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ORIGINAL RESEARCH

An intronic genetic variation of *MGMT* affects enhancer activity and is associated with glioma susceptibility

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Purpose: O[6]-methylguanine-DNA methyltransferase (MGMT) plays a crucial role in repairing damaged DNA caused by alkylating agents. A number of cancer susceptibility loci have been recognized as enhancer variants. This study aimed to explore the significance of enhancer variants of *MGMT* in glioma susceptibility.

Patients and methods: A retrospective case-control study consisting of 150 glioma patients and 327 controls was conducted to test whether enhancer variants of *MGMT* are associated with glioma susceptibility. Genotypes were determined by Sequenom MassARRAY technology. Associations were estimated by logistic regression. Biochemical assays were used to examine the function of glioma susceptibility locus.

Results: We found that the A allele of rs10764901, an intronic variant of *MGMT*, was associated with a significantly decreased risk of glioma. The rs10764901 AA genotype carriers had an OR of 0.49 (95% CI, 0.24–0.98; *P*=0.045) compared with the rs10764901 GG genotype. When the rs10764901 AG and AA genotypes were pooled for analysis, a significantly decreased risk of glioma was also found (OR, 0.63; 95% CI, 0.43–0.93; *P*=0.021). Functional analyses showed that the rs10764901 A allele drove a lower luciferase expression and had higher transcription factor binding affinity than the G allele.

Conclusion: An enhancer variant of *MGMT* rs10764901 affects the regulatory activity of enhancer by altering the binding affinity of transcription factors and is associated with glioma susceptibility.

Keywords: glioma, *MGMT*, enhancer, genetic variation, susceptibility

Introduction

O[6]-methylguanine-DNA methyltransferase (MGMT) is an important DNA repair enzyme that plays a crucial role in protection against the carcinogenic effects of alkylating agents.[1] MGMT transfers mutagenic and cytotoxic alkyl groups from DNA to its active site and then loses activity irreversibly.[2] Therefore, a certain amount of MGMT is required to maintain its DNA repair activity. Previous studies have shown that aberrant expression of *MGMT* plays an important role in carcinogenesis.[3–5] The increased frequency of oncogene mutations might be the pivotal carcinogenic mechanism of *MGMT* inactivation.[6,7] On the other hand, the MGMT level was also reported to be associated with response to alkylating drugs in cancer.[8,9] Promoter methylation, which is the most frequent mechanism of inactivation of *MGMT* in glioma,[10] has been well established as a major prognostic factor and predictor of chemotherapy response for glioma.[11,12] However, the expression level of *MGMT* is not exactly matched to the promoter methylation status,[13,14] which indicates that other mechanisms also play a non-negligible role in *MGMT* expression regulation.

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Some studies have revealed that several genetic variations of *MGMT* affect gene expression and then play an important role in carcinogenesis and progression of several cancers.[15–17] For example, the T allele of a promoter genetic variation rs16906252 was found to reduce expression of *MGMT* and be associated with increased risk of *MGMT*-methylated colorectal cancer and improved survival of patients with glioma.[15,17] In recent years, some novel significant genetic susceptibility factors of cancers have been identified by unbiased genome-wide association studies (GWAS). It is worthy to note that most of them are located in non-coding regions of genome.[18] Furthermore, a number of non-coding genetic variations identified by GWAS occur in regions that act as enhancers and have functional effects on enhancer activity.[18–20]

In order to survey the role of enhancer variants of *MGMT* in glioma, we investigated their associations with glioma susceptibility and explored their functional relevance. Our data demonstrate that an intronic variant of *MGMT* rs10764901 affects the regulatory activity of an enhancer by altering the binding affinity of transcription factors and is associated with glioma susceptibility.

Patients and methods

Selection of candidate genetic variations

Several tracks of Ensembl Genome Browser (http://www.ensembl.org/index.html, Release 75) were used to select candidate genetic variations.[21] First, we used the VISTA track to explore potential enhancers of *MGMT*.[22] Not only the gene body of *MGMT* but also its 100 kb upstream and downstream flanking regions were analyzed. Then, the Genomic Evolutionary Rate Profiling (GERP) conservation scores based on the 37 eutherian mammals were used to measure the evolutionary conservation of sequences of the potential enhancers of *MGMT*.[23] In order to screen the common genetic variations in our study population, the 1000 Genomes Project East Asian (1 KG EAS) common short variants (single-nucleotide polymorphisms [SNPs] and indels) track, which displays the variants with minor allelic frequency (MAF) >1% genotyped in East Asian individuals by the 1000 Genomes project, was used to analyze the variants located in the potential enhancers of *MGMT*.[24] Because of the relatively small sample size, only the common variants with MAF >10% in Southern Han Chinese (CHS) population were selected for genotyping in this study.

