



REVIEW ARTICLE

Clinical and Prognostic Significance of O⁶-Methylguanine-DNA Methyltransferase Promoter Methylation in Patients with Melanoma: A Systematic Meta-Analysis

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Tumor suppressor gene O⁶-methylguanine-DNA methyltransferase (*MGMT*) promoter methylation has been reported in melanoma. However, the clinical and prognostic significance of *MGMT* promoter methylation in patients with melanoma remained to be determined. A systematic search was performed to identify eligible papers published. The overall odds ratios (ORs) or hazard ratios and their 95% confidence intervals were calculated. Final 12 eligible publications involving Caucasian population were performed in this study, including 1,071 metastatic melanoma patients, 154 primary melanoma patients, and 211 normal controls. *MGMT* promoter methylation was significantly higher in primary or metastatic melanoma than in normal controls ($p < 0.05$). No difference of *MGMT* promoter methylation was found in primary and metastatic melanoma ($p = 0.432$). When metastatic melanoma was compared to normal controls, subgroup analysis showed the correlation between *MGMT* promoter methylation and different sample materials (tissue: OR = 7.01, $p < 0.001$ and blood: OR = 12.04, $p = 0.005$). *MGMT* promoter methylation was not associated with response to drug therapy and the prognosis in overall

survival and progression-free survival for multivariate analysis. Our results show that *MGMT* promoter methylation may be correlated with the increased risk of primary or metastatic melanoma. Based on blood samples, *MGMT* promoter methylation may become a noninvasive biomarker for the detection of metastatic melanoma. Further additional clinical studies are necessary. (**Ann Dermatol 30(2) 129~135, 2018**)

-Keywords-

Blood, Melanoma, Methylation, *MGMT*, Prognosis, Therapeutics

INTRODUCTION

Increasing evidence suggests genetic and epigenetic events, and environmental factor contribute to the development of melanoma¹⁻³. DNA methylation, a frequent epigenetic modification, is closely associated with the tumorigenesis, progression and prognosis of many types of human cancer^{4,5}. Mapped to human chromosome 10q26, O⁶-methylguanine-DNA methyltransferase (*MGMT*) belongs to a DNA repair gene and counteracts the chemotherapeutic effects of alkylating agent, which protects tumor cells^{6,7}. *MGMT* gene within the promoter region is frequently methylated in various cancers, including primary or metastatic melanoma^{5,8,9}. However, there are some conflicting and different data about *MGMT* promoter methylation in patients with melanoma. For example, Marini et al.¹⁰ reported that *MGMT* promoter methylation had a different frequency in primary and metastatic melanoma (64% vs. 15%). Hoon et al.¹¹ reported that

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MGMT gene had a different promoter methylation level in primary and metastatic melanoma (10% vs. 34%).

Therefore, the present meta-analysis was first conducted to analyze the relationship of *MGMT* promoter methylation between primary or metastatic melanoma and normal controls. In addition, we analyzed the correlation between *MGMT* promoter methylation and response to drug therapy in melanoma. Finally, we evaluated the prognostic effect of *MGMT* promoter methylation in melanoma patients with multivariate analysis.

MATERIALS AND METHODS

Literature search

We systematically searched a range of online digital databases to identify eligible articles published in English up until May 9, 2017, including PubMed, Cochrane Library, Embase and EBSCO. We used the following key words and text word strategy in this meta-analysis: (*O*⁶-methylguanine-DNA methyltransferase OR *MGMT*) AND (methylation OR hypermethylation OR methylated OR epigene*) AND (melanoma OR melanotic cancer OR black cancer). Additionally, a manual reference search for the included articles was also conducted to get other potential publications.

Selection criteria

The eligible articles had to satisfy the following inclusion criteria: 1) the patients were clinically diagnosed with primary or metastatic melanoma; 2) studies involved *MGMT* promoter methylation in melanoma; 3) studies reported sufficient data to evaluate the association between *MGMT* promoter methylation and melanoma (primary or meta-

static melanoma vs. normal controls and metastatic melanoma vs. primary melanoma); 4) studies reported sufficient data to assess the relationship of *MGMT* promoter methylation clinical features and the prognostic role using multivariate analysis if possible. The study with the most complete information was selected to exclude duplicated publications in the present meta-analysis when authors published more than one article using the overlapping sample data.

