

Role of MGMT in Tumor Development, Progression, Diagnosis, Treatment and Prognosis

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Abstract. *O⁶-Methylguanine-DNA-methyltransferase (MGMT) is a unique protein, which both repairs O⁶-alkylguanine lesions stoichiometrically without a multi-enzymatic pathway and self-inactivates. It has recently been linked to the therapeutic success of alkylating agent chemotherapy, specifically temozolomide treatment. This drug affects the MGMT pathway to induce cell death in tumor tissue. Low levels of functional MGMT have been correlated with success of treatment, while high levels bring about failure of therapy. Expression of MGMT protein varies in normal and tumoral tissue. Furthermore, its epigenetic silencing due to promoter methylation has been linked to its lack of expression in many types of tumor, including gliomas. Great enthusiasm surrounds the utility of this protein in cancer treatment. Not only has there been success in manipulating MGMT levels to enhance alkylating agent therapy, but studies also suggest a possible role of MGMT in protecting hematopoietic cells from the myelosuppressive effects of high-dose chemotherapy. Innovative research into this protein will no doubt be rewarding. This review presents a summary of what is known about this unique protein, including its structure, function in its pathway, polymorphisms, expression in normal and tumoral tissue, relation to alkylating agent therapy, and possible future applications.*

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Cells possess a number of protection mechanisms directed against DNA damage caused by endogenous and exogenous mutagens. They include base excision repair (BER), mismatch repair (MMR), nucleotide excision repair (NER), and the function of O⁶-methylguanine-DNA-methyltransferase (MGMT) (1-3). The latter, unlike other mechanisms, is a single-enzymatic pathway which repairs DNA adducts at the O⁶ position of guanine in a stoichiometric fashion. It is self-inactivated and has thus been termed a 'suicide enzyme' (1, 2). Several clinical trials have taken place to test efficacy of alkylating agents with regards to MGMT expression and promoter region methylation (2, 4, 5). The aim of this review is to provide a concise overview of the biological importance of MGMT protein with a focus upon its structure, function in the MGMT pathway, polymorphisms, and its expression in normal and neoplastic tissues. Also discussed are drugs affecting the MGMT pathway.

MGMT Structure and Function

MGMT is a protein unique in its ability to stoichiometrically repair DNA adducts and to self-inactivate. Its gene sequence was first cloned in 1988. Located on chromosome 10 at the 10q26 position, it consists of 5 exons and 4 introns and spans greater than 300 kb (6). Its encoded protein, MGMT, is 207 amino acids in length and has been conserved through evolution (1). All species of MGMT have conserved the active site sequence of proline-cysteine-histidine-arginine (1). The promoter region is both TATA-box and CAAT-box free and is rich in repetitive GC sequences comprising a CpG island (6). The promoter region spans 1.2 kb and includes the first exon and part of the first intron (6). Expression of MGMT can be induced by glucocorticoids, cyclic AMP, protein kinase C, DNA damage, and through interaction of several transcriptional factors, including SP1, activator

proteins 1 and 2 (AP-1, and AP-2), with its promoter region (1). The protein binds a zinc atom which, where present, increases its rate of repair. Even without the zinc atom, MGMT is still active (1). It functions as a transferase and as an acceptor of an alkyl-group. Upon recognition of an alkyl DNA adduct at the O^6 position of guanine, MGMT transfers the alkyl group to the sulfur of its internal acceptor site (1).

The primary substrate of MGMT is O^6 -methylguanine (O^6 -MG), however, it can repair adducts of greater size, such as O^6 -ethylguanine (O^6 -EG). It can also repair adducts at the O^4 position of thymine, preferentially O^4 -methylthymine (1-4, 6).