Study subjects

This study recruited 150 incident glioma patients and 327 healthy controls. A portion of patients and controls was enrolled in our previous studies on glioma.[25,26] In the present study, we extended the sample size of glioma patients from 138 to 150. All subjects were unrelated CHS living in Fujian, Zhejiang, and surrounding provinces. Patients with histopathologically confirmed glioma were recruited from January 2010 to October 2014 at the First Affiliated Hospital, Fujian Medical University (Fuzhou, n=122) and the First Affiliated Hospital, College of Medicine, Zhejiang University (Hangzhou, n=28). Healthy controls were cancer-free individuals recruited during the same period. At recruitment, written informed consent was obtained from each subject, and the detailed information on demographic and clinical characteristics were collected. This study was approved by the Institutional Review Board of the First Affiliated Hospital, Fujian Medical University.

Genotype analysis

Genomic DNA samples of 138 glioma patients and 327 healthy controls enrolled in our previous studies were isolated from the peripheral blood lymphocytes using a commercial Tiangen TIANamp Genomic DNA kit (Tiangen Biotech., Beijing, China).[25,26] To increase the sample size of cases, 8.0% (12/150) of genomic DNA samples of cases were isolated from paraffin-embedded normal tissue adjacent to cancer specimens using KAPA Express Extract Kits (KAPA Biosystems, Wilmington, MA, USA). Genotypes of the candidate enhancer variants were determined by the Sequenom MassARRAY iPLEX platform (Sequenom Inc., San Diego, CA, USA). The information of primers is shown in Table S1. To control the data quality, genotyping was performed without knowledge of the case/control status of the subjects. And a 5% random sample was tested twice by different persons, and the reproducibility was 100%.

Functional analyses of genetic variation

In the present study, we performed functional studies only for rs10764901 which was identified to be associated with glioma susceptibility significantly.

For luciferase reporter gene assays, two 1,366-bp DNA fragments corresponding to the potential enhancer (hs589) and containing rs10764901 G or A allele were amplified by polymerase chain reaction using same DNA sample template (primers are available upon request) and subcloned into the pGL3-promoter vector (Promega Corporation, Fitchburg, WI, USA). The resultant plasmid that contains rs10764901 G allele was designated as P-G, while the other one was designated as P-A. The two constructs were identical except for the different allele at rs10764901 polymorphic site. They

were restriction mapped and sequenced to confirm their authenticity. U251 cell was used for luciferase assays. The constructed reporter plasmid or the blank pGL3-promoter plasmid was co-electrotransfected with pRL-TK (Promega Corporation) to U251 cell, using Celetrix Electroporator (Celetrix Biotech., Manassas, VA, USA). Three independent transfection experiments were done, and each was performed in triplicate. The luciferase activity was analyzed by a Dual-Luciferase Reporter Assay System (Promega Corporation).

For electrophoretic mobility shift assays (EMSA), synthetic double-stranded and 3' biotin-labeled oligonucleotides corresponding to rs10764901 G (Probe-G) or A (Probe-A) sequences and U251 cell nuclear extract were incubated at 25°C for 20 minutes using the Light Shift Chemiluminescent EMSA Kit (Pierce, Rockford, IL, USA). For competition assays, non-labeled oligonucleotides at 150-fold molar excess were added to the reaction mixture before the addition of biotin-labeled probes. The reaction mixture was separated on 7% polyacrylamide gel electrophoresis, and the products were detected by Stabilized Streptavidin-Horseradish Peroxidase Conjugate (Pierce). Densitometric analyses of EMSA images were performed by ImageJ software (version 1.50i, NIH). The information of probes is shown in Table S2.