Data extraction

Two authors (F.Q. and Z.Y.) independently scanned the eligible publications and extracted the following data. Any disagreements regarding the data were discussed by all authors. The following information was collected from the included studies: the first author's last name, year of publication, mean or median age, tumor stage, treatment information of the patients, country, ethnicity, detection method of *MGMT* promoter methylation, methylation frequency, the number of cases and controls, treatment response (responder vs. non-responder group), and clinical prognostic information for multivariate analysis.

Statistical analysis

Stata software (ver. 12.0; Stata Corporation, College Station, TX, USA) was used for the pooled analysis. The pooled odds ratios (ORs) and the corresponding 95% confidence intervals (CIs) were calculated to evaluate the correlation between *MGMT* promoter methylation and melanoma. In addition, the overall hazard ratios (HRs) and their 95% CIs were also calculated to assess the prognostic role of *MGMT* promoter methylation in melanoma for multivariate analysis if possible. The Cochran's Q test was used

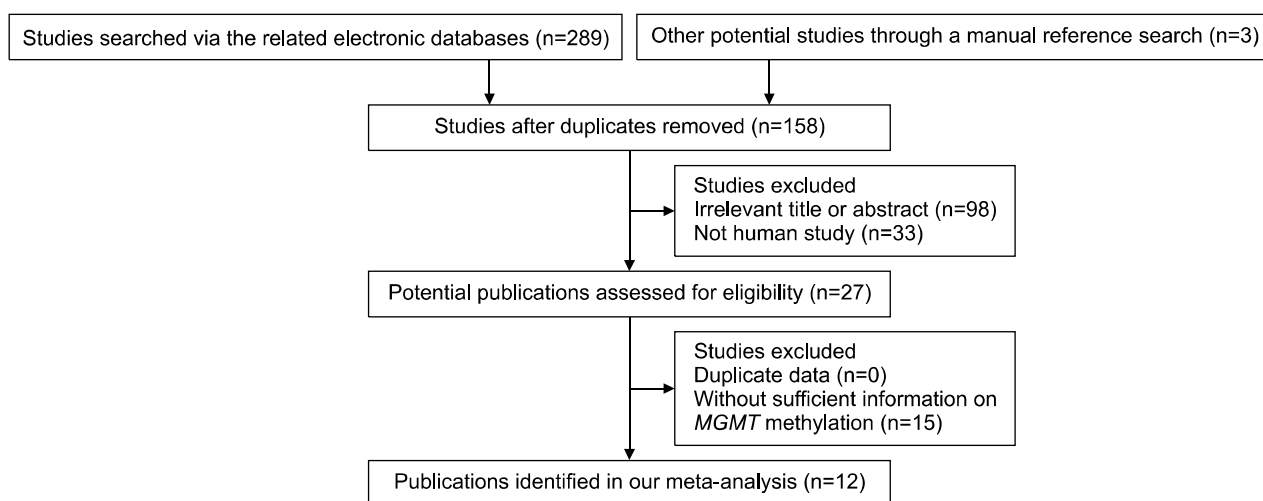


Fig. 1. Flow chart of a systematic literature search. *MGMT*: *O*⁶-methylguanine-DNA methyltransferase.

to examine the heterogeneity in the meta-analysis¹². Data analysis was performed using the random-effects model. A *p*-value of <0.1 stands for substantial heterogeneity when heterogeneity was measured^{13,14}. Subgroup analyses were conducted to analyze the different association among subgroups.

RESULTS

Study characteristics

Through the detailed steps of the systematic literature search, according to the above selection standards (Fig. 1), we finally selected 12 eligible publications in this meta-analysis^{8-11,15-22}. Articles published in English from 2004 to 2015 included a total of 1,071 patients with metastatic melanoma, 154 patients with primary melanoma, and 211 normal controls. All studies were conducted in Caucasian population. The baseline characteristics of the eligible publications are listed in Table 1.

