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RESEARCH ARTICLE

# Clinicopathological significance and potential drug target of O6-methylguanine-DNA methyltransferase in colorectal cancer: a meta-analysis

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**Abstract** Emerging evidence indicates that $O^6$-methylguanine-DNA methyltransferase (MGMT) is a candidate for tumor suppression in several types of human tumors including colorectal cancer (CRC). However, the correlation between *MGMT* hypermethylation and clinicopathological characteristics of CRC remains unclear. In this study, we conducted a systematic review and meta-analysis to quantitatively evaluate the effects of *MGMT* hypermethylation on the incidence of CRC and clinicopathological characteristics. A comprehensive literature search was done from Web of Science, the Cochrane Library Database, PubMed, EMBASE, CINAHL, and the Chinese Biomedical Database for related research publications written in English and Chinese. Methodological quality of the studies was also evaluated. Analyses of pooled data were performed with Review Manager 5.2. Odds ratio (OR) and hazard ratio (HR) were calculated and summarized, respectively. Final analysis from 28 eligible studies was performed. *MGMT* hypermethylation is found to be significantly higher in CRC than in normal colorectal mucosa, the pooled OR from 13 studies including 1085 CRC and 899 normal colorectal mucosa, OR=6.04, 95 % confidence interval (CI)=4.69–7.77, $p<0.00001$. *MGMT* hypermethylation is also significantly higher in colorectal adenoma than in normal colorectal mucosa, but it is significantly less compared to that in CRC patients. Interestingly, *MGMT* hypermethylation is correlated with sex status and is significantly higher in female than in male. *MGMT* hypermethylation is also associated with high levels of microsatellite instability (MSI). The pooled HR for overall survival (OS) shows that *MGMT* hypermethylation is not associated with worse survival in CRC patients. The results of this meta-analysis suggest that *MGMT* hypermethylation is associated with an increased risk and high levels of MSI and may play an important role in CRC initiation. However, *MGMT* hypermethylation may play an important role in the early stage of CRC progression and development, as well as having limited value in prediction of prognosis in CRC patients. We also discussed that MGMT may serve as a potential drug target of CRC.

**Keywords** Colorectal cancer · $O^6$-methylguanine-DNA methyltransferase (MGMT) · Tumor suppressor gene · Methylation · Meta-analysis · Odds ratio · Hazard ratio

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## Introduction

Colorectal cancer (CRC) is one of the most malignant types of cancers. In the USA and Europe, CRC is the second most frequent cancer that leads to death, which ranks only below lung cancer [1]. It is expected that there will be approximately