The majority of alkylating agents, including those used for therapeutic purposes, utilize MGMT pathways. Most alkylating agents induce cell death by targeting O^6 -alkylguanine adducts and, at a lesser frequency, O^4 -alkylthymine (1). The BER mechanism repairs *N* 7-methylguanine and *N* 3-methyladenine adducts whereas the MGMT mechanism repairs both the O^6 and O^4 position adducts (1, 7). Both exogenous and endogenous alkylating agents form O^6 -alkylguanine adducts. Exogenous methylating agents include methylnitrosourea (MNU) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), a substance found in tobacco; endogenous methylating agents include S-adenosylmethionine. The products of exogenous ethylating agents are those targeted by MGMT. These include ethylnitrosourea, diethylnitrosamine, and related compounds (1, 2, 6-8). MGMT can also repair O^6 -chloroethylguanine adducts formed by such chemotherapeutic agents as *bis*-(2-chloroethyl)-nitrosourea (BCNU), used in the treatment of gliomas (1-4, 8). Other chemotherapeutic agents also known to target the MGMT pathways include temozolomide, streptozotocin, procarbazine, and dacarbazine used in the treatment of gliomas, melanomas, carcinoid tumors, and Hodgkin lymphomas (1-10).

Although MGMT represents one independent repair mechanism, alkylating agents have taken advantage of other multi-enzymatic pathways to induce cell death. One example is the MMR mechanism, which repairs mismatched base pairs in DNA (7, 10). It has been suggested that the therapeutic action of alkylating agents would be more effective in an MGMT-deficient environment (1, 2, 4, 8, 11). For example, alkylating agents act through the formation of O^6 -MG due to preferential pairing of the latter with thymine, as opposed to preference of unmethylated guanine for cytosine, thereby forming a mismatch. When the O^6 -MG mismatch is formed, the MMR mechanism attempts to repair it by excising the mismatched thymine. In so doing, however, it repairs the O^6 -MG:T mismatch with another thymine residue, the result being what is termed 'the futile cycle' (1, 3-5, 8, 10). In other words, MMR will continue to repair O^6 -MG:T with thymine molecules, leading to several rounds of unsuccessful repair, single-strand breaks and eventually apoptosis. However, the O^6 -MG:T mismatch can be repaired

by MGMT. If MGMT repairs the O^6 -MG lesion, the MMR mechanism can easily repair the G:T mismatch (1-5, 8, 10).

Loss of MGMT expression has been reported to occur in many tumor types, including glioma, lymphoma, breast and prostate cancer, as well as retinoblastoma (1, 5, 6, 9, 11-27). This silencing is thought to be due to promoter methylation, a process also observed *in vitro* in tumors without MGMT activity. Following promoter methylation, transcriptional factors cannot bind and initiate replication of the gene (5, 8, 28, 29). As MGMT protects against mutagenic DNA adducts, it is plausible that loss of MGMT is a pre-tumorigenic mechanism (1, 5, 8). Although there is no clear chronological order to methylation of genes in carcinogenesis, nor a relationship between *MGMT* promoter silencing and silencing of other genes, *MGMT* silencing has been linked to mutations in other tumor-related genes. These include a) silencing of p53 in non-small cell lung cancer and astrocytic tumors (1, 2), b) *k-ras* gene mutations in gastric and colorectal cancer (1, 3, 30-35), and c) methylation of both the *CDKN1A* gene which encodes p21, and the *CDKN2A* gene, which encodes p16 (1, 5). These mutations as well as loss of MGMT are markers of a poor prognosis in lung, hepatobiliary, gastric and breast cancer (1-4, 6, 9, 36-40). On the other hand, lack of MGMT in various tumor types has also been associated with improved outcomes of alkylating agent therapy. Indeed, several trials with MGMT inhibiting agents such as O^6 -benzylguanine (O^6 -BG) and Lomeguatrib have recently been performed (1, 4, 31).

