



O⁶-Methylguanine-DNA Methyltransferase (MGMT): Challenges and New Opportunities in Glioma Chemotherapy

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Chemoresistance has been a significant problem affecting the efficacy of drugs targeting tumors for decades. MGMT, known as O⁶-methylguanine-DNA methyltransferase, is a DNA repair enzyme that plays an important role in chemoresistance to alkylating agents. Hence, MGMT is considered a promising target for tumor treatment. Several methods are employed to detect MGMT, each with its own advantages and disadvantages. Some of the detection methods are; immunohistochemistry, methylation-specific PCR (MSP), pyrophosphate sequencing, MGMT activity test, and real-time quantitative PCR. Methylation of MGMT promoter is a key predictor of whether alkylating agents can effectively control glioma cells. The prognostic value of MGMT in glioma is currently being explored. The expression of MGMT gene mainly depends on epigenetic modification—methylation of CpG island of MGMT promoter. CpG island covers a length of 762 bp, with 98 CpG sites located at the 5' end of the gene, ranging from 480 to 1,480 nucleotides. The methylation sites and frequencies of CpG islands vary in MGMT-deficient tumor cell lines, xenografts of glioblastoma and *in situ* glioblastoma. Methylation in some regions of promoter CpG islands is particularly associated with gene expression. The change in the methylation status of the MGMT promoter after chemotherapy, radiotherapy or both is not completely understood, and results from previous studies have been controversial. Several studies have revealed that chemotherapy may enhance MGMT expression in gliomas. This could be through gene induction or selection of high MGMT-expressing cells during chemotherapy. Selective survival of glioma cells with high MGMT expression during alkylating agent therapy may change MGMT status in case of recurrence. Several strategies have been pursued to improve the anti-tumor effects of temozolomide. These include the synthesis of analogs of O⁶-meG such as O⁶-benzylguanine (O⁶-BG) and O⁶-(4-bromothienyl) guanine (O⁶-BTG), RNAi, and viral proteins. This review describes the regulation of MGMT expression and its role in chemotherapy, especially in glioma. Targeting MGMT seems to be a promising approach to overcome chemoresistance. Further studies exploring new agents targeting MGMT with better curative effect and less toxicity are advocated. We anticipate that these developments will improve the current poor prognosis of glioma patients.

Keywords: MGMT, methylation, alkylating agents, target therapy, chemotherapy

INTRODUCTION

O⁶-methylguanine-DNA methyltransferase, known as MGMT, is a DNA “suicide” repair enzyme. It repairs damaged guanine nucleotides by transferring the methyl at O⁶ site of guanine to its cysteine residues, thus avoiding gene mutation, cell death and tumorigenesis caused by alkylating agents. MGMT gene is located on chromosome 10q26.3 (**Figure 1A**), with a total length of 300,437 bp (3, 4). The expression of MGMT gene is mainly regulated by epigenetic modification. Many studies have shown that the loss of MGMT expression is not due to gene deletion, mutation, rearrangement or unstable RNA, but due to methylation of CpG island of MGMT promoter (5–9). In 1987, Gardiner and Frommer discovered that the human MGMT gene has a CpG island with a length of 762 bp, with 98 CpG sites located at the 5' end of the gene, ranging from 480 to 1,480 nucleotides (nt) (**Figure 1B**). The transcription initiation site of the gene is nt 956, and the CpG island spans about 500 bp (1) at the 5' and 3' ends of the transcription initiation site. The nt naming was initiated by Harris et al. beginning from the recognition site (2) of the restriction enzyme BamH1. In the non-methylated state, the transcriptional initiation sites of MGMT adhere to four precisely located nucleosome-like structures, which fine-tunes the transcription of the gene. Methylation of CpG islands leads to heterochromatinization, accompanied by rearrangement and random localization of nucleosomes, thus obscuring the transcription initiation sites and making transcription devices unable to bind (10, 11). Other studies have shown that methylation and chromatin status modulate the transcription of MGMT gene by determining whether Sp1 and other transcription factors access the MGMT promoter (12).