Statistical analysis

The differences in sex and age between cases and controls were examined by two-sided chi-squared test and Wilcoxon rank sum test, respectively. The associations of the candidate enhancer variants with glioma susceptibility were estimated by ORs and their 95% CIs computed by multivariate logistic regression model and adjusted for sex and age. Bonferroni correction was used to adjust the *P*-value for multiple testing. Meanwhile, the false-positive report probability (FPRP) was also calculated to assess the significance as described by Wacholder et al.[27] Because of the relatively small sample size, we set the significance value of FPRP at 0.50 and the prior probability at 0.10 in this study. The statistical power to detect an OR of 1.50 (or its reciprocal, 0.67) was used to calculate FPRP value. We also performed the subgroup analyses by sex, age, and WHO grade. A *t*-test was used to examine the differences in luciferase activity between P-G, P-A, and the blank pGL3-promoter. The differences in densitometries of DNA-protein complexes formed with Probe-A and Probe-G were also analyzed by *t*-test. These statistical analyses were implemented in Statistic Analysis System software (version 9.4, SAS Institute, Cary, NC, USA) and STATA statistical software (version 14.0; StataCorp LP, College Station, TX, USA). *P*-value of <0.05 was used as the criterion of statistical significance, and all statistical tests were two sided.

Results

We first used Ensembl Genome Browser to choose candidate variants located in the potential enhancers of *MGMT*. As shown in Figure 1, four potential enhancers (hs656, hs696, hs331, and hs589) located in the intron of *MGMT* were identified by VISTA track. The potential enhancers hs656 and hs331 were excluded from further analyses because of the absence of common variants with MAF >10% in CHS population. The remaining two potential enhancers hs696 and hs589 were shown to have extreme evolutionary sequence conservation by the GERP conservation scores track. Two common variants rs577227 (MAF=0.39) and rs10764901 (MAF=0.41) were found in the potential enhancers hs696

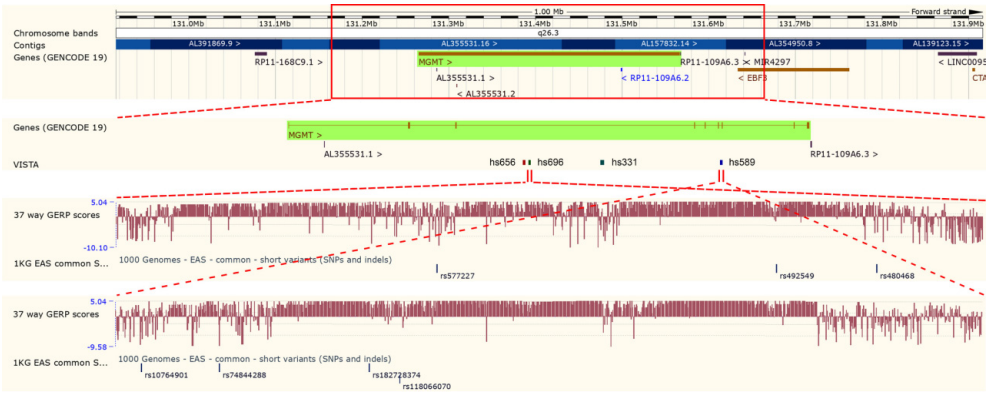

Figure 1 Selection of candidate enhancer variants of *MGMT*.
Notes: The VISTA track of Ensembl Genome Browser displays the potential enhancers of *MGMT*. The GERP scores represent the evolutionary conservation of sequences. The 1 KG EAS common short variants (SNPs and indels) track displays the variants with frequency >1% in East Asian individuals.
Abbreviations: MGMT, O⁶-methylguanine-DNA methyltransferase; SNP, single-nucleotide polymorphism. GERP, Genomic Evolutionary Rate Profiling; 1KG EAS, 1000 Genomes Project East Asian.

and hs589, respectively. They were both selected as candidate enhancer variants for further genotyping.