Association between MGMT promoter methylation and melanoma

The data from four studies included 66 patients with primary melanoma and 54 normal controls, as shown in Fig. 2, the pooled OR was 7.49 (95% CI=1.13~49.64, *p*=0.037), which indicted that MGMT promoter methylation was correlated with an increased risk of primary melanoma.

The data from seven studies consisted of 301 patients with metastatic melanoma and 211 normal controls, as shown in Fig. 2 (OR=7.84, 95% CI=3.58~17.17, *p*<0.001), that demonstrated that a significant association was found between MGMT promoter methylation and metastatic melanoma.

When 631 patients with metastatic melanoma were compared to 154 patients with primary melanoma, the result from six studies demonstrated that no correlation of MGMT promoter methylation was observed between metastatic and primary melanoma (Fig. 2) (OR=1.63, 95% CI=0.48~5.50, *p*=0.432).

Subgroup analyses of MGMT promoter methylation in metastatic melanoma vs. normal controls

According to sample type (tissue and blood) and detection method (methylation specific polymerase chain reaction [MSP] and quantitative methylation-specific polymerase chain reaction [QMSP]), we performed subgroup analyses to find the difference among different subgroups.

Subgroup analysis of sample type showed that MGMT promoter methylation was correlated with metastatic melanoma in tissue and blood samples (tissue: OR=7.01,

Table 1. General characteristics of the eligible publications

Author	Country	Ethnicity	Therapy	Mean or median (yr)	Stage	Method	Sample	Metastatic		Primary	Normal	Responders	Non-responders	MA-survival
								M	N					
Hoon et al. ¹¹ (2004)	USA	Caucasians	NA	NA	3~4	MSP	Tissue	29	86	2	20	0	10	
Hoon et al. ¹¹ (2004)	USA	Caucasians	NA	NA	3~4	MSP	Blood	6	31		33	0		
Mori et al. ²² (2005)	USA	Caucasians	Therapy	NA	4	MSP	Blood	5	50		40	2	23	3/24
Marini et al. ¹⁰ (2006)	Germany	Caucasians	NA	61.8	NA	QMSP	Blood	2	13	18	28	0	23	
Kohonen-Corish et al. ²¹ (2006)	Australia	Caucasians	NA	NA	NA	MSP	Tissue	26	84		84	5		
Rastetter et al. ²⁰ (2007)	Germany	Caucasians	NA	NA	NA	MSP	Tissue	2	5	1	10	0	18	
Liu et al. ¹⁹ (2008)	USA	Caucasians	Therapy	NA	NA	QMSP	Tissue	4	32	1	8	0	3	
Busch et al. ¹⁸ (2010)	Norway	Caucasians	Therapy	63	3~4	MSP	Tissue	9	85			7	75	2/10
Hassel et al. ⁹ (2010)	Germany	Caucasians	Therapy	NA	NA	COBRA	Tissue	185	421	24	59	11	23	32/94 OS, PFS
Cesinaro et al. ¹⁷ (2012)	Italy	Caucasians	Therapy	NA	NA	MSP	Tissue	22	74	1	29			
Schraml et al. ¹⁶ (2012)	Switzerland	Caucasians	Therapy	NA	NA	MSP	Tissue	11	42			6	11	5/31
Tuominen et al. ¹⁵ (2015)	Sweden	Caucasians	Therapy	NA	3~4	MSP	Tissue	28	130			16	37	12/93 OS
Beasley et al. ⁸ (2015)	USA	Caucasians	Therapy	NA	3	PSQ	Tissue	14	18			2	3	12/15

M: methylation, N: study population, MA: multivariate analysis, NA: not applicable, MSP: methylation specific polymerase chain reaction, QMSP: quantitative methylation-specific polymerase chain reaction, COBRA: combined bisulphite restriction analysis, OS: overall survival, PFS: progression-free survival, PSQ: pyrosequencing.

