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Review

Implications of Advances in Studies of O^6-Methylguanine-DNA-Methyltransferase for Tumor Prognosis and Treatment

Yuexia Chen[1], Wei Qu[1], Jianhong Tu[1], Hongyan Qi[2,*]

[1]Department of Pathology, The Third Hospital of Nanchang, 330008 Nanchang, Jiangxi, China
[2]Department of Pathology and Pathophysiology and Department of Radiation Oncology of the Second Affiliated Hospital, University School of Medicine, Zhejiang University, 310058 Hangzhou, Zhejiang, China
*Correspondence: qihongyan@zju.edu.cn (Hongyan Qi)

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Abstract

O^6-methylguanine-DNA-methyltransferase (MGMT) is a DNA repair enzyme, which reverses the alkylation of guanine O^6 through directtransfer of the methyl group, maintains the gene stability and avoids tumor occurrence. Studies have shown that *MGMT* gene methylation, polymorphism and protein expression are involved in the process of various tumor development, such as colon cancer, gastric carcinoma, etc. *MGMT* gene promotes methylation, protein expression and enzyme activity from various tissues, which resultsin different effects on the prognosis of patients. MGMT promoter methylation is a positive factor for the prognosis of Glioblastoma (GBM), which can prolong overall survival and progression-free survival, reduce the resistance of tumor cells to temozolomide treatment, and improve the prognosis. The treatment of tumors based on MGMT focuses on three aspects: targeting MGMT to increase the sensitivity of alkylated drug therapy in tumors, immunotherapy combined with alkylated agents on tumor treatment, and treatment for patients with MGMT promoter non-methylation. Similarly, a number of studies have targeted MGMT to reduce alkylated agent resistance in other systems. Although numerous studies on MGMT in tumors have been reported, there are problems that need to be solved, such as selection and consensus of MGMT promoter methylation detection methods (CpG detection sites, cut-off value) and the treatment of MGMT non-methylated GBM patients, especially elderly patients. In this review, we describe the regulation of MGMT expression and its role inchemotherapy, especially in gliomas. Further studies exploring new methods targeting MGMT with better curative effect and less toxicity are advocated. We anticipate that these developments will be progressive and sufficiently used for clinical application.

Keywords: MGMT; expression; prognosis; treatment; tumor

1. Introduction

O^6-methylguanine-DNA-methyltransferase (MGMT), located on chromosome 10q26, is over 170 kb in length, including 5 exons and 4 introns. mRNA is 866 bp in length, encodes 207 amino acids and contains a conserved active site [1]. The promoter region is approximately 1.2 kb and contains enhancers, binding sites for transcription factors such as GR, Sp1, AP1, AP2 [2–4]. The promoter region lacks TATA and CAAT boxes, but is rich in GC sequences, forming 98 CpG sites. These sites are where cytosines are easily methylated, especially in the regions –186 to –172 and +93 to +153 where CpG methylation plays a major role in transcriptional regulation. MGMT expression is mainly regulated by transcriptional and epigenetic regulation, the former including SP1, AP1, NF-κB, GRE, p53, etc., while the latter contains DNA methylation in the promoter region and post-translational modifications of histones [5]. With cytosine methylation, the chromosome structure changes from loose and active euchromatin to compressed and aggregated heterochromatin, which prevents transcription factors from binding to the promoter region and inhibits transcription. The amino termini of histones could be modified by methylation, acetylation, ubiquitination, and poly-ADP-ribosylation, and modification of H3K9 was associated with MGMT silencing [6]. With DNA methylation, methylated CpG island binding proteins, especially MeCP2, bind to the methylated CpG island and then recruit histone deacetylases and H3K4 demethylases (such as LSD1), as well as histone methyltransferase to form heterochromatin like protein factors (HP1), eventually preventing the transcription process [5]. miRNAs, such as miR-181b, miR-181d, miR-221, miR-222, miR-767-3p and miR-648n [7–10], bind to the 3′ untranslated region of MGMT to reduce mRNA stability and affect protein translation.