**Table 1** Basic characteristics of the included studies

| Study | Country | Patients | Methods | Primary aim | Methylation site | MGMT expression |
|---|---|---|---|---|---|---|
| Whitehall et al. [17] | Australia | 90 | MSP, IHC | To determine the methylation status of *MGMT* methylation in subset of CRC and in precursor lesions | Promoter, CpG islands | + |
| Nagasaka et al. [18] | Japan | 90 | MSP, IHC | To determine the methylation status of *MGMT* methylation in CRC patients | Promoter, CpG islands | + |
| Kohonen-Corish et al. [19] | Australia | 183 | MSP, IHC | Analysis of the prognostic significance of MSI-L and loss of *MGMT* expression in CRC patients | Promoter, CpG islands | + |
| Shen et al. [20] | USA | 95 | MSP, IHC | To determine if *MGMT* methylation could be one of the mediators of field cancerization in the colon mucosa | Promoter, CpG islands | + |
| Qi et al. [21] | China | 62 | MSP, IHC | To investigate the functions of promoter hypermethylation of *MGMT* in CRC tumorigenesis and progression. | Promoter, CpG islands | + |
| Krtolica et al. [22] | Belgrade | 46 | MSP | To investigate the significance of *p16* and *MGMT* in CRC tumorigenesis and progression. | Promoter, CpG islands | − |
| Mikami et al. [23] | Japan | 60 | MSP, IHC | To examine the methylation profiles and expression of four genes in CRC patients | Promoter, CpG islands | + |
| Menigatti et al. [24] | Italy | 9 | MSP | Analysis of methylation of CpG islands in the *p16* and *MGMT* in CRC progression | Promoter, CpG islands | − |
| Ahlquist et al. [11] | Norway | 150 | q-MSP | To determine methylation status of 11 genes in CRC and adenomas | Promoter, CpG islands | − |
| Nagasaka et al. [12] | Japan | 593 | MSP, IHC | To determine *MGMT* promoter that leads to loss of its protein expression and if MGMT methylation correlates with KRAS mutation in CRC patients | Promoter, CpG islands | + |
| Krakowczyk et al. [25] | Poland | 68 | MSP | Analysis of methylation of CpG islands in the *MGMT* and *p16* genes in CRC patients | Promoter, CpG islands | − |
| Zhang et al. [35] | China | 24 | MSP, IHC | To examine *MGMT* expression profile and methylation status in CRC patients | Promoter, CpG islands | + |
| Chen et al. [26] | China | 117 | MSP | To investigate the association of methylation in the promoter regions of *APC* and *MGMT* in CRC patients | Promoter, CpG islands | − |
| Hawkins et al. [27] | Australia | 1123 | COBRA, RT-PCR | To define the clinicopathological profiles associated with *MGMT* methylation in CRC patients | Promoter, CpG islands | + |
| Balic et al. [28] | Austria | 66 | MethyLight | To establish and validate HRM analysis for detection of promoter methylation on archival formalin-fixed paraffin-embedded tissues from CRC patients | Promoter, CpG islands | − |
| Vogel et al. [29] | USA | 734 | MSP | To study how caretaker gene silencing relates to gatekeeper mutations in CRC patients | Promoter, CpG islands | − |
| Huang et al. [30] | China | 151 | MSP | To verify whether *MGMT* methylation was linked to the occurrence of K-RAS mutation in CRC patients | Promoter, CpG islands | − |
| Kim et al. [31] | Korea | 570 | Pyrosequencing | To determine methylation status of 11 genes in CRC patients | Promoter, CpG islands | − |
| Abouzeid et al. [32] | Egypt | 36 | MSP | To determine methylation status of *RASSF1A*, *MGMT*, and *HIC-1* in CRC patients and precursor lesions | Promoter, CpG islands | − |
| Lee et al. [33] | Korea | 336 | MSP | To determine methylation status of *hMLH1*, *hMSH2*, and *MGMT* in CRC patients and precursor lesions | Promoter, CpG islands | − |
| Shima et al. [34] | USA | 124 | q-MSP, IHC | To determine prognostic significance of *MGMT* alterations in CRC patients | Promoter, CpG islands | + |
| Mokarram et al. [36] | Iran | 92 | MSP | To identify which CpG sites are critical for its downregulation of the *MGMT* gene in CRC patients | Promoter, CpG islands | − |
| Nilsson et al. [13] | Sweden | 163 | Pyrosequencing | To determine the methylated fraction of CpG sites in promoters of five genes in CRC patients | Promoter, CpG islands | − |
| Sinha et al. [37] | India | 855 | MSP | To evaluate the role of Kras gene mutation and RASS F1A, FHIT, and MGMT gene promoter hypermethylation in CRC patients | Promoter, CpG islands | − |
| Li et al. [38] | China | 44 | MSP | To analyze and compare the levels of *MGMT* and *MLH1* gene methylation in CRC patients | Promoter, CpG islands | − |
| Farzanehfar et al. [39] | Iran | 40 | q-MSP | To determine if MGMT methylation may be one of the candidate mediators of field cancerization in the colon mucosa | Promoter, CpG islands | − |
| Wu et al. [40] | China | 132 | MSP | To determine the DNA methylation status of *MGMT*, *CDKN2A*, and *MLH1* in CRC patients | Promoter, CpG islands | − |

**Table 1** (continued)

| Study | Country | Patients | Methods | Primary aim | Methylation site | MGMT expression |
|---|---|---|---|---|---|---|
| Gao et al. [41] | China | 32 | MSP, IHC | To verify the effect of hypermethylation of *MGMT* on CRC tumorigenesis and progression | Promoter, CpG islands | + |

MGMT expression (+): MGMT protein was detected in the study. MGMT expression (−): MGMT protein was not detected in the study

*MSP* methylation-specific PCR, *RT-PCR* reverse transcription followed by conventional PCR

8 % of new CRC cases in the USA in 2014 [2]. Surgical resection can be performed to remove the tumor if neither lymph node nor distant metastasis was present, and the recurrence rate after surgery remains high [3, 4]. Thus, further study on the mechanism of initiation, progression, and identification of prognostic marker and potential drug target is still needed and will help select the patients with high chance of CRC recurrence and provide better prognosis and individualized treatment. Epigenetic modification of gene expression, such as DNA methylation, plays an important role in carcinogenesis. Aberrant methylation of CpG dinucleotides, such as DNA hypermethylation and DNA hypomethylation, is a commonly observed epigenetic modification in human cancer [5–7]. Therefore, analysis of gene methylation as a tool for diagnosis of tumors and its use as a prognostic marker have been widely used for various types of cancer including CRC.