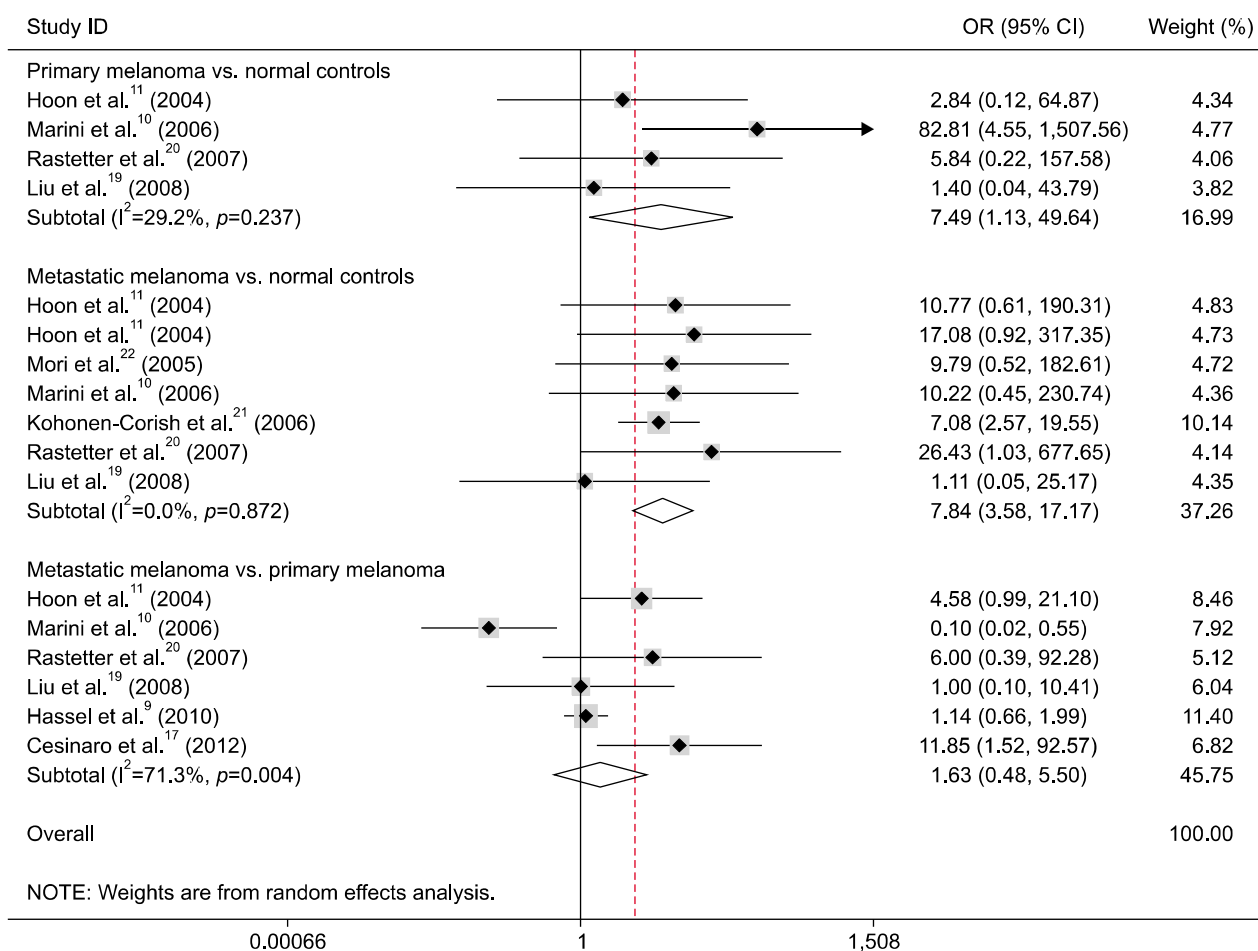


Fig. 2. Forest plot of the association between O⁶-methylguanine-DNA methyltransferase (*MGMT*) promoter methylation and melanoma. OR: odds ratio, CI: confidence interval.