DETECTION METHODS FOR MGMT

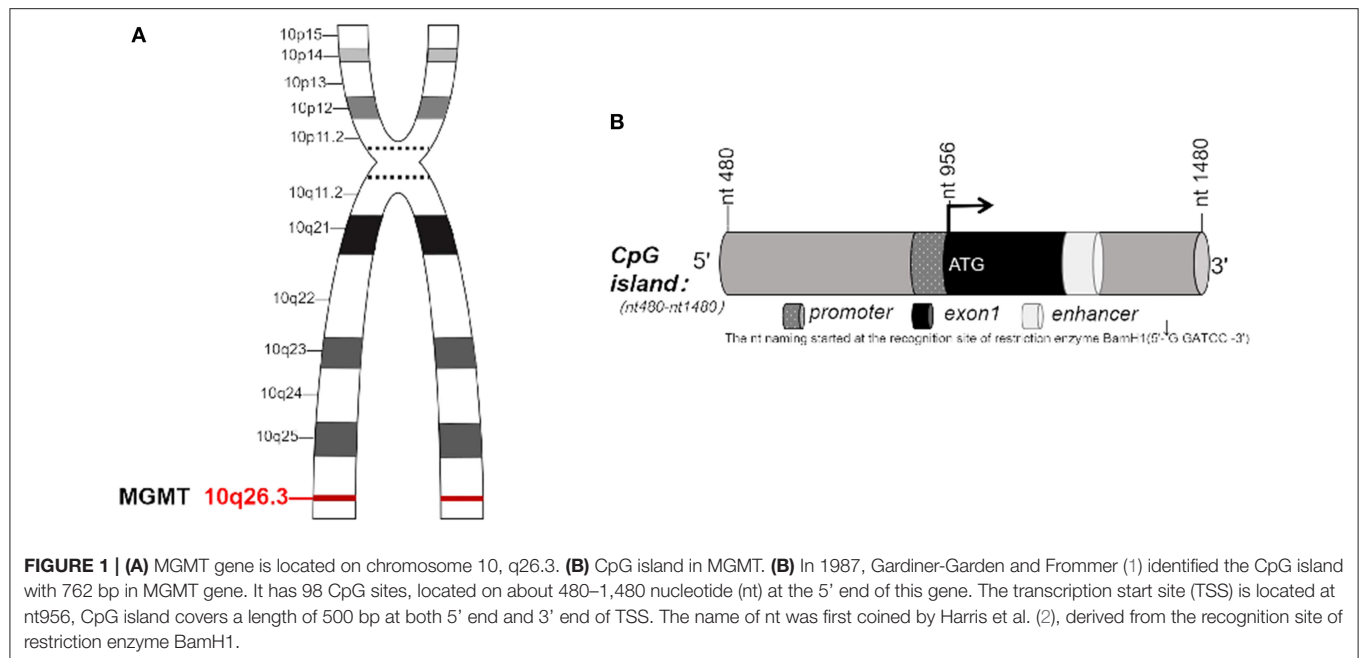
Immunohistochemistry is a semi-quantitative method used to detect the expression of MGMT protein (13–15). It can distinguish between tumor cells and non-tumor cells, and thus the results are not confounded by the heterogeneity of tumors, but this method is greatly influenced by the subjectivity of the observer (16). In clinical research, many methods are used to detect the methylation status of MGMT promoter in glioma. The most frequently used method is methylation-specific PCR (MSP) (17, 18). This method was first established in 1996 for detection of promoter methylation (19). However, it can only detect the methylation of small fragments complementary to primers, and cannot determine the exact location of promoter methylation. Therefore, it is a semi-quantitative method (20). Whether conventional MSP primers (+120 to +143, +173 to +196) bind to the key regions that regulate MGMT expression remains unclear. Pyrophosphate sequencing is a comprehensive

sequencing method. In this method, methylation level of a single CpG site modified by sulfite can be quantitatively evaluated by efficient PCR amplification and sequencing. Therefore, Pyrophosphate sequencing is more reliable than MSP (21). However, the role of CpG island hypermethylation in gene silencing remains controversial (22–25). Many researchers hold the view that methylation of MGMT promoter directly inhibits gene transcription, thus detection of promoter methylation may serve as an indicator of susceptibility to alkylating agents. In fact, the most direct method used to detect the sensitivity of alkylating agents is MGMT activity test. The number of MGMT active molecules per unit protein or DNA detected by MGMT activity test can reflect the level of MGMT protein and RNA. However, because the test requires fresh or frozen tissues and *in situ* hybridization, it is not feasible for daily application. In contrast, quantitative detection of MGMT RNA expression by real-time quantitative PCR seems more suitable and highly sensitive, but few studies have applied it in MGMT RNA detection. Recently, Wang et al. found that a combination of immunohistochemistry and qMSP assays can provide high sensitivity and specificity for the prediction of MGMT status (26).

THE PREDICTION AND PROGNOSTIC VALUE OF MGMT PROMOTER STATUS IN GLIOMA

The MGMT gene encodes a DNA damage repair protein that removes alkylating agents resulting in resistance to chemotherapy. Because DNA methylation can inhibit transcription, methylation of MGMT promoter increases sensitivity to alkylating agents (27). Several studies have shown that methylation of MGMT promoter can predict whether alkylating agents can be of benefit in glioblastoma and low-grade gliomas (28–37). Two other clinical trials have revealed that methylation status of MGMT promoter can predict the prognosis of glioma patients. In these two studies, retrospective analysis of MGMT promoter methylation in elderly patients found that it could predict good prognosis in temozolomide (TMZ) group, but not in radiotherapy alone group (38, 39). The EORTC26951 clinical trial retrospectively analyzed the methylation status of MGMT promoter in anaplastic oligodendroglioma patients. It was found that methylation of MGMT promoter in anaplastic oligodendroglioma patients predicted better overall survival (OS) and PFS, whether in radiotherapy alone or in sequential radiotherapy and chemotherapy group [chemotherapy regimen: procarbazine, lomustine (CCNU), vincristine (PCV)]. But it had no prognostic value in glioblastoma patients. Elsewhere, it has been reported that methylation of MGMT promoter has no predictive value for chemosensitivity of anaplastic oligodendroglioma patients undergoing adjuvant PCV chemotherapy (40). Another phase III randomized clinical trial, NOA-04, drew a similar conclusion that methylation of MGMT promoter and IDH1 mutation reduces the risk of progression in anaplastic glioma patients, and patients with MGMT promoter methylation have a longer PFS (41) in both radiotherapy and chemotherapy groups (PVC). In addition,

Abbreviations: MGMT, O⁶-methylguanine-DNA methyltransferase; MSP, methylation-specific PCR; IHC, immunohistochemistry; PCV, methylbenzylhydrazine, cyclohexanisolone and vincristine; OS, overall survival; PFS, progression-free survival; IDH1, isocitrate dehydrogenase; LMPER, Linker-mediated PCR; TSS, transcription initiation site; BSP, Bisulfite Sequencing PCR; O⁶-BG, O⁶-benzylguanine; TMZ, temozolomide; CSF, cerebrospinal fluid; O⁶-BTG, O⁶-(4-bromothienyl) guanine; DSF, disulfiram.



results from a phase III clinical trial prospectively indicate that MGMT promoter methylation status can be used as a biomarker to predict good prognosis of glioblastoma patients treated with TMZ (42) (Table 1).