MGMT is not the only repair mechanism that acts on DNA adducts formed by alkylating agents. Other mechanisms include NER, BER, and the previously mentioned MMR (1, 7, 41, 42). NER is a multi-enzymatic pathway that repairs DNA damage by bulky adducts. It is a transcriptional method involving recognition and repair of damaged DNA, and subsequent synthesis of new DNA (1, 41). Like NER, BER is a multi-enzymatic pathway that repairs single DNA base damage *via* recognition, removal and synthesis of single bases. Key enzymes in this process are DNA polymerase β (pol β) and DNA glycosylases (3, 7, 41, 42).

When O^6 -guanine is ethylated, the resulting O^6 -EG lesions can be converted into G:C interstrand crosslinks, which are repaired by NER. However, prior to the creation of the interstrand crosslinks, either MGMT or NER can repair the O^6 -EG lesions (1, 41). NER is better able to repair lesions in transcribing genes than is MGMT (1). Bulkier adducts such as O^6 -chloroethylguanine can also be formed by exposure of DNA to BCNU (43), which results in intramolecular rearrangement of DNA to form *N* 1-guanine-*N* 3-cytosine interstrand crosslinks (1, 8). MGMT is capable of repairing the lesion before a crosslink is formed by removing the chloroethyl group. However, in cases in which an interstrand crosslink is formed during the

S-phase, DNA double-strand breaks are formed, producing sites at which the interstrand crosslinks stall the replication process. If these double-strand breaks are not repaired, apoptosis follows (1, 3). Both NER and BER are capable of restoring the DNA to normalcy. NER is able to excise 25 to 30 nucleotides, including nucleotides that contain double-strand breaks. BER is able to repair single bases or short fragments of bases containing damaged DNA. Thus, both BER and NER are capable of repairing toxic DNA lesions (1, 3, 7, 8, 41-43).

BER is of importance in the repair of lesions induced by several alkylating agents. The *N*-methylation lesions repaired include *N* 7-methylguanine, *N* 3-methyladenine and *N* 3-methylguanine (1, 43). It is possible that intermediate proteins part of the BER pathway possess both cytotoxic and mutagenic effects (42); however, due to its over-riding protective function relative to alkylating agents, such damage is relatively minor. As previously mentioned, DNA glycosylases are important to the function of BER as well as pol β . Indeed, it is known that mice with defective pol β are hypersensitive to alkylating agents (42). Another important protein, poly(ADP-ribose) polymerase 1 (PARP-1), is involved in the repair of DNA single-strand breaks. Mice lacking PARP-1 are hypersensitive to MNU, suggesting that PARP-1 deficiency could result in a better response to MNU therapy (2). Recent studies have demonstrated that BER plays an important role in protecting non-replicating cells from DNA damage (42).

MGMT Polymorphisms

Several *MGMT* polymorphisms have been discovered. These slightly altered forms of *MGMT* may be useful as diagnostic tools, pre-treatment factors and indicators of risk of having a tumor. Some of the forms act as pre-treatment assessment tools as they are less sensitive to the action of certain *MGMT* inhibitors (6, 9). In addition, they do have risk associations in the setting of breast, lung, head and neck, colorectal and other types of cancer (6, 9, 33, 38). To date, 438 polymorphisms have been found (38). They showed minor allele frequencies, defined as the relative abundance of a particular allele type among all individuals of a population, usually over 0.05%, and most affecting 5'-UTR, 3'-UTR and introns (6, 9, 38). The most common variants of *MGMT* are Ile143Val and Lys178Arg. Existing in nearly perfect disequilibrium with one another, they comprise 20% of observed variations (9). Other known variations affecting the primary structure of *MGMT* include Trp65Cys, Leu84Phe, Gly160Arg, Gly135Thr, Gly290Ala, Cys485Ala, Cys575Ala, Gly666Ala, Cys777Ala, Gly795Cys, Ala1034Gly and Cys1099Thr (6). Both Leu84Phe and Ile143Val are of interest as they are situated close to the Cys145 active site and may affect *MGMT* function (6, 9). This review will

focus on 5 of the polymorphisms: Ile143Val, Lys178Arg, Leu84Phe, Trp65Cys and Gly160Arg. Their frequency of occurrence, biological significance, and effect on patient outcomes will be discussed.