The detailed baseline characteristics of study subjects are presented in Table 1. There was no significant differences of age and sex distributions between glioma patients and controls, which suggested that the frequency matching of cases and controls was adequate. Of the 150 patients, 80 (53.3%) patients were classified into astrocytic tumors, 37 (24.7%) were classified into oligodendroglial tumors, 16 (10.7%) were classified into oligoastrocytic tumors, and 17 (11.3%) were other histological types. The detailed tumor WHO classification data were successfully collected from 136 (90.7%) patients. Among them, seven (4.7%) patients were WHO grade I, 45 (30.0%) were WHO grade II, 37 (24.7%) were WHO grade III, and 47 (31.3%) were WHO grade IV. There are no clear grade records for the remaining 14 (9.3%) patients. Maximal safe resection or subtotal resection were performed on all patients. Meanwhile, 101 (67.3%) and 112 (74.7%) patients also underwent radiotherapy- and alkylate-based chemotherapy, respectively.

The genotype distributions of rs10764901 and rs577227 in controls were in agreement with that expected under Hardy-Weinberg equilibrium. The multivariate logistic regression was used to investigate the associations between the candidate enhancer variants and glioma susceptibility with adjustment for sex and age (Table 2). As a result, a significant association between rs10764901 and glioma susceptibility was found. Subjects carrying rs10764901 AG or AA genotype had an adjusted OR of 0.67 (95% CI, 0.44–1.02; P=0.059) or 0.49 (95% CI, 0.24–0.98; P=0.045), respectively, compared with individuals carrying GG genotype. These results suggest that rs10764901 A allele may be a protective allele and acts in an allele dose-dependent manner (trend test; P=0.018). A more significant decreased risk of glioma was seen when groups of rs10764901 AG and AA genotype were pooled for analysis (adjusted OR, 0.63; 95% CI, 0.43–0.93; P=0.021). The association for rs10764901 was still significant after Bonferroni correction for two testings. Moreover, the estimated FPRP value for rs10764901 was 0.332 which is below the prespecified FPRP value of 0.50. It also suggests that our finding is noteworthy. We also examined whether genotype frequencies of rs10764901 differed between blood DNA and adjacent normal tissue DNA. No significant difference was found (Table S3). The significant association between rs10764901 and glioma susceptibility was also found when we repeated the association analyses without the 12 cases with adjacent normal tissue DNA (Table S4). These results verified that there was no significant heterogeneity in genotype frequencies for rs10764901 between different DNA sources. The other candidate enhancer variant rs577227 was not associated with glioma susceptibility significantly. Compared with subjects carrying rs577227 TT genotype, the adjusted ORs for TC or CC genotype were 1.33 (95% CI, 0.87–2.03; P=0.193) or 1.32 (95% CI, 0.69–2.53; P=0.410), respectively. When we combined rs577227 TC and CC genotype for analysis, the adjusted OR was 1.34 (95% CI, 0.89–2.01; P=0.156).

Table 1 Selected characteristics of glioma patients and controls

Characteristics	Cases (n=150)	Controls (n=327)	*P*-value
Age (years), mean (SD)	45.0 (15.6)	48.4 (12.5)	0.160[a]
Sex, n (%)			0.754[b]
Male	99 (66.0)	211 (64.5)	
Female	51 (34.0)	116 (35.5)	
Pathology, n (%)			
Astrocytic tumors	80 (53.3)		
Oligodendroglial tumors	37 (24.7)		
Oligoastrocytic tumors	16 (10.7)		
Others	17 (11.3)		
WHO grade, n (%)			
I	7 (4.7)		
II	45 (30.0)		
III	37 (24.7)		
IV	47 (31.3)		
Unknown	14 (9.3)		
Surgery	150 (100.0)		
Radiotherapy	101 (67.3)		
Chemotherapy	112 (74.7)		

Notes: [a]Wilcoxon rank sum test; [b]Two-sided chi-squared test.

We then carried out subgroup analyses for rs10764901 based on sex, age, and WHO grade in a dominant model (Figure 2). The protective effect of rs10764901 A allele was observed across all subgroups, especially in males and patients with WHO grade III glioma. Significantly decreased risk of glioma was observed in males (adjusted OR, 0.53; 95% CI, 0.32–0.86; *P*=0.011). Similarly, it was found that rs10764901 was significantly associated with deceased risk of WHO grade III glioma (adjusted OR, 0.49; 95% CI, 0.25–0.99; *P*=0.046). No significant association was found in other subgroups although the trend is similar in all subgroups. It might be due to the small sample sizes within a stratum.