EPIGENETIC REGULATION OF MGMT CPG ISLAND

The methylation sites and frequencies of CpG islands vary among MGMT-deficient tumor cell lines, xenografts of glioblastoma and *in situ* glioblastoma. Pieper et al. used Linker-mediated PCR (LMPCR) to detect the methylation status of MGMT promoter. It was found that the changes in methylation level of MGMT promoter mainly occurred at four CpG loci in cell lines expressing MGMT and those lacking MGMT, rather than being distributed uniformly throughout the CpG island. Two of them are located at about 130 nucleotides (+130) downstream of the transcription initiation site (TSS), including the sites recently studied using MPS. Two other nucleotides (−200) are located upstream of the transcription initiation site, and the transcription factor binding sites in both cell lines are not methylated. The transcription initiation site is defined as 0 (43). Watts et al. performed bisulfite sequencing PCR (BSP) on 108 CpG loci of 8226/s and 8226/v promoter CpG islands, respectively, and found that 8226/v has three methylation-rich regions which differs from those of 8226/s: −446 to −353, −265 to −162 and +112 to +212 (11). Costello et al. analyzed the methylation status of CpG loci in MGMT promoter −252 to −155 and −90 to +65 regions of glioma cell line by LM-PCR, and found that 21 of 25 loci were negatively correlated with MGMT gene expression (12). Because the authors detected the methylation level in high, low and non-expressing cell lines in these two

regions, a quantitative relationship could not be established. Qian et al. used BSP method to detect the methylation level of CpG loci from −249 to +259 in MGMT CpG island region. It was found that HT29, a cell line expressing MGMT, was almost not methylated in this region, whereas BE, a cell line not expressing MGMT, was heavily methylated in each clone in this region. The most frequently methylated regions ranged from −249 to −103, +107 to +196 (44). Malley et al. used pyrophosphoric acid sequencing to detect the methylation of CpG islands in the entire MGMT promoter of glioblastoma cell lines, xenografts and normal brain tissues (41 samples). It was found that the +152 to +214 were the key regions promoting the transcription of MGMT (45). Subsequently, methylation of MGMT promoter was studied in human glioma samples. It was found that the methylation of CpG loci at −186 to −172 and +93 to +153 regions was most correlated with MGMT gene expression, but previous MSP loci were not found in this region, although the methylation level of MSP loci was similar to that reported previously (46). Bady et al. used human methylation 450 gene chip (HM-450K) to detect 14 CpG loci of MGMT promoter in 63 glioblastoma samples. It was found that the methylation of −193 and +173 CpG loci was negatively correlated with gene expression and had a good predictive accuracy for prognosis (47). Similarly, Mur et al. obtained genome-wide methylation profiles of 247 glioma samples from HM-450K platform, including 25 CpG loci in CpG island of MGMT promoter region. The methylation of +173 CpG loci was significantly associated with overall survival (48). These researchers also found that MGMT promoter CpG islands are not suitable for methylation and this do not regulate expression or predict the prognosis of patients. Everhard et al. found that MGMT promoter regions −452 to −399 were highly methylated in both tumors and normal brain tissues. The region −90 to +69 is the first CpG region of small

TABLE 1 | Summary of the OS and PFS of patients receiving different treatments and characterized by non-methylated and methylated MGMT promoters in different studies.