Ile143Val. Ile143Val, a polymorphism found in 18% of individuals of European origin, is not present in Nigerian and Japanese descendants (38). The allele frequency is approximately 0.15% in Caucasians and is only rarely found in Asians and Africans (9). Located in a region of high linkage disequilibrium with 19 other polymorphisms, it has no effect upon pancreatic, prostate, oral and gastric cancer nor on melanoma risk, but is associated with a reduced risk in women for colorectal as well as head and neck cancer (6, 9). Its correlation with lung, breast and endometrial cancer is debated (6, 9). Some studies suggest it has no effect upon lung cancer (44-46), whereas another found it to be weakly associated with an increased risk (47). Yet a third study reported a two-fold increased risk of lung cancer, particularly adenocarcinoma, in Caucasians and African-Americans with the Ile143Val (48). Similarly, one study noted that this variation had no effect upon breast cancer (49), while another suggested that fruit and vegetable consumption resulted in a reduced breast cancer risk in women (50). A recent study suggested that there is a reduced risk of endometrial cancer in smokers with the Ile143Val polymorphism. Interestingly, the same study found that Ile143Val carriers who had been smoking more than 30 pack-years had a significantly lower risk for endometrial cancer as compared to nonsmoking women homozygous for Ile143Val (51).

The Ile143Val polymorphism results in *MGMT* equally capable of repair as is wild-type *MGMT* (9). In fact, after being incubated with *O*⁶-(4-bromophenyl) guanine (Patrin-2, an *MGMT* inhibitor), it has been shown to be 1.3 times more active in repairing *O*⁶-MG adducts than is the wild type (9). It is also able to repair *O*⁶-[4-oxo-4-(3-pyridyl)butyl] guanine (*O*⁶-pobG) more effectively than both the wild-type and the Leu84Phe polymorph *MGMT*. (6). Ile143Val *MGMT* is less sensitive to *O*⁴-benzylfolate (BF), a powerful *MGMT* inhibitor and slightly less sensitive to Patrin-2, the *MGMT* inhibitor mentioned above (6). The Ile143Val variant has reduced activity towards low molecular weight inhibitors, such as *O*⁶-BG and Patrin-2 (6). Ile143Val tumors respond poorly to chemotherapy (9). The role of Ile143Val expression is of importance as a pre-treatment marker since patients with this phenotype express reduced sensitivity to *MGMT* inhibitors, a factor that may affect treatment.

Lys178Arg. This polymorphism has no effect upon the risk of developing breast, pancreatic, prostate and gastric carcinoma or melanoma (6, 44, 46, 49, 50, 52-54). Some studies showed a weak association between Lys178Arg and

increased lung cancer risk (6, 47, 48), whereas another study showed it to be associated with a lower lung cancer risk (38). Lys178Arg is associated with reduced risk of colorectal, as well as head and neck cancer in women, and endometrial cancer in heavy smokers (6, 9, 46). This polymorphism repairs O^6 -[4-oxo-4-(3-pyridyl)butyl] guanine (O^6 -pobG) more effectively than does either the wild-type or the Leu84Phe polymorphism MGMT (6). The Lys178Arg variant is less sensitive to the inhibitor BF and slightly less sensitive to Patrin-2 (6). Thus, Lys178Arg is also linked to an overall poorer response to chemotherapy (6). As it is genetically related to the Ile143Val variant (6, 9), there are studies on the association of the two forms. One found that the Ile143Val and Lys178Arg polymorphism carriers are better protected against the mutagenic effects of alkylating agents in comparison to wild-type carriers (6). As a prognostic factor, Lys178Arg expression appears to be useful in differentiating between patients in whom chemotherapy and MGMT inhibitor therapy would or may not be successful, since this polymorphism is less sensitive to MGMT inhibitors and is associated with a poorer chemotherapeutic response.

