=0.278), age (P=0.332), stage (P=0.149), or primary tumor site (P=0.247).

Correlation of MGMT promoter methylation with TP53 mutation

To evaluate the potential association of *MGMT* promoter methylation with *TP53* mutation in melanoma, we subjected DNA samples from the same 205 tumors to PCR followed by Sanger sequencing to examine *TP53* mutations in exons 5–8, which are commonly affected. We identified 37 cases with *TP53* mutations. Of these, the *MGMT* promoter was methylated in 29 cases (30% of 97 samples with methylation) and unmethylated in only 8 (7% of 108 samples without methylation) (*Table 3*). Accordingly, we found a significant association of *MGMT* promoter methylation with *TP53* mutation (P=0.004), with an odds ratio of 4.463 [95% confidence interval (CI): 1.879–22.664] When separating the patient by the primary site (mucosal *vs.* acral *vs.* skin), there is a significant correlation between *MGMT* promoter methylation status and *TP53* mutation (P=0.01).

A further analysis revealed that the melanomas harbored different *TP53* mutation types, such as T:G, A:G, and C:G. The G:C to A:T mutation at exon 5 was identified in 8 of 97 (8%) cases with a methylated *MGMT* promoter and none of the cases with an unmethylated *MGMT* promoter, indicating that this type of mutation tends to occur under conditions of *MGMT* promoter methylation (P<0.01). Moreover, the G:C to A:T mutation at exon 5 was detected in 8 of 148 (5%) patients with MGMT protein expression and none of the cases without MGMT protein expression, indicating an increased tendency of this mutation to occur in melanomas expressing MGMT protein. However, this latter association was not statistically significant (P=0.109).

Association of MGMT promoter methylation and TP53 mutation with OS among patients with melanoma following treatment with alkylating agents

In our univariate Cox analyses, we identified stage and *MGMT* promoter methylation as factors significantly associated with OS among melanoma patients treated with alkylating agents (*Table 4*). The multivariate Cox regression model subsequently identified both factors as significantly predictive of OS (*Table 4*).

The median OS time of all 205 patients was 38.9 months, and a Kaplan-Meier analysis showed that patients with *MGMT* promoter methylation had a significantly greater median OS, compared to those without *MGMT* promoter methylation (36.7 *vs.* 23.1 months, P=0.021, *Figure 3A*). Although patients without MGMT protein expression also had a greater OS than did those with MGMT protein expression, this difference was not significant (34.5 *vs.* 25.9 months, P=0.092). Similarly, when patients were grouped by MGMT IHC staining scores, those with scores of 0 had poorer OS relative to those with scores of 1 or 2, although this difference was not statistically significant (36.7 *vs.* 26.1 and 21.5 months, respectively, P=0.086, *Figure 3B*).

Although patients harboring a *TP53* mutation or the combination of a *TP53* mutation and *MGMT* promoter methylation had a relatively better OS outcome, this difference was also not significant (33.4 *vs.* 28.5 months, P=0.247 and 35.1 *vs.* 27.2 months, P=0.259, respectively). Finally, the stratification of patients by primary tumor site

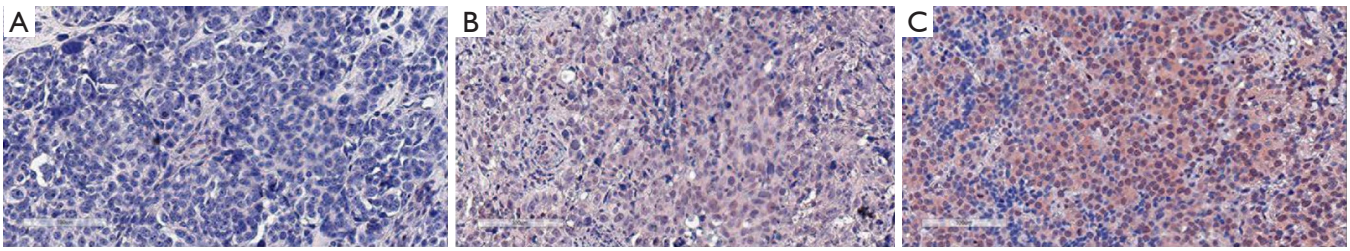

Figure 2 Representative images of melanoma tumor cells of MGMT expression (IHC; cell scale bar: 100 μm). (A) score 0; (B) score 1; (C) score 2.