MGMT is a highly conserved enzyme involved in DNA damage repair with a conserved amino acid sequence-(I/V) PCHR (Proline, cysteine, histidine, arginine) (V/I) located at the active center. During repair, MGMT independently transfers methyl groups from the guanine O^6 site directly to its own 145 cysteine residue, which is then accompanied by irreversible ubiquitination degradation [11] (Fig. 1). If O^6MeG is not repaired, it leads to a G:C $\rightarrow$ A:T mutation that can be recognized by the mismatch repair system (MMR), which then initiates an apoptotic signaling pathway, leading to an ineffective replication cycle, DNA

fragmentation, and eventually apoptosis [12]. MGMT can repair various types of damage caused by alkylated agents from a wide range of sources, including endogenous and exogenous damage, the latter of which encompasses the DNA causing methylation damage at the guanine O^6 site, such as temozolomide (TMZ), and drugs that cause O^6-CLG damage at the guanine O^6 site on DNA, such as carnitine mustard (BCNU, BiCNU), ronazine mustard (CCNU and CeeNU) and other chemotherapeutic agents [13,14]. MGMT transfers methyl from the guanine O^6 site directly to its cysteine residue at position 145 to complete repairing O^6-mG damage of DNA.

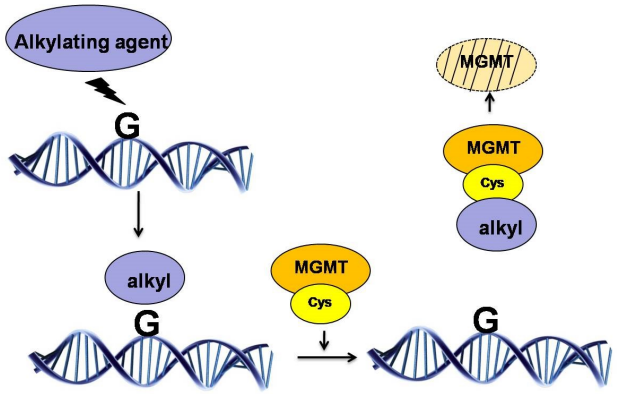

Fig. 1. The process of (MGMT) repairing damaged DNA. MGMT, O^6-methylguanine-DNA-methyltransferase; Cys, Cysteine.

2. Role of MGMT in Tumorigenesis and Progression

The G:C → A:T mutation in *K-ras*, *P53*, and other oncogenes or tumor suppressor genes is caused by MGMT down-regulation of protein expression as a result of promoter methylation, which favors the emergence and growth of malignancies. With this modification, a clear connection has been made between MGMT decrease and tumorigenesis [15]. In gastric cancer, colorectal cancer, breast cancer, oral squamous cell carcinoma and cervical squamous cell carcinoma tissues, MGMT promoter methylation is higher than that of surrounding normal mucosa or tissues. Therefore, MGMT promoter methylation might be considered a marker of precancerous lesions and a biomarker for early tumor diagnosis [16–19]. Another meta-analysis revealed that MGMT promoter hypermethylation was strongly linked to a higher risk of developing gastric cancer and may be connected to the spread of gastric cancer to distant sites and lymph nodes [20]. In our previous study to explore the molecular mechanism of MGMT in the malignant transformation and tumorigenesis of gastric cells induced by amide compounds, we found that MGMT up-regulation was induced by promoter hypomethylation. High expression of MGMT can prevent malignant transformation and tumorigenesis induced by amide compounds. In addition, in normal gastric tissues and gastric cancer patient specimens, MGMT was up-regulated in precancerous lesions and metaplastic tissues, and down-regulated in gastric cancer tissues, suggesting that MGMT may be involved in the occurrence and development of gastric cancer [21]. Additionally, MGMT has been shown to have a role in the development of other pathologies such as liver cancer, cholangiocarcinoma, lung cancer and other tumors [22–24]. *MGMT* gene polymorphisms have also been shown to be involved in tumor formation. A Mexican study demonstrated that MGMT rs12917 may contribute to the occurrence and progression of lung cancer [25]. It has also been revealed that women having the *MGMT* gene polymorphism Ile143Val have a lower chance of developing rectal cancer [26]. Single nucleotide polymorphisms in the *MGMT* gene can also affect the duration of TMZ-induced myelotoxicity and the side effects of antitumor drugs in adult patients with diffuse glioma [27]. MGMT polymorphism rs12917 might affect the response to chemotherapy in pediatric Hodgkin lymphoma [28]. Several investigations have discovered a link between the MGMT V1/W genotype and glioma recurrence [29] with other studies on the association between *MGMT* gene polymorphisms and tumors being performed.