Leu84Phe. The Leu84Phe variation has an allele frequency of 0.15% (9). Its expression does not vary significantly by race, but it is somewhat more prevalent among Caucasians than Chinese (6). One study demonstrated a correlation between this polymorphism and cancer risk, alcohol intake, body mass index (BMI) and post-menopausal hormone (PMH) use (55). This polymorphism has no effect upon lung, oral and gastric cancer or melanoma risk (44). It is, however, associated with an increased risk of breast cancer in heavy smokers (50), an increased incidence of glioblastoma multiforme (56) as well as an increased risk of prostate (53) and bladder carcinoma (57). Leu84Phe polymorphism carriers have a lower risk of endometrial cancer (51), a lower incidence of head and neck cancer (46) and colorectal cancer associated with a better prognosis (6, 58). Furthermore, women who consumed more than 0.5 alcoholic beverages per day with the Leu84Phe polymorphism had an increased risk of colorectal cancer, while those with the allele who drank less had a lower risk (55). In addition, women with the polymorphism and a body mass index (BMI) ≥ 25 had a lower risk of colorectal cancer than women with a BMI < 25 who were homozygous for the wild-type allele (55). In those homozygous for the wild-type allele, PMH use was inversely proportional to colorectal cancer risk (55). Interestingly, no such correlation was noted in carriers of the Leu84Phe polymorphism. The same study found no association between colorectal cancer, polymorphism, BMI, and environmental factors including smoking and alcohol intake (55).

Leu84Phe is as capable as wild-type MGMT in the repair of O^6 -MG adducts (6). One study found Leu84Phe variant

proteins to be more susceptible to NNK-induced aberrations than the wild type (9). It was also suggested that the Leu84Phe polymorphism may affect Zn^{2+} binding to MGMT, which is known to enhance MGMT activity (9). Although the function of this polymorphism is very similar to that of the wild type, its expression may be of prognostic utility in heavy smokers, since NNK is primarily found in tobacco smoke (6, 9).

Trp65Cys. This form of MGMT is very rare. At 37°C, it is unstable, both *in vivo* and *in vitro*. Its rapid degradation is thought to be due to the Trp65 side chain located in the first helix of the N-terminal domain (6). It is not as effective in protecting against the negative effects of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), a harmful alkylating agent, as is wild type MGMT or that of the Leu84Phe polymorphism (6).

Gly160Arg. In this form of MGMT, the Gly160 residue lies nearby the Cys145 active site (9). It is a rare variant found in less than 1% of Caucasians, but it is expressed in approximately 15% of Japanese (9). This polymorphism has generated interest due to its strong resistance to the MGMT inhibitor O^6 -BG. The ED50, the dose that causes a desirable outcome in 50% of a population, is 40-fold stronger than that of wild-type MGMT (6). Thus, patients with this form of MGMT would not be candidates for O^6 -BG therapy combined with alkylating agent treatment. The protein with this polymorphism also poorly repairs and discriminates against bulky adducts, due to the proximity of the large, highly charged side chain of arginine to the Cys145 active site (6). In comparison to the wild type, this polymorphism is less efficient in repair of O^6 -MG and O^6 -EG (6), and is compromised in the repair of O^6 -pobG (9). In contrast, it is equally as effective as the wild-type in providing protection against MNNG and BCNU (6). Although the polymorphism is rarely encountered, pretreatment testing may be useful, given its lesser ability to repair both small and bulky adducts at the O^6 position of guanine. As alkylating agents use this pathway to induce cell death, an already weakened repair mechanism would be advantageous. However, as the Gly160Arg polymorphism is highly resistant to O^6 -BG (9), it would be unwise to use MGMT inhibitors in patients expressing this polymorphism.

MGMT Expression in Normal and Neoplastic Tissues

Due to the important role of MGMT in DNA repair, several studies have been undertaken to find correlations between MGMT expression, tumorigenesis, immunohistochemistry and methylation status. MGMT expression in normal and neoplastic brain tissue is summarized in Table I.