To investigate the regulatory activity of the potential enhancer hs589 and whether rs10764901 has a functional effect on the regulatory activity of hs589, we constructed two luciferase reporter gene plasmids (P-G and P-A) encompassing hs589 with rs10764901 G or A allele, respectively, and then transiently transfected into U251 cells. As shown in Fig-

Table 2 Genotype frequencies of rs10764901 and rs577227 in glioma patients and controls and their contributions to the risk of glioma

Variants	Genotype	Patients (n=150) No. (%)	Controls (n=327) No. (%)	OR[a] (95% CI)	*P*-value
rs10764901	GG	79 (52.67)	136 (41.59)	1.00 (reference)	
	AG	59 (39.33)	150 (45.87)	0.67 (0.44–1.02)	0.059
	AA	12 (8.00)	41 (12.54)	0.49 (0.24–0.98)	0.045
	AG+ AA	71 (47.33)	191 (58.41)	0.63 (0.43–0.93)	0.021
	P_{trend}[b]			0.018	
rs577227	TT	51 (34.00)	136 (41.59)	1.00 (reference)	
	TC	78 (52.00)	154 (47.09)	1.33 (0.87–2.03)	0.193
	CC	21 (14.00)	37 (11.32)	1.32 (0.69–2.53)	0.410
	TC+ CC	99 (66.00)	191 (58.41)	1.34 (0.89–2.01)	0.156
	P_{trend}[b]			0.117	

Notes: [a]Data were calculated by logistic regression, adjusted for age and sex; [b]Tests for trend of odds were based on likelihood ratio tests assuming a multiplicative model.

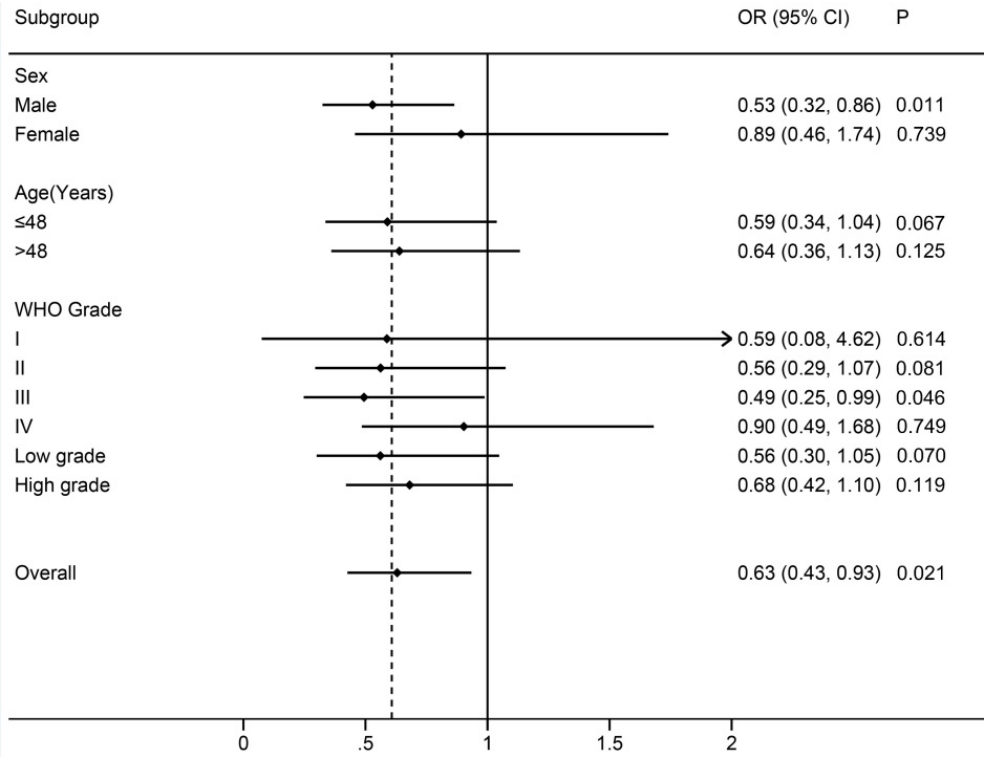

Figure 2 Subgroup analyses for rs10764901 based on sex, age, and WHO grade in a dominant model.
Note: The central black dot represents the OR, and the horizontal line indicates the 95% CI.

ure 3A, about six- or threefold higher reporter gene expression was observed for the plasmid P-G or P-A, respectively, than the pGL3-promoter plasmid (P-G vs pGL3-promoter, $P<0.001$; P-A vs pGL3-promoter, $P<0.001$). These results suggest that hs589 might be a real functional enhancer element. Then, we compared the transcriptional activity of rs10764901 G and A alleles. The P-G construct drove about twofold higher luciferase expression than the P-A construct ($P=0.002$). This finding indicates that rs10764901 G>A change affects the enhancer activity of hs589.