3. Effect of MGMT on Tumor Prognosis

3.1 Effect of MGMT Promoter Methylation on Prognosis of Different Tumors

MGMT promoter methylation varied among different tumors. Approximately 38% of brain tumors were prone to MGMT promoter methylation, as were 28% of head and neck tumors, 26% of colon cancer, 25% of lymphoma, and 24% of lung cancer, while tumors of other organs exhibited a lower incidence of MGMT promoter methylation, such as pancreatic cancer, melanoma, kidney cancer, bladder cancer, and leukemia [30].

Numerous studies have demonstrated that MGMT promoter methylation is a beneficial factor for tumor prognosis. It has been proven to be related to improved patient prognosis, particularly in Glioblastoma (GBM), as well as prolonged overall survival (OS) and progression-free survival (PFS).

In a prospective clinical experiment conducted in 2004, Hegi *et al.* [31] showed the prognostic significance of MGMT promoter methylation for the prognosis of GBM patients receiving TMZ, an alkylating drug. The study revealed that MGMT promoter methylation leading to *MGMT* gene inactivation was associated with longer survival in GBM patients, suggesting that MGMT promoter methylation remains the only significant predictor for GBM patients [31]. In a subsequent study of 206 GBM patients, the re-

searchers found that GBM patients with MGMT promoter methylation responded to TMZ, while those who did not were more likely to develop treatment resistance [32]. Subsequently, other researchers have carried out a large number of studies on the correlation between MGMT promoter methylation and prognosis of GBM patients and found the prognosis of grade II and III gliomas to be positively impacted by MGMT promoter methylation [33,34].

Szylberg *et al*. [35] collected data on 41 newly diagnosed GBM patients treated at the 10th Military Research Hospital and Polyclinic in Poland between 2011 and 2014 to assess the impact of MGMT promoter methylation and other clinical factors on survival in patients with primary GBM (mean age, 53 years). They discovered that MGMT promoter methylation, which may be correlated with age and surgical resection technique, was also a significant positive predictive biomarker in GBM patients [35].

The results are still debatable even though the prognostic value of MGMT promoter methylation in GBM patients are well acknowledged. Some studies have not shown a significant correlation between MGMT promoter methylation and OS or PFS. In a multicenter Portuguese investigation, neither univariate nor multivariate analysis revealed a statistically significant relationship between MGMT promoter methylation and overall survival or PFS in patients with GBM receiving TMZ-based chemoradiotherapy [36]. Egaña *et al*. [37] also concluded that MGMT promoter methylation did not affect patient survival in the cohort studied. However, around 9 months after the diagnosis of GBM, Dahlrot *et al*. [38] discovered the connection between MGMT promoter methylation and overall survival, as prior to this, there was no correlation between MGMT promoter methylation and OS. In addition, Caccese *et al*. [39] found there was a nonlinear correlation between MGMT promoter methylation and OS, with longer OS with increasing MGMT promoter methylation. This study showed a median OS of 14.8 months for MGMT 0–4%, 18.9 months for MGMT 4–40%, and 29.9 months for MGMT 40–100% [39]. MGMT promoter methylation has also been used to stratify malignant gliomas in GBM and has been used as a prognostic marker for overall survival and as a predictor of chemotherapy response in GBM patients [40].

Numerous studies have been conducted in other tumors in addition to the one on MGMT promoter methylation and GBM, and the effects of tumor prognosis varied. MGMT promoter methylation in succinate dehydrogenase defect of gastrointestinal stromal tumors and epithelioid/mixed phenotype is particularly common in the wild type of gastrointestinal stromal tumor and this MGMT methylation pattern may provide a new potential treatment option for wild-type gastrointestinal stromal tumor [41]. In well-differentiated pancreatic neuroendocrine tumors, PFS is longer in patients with high MGMT promoter methylation and low MGMT expression [42]. In metastatic colorectal cancer, melanoma, central nervous system lymphoma and other tumors, MGMT promoter hypermethylation increased the sensitivity of alkylating agents, thus prolonging the survival of patients [43–45]. Numerous studies in esophageal, cervical, and lung cancers have shown controversial results [24,46,47]. These differences may be caused by: differences in MGMT promoter methylation detection assays or different methylation sites in the detected MGMT promoter region, different samples, different numbers contained in different tumor cells, and patient age [48].