(mucosal *vs.* acral *vs.* skin) revealed that patients without *MGMT* promoter methylation exhibited worse OS, compared to those with *MGMT* promoter methylation (P=0.015).

Discussion

Although the DNA alkylating agents TMZ and DTIC are considered standard components of chemotherapy for melanoma, patients who receive TMZ for stage IV melanoma have a low response rate (13–15%) and dismal prognosis (OS, 7.7–8.4 months) (6). In addition, only 5% of patients achieve a complete response with TMZ or DTIC treatment, and few achieve a sustained response to either drug (25,26). However, the factors that might better identify patients who may benefit from TMZ or DTIC chemotherapy remain largely unknown. Therefore, in this study, we aimed to evaluate the prognostic role of *MGMT* promoter methylation and *TP53* mutation in a large cohort of Chinese patients treated with alkylating agents for melanoma. In our population of 205 patients, we observed a *MGMT* promoter methylation rate of 47%, which was demonstrably higher than the corresponding frequency among Caucasian patients (21.5–26%) (15,27). This discrepancy may reflect differences in the pathogenesis and pathological subtypes of melanoma between non-Caucasians and Caucasians.

Additionally, our analysis of correlations between *MGMT* promoter methylation and clinicopathological features found that *MGMT* methylation only associated significantly with tumor stage, but not with sex, age, or primary tumor site. Specifically, *MGMT* promoter methylation was significantly more frequent among high-grade (stages III, IV) melanomas, compared to low-grade (I, II) melanomas (51% *vs.* 24%, P=0.004). However, we note that the large difference in the numbers of low-grade and high-grade melanomas (34 *vs.* 171) included in this analysis might have led to statistical bias. Still, our findings are consistent with a previous study, which found that *MGMT* promoter methylation was more common in advanced-stage tumors relative to early-stage tumors (28). Taken together, these findings suggest that *MGMT* promoter methylation and consequent inactivation may play a crucial role in melanoma progression.

Immunohistochemistry revealed a lack of MGMT expression in 82 of 97 (85%) tumors exhibiting *MGMT* promoter methylation, compared to 66 of 108 (61%) tumors with an unmethylated *MGMT* promoter. This finding indicates differences in MGMT protein expression between melanomas that do and do not exhibit *MGMT* promoter methylation (P=0.021); specifically, promoter methylation is associated with a loss of protein expression. However, the high percentage of melanomas lacking both *MGMT* promoter expression and detectable MGMT protein expression suggests that the latter process may be regulated by additional factors. Additionally, technical issues and other factors, such as tumor heterogeneity, may have influenced the results, as a previous study indicated that the use of different methods to assess the *MGMT* promoter methylation status might affect the results (29). In a phase II study of extended-dose temozolomide in patients with melanoma, Petra performed MS-PCR to detect the MGMT promoter methylation rate of 26%, and Rainer and his colleague determined a MGMT promoter methylation frequency of 21.5% via methylation-sensitive high-resolution melting (15,27). In our cohort, we used MS-PCR to detect the MGMT promoter methylation status. Petra reported that IHC staining for MGMT could not predict the responses of melanoma patients treated with TMZ (30). However, Augustine profiled the expression of 38,000 genes using an oligonucleotide-based microarray platform, and found a significant correlation of *MGMT* expression with TMZ sensitivity in the context of melanoma (P≤0.0001) (31). In our cohort, we used IHC to evaluate the expression of MGMT protein in melanomas but found