To explore whether the differences in enhancer activity between rs10764901 G and A alleles are due to their

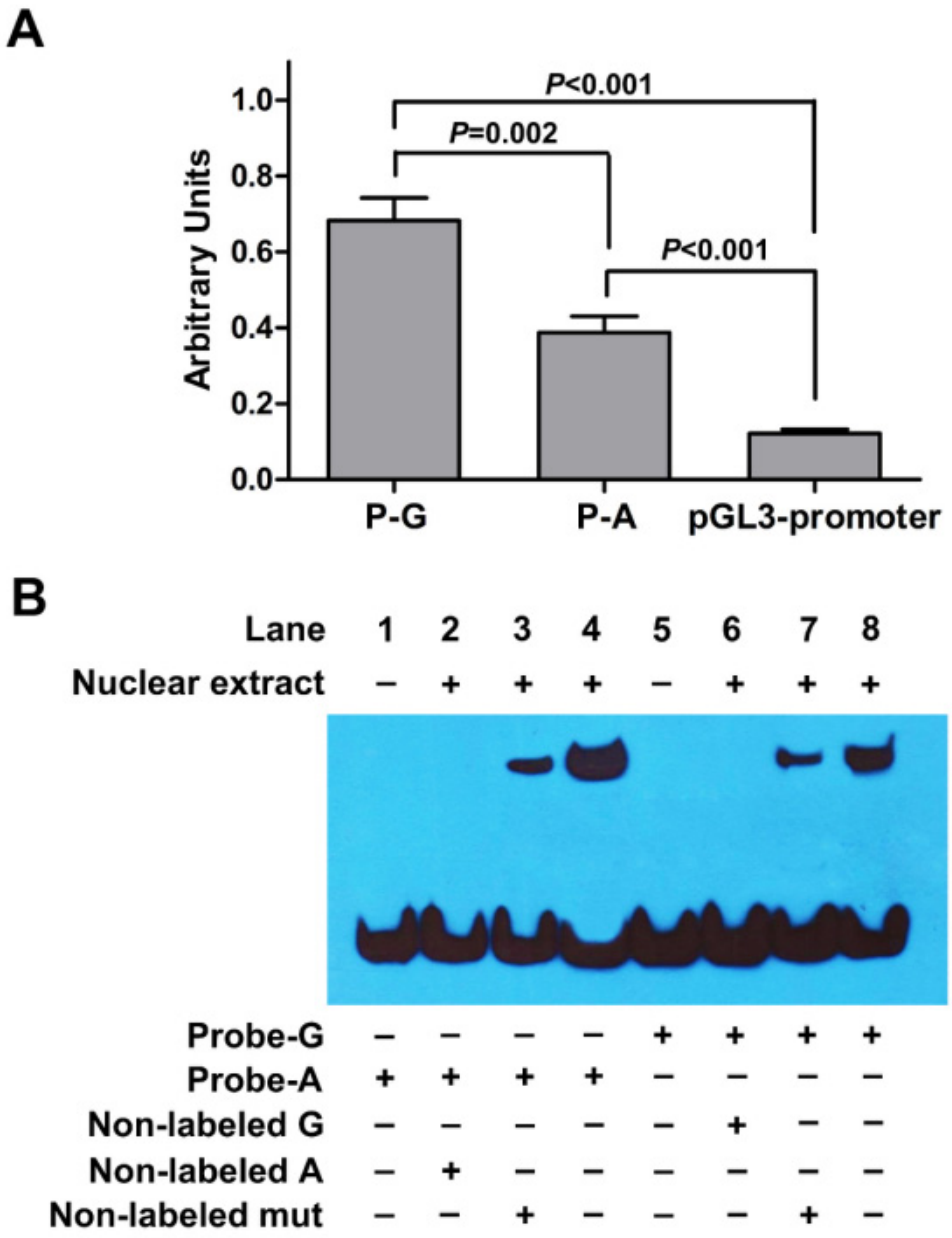

Figure 3 Results of luciferase reporter gene and EMSA.