References	Pathology	Treatment	OS (months)			PFS (months)		
			MGMTm	MGMTu	ALL	MGMTm	MGMTu	ALL
Criniere et al. (28)	GBM	RT+BCNU	17.1 (14.5–26.5)	13.1 (10.1–17.2)	NG	NG		
		RT	15.1 (9.8–n.r.)	10.2 (3.33–21.9)	NG			
		ALL☆	14.4 (13–16.1)	13.6 (11.4–15.7)	13.9 (12.5–15.3)	7.33 (5.8–8.43)	7.63 (6.47–8.63)	7.37 (6.5–8.43)
Hegi et al. (29)	GBM	RT+TMZ	21.7 (17.4–30.4)	12.7 (11.6–14.4)	NG	10.3 (6.5–14.0)	5.3 (5.0–7.6)	NG
		RT	15.3 (13.0–20.9)	11.8 (9.7–14.1)		5.9 (5.3–7.7)	4.4 (3.1–6.0)	
		ALL	18.2 (15.5–22.0)	12.2 (11.4–13.5)		NG	NG	
Reifenberger et al. (30)	GBM (age ≥ 70)	RT+TMZ	13.1 (11.0–15.3)	10.4 (8.4–12.4)	12.3(11.2–13.4)	7.3 (6.2–8.5)	7.2 (5.6–8.7)	7.2 (6.3–8.0)
		TMZ	7.2 (5.6–8.9)	2.6 (n.r.)	6.8(4.8–8.8)	6.8 (2.5–11.0)	0.5 (n.r.)	5.3 (0.1–10.5)
		RT	7.8 (3.4–12.2)	8.8 (7.5–10.1)	8.7(7.0–10.4)	4.5 (3.5–5.4)	5.2 (4.3–6.2)	5.0 (4.4–5.6)
		No treatment	2.3 (0.8–3.8)	2.0 (0.6–3.7)	2.3(0.9–3.7)	1.8 (1.1–2.4)	1.7 (0.4–3.1)	1.8 (1.0–2.5)
		ALL	8.4 (6.7–10.1)	6.4 (3.9–8.9)	7.7(6.3–9.0)	5.2 (4.3–6.1)	4.7 (3.8–5.5)	4.8 (4.3–5.3)
Esteller et al. (31)	AA/GBM	ALL (RT+BCNU)	MGMTu/MGMTm: HR = 9.5 (95% CI: 3.0–42.7, $p < 0.001$)			MGMTu/MGMTm: HR = 10.8 (95% CI: 4.4–30.8, $p < 0.001$)		
Hegi et al. (32)	GBM	ALL (RT+TMZ after surgery)	MGMTu/MGMTm: The risk of death within 18 months after surgery: 92% vs. 38%; $p = 0.002$			NG		
Everhard et al. (33)	LGG	ALL (TMZ)	NG			29.5 (21.5–n.r.)	6 (5–n.r.)	28 (20–n.r.)
Pandith et al. (37)	Gliomas	RT+TMZ	40.1 (29.8–50.3)	6.8 (3.8–9.6)	43.4 (32.5–54.1)	23.9 (20.0–27.7)	3.2 (0.6–5.8)	25.8 (21.9–29.6)
Malmstrom et al. (38)	GBM (age ≥ 60)	TMZ	9.7 (8.0–11.4)	6.8 (5.9–7.7)	8.3 (7.1–9.5)	NG		
		Standard RT (60 Gy)	8.2 (6.6–9.9)*	7.0 (5.7–8.3)*	6.0 (5.1–6.8)			
		Hypofractionated RT (34 Gy)			7.5 (6.5–8.6)			
		ALL	9.0 (8.0–10.0)	6.9 (5.9–7.9)	NG			
Wick et al. (39)	AA/GBM (age ≥ 65)	TMZ	n.r. (10.1–n.r.)	7 (5.7–8.7)	8.6 (7.3–110.2)	8.4 (5.5–11.7)	3.3 (3.0–3.5)	3.3 (3.2–4.1)
		RT	9.6 (6.4–n.r.)	10.4 (8–11.6)	9.6 (8.2–10.8)	4.6 (4.2–5.0)	4.6 (3.7–6.3)	4.7 (4.2–5.2)
		ALL	11.9 (9.0–n.r.)	8.2 (7.0–10.0)	NG	5.7 (5.0–7.4)	3.5 (3.3–3.7)	NG
van den Bent et al. (40)	AOD/AOA (≥25% oligodendroglioma elements)	RT RT+PVC	59.3 (30.0–66.2) n.r. (n.r.)	12.3 (11.5–28.5) 19.0 (12.3–34.5)	NG	17.9 (11.9–43.4) 49.0 (19.1–71.2)	7.8 (7.1–17.6) 10.5 (5.2–23.0)	NG
Wick et al. (41)	Anaplastic gliomas (WHO III)		NG			MGMTu/MGMTm:		
		RT			72.1 (n.r.)	HR = 2.0 (95% CI: 1.1–3.6, $p < 0.03$)		30.6 (16.3–42.8)
		TMZ/PCV			82.6 (n.r.)	HR = 2.7 (95% CI: 1.4–5.1, $p < 0.003$)		31.9 (21.1–37.3)
Gilbert et al. (42)	GBM	Standard dose TMZ	21.4 (17.6–29.0)	14.6 (13.2–16.5)	16.6 (14.9–18.0)	6.5 (4.1–9.6)	5.1 (4.3–5.7)	5.5 (4.7–6.1)
		Dense dose TMZ	20.2 (15.4–25.1)	13.3 (12.3–14.3)	14.9 (13.7–16.5)	10.1 (7.9–12.4)	6.0 (5.5–6.5)	6.7 (6.2–7.7)
		ALL	21.2 (17.9–24.8)	14.0 (12.9–14.7)	NG	8.7 (6.6–11.2)	5.7 (5.1–6.1)	NG

☆, included other treatments: BCNU alone or supportive care; *, standard RT and hypofractionated RT is grouped together; GBM, glioblastoma; LGG, low-grade gliomas; AA, anaplastic astrocytoma; AOD, anaplastic oligodendroglioma tumors; AOA, anaplastic oligoastrocytoma; RT, radiotherapy; TMZ, temozolomide; BCNU, carmustine; PCV, procarbazine, lomustine, and vincristine; OS, median overall survival; PFS, median progression-free survival; MGMTm, MGMT promoter methylated status; MGMTu, MGMT promoter unmethylated status; NG, not given; n.r., not reached; HR, hazard ratio; 95%CI, 95 percent confidence interval.

promoter, TSS and non-coding exon, which is equivalent to the methylation-free region (46) in both normal brain tissue and tumors. Thus, transcriptional silencing does not require methylation of the entire CpG island, but only methylation of several gene-specific core CpG sites. Therefore, methylation at some regions of promoter CpG islands is particularly associated with gene expression.