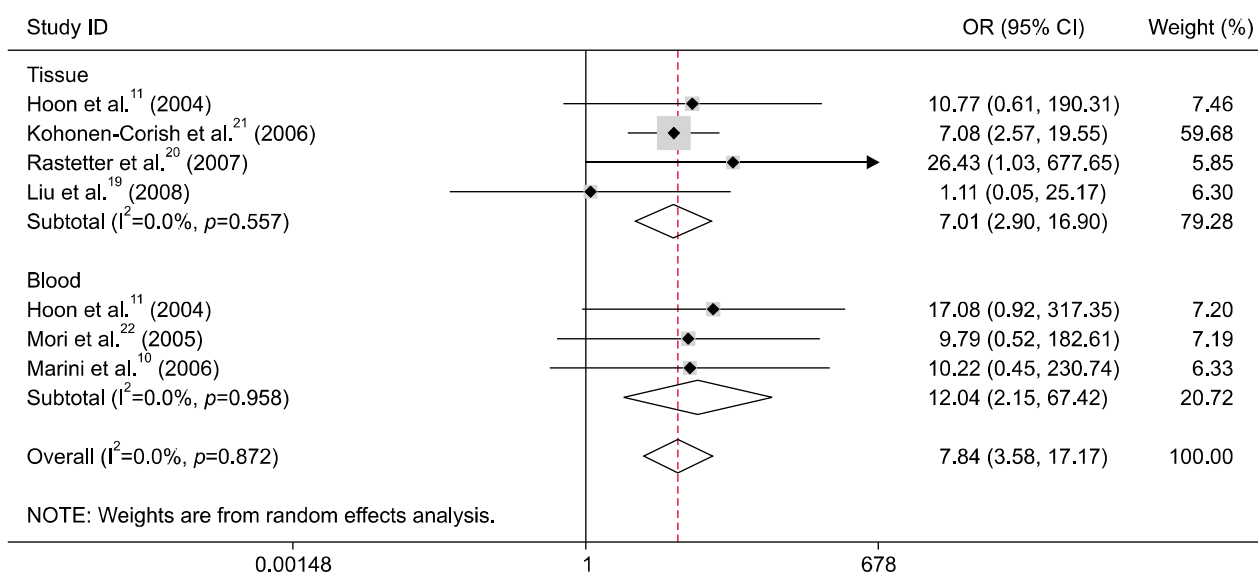


Fig. 3. Subgroup analysis of O⁶-methylguanine-DNA methyltransferase (*MGMT*) promoter methylation by sample type in metastatic melanoma vs. normal controls. OR: odds ratio, CI: confidence interval.

95% CI=2.90~16.90, $p<0.001$ and blood: OR=12.04, 95% CI=2.15~67.42, $p=0.005$ (Fig. 3).

Subgroup analysis by detection method demonstrated that *MGMT* promoter methylation was associated with metastatic melanoma in the MSP method (OR=8.85, 95% CI=3.83~20.49, $p<0.001$), but not in the QMSP method (OR=3.37, 95% CI=0.37~30.64, $p=0.281$) (Fig. 4).

Subgroup analysis of *MGMT* promoter methylation in metastatic vs. primary melanoma

We performed subgroup analysis by therapeutic information (yes vs. not clear) to evaluate the influence of *MGMT* promoter methylation if patients received drug therapy (heterogeneity, $p=0.004$). The result showed that no correlation was observed between *MGMT* promoter methylation and therapeutic information in metastatic vs.

primary melanoma (all $p>0.1$; data not shown).

Association between *MGMT* promoter methylation and response to therapy in melanoma

The pooled OR from six studies including 439 patients with melanoma receiving therapy demonstrated that no relationship was found between *MGMT* promoter methylation and response to drug therapy (OR=1.82, 95% CI=0.74~4.43, $p=0.191$) (Fig. 5).

Prognostic role of *MGMT* promoter methylation using multivariate analysis

One study involving 122 patients with melanoma receiving therapy reported that *MGMT* promoter methylation was not associated with overall survival (OS) (HR=0.955, 95% CI=0.63~1.45) and progression-free survival (PFS)