Notes: (**A**) Luciferase expression of constructs containing the potential enhancer (hs589) with rs10764901 G allele (P-G) or A allele (P-A) in U251 cells. P-G, P-A, or pGL3-promoter construct was co-electrotransfected with pRL-TK to standardize transfection efficiency, respectively. Data shown are the means ± SE from three independent transfection experiments, each performed in triplicate. (**B**) EMSA with biotin-labeled oligonucleotides containing the rs10764901 G allele (Probe-G) or A allele (Probe-A) and nuclear extracts from U251 cells. Lanes 1 and 5 show mobilities of the labeled oligonucleotides without nuclear extracts; lanes 2 and 6 show mobilities of the labeled oligonucleotides with nuclear extracts in the presence of non-labeled G or non-labeled A competitors; lanes 3 and 7 show mobilities of the labeled oligonucleotides with nuclear extracts in the presence of non-labeled mut competitor; lanes 4 and 8 show mobilities of the labeled oligonucleotides with nuclear extracts in the absence of competitor.

Abbreviation: EMSA, electrophoretic mobility shift assay.

ability to bind transcriptional activators or inhibitors, we conducted EMSA using U251 cell nuclear extract. As shown in Figure 3B, U251 cell nuclear extract was able to bind both Probe-G and Probe-A, and the binding patterns were similar. However, more DNA-protein complex was formed when Probe-A was incubated with U251 cell nuclear extract (Lane 4) compared to Probe-G (Lane 8) under the same experimental conditions, with the relative densitometries (mean ± SE) from three independent experiments being 1.08±0.06vs 0.73±0.07 (P=0.016). It indicates that the protein binding affinity of Probe-A was higher than that of Probe-G. In competition assays, 150-fold excess of non-labeled A or non-labeled G probe could completely eliminate the DNA-protein interaction (Lane 2 and Lane 6, respectively). Meanwhile, the DNA–protein interaction was partly eliminated by 150-fold excess of non-labeled mut probe (Lane 3 and Lane 7). These results indicate that the binding is sequence specific.

Discussion

In the present study, we demonstrated that an intronic variant of *MGMT* rs10764901 is significantly associated with glioma susceptibility by a retrospective case-control study involving 150 glioma patients and 327 controls. The rs10764901 A allele carriers were found to have a significantly decreased risk of glioma. Functional analyses revealed that the rs10764901 A allele had higher transcription factor binding affinity than the G allele and drove a lower luciferase expression.

MGMT is well known as a unique DNA repair gene that reverses DNA alkylation damage alone.[1] It is widely considered to be a tumor suppressor gene because some studies have found that overexpression of *MGMT* is associated with decreased risk of several cancers.[3,4] However, we found an interesting result that rs10764901 A allele, which drove a lower gene expression, significantly reduced the risk of glioma. It indicates that *MGMT* may function as an oncogene under certain circumstances. Consistent with our results, a study on colorectal cancer found that the T allele of *MGMT* promoter variant rs16906252 is associated with constitutively reduced gene expression and reduces the risk of *MGMT*-unmethylated colorectal cancer.[17] These results indicate that *MGMT* overexpression may be a risk factor for *MGMT*-unmethylated cancer. Interestingly, a converse result that rs16906252 T allele is associated with an elevated risk of *MGMT*-methylated colorectal cancer was observed.[17] These clues indicate that the role of *MGMT* in carcinogenesis is far more complex, and it might be completely opposite in cancer subclassifications with different methylation status of *MGMT*. Moreover, it was also reported that, in the context of different methylation status of *MGMT*, the prognostic effect of *TERT* gene is dichotomous,[28] which supports the hypothesis that the mechanism of carcinogenesis is different for cancer subclassifications with different methylation status of *MGMT*. This postulation can partly explain the inconclusive results reported by previous published studies on *MGMT* genetic variations.[29] The studies enrolling more patients with *MGMT* methylation might reach the opposite conclusion to those recruiting more *MGMT*-unmethylated patients.