THE INFLUENCE OF CHEMOTHERAPY AND RADIOTHERAPY ON THE METHYLATION STATUS, ACTIVITY AND PROTEIN EXPRESSION OF MGMT

Methylation of the promoter region of the MGMT gene is known to predict the response to alkylating agent's treatment in glioma patients. However, knowledge about the change in the methylation status of the MGMT promoter after chemotherapy, radiotherapy or both is still incomplete. Wiewrodt et al. analyzed MGMT activity in 40 paired primary and recurrent glioblastomas, 16 patients after RT only, 24 patients with RT combined with chemotherapy (TMZ and/or CCNU or ACNU). In both recurrent groups, the MGMT activity was higher than in primary tumors. In contrast, for patients who received RT only, there was no significant difference between primary glioblastomas and recurrences. The MGMT activity was significant, however, in patients with primary glioblastomas and recurrences that received RT plus alkylating agent therapy (49). Brandes et al. analyzed MGMT promoter methylation status of 38 paired primary and recurrent glioblastomas treated with adjuvant radiotherapy and chemotherapy. They found that MGMT methylation status was changed in 14 patients (37%) who had recurrent tumors and more frequently in those with methylated MGMT than in unmethylated patients (50). Christmann et al. compared MGMT activity and MGMT promoter methylation in 46 primary glioblastoma samples and 19 recurrent glioblastoma samples. They found that MGMT activity increased after treatment, and methylation of MGMT promoter was detected in 39% primary tumors, while only 5.3% recurrent glioblastomas displayed MGMT promoter methylation (17). Elsewhere, Felsberg et al. analyzed the methylation status of MGMT promoter in 80 paired primary and recurrent glioblastomas, of which 16 patients received radiotherapy alone and 64 patients received radiotherapy and TMZ chemotherapy. They found the MGMT methylation status of 89% patients was not altered (51). It is worth noting, besides, that the response of the human MGMT promoter to genotoxic stress may be weak. Although Fritz et al. and Chan et al. had reported MGMT mRNA transcription can be induced by DNA-damaging treatments, both of their experiments were limited in rat H4IIE hepatoma cells (52, 53). As for human MGMT promoter, Grombacher et al. found that it could be induced by dexamethasone when transfected into rat H4IIE and human HeLa S3 (Mex+) cells, but methylating agents and ionizing radiation only worked in H4IIE cells (54). Boldogh et al. analyzed the mechanism of human MGMT expression induction, they found that protein kinase C-mediated signaling played an important role, involving

activation of AP-1 sites on MGMT promoter by TPA (55). Aasland et al. further identified that human MGMT promoter can be induced by glucocorticoids, but not by genotoxic stress, in human malignant glioblastoma cells (56). They put forward that a cluster of SP1 sites in human MGMT promoter prevented transcriptional up-graduation and overshadowed activation signals from other weaker transcriptional factors. The transcription factor SP1 was sequestered by p53, which was induced following radiochemotherapy (57). Coincidentally, an earlier retrospective clinical data from Pitter et al. showed longer survival of no glucocorticoid usage GBM patient cohorts, alongside corresponding data in animal models (58). Thus, radiation and chemotherapy may have minor influence on transient transcriptional activation of human MGMT. The finding of a protection of tumor cells by dexamethasone and other steroids suggests that a controlled use of glucocorticoids in GBM therapy is desirable. In conclusion, these studies revealed that chemotherapy may provoke an up-regulation of MGMT expression in gliomas through selection of high MGMT expressing cells during chemotherapy. Selective survival of glioma cells with high MGMT expression during alkylating agent therapy may change MGMT status when recurrence.

MGMT IN GLIOBLASTOMA STEM CELLS

Cancer stem cells have been implicated in the progression and recurrence of GBMs. It has been recognized that even after effective treatment of tumors, minimal residual stem cells may be activated to enter a new stage of differentiation and proliferation. In this way, cancer stem cells promote the recurrence of tumors. Thus, we postulate that glioblastoma stem cells may cause resistance to TMZ, which enables them survive during chemotherapy. Liu et al. and Pistollato et al. revealed that glioblastoma (GBM) stem cells, identified with the stem cell marker CD133, express high level of MGMT and displayed strong tumor resistance to TMZ (59, 60). Beier et al. reported that there are distinct stem cell populations that, despite having similar MGMT promoter methylation status, differ in MGMT protein expression. And they also found that TMZ preferentially kills cancer stem cells in glioblastoma in MGMT-negative cell lines (61, 62). Mantwill et al. stated that MGMT is not expressed in all stem cell lines, which indicates that these cells have different grades of TMZ resistance (63). Happold et al. observed that differentiation of glioma stem cells resulted in a gradual loss of MGMT expression and increased TMZ sensitivity (64, 65). Although MGMT is highly expressed in stem cells, it is not clear why the alkylating agents are not effective in recurrent GBMs. Do the differentiated cells retain the TMZ resistance features of stem cells? These challenges necessitate the search for the mechanisms that regulate the expression of MGMT in different cell stages.

TARGETING MGMT PROTEIN

O⁶-benzylguanine (O⁶-BG) is the analog of O⁶-meG which is a low molecular weight pseudosubstrate for MGMT. It inactivates MGMT through alkyl group transfer (**Figure 2**). It can pass the