MGMT gene polymorphism was also involved in predicting the prognosis of many tumors. Researchers have found that MTNR1B single-nucleotide polymorphisms (SNPs) combined with CDKN2A and MGMT promoter methylation status can be used to predict shorter survival in colon cancer [49].

3.2 Effect of MGMT Protein Expression on Prognosis of Different Tumors

MGMT, a DNA repair enzyme, is expressed in many organs of the body and its expression varies from one organ and tissue to another. The expression of MGMT protein was highest in the liver, followed by lung, kidney and colon, and lowest in the pancreas, hematopoietic cells, lymphoid tissues and brain. It was decreased in tumors such as gliomas, lymphomas, breast cancer, prostate cancer, and retinoblastoma, most likely related to the methylation status of its promoter region [50].

While MGMT protein expression detection methods mainly include immunofluorescence and immunohistochemistry (IHC), the latter predominates. In the early 1990s, Belanich *et al*. [51] used immunofluorescence method to detect the expression of MGMT protein in 99 cases of glioma tissues and found that patients with high MGMT protein expression were less sensitive to BCNU, resulting in shorter overall survival and progression-free survival. This was subsequently demonstrated in astrogliomas [51]. With various tumor types, there are differences in the expression of the MGMT protein as identified by the IHC approach and the prognosis of the tumor. A report of 73 cases of patients with newly diagnosed GBM utilized immunohistochemical methods to analyze MGMT protein expression as an auxiliary for TMZ and radiation treatment of GBM. In patients with detected prognostic markers, it was found that low MGMT protein expression in patients (15%) compared with patients with high expression of MGMT protein, overall survival and PFS were significantly improved. This suggests that MGMT protein expression is an independent and a positive prognostic factor in GBM patients [52]. MGMT immunohistochemistry expression has been demonstrated to be substantially correlated with various glioma grades and subtypes [53] including lymphomas, thymic tumors, and pituitary tumors [54–56].

Other investigations have reached the opposite conclusion. Aanchal *et al*. [57] collected the tissues of pa-

tients with meningeal hemangiopericytoma from 2002 to 2011. Immunohistochemistry was used to detect expression of MGMT protein and methylation-specific PCR (MSP) was adopted to detect MGMT promoter methylation. In investigating the relationship between MGMT expression and the prognosis of patients with meningeal hemangiopericytoma, they found no significant correlation between MGMT protein expression and progression-free survival [57]. In esophageal and gastrointestinal neuroendocrine tumors, MGMT protein expression level was also shown to have no specific correlation with prognosis, whereas in pancreatic neuroendocrine tumors, colorectal cancer and salivary gland carcinoma, patients with low MGMT protein expression had a poorer prognosis [58–61]. A recent study found that decreased MGMT expression in pancreatic neuroendocrine tumors was associated with a higher risk of progression [62]. The reasons for these discrepancies may be related to tumor type, the number of patients studied, individual differences, tissue size, medication differences, and diagnostic staining.

3.3 Effect of MGMT Enzyme Activity on Prognosis of Different Tumors

The enzyme activity of MGMT varies with different tissues, individuals and individual stages. In normal tissues, MGMT enzyme activity in the liver was highest, and lowest in brain tissue. While MGMT activity was highest in liver, ovarian, and colon tumors, it was still very low in gliomas, which may have contributed to the sensitivity of glioma cells to TMZ therapy. The activity varied according to the classification of gliomas, being lowest in astrocytomas and malignant gliomas, with an average of 111 fmol/mg, and up to 270 fmol/mg in non-glioma brain tumors, such as meningiomas [63]. MGMT activity in tumor tissues was higher than that in corresponding normal organ tissues, but in the testes and liver, the activity of tumor tissues was lower than that in normal tissues. There is minimal research examining the connection between MGMT activity and tumor prognosis. Early research has suggested that elevated MGMT activity may reduce the effectiveness of alkylated anticancer medications and have an adverse effect on patient prognosis [64,65].