Unfortunately, detailed information on methylation status of *MGMT* of glioma patients enrolled in this study is scant. Thus, we have no hard evidence to indicate that the *MGMT*-unmethylated glioma constituent ratio is higher than *MGMT*-methylated glioma in this study, which might partly explain why a deleterious role of *MGMT* overexpression in glioma carcinogenesis was inferred from our study. However, it is worthy to note that nearly two thirds of patients enrolled in this study are males. *MGMT* unmethylation has been found to be significantly higher in male patients with glioma.[30,31] Therefore, it is plausible to deduce that *MGMT*-unmethylated glioma is in the majority in our study.

Still some other clues indicate that the physiological role of *MGMT* is far more than DNA repair. For instance, *MGMT* is expressed ubiquitously and its expression level far exceed its need to repair DNA damage.[1] The exact role of phosphorylated MGMT is still an enigma.[1] All of these and our findings indicate a broad function of *MGMT* needed further investigation.

A major limitation of this study is the relatively small sample size which might limit the statistical power. In addition, the lack of information on methylation status of *MGMT* results in the absence of subgroup analyses based on methylation status. Therefore, we could not explore directly whether rs10764901 has a converse role in developing glioma with different methylation status of *MGMT*.

Conclusion

We identified an enhancer variant of *MGMT* rs10764901 as a novel susceptibility locus for glioma for the first time. Our data revealed that rs10764901 A allele drove a lower gene expression and significantly reduced the risk of glioma. These results indicate that the underlying role of *MGMT* in glioma carcinogenesis needs further investigation. In addition, they warn us of the need for cancer risk stratification in study on genetic predisposition of cancer.

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Author contributions

LH and XS designed this manuscript; LH, WX, LD, DY, and SZ conducted the study and collected glioma tissues and blood samples as well as corresponding clinical characteristics of cases and controls; LH and XS analyzed the data and wrote this manuscript. All authors contributed toward data analysis, drafting, and critically revising the paper and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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Supplementary material

Table S1 Information of primers for Sequenom MassARRAY iPLEX assays

Variants	Primer	Sequence (5′-3′)
rs10764901	Second PCR primer	ACGTTGGATGCTGAAAAGAGGAAGAGACGC
	First PCR primer	ACGTTGGATGAGAGACACGTCAGGACTTAC
	Extend primer	ACGCGAATTTTCTATGAAAAGTA
rs577227	Second PCR primer	ACGTTGGATGAGTATAAACAGGAACGGAGC
	First PCR primer	ACGTTGGATGAGGGCACTTGAAATGCTGAC
	Extend primer	GGGGCAGACAGGCAGGCAG

Table S2 Information of probes for electrophoretic mobility shift assays

Probe	Sequence (5′-3′)
Probe-G	gcttgcccaagacatgtacttttcatagaaa-biotin
Probe-A	gcttgcccaagacatatacttttcatagaaa-biotin
Non-labeled G	gcttgcccaagacatgtacttttcatagaaa
Non-labeled A	gcttgcccaagacatatacttttcatagaaa
Non-labeled mut	gcttgcccaagacatctacttttcatagaaa

Table S3 Genotype frequencies of rs10764901 genotyped using blood DNA or adjacent normal tissue DNA in glioma patients

Genotype	Blood (n=138) No. (%)	Tissue (n=12) No. (%)	*P*-value
GG	72 (52.17)	7 (58.33)	0.371[a]
AG	56 (40.58)	3 (25.00)	
AA	10 (7.25)	2 (16.67)	

Notes: [a]Two-sided χ^2 test.

Table S4 Genotype frequencies of rs10764901 genotyped using blood DNA in glioma patients and controls and their contributions to the risk of glioma

Genotype	Patients (n=138) No. (%)	Controls (n=327) No. (%)	OR[a] (95%CI)	*P*-value
GG	72 (52.17)	136 (41.59)	1.00 (reference)	
AG	56 (40.58)	150 (45.87)	0.70 (0.46–1.07)	0.097
AA	10 (7.25)	41 (12.54)	0.44 (0.21–0.94)	0.034
AG+AA	66 (47.83)	191 (58.41)	0.64 (0.43–0.96)	0.031
P_{trend}[b]			0.019	

Notes: [a]Data were calculated by logistic regression, adjusted for age and sex; [b]Tests for trend of odds were based on likelihood ratio tests assuming a multiplicative model.