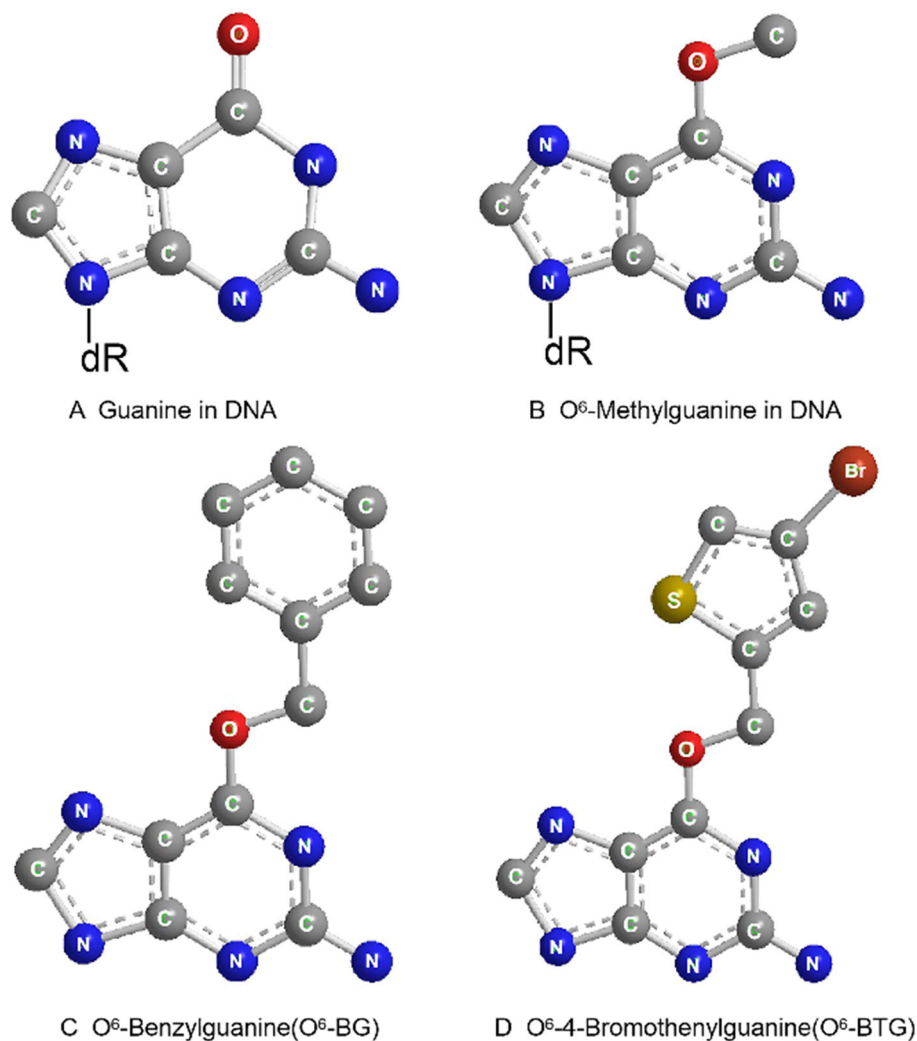


FIGURE 2 | Molecular structure of guanine in DNA (A), O⁶-Methylguanine in DNA (B), O⁶-Benzylguanine (C), and O⁶-4-Bromophenylguanine (D).

blood brain barrier and has, therefore, the potential to be a treatment for gliomas. It has been widely used as an MGMT inhibitor and as a sensitizer of glioma cells to alkylating agent TMZ (66, 67). Koch et al. found that local intracranial interaction of O⁶-BG with TMZ after intraoperative removal of brain tumors might delay tumor recurrence without any side effect (68). Phase I, II and III clinical trials of O⁶-BG combined with TMZ have revealed that this combination successfully abrogates other tumors, such as brain tumor, melanoma, lymphoma and colon cancer (69–72). A later phase II clinical research by Quinn et al. found that O⁶-BG combined with gliadel wafer prolonged the survival time of patients. However, it also increased the risk of hydrocephalus, cerebrospinal fluid (CSF) leak, and CSF/brain infection (73).

Another pseudosubstrate, O⁶-(4-bromophenyl) guanine (O⁶-BTG), has 10-fold higher potency than O⁶-BG in inactivating MGMT protein and is orally bioavailable without inherently toxic (Figure 2). It has been reported that O⁶-BTG efficiently

and rapidly inactivates MGMT in various tumors *in vivo* and *in vitro* and significantly increases tumor sensitivity to TMZ (74–77). A phase I trial of O⁶-BTG in combination with TMZ in advanced solid tumors established an oral ATase-depleting dose of lomeguatrib (Trade name for O⁶-BTG) and developed a combination regimen with TMZ that was 75% of the maximum tolerable dose of the single agent. The dose-limiting toxicity of O⁶-BTG was myelosuppression (78). Papachristodoulou et al. reported that a liposomal O⁶BTG can efficiently target MGMT, thereby sensitizing murine and human glioma cells to TMZ *in vitro* and magnetic resonance image-guided microbubble-enhanced low-intensity pulsed focused ultrasound mediates the delivery of the stable liposomal MGMT inactivator into the tumor region resulting in complete MGMT depletion *in vivo* (79).

Although the developed MGMT inhibitors, O⁶-BG and O⁶-BTG, are effective, their systemic toxicity due to non-specific targeting to normal cells cannot be ignored. Going forward, the