$O^6$-methylguanine-DNA methyltransferase (MGMT) is a DNA repair protein with the ability to remove mutagenic and cytotoxic adducts from $O^6$-guanine in DNA [8]. MGMT transfers the alkyl group from the $O^6$-guanine in DNA to an active cysteine within its own sequence. One MGMT molecule is inactivated for each lesion repaired [9]. The active site of MGMT molecule cannot be recovered after such an enzymatic reaction [10]. The role that MGMT losses in colorectal tumorigenesis is complex and not well characterized. Low level of *MGMT* methylation has been detected in the normal colorectal mucosa taking from the margin of the resected CRC as well as individuals without CRC [11, 12]. *MGMT* methylation has also been detected more frequently in colorectal adenomas than in normal tissues. These findings suggest that *MGMT* methylation may play a role in preceding the development of CRC [11, 13]. Therefore, *MGMT* methylation status might be a valuable biomarker for early detection and prediction of prognosis in CRC. Some studies have demonstrated that *MGMT* methylation is a weak prognostic marker or has no survival advantage, but the studies fail to show any significance of *MGMT* methylation in CRC due to limited power. We conducted this systematic meta-analysis with a total of 28 inconsistent observational studies to assess the value of *MGMT* methylation as an early detection and prognosis biomarker in CRC (Table 1).

## Material and methods

### Articles search strategy and selection criteria

The following electronic databases were searched for relevant articles without any language restrictions: Web of Science (1945~2014), the Cochrane Library Database (Issue 12, 2014), PubMed (1966~2014), EMBASE (1980~2014), CINAHL (1982~2014), and the Chinese Biomedical Database (CBM) (1982~2014). We also checked Google scholar for additional articles.

We searched articles using following terms: *MGMT* promoter methylation and CRC, adenoma, and normal tissue. We included studies that met the following criteria: (1) the association between *MGMT* methylation and CRC and/or adenoma and (2) the association of *MGMT* methylation and prognosis in CRC patients. The exclusion criteria included the following: (1) letters, reviews, case reports, conference abstracts, editorials, and expert opinion; (2) all publications regarding in vitro/ex vivo studies, cell lines, and human xenografts were also excluded; and (3) if the study utilized the same population or overlapping database.

### Data extraction

Two reviews independently extracted data using a standardized form. The information extracted from each article included the following: first author, year of publication, countries, number of patients, methods to detect *MGMT* methylation, stages, prognosis, hazard ratios with corresponding 95 % confidence intervals for patients with methylated *MGMT*, and the total number of participants.

### Statistics analysis

Hazard ratio (HR) with a 95 % confidence interval was calculated for the association between MGMT methylation and prognosis. Odds ratios (ORs) with 95 % confidence intervals

were calculated by using a fixed or random effect model depending on heterogeneity (a fixed effect model for $I^2$ <50 %, a random effect model for $I^2$ >50 %) [14]. The $I^2$ index was proposed to quantify the degree of heterogeneity in a meta-analysis [15]. Subgroup analysis was performed to compare *MGMT* methylation between normal tissues and CRC tissues, normal tissues and adenoma, early and late stages of CRC, male and female, and adenoma and CRC. All *p* values were two sided. Funnel plots were designed for publication bias. All analyses were performed with Review Manager 5.2 [16].

## Results

### Identification of relevant studies

One hundred and twenty publications were identified by the search method as described above. Ninety-eight of those were excluded due to laboratory studies, non-original articles (review), or studies irrelevant to the current analysis. Eventually, there were 28 studies included in the final meta-analysis [11–13, 17–41] as shown in Fig. 1. The frequency of *MGMT* methylation ranged from 22.7 to 77.8 % (average 41.2 %) in cancer tissues. The frequency of *MGMT* methylation in cancer tissues ranged from 25.0 to 77.8 % (average 42.7 %) and from 22.7 to 60.7 % (average 39.5 %) in Caucasian and Asian, respectively.

### The correlation of MGMT hypermethylation with clinicopathological features

#### *The inactivation of MGMT through hypermethylation in CRC and adenoma*

*MGMT* hypermethylation is significantly higher in CRC than in normal colorectal mucosa. The pooled OR from 13 studies including 1085 CRC and 899 normal colorectal mucosa is shown in Fig. 2 (OR=6.04, 95 % confidence interval (CI)= 4.69–7.77, $p<0.00001$), which indicates that *MGMT* inactivation through hypermethylation plays an important role in the pathogenesis of CRC. *MGMT* hypermethylation also occurs in colorectal adenoma, but significantly less compared to that in CRC. The pooled OR from nine studies including 709 CRC and 450 colorectal adenoma is shown in Fig. 3 (OR=1.30, 95 % CI=1.00–1.69, $p=0.05$). In addition, *MGMT* hypermethylation is also significantly higher in colorectal adenoma than in normal colorectal mucosa. The pooled OR from seven studies including 373 colorectal adenoma and 511 normal colorectal mucosa is shown in Fig. 4 (OR=4.81, 95 % CI= 3.28–7.04, $p<0.00001$).