4. Effect of MGMT on Tumor Therapy

MGMT is a DNA repair that inhibits the cross-linking of double-stranded DNA through alkylated agents, reverses the alkylation of guanine O^6 position, repairs DNA damage caused by drugs (such as alkylating agents), and leads to resistance to alkylated drugs [66]. It is also involved in the resistance of DNA to alkylated anticancer drugs such as TMZ, which has been studied extensively to overcome these therapeutic difficulties.

4.1 Targeting MGMT to Increase the Sensitivity of TMZ Therapy in Tumors

Although previous clinical trials have enhanced the therapeutic effect of TMZ by reducing MGMT protein expression, certain trials did not achieve clinical benefits [67]. A growing number of studies have focused on increasing the sensitivity of tumors (especially GBM) to TMZ treatment by targeting MGMT through various axes. Geng *et al*. [68] found that exosome-mediated circWDR62 promoted TMZ resistance and progression in glioma by targeting the Mir-370-3p/MGMT axis, suggesting that exosomal circWDR62 in human serum may be a therapeutic target for glioma. Zhou *et al*. [69] found that cyanidin-3-o-glucoside promoted the treatment of MGMT-induced glioma cell resistance through the potential signaling mechanism of miR-214-5p-mediated inhibition of TMZ resistance in LN-18/TR cells. Other studies have demonstrated that the lncRNA UCA1/miR-182-5p/MGMT axis regulates the sensitivity of glioma cells to TMZ through the MGMT-related DNA damage pathway [69,70]. In addition to gliomas, other tumors that have been studied include melanoma, lymphoma, and ovarian cancer [71–73].

Other potential targets have been investigated. The MGMT substrate analogue, O^6-benzylguanine, was a specific inhibitor of MGMT, which binds the benzoyl group to the 145th cysteine residue in the active center of MGMT protein, preventing the binding of the latter to DNA. It effectively reduces the ability of MGMT to repair alkyl-adduct DNA and inhibits MGMT activity. However, it has never been used clinically due to its side effects [74]. Pinto *et al*. [75] designed a hybrid drug that covalently binds BG residues to the interacting part of DNA (6-chloro-2-methoxy-9-aminoacridine) and found that compound 19a inhibits MGMT activity without inducing significant levels of DNA damage, providing a new therapeutic opportunity for GBM patients with TMZ resistance.

Numerous studies have investigated MGMT and tumor medication resistance by manipulating additional upstream and downstream signaling pathways in addition to the use of the aforementioned inhibitors. A study from China found that BanxiaXiexin decoction regulates MGMT expression through IL6/JAK/ STAT3-mediated PDL1 activity, which affects the sensitivity of gastric cancer cells to drugs. As a result, inhibiting MGMT offers a fresh approach to treating gastric cancer [76]. Additionally, it has been demonstrated that MGMT has a role in the chemosensitivity of cisplatin in gastric cancer [77]. MAPK/ERK inhibitor (U0126) combined with TMZ can be used in patients with advanced hepatocellular carcinoma as the former can block the MAPK/ERK signaling pathway to down-regulate MGMT expression and increase the sensitivity of HCC cells to TMZ [78]. MGMT was involved in the resistance process of dacarbazine treatment in uveal melanoma, but TRIM72 increased the sensitivity of dacarbazine treatment by ubiquitination and degradation of

Table 1. A collection of anti-tumor agents targeting MGMT.