Table I. *MGMT correlations in normal and neoplastic brain tissue.*

Study (ref)	Tumour type	n	Significant findings
Nagane <i>et al.</i> , 2007 (13)	Recurrent GBM	30	↓ MGMT and ↑ PF survival
	GBM	19	↑ MGMT activity and MGMT protein expression
Pollack <i>et al.</i> , 2008 (14)	Pediatric malignant gliomas	109	↓ MGMT and ↑ PF survival ↑ MGMT in tumors vs. normal tissue
Hegi <i>et al.</i> , 2005 (11)	GBM	206	MGMT methylation: 55% ↓ risk of death Longest overall survival in patients with promoter methylation + TMZ (21.7 months)
Wick <i>et al.</i> , 2007 (15)	Recurrent glioma	90	No correlation with MGMT promoter methylation and treatment efficacy
Chinot <i>et al.</i> , 2007 (16)	GBM	26	↓ MGMT expression and ↑ PF survival
Weaver <i>et al.</i> , 2006 (18)	Glioma	10	40% patients (4/10) methylated MGMT 55-100% of patients demonstrated hypermethylation of at least one CpG promoter in their primary tumor 42-83% demonstrated presence of same hypermethylated promoter in the blood
Nakasu <i>et al.</i> , 2007 (20)	Diffuse astrocytomas	28	MGMT negativity and ↑ malignant transformation
Bobola <i>et al.</i> , 2007 (59)	Developing brain	71	↑ MGMT expression/activity in developing brain and with gestational age
Preusser <i>et al.</i> , 2008 (19)	GBM	164	MGMT methylation and ↑ patient survival
Silber <i>et al.</i> , 1996 (60)	Normal and tumorous brain	117	No significant correlation between IHC and patient outcome Normal tissue MGMT negativity: 12% Tumor MGMT negativity: 55% in normal brain tissue of patients with primary brain tumors
Capper <i>et al.</i> , 2008 (26)	Astrocytoma	162	↓ MGMT expression and ↑ tumor malignancy

↓ = Decrease; ↑ = increase; PF, progression-free; TMZ, temozolomide; GBM, glioblastoma multiforme; IHC, immunohistochemistry.

MGMT in non-tumorous tissues. As a repair enzyme, MGMT is expressed in every human tissue, but its level in individual tissues varies, even in subpopulations. Some trends are apparent. For example, its expression is highest in the liver, relatively high in lung, kidney and colon, and the lowest in pancreas, hematopoietic cells, lymphoid tissues and brain (1-8). Furthermore, MGMT expression levels in tissues remain stable with age (59). The study of Bobola *et al.* examined MGMT activity in 71 brains at 6 to 19 weeks of gestation and found activity in such developing brains to be directly correlated with gestational age and that the proportion of specimens negative for MGMT at 19 weeks was approximately that observed in adults. Prior to 19 weeks of age, fetal brain levels of MGMT were low, suggesting that exposure to exogenous and endogenous alkylators prior to that time may, due to vascular permeability, such as the blood-brain barrier, result in increased susceptibility to tumor formation in later life (59).

MGMT in tumor tissues. Several studies have found that MGMT levels are higher in tumors than in their normal tissue counterparts (1-8). For example, Silber *et al.* assessed levels of MGMT in normal brain tissue and in primary brain tumors. They found relatively but significantly lower amounts of MGMT in tumors (60). Also of interest were the observations of Sawhney *et al.* who found significant loss of MGMT expression in the transition of hyperplasia to dysplasia in patients with oral squamous cell carcinoma (61).

This suggested that diminution in MGMT expression is an early step in oral tumorigenesis.