Table 3 *TP53* mutation and methylation status of the *MGMT* promoter

Tumor	Gender	Age	Methylation status	MGMT protein expression	TP53 mutation	P value
481	M	63	U	(–)	exon5 T:G M169R	0.004
487	F	29	U	(+)	exon6 G:T R213L	
739	M	59	U	(+)	exon5 C:G P153P	
859	M	57	U	(–)	exon6 T:G H193Q	
941	M	41	U	(–)	exon6 A:G H214R	
974	F	61	U	(–)	exon8 T:G L299R	
966	M	69	U	(–)	exon6 G:T R213L	
1048	F	55	U	(–)	exon5 T:G M169R	
508	M	57	M	(–)	exon8 A:G H297R	
511	M	33	M	(–)	exon5 T:C S166P	
565	M	49	M	(–)	exon5 A:G T170A	
610	F	41	M	(+)	exon8 T:G L299R	
634	F	40	M	(–)	exon5 G:A D184N	
692	F	29	M	(–)	exon8 T:G F270V	
693	M	36	M	(–)	exon5 A:G T170A	
761	F	46	M	(–)	exon8 C:G L264V	
770	M	60	M	(–)	exon6 A:G H214R	
818	M	56	M	(–)	exon5 G:A D184N	
820	M	43	M	(–)	exon7 C:A L257M	
821	M	62	M	(–)	exon5 G:A D184N	
837	F	36	M	(–)	exon8 A:G K305E	
866	F	61	M	(–)	exon5 G:A D184N	
875	M	37	M	(–)	exon5 A:G T170A	
897	F	48	M	(–)	exon8 A:G H297R	
903	M	52	M	(–)	exon5 G:A D184N	
905	M	57	M	(–)	exon8 A:G H297R	
917	F	61	M	(–)	exon5 G:A D184N	
922	M	39	M	(–)	exon5 G:A D184N	
934	M	43	M	(–)	exon8 C:G L264V	
955	F	56	M	(–)	exon6 A:G H214R	
961	F	58	M	(+)	exon8 T:G L299R	
978	M	64	M	(–)	exon8 T:G F270V	
994	F	66	M	(–)	exon7 C:A L257M	
1,011	M	58	M	(–)	exon5 T:C S166P	
1,064	M	52	M	(–)	exon5 G:A D184N	
1,087	F	41	M	(–)	exon8 A:G K305E	
1,093	F	47	M	(–)	exon5 A:G T170A	

The numbers are the blind number of the tumour sample. *MGMT*, O6-methylguanine-DNA methyltransferase; (–), negative; (+), positive.

Table 4 Cox regression analysis of *MGMT* promoter methylation and clinicopathologic factors with overall survival

Variable	Univariate analysis		Multivariate analysis	
	Hazard ratio (95% CI)	P	Hazard ratio (95% CI)	P
Age	1.124 (0.874–2.684)	0.113	1.618 (0.922–2.839)	0.094
Gender	1.201 (0.547–1.976)	0.941	1.068 (0.637–1.790)	0.802
Stage	1.634 (0.994–2.263)	0.013	1.509 (1.071–2.126)	0.019
Primary site	0.967 (0.713–1.744)	0.611	1.114 (0.809–1.536)	0.508
MGMT promoter unmethylation	1.121 (0.691–2.653)	0.021	1.443 (0.731–2.342)	0.015
MGMT protein expression	1.478 (0.877–2.653)	0.092	1.653 (0.932–2.932)	0.086
TP53 mutation	1.349 (0.627–2.986)	0.247	1.634 (0.783–3.411)	0.191
MGMT promoter unmethylation & *TP53* mutation	1.427 (0.674–2.991)	0.259	1.713 (0.761–3.579)	0.211

MGMT, O6-methylguanine-DNA methyltransferase; 95% CI, 95% confidence interval.