Compound	Mechanism	Tumor type	Ref
circWDR62	Targeting miR-370-3p/MGMT axis promotes TMZ resistance	glioma	Geng *et al*. [68]
Cyanidin-3-O-glucoside	Down-regulation of β-catenin and MGMT by miR-214-5p inhibited TMZ resistance	glioma cell	Zhou *et al*. [69]
lncRNA UCA1	lncRNA UCA1/miR-182-5p/MGMT axis modulates glioma cell sensitivity to TMZ	glioma cell	Cheng *et al*. [70]
lncRNA POU3F3	lncRNA POU3F3/miR-650 axis upregulates MGMT expression to promote drug resistance	melanoma	Wu *et al*. [71]
miR-370	miR-370 inhibited MGMT expression and increased sensitivity to TMZ	primary central nervous system lymphoma	Li *et al*. [72]
MCL1	HDACis combined with PaTrin-2 overcomes resistance via the MGMT-DUB3-MCL1 axis	ovarian cancer	Wu *et al*. [73]
NCT503	Modulation of Wnt/β-catenin axis reduces MGMT expression to overcome drug resistance	glioblastoma	Jin *et al*. [80]
Tubeimoside-I (TBMS1)	Induced apoptosis in GBM cells through reducing MGMT expression and inhibiting the EGFR induced PI3K/Akt/mTOR/NF-κB signaling pathway	glioblastoma multiforme	Tang *et al*. [81]
GNA13	GNA13/PRKACA/MGMT modulates glioma sensitivity to TMZ	glioma	Liu *et al*. [82]
Pyrvinium pamoate	The AKT/GSK3β/β-catenin signaling axis regulates MGMT expression	glioblastoma	Li *et al*. [83]
DEC1	Control TMZ resistance via the SP1-MGMT axis	glioma cell lines	Lv *et al*. [85]
METTL3	Silencing MeTTL3-mediated inhibition of total methylation improves TMZ resistance	glioblastoma cell lines	Shi *et al*. [84]
Banxia xiexin decoction	Influence the drug sensitivity of GC cells by regulating the expression of MGMT	gastric cancer cells	Feng *et al*. [76]
Cisplatin (DDP)	Inhibits MGMT-mediated autophagy suppression to decrease chemosensitivity in GC	gastric cancer	Lei *et al*. [77]
U0126	Downregulate MGMT expression via blocking MAPK/ERK pathway increased sensitivity to TMZ	hepatocellular carcinoma cells	Li *et al*. [78]
Acridine-O^6-benzylguanine hybrids	Compound 19a inactivates MGMT to increase TMZ sensitivity	glioblastoma cell lines	Franco *et al*. [75]

TMZ, temozolomide; GBM, Glioblastoma; GC, guanine-cytosine.

MGMT [79]. NCT503, Tubeimoside-I, GNA13, Pyrviniumpamoate, DEC1, METTL3, MMR have been confirmed to increase the sensitivity of GBM to TMZ treatment by regulating MGMT [80–86], as summarized in (Table 1, Ref. [68–73,75–78,80–85]).

4.2 Immunotherapy Combined with Alkylating Agents for Tumor Treatment

In recent years, immunotherapy has gained popularity as a cancer treatment option, although certain clinical trials have not produced positive outcomes. In phase 3 trials of TMZ plus nivolumab in newly diagnosed MGMT promoter methylated GBM, radiation therapy (RT) + TMZ plus the immune checkpoint inhibitor nivolumab did not improve survival [87]. Absent longer OS was seen compared to RT plus TMZ in a further phase 3 study of TMZ with nivolumab in GBM patients lacking MGMT methylation [88].

4.3 Treatment of MGMT Promoter Non-Methylated GBM

Different randomized trials have shown that GBM patients with MGMT promoter methylation were associated with significantly higher survival when treated with combined radiotherapy and TMZ. Even when disease relapses, TMZ was increasingly beneficial for GBM patients with MGMT promoter methylation [89]. However, TMZ therapy was ineffective and had a dismal prognosis for MGMT non-methylated individuals.

Barazzuol *et al*. [90] found at the cytological level that PARP2, an important enzyme involved in DNA repair, and its inhibitor ABT-888, when used in combination with

X-ray and TMZ, could enhance radio sensitivity and chemical sensitivity in MGMT methylated cell lines as well as in MGMT non-methylated cell lines. These findings suggest that ABT-888, in combination with traditional chemotherapy and radiation, has the clinical potential to enhance the currently recommended course of treatment for GBM, particularly for individuals who lack MGMT methylation [90]. Jue *et al*. [91] tested the PARP inhibition of veliparib (ABT-888) in a group of patient-derived cell lines and patient-derived xenograft models, which confirmed that the use of veliparib and RT was an effective treatment for GBM patients with non-methylated MGMT promoter. In a randomized phase II trial involving patients with newly diagnosed MGMT-unmethylated GBM, Veliparib treatment was generally well tolerated, although there was insufficient proof for a therapeutic benefit [92]. In addition to the use of PARP inhibitors, Meclofenamate, a nonsteroidal anti-inflammatory drug, as a gap junction inhibitor, could enhance the vulnerability of GBM cells to lomustine induced cell death. However, it did not depend on the methylation status of the MGMT promoter, which provides a new therapeutic possibility for GBM patients without MGMT promoter methylation [93]. Also, miRNA has been shown to increase the sensitivity of MGMT promoter non-methylated GBM patients to TMZ. Kirstein *et al*. [94] found that miRNAs may be a promising and innovative treatment to improve TMZ sensitivity and increase progression-free and long-term survival in MGMT non-methylated GBM patients.