#### *Relationship between the frequency of MGMT hypermethylation and sex status*

*MGMT* hypermethylation rate in female CRC patients is significantly higher than that in male patients. The pooled OR

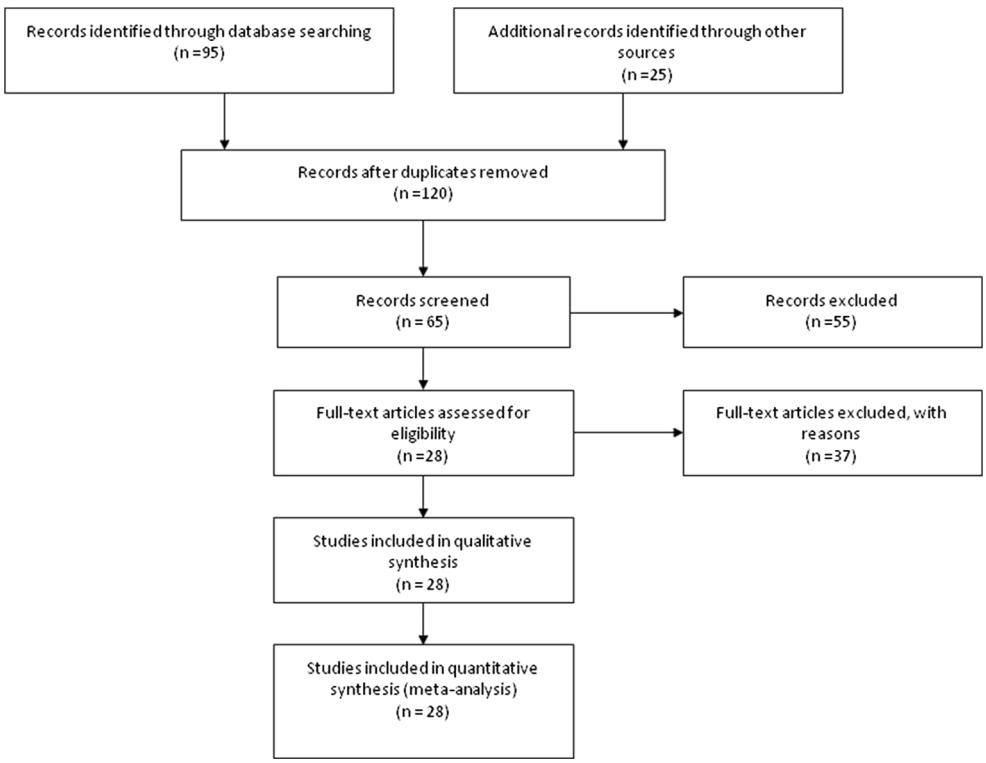


**Fig. 1** Schematic flow diagram for selection of included studies

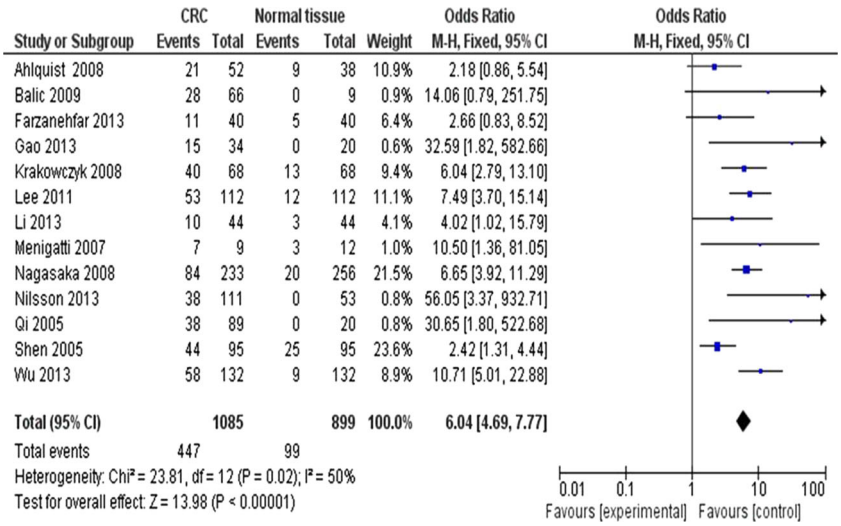


**Fig. 2** Forest plot for *MGMT* methylation in CRC and normal tissue

from ten studies including 1824 male and 1634 female CRC is shown in Fig. 5 (OR=0.86, 95 % CI=0.74–0.99, $p$=0.03), indicating that *MGMT* hypermethylation is strongly associated with sex status in CRC patients.