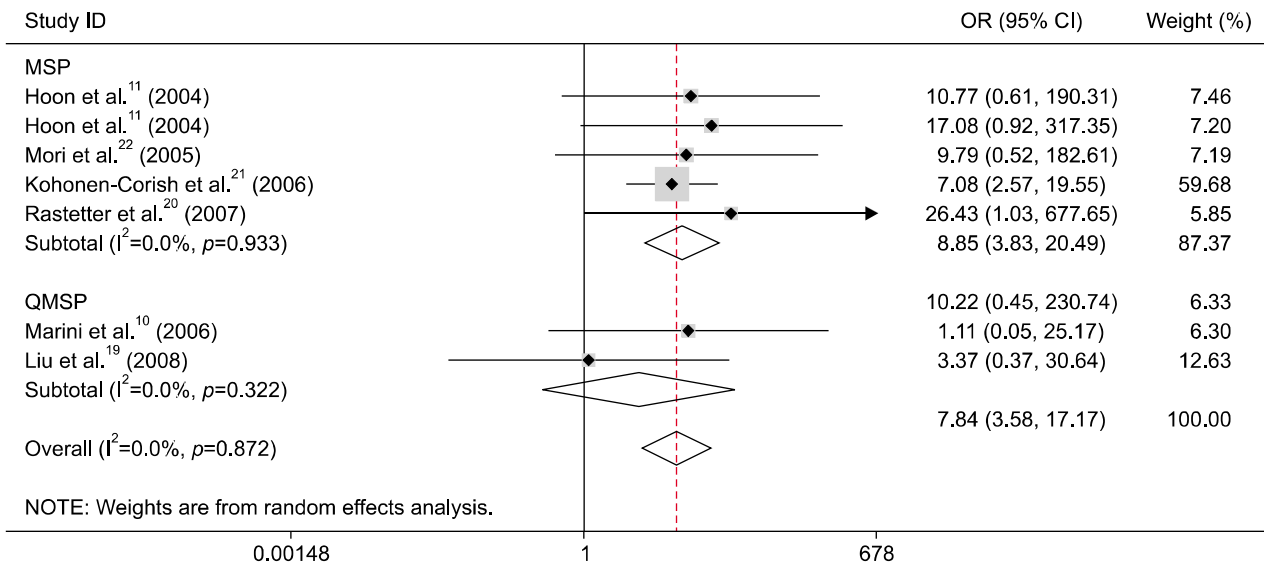


Fig. 4. Subgroup analysis of O⁶-methylguanine-DNA methyltransferase (*MGMT*) promoter methylation by detection method in metastatic melanoma vs. normal controls. OR: odds ratio, CI: confidence interval, MSP: methylation specific polymerase chain reaction, QMSP: quantitative methylation-specific polymerase chain reaction.

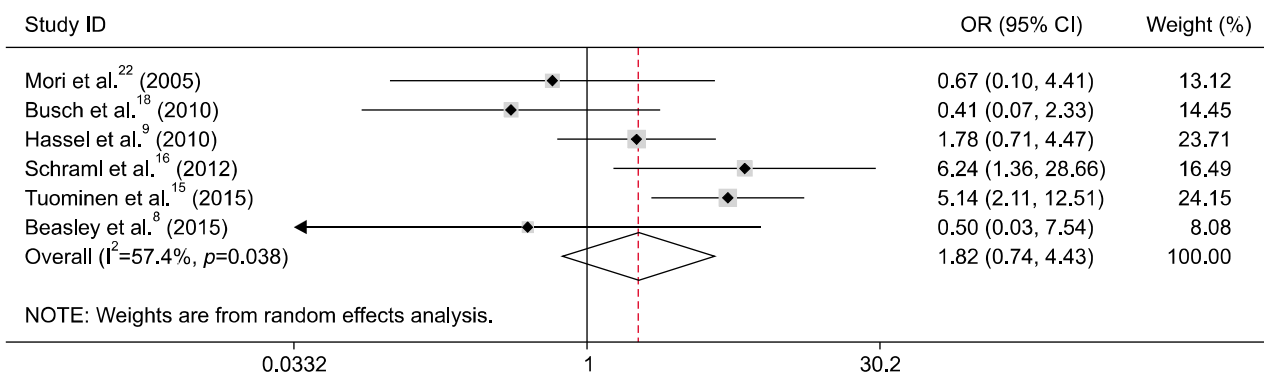


Fig. 5. Forest plot of the association between O⁶-methylguanine-DNA methyltransferase (*MGMT*) promoter methylation and response to therapy in melanoma. OR: odds ratio, CI: confidence interval.