The survival benefit of a 70% degree of resection threshold in GBM patients with non-methylated MGMT promoter supports the maximization of safe resection rather than the "all or nothing" approach, according to research by Katsigiannis *et al*. [95]. Based on a study of 175 newly diagnosed patients with primary GBM [96], MGMT promoter methylation combined with complete resection was an independent predictor for improved overall and PFS in newly diagnosed isocitrate dehydrogenase (IDH) wild-type GBM. These studies suggest that the effect of the scope of surgical resection on the prognosis of GBM patients may be related to the methylation level of the MGMT promoter.

The most widely used prognostic procedures for GBM exclude individuals older than 70 as it is perceived that older patients have a decreased tolerance to surgery and chemotherapeutic treatments and have a poorer prognosis [97,98]. When older GBM patients over 70 years old were combined with MGMT promoter non-methylation, the treatment was more difficult. Yuen *et al*. [99] analyzed the historical evidence-based data of GBM treatment in older patients and proposed that for these patients with MGMT promoter methylation, single-dose TMZ may be considered, while for older patients with MGMT promoter non-methylation, subfractionated radiotherapy alone may be sufficient (Table 2, Ref. [90,91,93–95,99,100]).

Moreover, MGMT expression may affect the chemotherapy drugs and lessen their therapeutic effect. At the same time, chemotherapy drugs also affect MGMT expression. Numerous investigations have revealed that MGMT methylation or activity in patients with GBM altered after receiving chemotherapy and that the expression of MGMT in certain patients with recurrence was different from that of the initial tumor. No mechanisms have been elucidated in these phenomena or in correlating with the selectivity of chemotherapeutic agents to cells with high MGMT expression [101].

5. New Progress in Other Aspects of MGMT

There have been many other aspects of research on MGMT, such as the effect of MGMT promoter methylation status combined with other genes on tumor prognosis or treatment, along with the update of MGMT methylation detection methods.

In addition to the single factor of MGMT promoter methylation status affecting tumor prognosis, the combination with other genes can also affect tumor prognosis, especially for GBM patients. According to certain studies, GBM patients' tumors had OS and PFS that were considerably greater than those of patients with wild-type *IDH1* GBM tumors that had an unmethylated MGMT promoter [102,103]. In addition to *IDH1* mutation, some researchers have studied the effect of *TERT* promoter mutation status and MGMT promoter methylation status of different patterns on the prognosis of GBM, but the results have been conflicting. Some studies have suggested that *TERT* promoter mutation combined with MGMT promoter methylation can prolong overall survival and progression free survival [104,105]. The prognosis of GBM with MGMT promoter methylation and *TERT* wild-type was found to be superior to that of other subtypes in another study [106]. This discrepancy might be brought on by various MGMT promoter methylation detection techniques employed in various laboratories, individual variations, or sample size.

Methylation-specific PCR, pyrosequencing, or methylation arrays are recommended for detecting MGMT promoter methylation assays according to the European Association of Neuro-Oncology guidelines while the European Society for Medical Oncology does not recommend immunohistochemistry to determine MGMT promoter methylation status [107]. Earlier studies have shown that IHC assays are not recommended for evaluating MGMT status [108]. Subsequently, pyrosequencing has also been proven to be an ideal choice for detecting MGMT promoter methylation status, with a recommended biological cutoff of 10% or 21% of the receiver operating characteristic [109–111]. In addition, it has recently been found that pyrosequencing (PSQ) to evaluate the percentage of MGMT promoter methylation is very important for predicting the volume response and prognosis of patients with residual tumor GBM [112]. Other methods used to detect MGMT promoter methylation include Lab-on-Chip com-

Table 2. MGMT non-methylated GBM treatment.