### *The role of MGMT hypermethylation in CRC development*

We analyzed 2320 CRC patients pooled from ten studies to assess whether or not the aberrant *MGMT* hypermethylation in CRC was associated with the advanced stage. As shown in Fig. 6, aberrant *MGMT* hypermethylation is not significantly high in advanced CRC (III and IV) than that in early staged CRC (I and II), OR=0.99, 95 % CI=0.84–1.18, $p$=0.94. These results suggest that epigenetic silencing of *MGMT* gene expression by promoter hypermethylation may play an important role in the early stage of CRC progression and development.

### *The correlation of MGMT hypermethylation with MSI in CRC*

We also determined 2506 CRC patients pooled from five studies to assess whether or not the aberrant *MGMT* hypermethylation in CRC was associated with microsatellite instability (MSI). As shown in Fig. 7, aberrant *MGMT* hypermethylation is significantly higher in MSI-positive CRC than that in MSI-negative CRC, OR=1.43, 95 % CI=1.14–1.80, $p$=0.002. These results suggest that epigenetic silencing of *MGMT* gene expression by promoter hypermethylation may play an important role in increasing levels of MSI.

### *MGMT hypermethylation as a prognostic factor for CRC*

Only three studies estimated the relationship between OS and *MGMT* hypermethylation in CRC. The pooled HR for OS shows that *MGMT* hypermethylation is not associated with

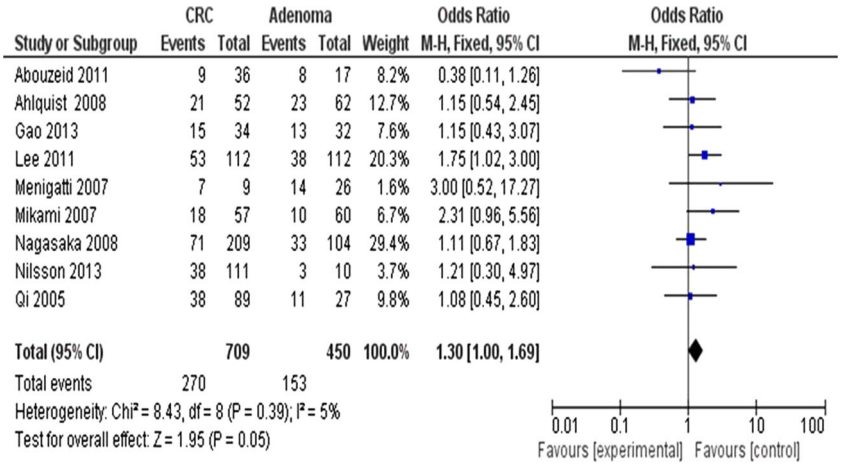


**Fig. 3** Forest plot for *MGMT* methylation in CRC and adenoma

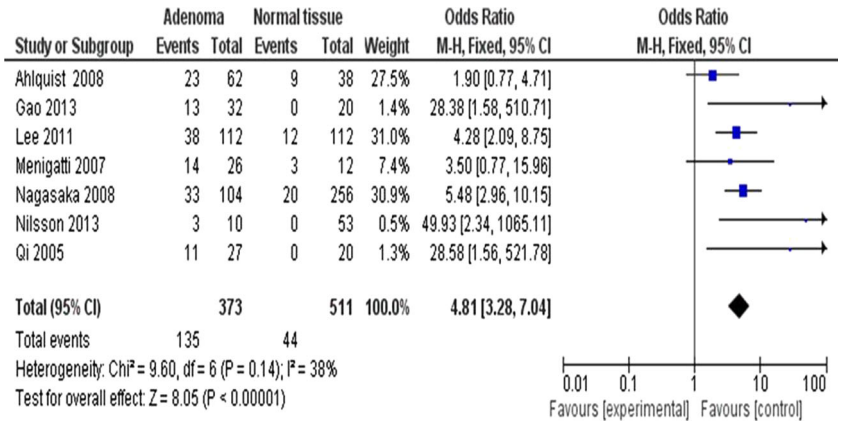


**Fig. 4** Forest plot for *MGMT* methylation in adenoma and normal tissue

worse survival in CRC patients as shown in Fig. 8 (HR=1.05, 95 % CI=0.91–1.21, $p$=0.51).

*Sensitivity analyses and publication bias*

Finally, we performed a sensitivity analysis to assess the result stability, in which one study is removed at a time. The stability of the analysis is acceptable, since the pooled ORs and HRs are not significantly become different. The results also show no publication biases in the meta-analysis of *MGMT* methylation and clinicopathological features, and the funnel plots are largely symmetric (Fig. 9).