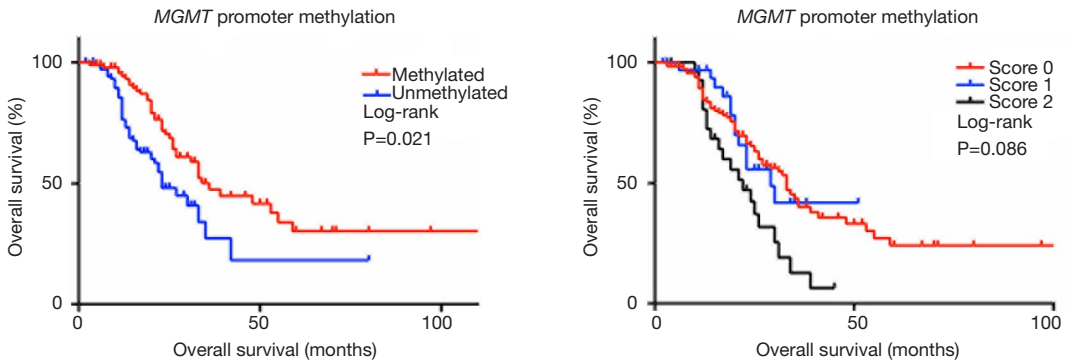

Figure 3 Overall survival of melanoma patients in relation to *MGMT* promoter methylation. (A) Kaplan-Meier curves showing the correlation of *MGMT* promoter methylation status with OS of melanoma patients (P=0.021); (B) Kaplan-Meier curves showing the correlation of *MGMT* expression with OS of melanoma patients (P=0.086).

that this factor could not predict the prognosis of patients treated with alkylating agents. Therefore, future studies should focus on gene expression profiling. Regarding tumor heterogeneity, promoter hypermethylation heterogeneity has been frequently observed in melanomas and glioblastomas (32,33). However, we could not explore the issue of heterogeneity in this study because of an inability to acquiring tumor tissues from in multiple sites in the body.

We further investigated the correlation of *TP53* mutation with MGMT expression, as such correlations have been previously identified in different cancer types, including melanoma and glioblastoma (34-36). In our cohort, we identified *TP53* mutations in 18% of melanoma cases, and observed that this mutation was more frequent among patients with *MGMT* promoter methylation (30%) than among those without *MGMT* promoter methylation (7%). Several previous studies have indicated an association between *MGMT* promoter hypermethylation and G:C to A:T transition mutations of the *TP53* gene in nervous system tumors and glioblastoma (37,38). Consistent with these earlier findings, we observed a significant incidence of *TP53* G:C to A:T mutation in patients with *MGMT* promoter methylation, (8 of 97, 8%, P<0.01). However, the relationship between the *MGMT* promoter methylation status and *TP53* mutation remains under debate (39-43). Our analysis indicated a significant correlation of *MGMT* promoter methylation with *TP53* mutation (P=0.004), suggesting that the former may increase the occurrence of the latter in melanoma. It is therefore reasonable to surmise that *MGMT* promoter methylation and *TP53* mutation

are not independent in the carcinogenesis of melanoma. However, the underlying mechanism and functional aspects require further investigation.

We believe that the most crucial finding of the present study is the association of *MGMT* promoter methylation with a significantly longer OS among patients with melanoma. In our multivariate Cox analyses, we identified the *MGMT* promoter methylation status as an independent prognostic variable associated with a longer OS. This is the first study to demonstrate the prognostic value of *MGMT* promoter methylation in non-Caucasian melanoma population treated with alkylating agents.

Although previous studies have established that the *MGMT* promoter methylation status can predict the efficacy of TMZ treatment in patients with glioblastoma (44,45), randomized clinical trials of a combination regimen comprising the MGMT inhibitor lomeguatrib (LM) and TMZ for metastatic cutaneous melanoma have not demonstrated any significant improvements in patient responses or OS when compared to TMZ monotherapy in a Caucasian population (46). As we noted previously, however, epidemiologic factors differ greatly between Asian and Caucasian populations. The predictive value of *MGMT* promoter methylation regarding response to alkylating agents needs to be supported by further validation. Moreover, the existing evidence merits additional basic research and clinical trials of *MGMT* promoter methylation.

In summary, we have demonstrated the prognostic significance of *MGMT* promoter methylation in melanoma patients treated with alkylating agents. Notably, the presence of *MGMT* promoter methylation was associated with the occurrence of *TP53* mutation in melanoma. Determination of the *MGMT* promoter methylation status might help to identify melanoma patients who will likely benefit from chemotherapy with alkylating agents.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tcr.2018.05.06). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was approved by the Medical Ethics Committee of the Beijing Cancer Hospital & Institute (2017KT21) and was conducted according to the principles of the Declaration of Helsinki (as revised in 2013). Written informed consent was obtained from all participants.

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