Treatment	Mechanism	Tumor type	Ref
Veliparib (ABT-888)	induced apoptosis and decreased cell proliferation in a PDX of MGMT unmethylated GBM	glioblastoma	Jue *et al*. [91]
ABT-888	might be significant in MGMT-unmethylated patients less benefit from TMZ	glioblastoma	Barazzuol *et al*. [90]
Meclofenamate	reinforces the antitumoral effects of chemotherapeutic agent lomustine, independent of MGMT promoter methylation status	glioblastoma	Schneider *et al.* [93]
miRNAs	enhance TMZ sensitivity in MGMT unmethylated patients	glioblastoma	Kirstein *et al*. [94]
70% extent of resection threshold	benefit for survival of patients with MGMT unmethylated GBM	glioblastoma	Katsigiannis *et al*. [95]
Chemotherapy drugs	combination of carelizumab, anlotinib, and oxitinib	glioblastoma	Wang *et al*. [100]
hypofractionated radiation therapy (hRT)	hRT alone can be considered	elderly patients with newly diagnosed glioblastoma	Yuen *et al*. [99]

PDX, patient-derived xenograft.

patible isothermal amplification, two-probe quantification of MSP, and methylation quantification of endonuclease resistant DNA [113–115]. IHC assays for MGMT protein or enzyme activity have also been used as a proxy for methylation status, especially in resource-limited settings, where PCR technology is lacking [116]. The MGMT promoter methylation, methylation site preference, and cut-off value could not be determined using a conventional approach, regardless of the detection method used.

The identification of methylation locations and threshold values continue to be established, despite evidence linking MGMT promoter methylation status to therapy and prognosis in GBM patients. Research has found that a cutoff of 9% for 74–78 CpG sites is better than a higher cutoff of 28% or 29% [110]. As the MGMT promoter methylation detection PSQ threshold depends on the average methylation CpGs threshold, the recommended value was 10%, which was divided into "transition zone" or "gray area", as it might confer some sensitivity to TMZ treatment [117]. IHC has proved to be a robust method for predicting the prognosis of patients in the gray area defined by PSQ [118].

Various researchers have turned their attention to radiomics imaging methods based on nuclear magnetic resonance imaging, trying to establish a preoperative, noninvasive MGMT promoter methylation detection [119]. Extreme Gradient Boosting feature selection model, genetic algorithm based packaging model, machine learning, intravoxel incoherent motion (IVIM) and dynamic susceptibility contrast (DSC), and T2-weighted image have all been proven to be effective in evaluating MGMT promoter methylation in GBM patients [120–124]. Patients can combine imaging prediction with surgical specimen detection. This combination can more precisely predict MGMT methylation levels and let patients choose a more effective treatment strategy. There have been other studies that used patient body fluids to detect MGMT promoters' methylation status, such as peripheral blood and cerebrospinal fluid [125,126].

6. Conclusions

In its capacity as a DNA repair enzyme, MGMT is independently involved in DNA damage repair and is crucial for the emergence and growth of malignancies. In the early stage of tumor development, the transfer methyl group of MGMT avoids gene mutations of oncogenes and tumor suppressor genes such as *K-ras*, *P53* and *PTEN*, and plays an anti-tumor role. MGMT performs a function in transferring the methyl group to protect the body throughout tumor formation when chemotherapy medications, primarily alkylated chemotherapeutic agents, are used. While MGMT increases the protective effect on cells, it also produces drug resistance, which may affect the patient's prognosis.

Although there are numerous studies on the influence of MGMT promoter methylation, protein expression, enzyme activity and gene polymorphism on tumor prognosis and treatment, the results have been controversial. Problems to be solved, especially for GBM patients, include the lack of consensus on the selection of MGMT methylation detection methods, the selection of methylation sites, and the determination of cut-off values. The use of different methods and standards in various laboratories can lead to inconsistent results. In addition, TMZ resistance caused by MGMT is still the key to the treatment of GBM, and the development of more targeted drugs that could be combined with TMZ or inhibit MGMT expression is also critical, especially for older patients who are more than 70 years old, as within this vulnerable group, it is urgent to develop more effective treatment regimens.

Abbreviations

MGMT, O^6-methylguanine-DNA-methyltransferase; GBM, Glioblastoma; OS, Overall survival; PFS, Progression free survival; TMZ, temozolomide; IHC, Immunohistochemistry.

Author Contributions

YC and HQ had the idea for the article; WQ and JT performed the literature search and drafted the work; and HQ critically revised the work. All authors reviewed the manuscript. All author read and approved the final manuscript.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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