## Discussion

MGMT is a DNA repair enzyme which can remove alkyl group from $O^6$ position of guanine. Alkylguanine adducts result in mispairing with thymine by leading to G:C to A:T transitions during DNA replication [9]. *MGMT* is genetically or epigenetically altered in different kinds of primary or advanced carcinomas. *MGMT* is critical to protect normal cells from exogenous carcinogens. Its inactivation by the promoter hypermethylation plays an important role in tumorigenesis in several types of tumors including CRC [42–46].

To date, there have been some studies describing the methylation status of *MGMT* in CRC; however, the roles of methylation of *MGMT* in CRC and clinical significance have not been thoroughly investigated. We conducted the meta-analysis to determine the correlation between *MGMT* hypermethylation and clinicopathological characteristics in CRC. Analysis of the pooled data showed that (1) CRC had a higher proportion of hypermethylation rate than colorectal mucosa; *MGMT* hypermethylation also occurs in colorectal adenoma, but its proportion of rate is significantly less compared to CRC. In addition, *MGMT* hypermethylation also had significantly higher proportion of hypermethylation in colorectal adenoma than in normal colorectal mucosa. (2) *MGMT* hypermethylation is correlated with sex status and significantly higher in female than in male. (3) Aberrant *MGMT* hypermethylation is not significantly higher in advanced CRC than that in early staged CRC. These results suggest that epigenetic