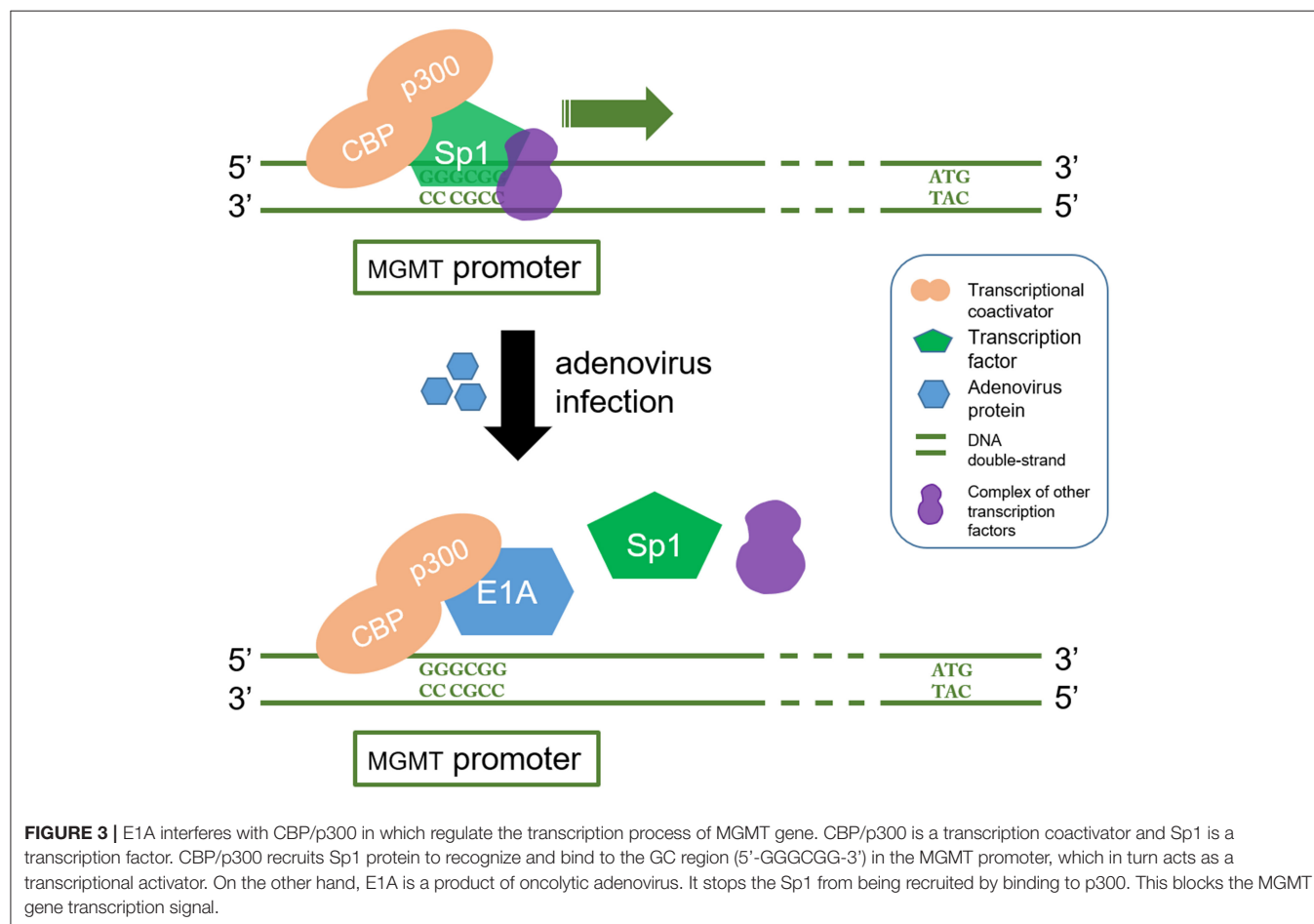
high glucose consumption hallmark of tumor cells presents a new avenue that can be exploited for development of selective inhibitors by conjugating agents to glucose. It has been reported that both O⁶BG-Glu and O⁶BTG-Glu are highly effective at inhibiting MGMT in several cancer cell lines, including T98G glioblastoma. These agents also enhance the cell-killing effect of temozolomide (80–82). Besides, Tomaszowski et al. found that glucose conjugates are subject of transport out of the cell by the ATP-binding cassette (ABC) transporter mediated efflux, which impacts the efficiency of MGMT inhibition. In this study, the importance of proper linker selection for a successful ligand-based drug delivery strategy was underscored (83). Similarly, conjugating pseudosubstrates to folate esters is another promising strategy to target tumor cells (84). So far, few studies have investigated the cellular effects of glucose or folate esters conjugated inhibitors. Further detailed studies should unravel the mechanisms of these inhibitors to provide better treatment agents.