(HR=0.91, 95% CI=0.61~1.36) for multivariate analysis⁹. Another study involving 130 patients with metastatic melanoma receiving therapy reported that *MGMT* promoter methylation was notably associated with PFS (HR=2.17, 95% CI=1.31~3.57) in multivariate analysis¹⁵. While the pooled HR from two studies showed no correlation between *MGMT* promoter methylation and PFS in multivariate analysis (HR=1.39, 95% CI=0.59~3.25).

DISCUSSION

DNA methylation of tumor suppressor genes (TSGs) within the promoter region is a major molecular mechanism of epigenomic regulation, which results in the dysfunction or loss of the related gene expression and may play a key role in cancer development^{23,24}. *MGMT* has been identified as a key TSG²⁵, loss of *MGMT* expression through promoter methylation has been reported in melanoma^{11,15}. However, the clinical effect of *MGMT* promoter methylation remains unclear in melanoma. We performed this meta-analysis to estimate whether *MGMT* promoter methylation provided valuable insight as an epigenetic biomarker for patients with melanoma.

Our meta-analysis included all eligible articles, which were performed in Caucasian population. The results shown that *MGMT* promoter methylation was notably higher in primary or metastatic melanoma than in normal controls, which suggested that *MGMT* promoter methylation may be correlated with the development of melanoma. Additionally, when metastatic melanoma was compared to primary melanoma, *MGMT* promoter methylation was not found to be statistically significantly higher in metastatic melanoma than in primary melanoma ($p=0.432$).

Further subgroup analyses were carried out based on the methylation detection method (MSP and QMSP) and sample type (tissue and blood) in the comparison of metastatic melanoma and normal controls. The result showed that the correlation between *MGMT* promoter methylation and metastatic melanoma was associated with sample type, and *MGMT* promoter methylation had a higher OR value in the blood (OR=12.04, $p=0.005$) than in the tissue (OR=7.01, $p<0.001$), indicating that detection of *MGMT* promoter methylation may become a potential biomarker using blood samples for metastatic melanoma. Subgroup analysis of testing method showed that *MGMT* promoter methylation was linked to metastatic melanoma in the MSP subgroup (OR=8.85, $p<0.001$), but not in the QMSP subgroup ($p=0.281$), which suggested that MSP may be a sensitive method. However, the subgroup results should be carefully considered as only small sample sizes (blood: 94 cases vs. 96 controls, tissue: 207 cases vs. 115 controls,

MSP: 256 cases vs. 185 controls, QMSP: 45 cases vs. 26 controls) were analyzed in this meta-analysis.

MGMT promoter methylation was significantly correlated with response to therapy among two studies^{15,16}, but the remaining four studies demonstrated no association between *MGMT* promoter methylation and response to therapy^{8,9,18,22}. Our results involving 439 patients with melanoma from above 6 studies showed that *MGMT* promoter methylation was not linked to response to therapy ($p=0.191$). Finally, we evaluated whether *MGMT* promoter methylation was associated with the prognosis of melanoma patients for multivariate analysis, promoter methylation of the *MGMT* gene was not linked to the prognosis in OS among one study⁹. The result from two studies indicated that *MGMT* promoter methylation was not correlated with PFS in multivariate analysis. Additional clinical studies with larger sample sizes are needed to further confirm the prognostic significance using multivariate analysis. Some potential limitations should be stated in this meta-analysis. First, our study only included Caucasian population, such as other study populations, such as Asian and African populations, were not included. Second, only one or two studies reported the prognostic information of *MGMT* promoter methylation using multivariate analysis. Third, the sample sizes of subgroup analyses were smaller, more studies with large population are essential in the future, especially in blood samples. Finally, in order to compare "future" Asian data, additional studies with the detailed clinical subtype of melanoma (such as acral lentiginous melanoma, superficial spreading melanomas, nodular melanomas, etc.) are needed.

In conclusion, the current findings indicate that *MGMT* promoter methylation in primary or metastatic melanoma has a higher level than in normal controls. *MGMT* promoter methylation had a similar frequency in metastatic and primary melanoma, and it may not be correlated with response to drug therapy and the prognosis in OS and PFS. *MGMT* promoter methylation may be a potential biomarker using blood samples for metastatic melanoma. More prospective studies using large sample sizes are essential to further validate our findings in the future.

CONFLICTS OF INTEREST

The authors have nothing to disclose.

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