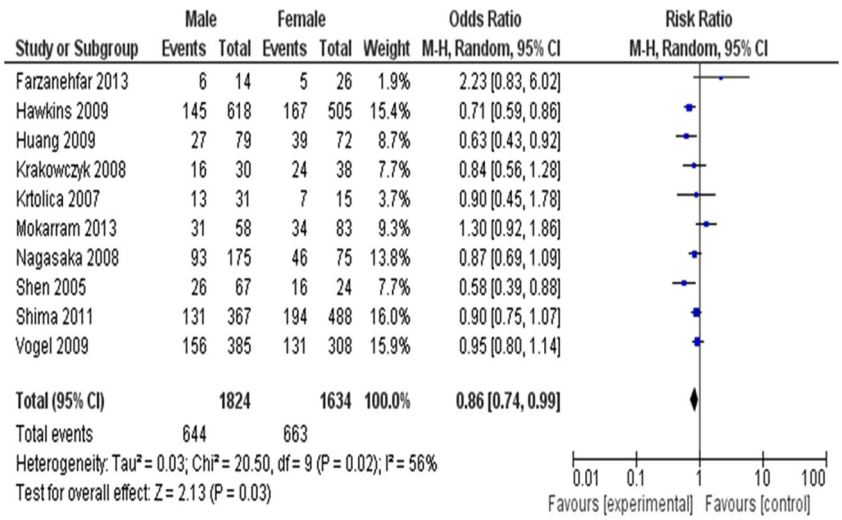


**Fig. 5** Forest plot for *MGMT* methylation in female and male CRC patients

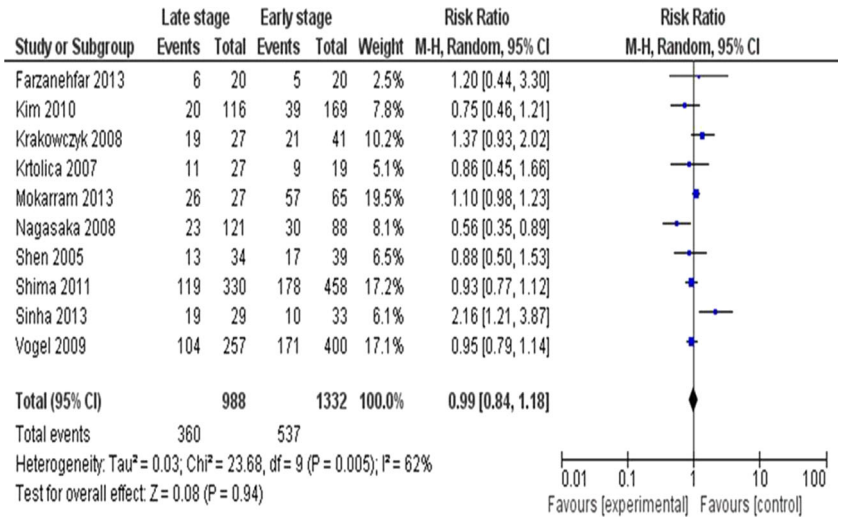


**Fig. 6** Forest plot for *MGMT* methylation in late and early stages of CRC

silencing of *MGMT* gene expression by promoter hypermethylation may play an important role in the early stage of CRC progression and development. (4) Aberrant *MGMT* hypermethylation is significantly higher in MSI-positive CRC than that in MSI-negative CRC, which indicates that epigenetic silencing of *MGMT* gene expression by the promoter hypermethylation may play an important role in increasing levels of MSI. (5) The pooled HR for OS shows that *MGMT* hypermethylation is not associated with worse survival in CRC patients. The results from the current study demonstrate that the hypermethylation rate of *MGMT* gene promoter in CRC is significantly higher than that in the normal colorectal mucosa, as well as colorectal adenoma, indicating that *MGMT* promoter hypermethylation is common in CRC. Since changes in *MGMT* promoter hypermethylation are reversible, drug treatment through demethylation may be useful to delay carcinogenesis and progression and to improve prognosis. This approach may bring new direction and hope for cancer treatment through gene-targeted therapy. Therefore, MGMT has a role as a potential drug target of CRC. The finding that *MGMT* hypermethylation is correlated with sex status and significantly higher in female than in male is also interesting. Female sex hormones have been implicated in the etiology of proximal CRC, and they may participate in different tumorigenic pathways that are associated with distinct DNA methylation-based molecular signatures and specific DNA methylation alterations [47].

Epigenetic alteration, particularly aberrant DNA methylation, is one of the best-characterized epigenetic modifications contributing to tumor initiation and progression [6, 7]. A cell only has limited resources to repair abnormal adducts since the active site cannot be regenerated after *MGMT* methylation. The DNA mutation repairing ability depends on the rate of MGMT synthesis. Therefore, the preciseness of MGMT protein expression indicates the capability of *MGMT* DNA mutation repairing during protection against tumorigenesis. *MGMT* methylation or loss of MGMT has been associated with unfavorable prognosis in brain tumor and B cell lymphoma [48–50]. However, in CRC, inconsistent results have shown the association between *MGMT* methylation and CRC. This is likely due to the previous studies that are limited by low statistical power. Another reason is that previous

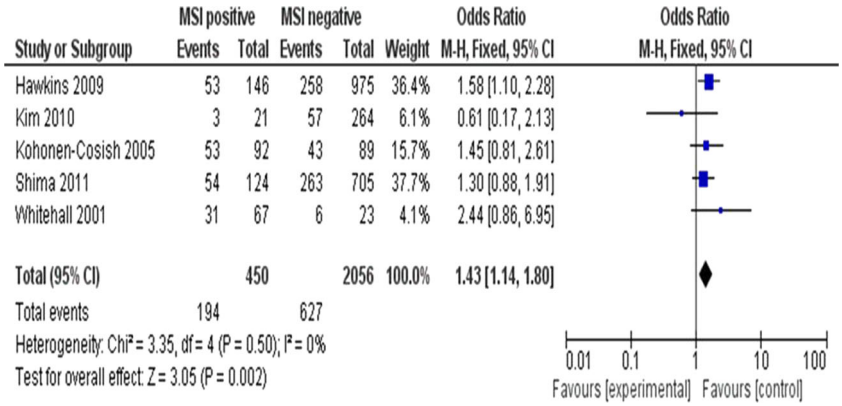


**Fig. 7** Forest plot for *MGMT* methylation and microsatellite instability (MSI) in CRC patients

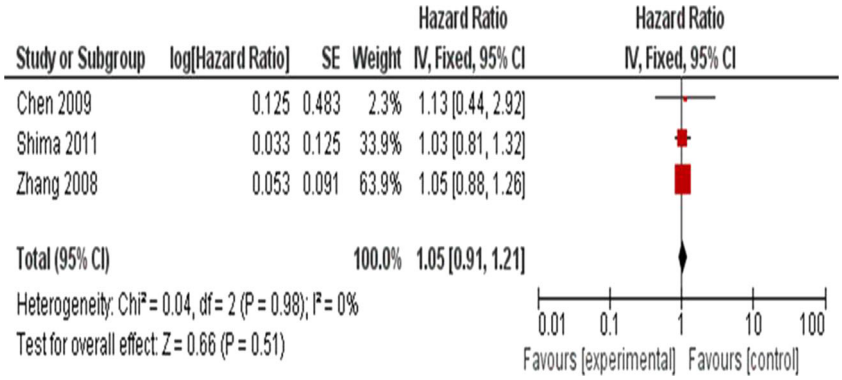


**Fig. 8** Forest plot for *MGMT* methylation and overall survival of CRC patients

studies use one or the other *MGMT* promoter regions to correlate methylation data with the loss of MGMT protein [17, 20, 51, 52]. Therefore, *MGMT* can be considered as a tumor suppressor, and its inactivation could contribute to tumor progression and poor prognosis. For the first time, we used a meta-analysis to assess *MGMT* methylation on CRC progression. Our result indicates that *MGMT* methylation is not significantly associated with the prognosis of CRC.