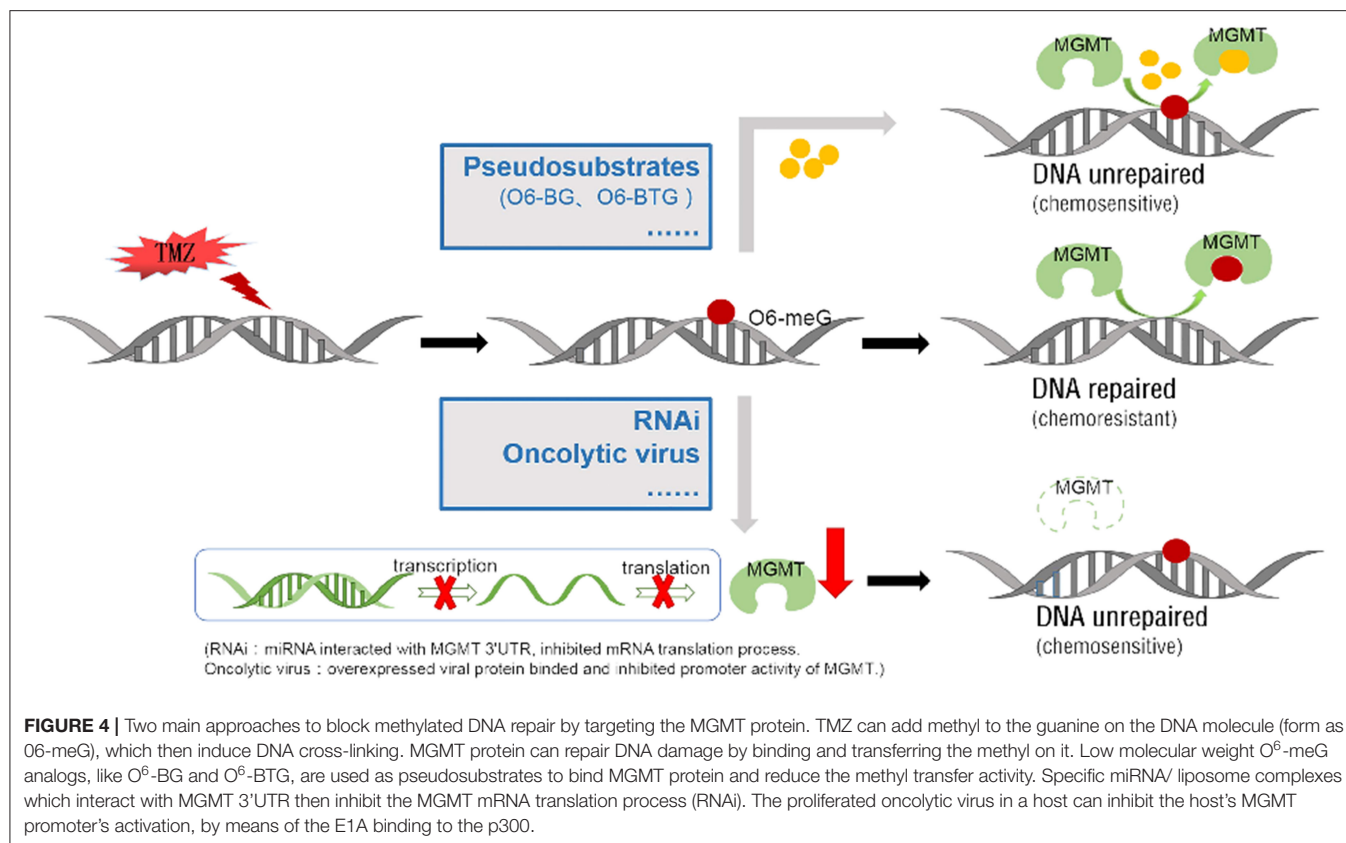
Watson et al. conducted phase II clinical trials to evaluate the efficacy of lomeguatrib in patients with melanoma. They found that lomeguatrib plus TMZ had a greater MGMT inactivation than did TMZ treatment alone (85). Another study by the same group also reported that lomeguatrib can be applied in prostate, primary CNS, and colorectal cancers to inactivate MGMT (86).

However, lomeguatrib increases myelosuppression, and other studies revealed that it did not improve the response rate to TMZ (69, 70, 74, 87–89).

RNA interference is another promising therapy targeting MGMT. Kato et al. reported that when combined with TMZ, the MGMT-siRNA/liposome complex exerted a strong synergistic antitumor effect (90). Zhang et al. found that miR-181d downregulated MGMT by directly interacting with MGMT 3'UTR, and this potentiated the TMZ sensitizer as an MGMT targeting therapy (91). Nie et al. found that miR-198 directly targeted MGMT by binding to the 3'-UTR of MGMT, thereby inhibiting the MGMT mRNA translation in GBM cells. MiR-198 restored the tumor sensitivity to TMZ in glioblastomas overexpressing MGMT (92).

Oncolytic viruses which inactivate or leverage the cellular DNA-repair machinery to achieve productive replication have also been exploited to design agents targeting MGMT. Adenoviruses express proteins which can downregulate MGMT expression. It has been reported that overexpression of adenovirus E1A, which binds p300, efficiently inhibits both basal and TSA inducible promoter activity of MGMT and may thus reduce chemoresistance (93, 94). CBP/p300 is a transcriptional coactivator which interacts with multiple transcription factors including those involved in MGMT gene. It plays an important





role in many cellular processes, and the structural and functional versatility of CBP/p300 are yet to be fully elucidated. For this reason, the utilization of adenovirus targeting MGMT is far from being clinically implemented (95) (**Figure 3**).

Jiang et al. reported that a combination of TMZ and viral therapy may overcome the chemoresistance of gliomas to TMZ (96). Further, it has been shown that oncolytic virus-mediated manipulation of DNA damage responses can also be applied to kill GSCs (97). The discovery of this oncolytic viral therapy opens a new era in cancer therapy. However, there are safety concerns regarding the use of virus-based therapy.

Other drugs that target MGMT protein such as disulfiram (DSF) have also been studied. For instance, Paranjpe reported that DSF directly suppressed MGMT protein expression through sole site Cys145 (98).

CONCLUSION AND PERSPECTIVES

MGMT is a DNA methyltransferase which repairs damaged DNA thus avoiding cell death caused by alkylating agents. The expression of MGMT gene is mainly regulated by epigenetic modification. Several methods have been developed for MGMT detection including immunohistochemistry, methylation-specific PCR, pyrophosphate sequencing, MGMT activity test, real-time quantitative PCR among others. Methylation of MGMT promoter can predict whether alkylating agents are effective for glioblastoma and low-grade gliomas. The

prognostic value of MGMT methylation is still controversial and calls for further clarification. Epigenetic regulation of specific sites of MGMT CpG island influences MGMT transcription. Chemotherapy and radiotherapy may modulate MGMT methylation status, activity and protein expression. TMZ is a promising chemotherapeutic agent for glioma, but the rapid development of drug resistance poses a huge challenge. Overexpression of MGMT is an important mechanism of TMZ resistance. Several strategies have been pursued to improve the anti-tumor effects of TMZ. These include development of pseudosubstrates, RNAi, viral proteins and many others agents (**Figure 4**). Given on-going research advancements in this field, the current poor prognosis of glioma patients is expected to improve.

AUTHOR CONTRIBUTIONS

AS designed the study and determined the final version. QW revised the manuscript and polished the language. WY drafted the manuscript. LZ made the figures and tables. All authors read and approved the final manuscript.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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