This study has several potential limitations. The different methods used to measure MGMT methylation may also affect the individual results, and the different methods were not able to measure the same CpG sites within MGMT promoter in individual study. There are other factors that may also affect *MGMT* methylation. For example, *MGMT* germline polymorphism was reported to be associated with somatic *MGMT* promoter methylation and gene silencing in CRC patients [53]. Age is also a factor that may affect *MGMT* methylation status in CRC patients. However, we were not able to do meta-analysis due to limited available data. Therefore, cautions should be taken when these analysis results are interpreted among the general populations.

In conclusion, our meta-analysis shows that *MGMT* may play an important role in CRC initiation, and *MGMT* hypermethylation is associated with high levels of MSI. *MGMT* hypermethylation may play an important role in the early stage of CRC progression and development. Further large-

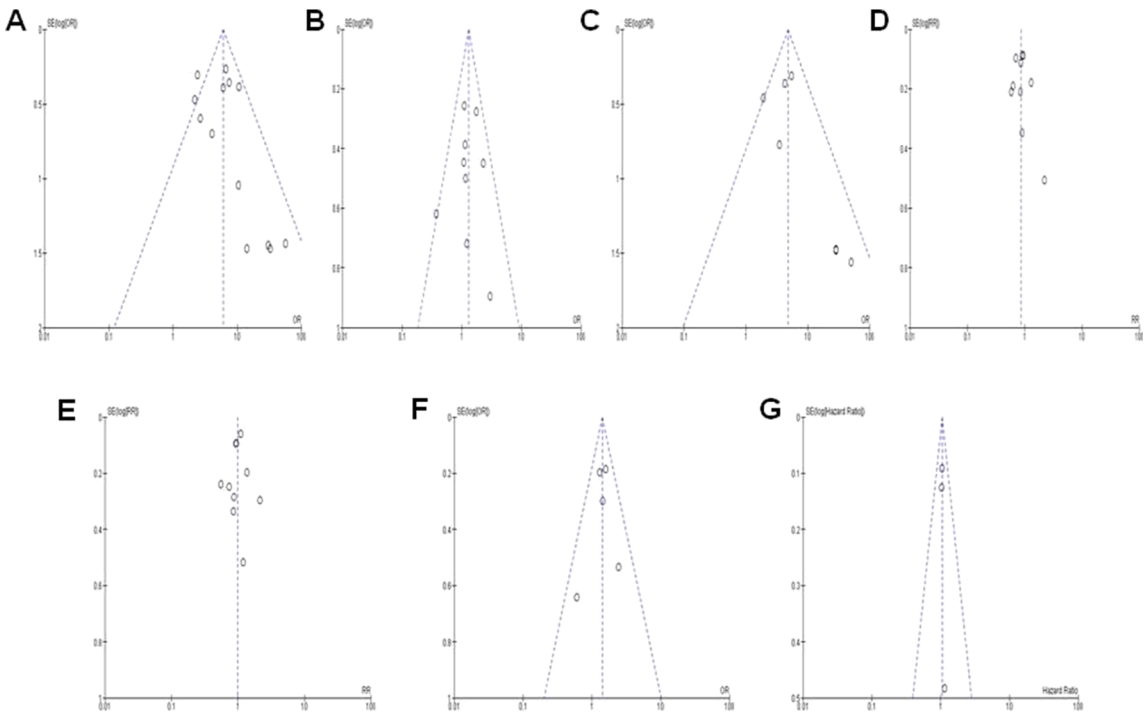


**Fig. 9** Funnel plot for publication bias. **a** *MGMT* methylation in CRC and normal tissue. **b** *MGMT* methylation in CRC and adenoma. **c** *MGMT* methylation in adenoma and normal tissue. **d** *MGMT* methylation in different genders of CRC. **e** *MGMT* methylation in late and early stages of CRC. **f** *MGMT* methylation in different levels of microsatellite instability (MSI) of CRC. **g** The association of *MGMT* methylation and overall survival of CRC patients. *X* axis: value of odds ratio (OR). *Y* axis: standard errors (SE) multiply log scale of OR

scale studies, especially multicenter and well-matched cohort research, will provide more insights into the role of *MGMT* in the prognosis and clinical implementation of CRC patients.

**Conflicts of interest** This work was supported by Wenzhou Municipal Science and Technology Project (Y20090028). The author reports no conflicts of interest in this work.

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