MGMT levels are very heterogeneously expressed, not only between individuals but in tumor tissues as well. Gene silencing, which contributes to this trend, blocks the ability of DNA to be replicated and effectively negates protein expression. Silencing of MGMT expression has been documented in a variety of tumor types (5). In contrast, increased levels of MGMT have been observed in others, including colorectal, pancreatic, breast and lung carcinomas, non-Hodgkin lymphoma and myeloma, as well as gliomas (1, 5, 62). Silencing of MGMT expression is most likely due to epigenetic silencing *via* promoter region methylation. Indeed, epigenetic changes occurring during tumor progression have been correlated with levels of MGMT expression (5). For example, Nakasu *et al.* found a relationship between loss of MGMT expression and anaplastic transformation in diffuse astrocytomas (20). In addition, Pike *et al.* found methylation of MGMT to be present in diffuse large B-cell lymphoma and concluded that methylation of specific CpG islands plays a major role in tumor development (27). Nonetheless, further investigation is required to validate whether MGMT epigenetic silencing plays a role in this and other tumor types.

Cooper *et al.* investigated the role between MGMT expression and promoter methylation on the one hand and survival of 108 small-cell lung cancer (NSCLC) patients on

the other. Although there was no correlation between expression of MGMT and survival, DNA repair protein expression was noted in every case. It was also evident that *MGMT* promoter methylation predicted a poor prognosis (37). Yet other studies found no relationship between *MGMT* promoter methylation and survival in NSCLC (63). In addition, Herath *et al.* found no relationship between methylated *MGMT* and reduced MGMT expression in 61 hepatocellular carcinomas (40). In colorectal cancer, Zhang *et al.* reported longer survival rates in patients with MGMT-negative surgical margins; however methylation did not have a significant effect upon overall survival in the 24-case series of colorectal cancer (30). Thus, it appears that methylation of the *MGMT* promoter region is an important predictor of patient survival in various tumor types.

MGMT expression has also been correlated with various lifestyle choices. For example, Cooper *et al.* found a significant difference in MGMT expression between smokers and nonsmokers with NSCLC (37). In addition, Sawhney *et al.* reported that oral ingestion of betel quid with tobacco was an important risk factor, as it was correlated with loss of MGMT expression in oral squamous cell carcinoma. Furthermore, loss of MGMT expression was associated with tobacco consumption in 63% of the patients with precancerous oral lesions (61).

Although a focus has been placed on *MGMT* promoter methylation, MGMT expression has also been studied by immunohistochemistry (IHC) (19, 26). Capper *et al.* evaluated MGMT expression as a prognostic factor in diffuse astrocytic tumors (n=162) ranging from low to high grade. The study found MGMT expression to be less prominent as the tumors became more malignant. In other words, high-grade gliomas showed less MGMT expression than low-grade gliomas, suggesting a loss of MGMT expression in the process of anaplastic transformation (26). Thus, it may be possible to use MGMT expression as a prognostic indicator, MGMT levels being a reflection of tumor grade. It may also be of utility in selecting treatment modalities, as high levels of MGMT are negatively correlated with response to alkylating agent therapy.

On the whole, knowledge of MGMT expression in tumor tissue is of importance. It is related to patient survival in being variously correlated with expression. It has also been associated with lifestyle habits such as tobacco smoking and oral ingestion of betel quid. Lastly, assessment of MGMT levels may be beneficial in the selection of treatment modalities.

MGMT and Temozolomide Treatment of Brain and Other Neoplasms

Several trials have been undertaken in an effort to understand the relationship between efficacy of alkylating agent chemotherapy and MGMT status. The drug most frequently

used is temozolomide (TMZ), an alkylating agent that exerts its action by its conversion at physiological pH, to monomethyl triazeno imidazole carboxamide (MTIC) (64). The latter produces *O*⁶-MG lesions, which are primarily repaired by MGMT. The effect of MTIC is schedule dependent in that doses which are given closer together are more effective in reducing MGMT levels (64). Lowered MGMT levels result in added MTIC cytotoxicity. Dacarbazine, primarily used to treat patients with melanoma, is also an MTIC pro-drug (64, 65). In combination with TMZ, the effect of dacarbazine, lomustine, procarbazine, cisplatin and others have also been investigated and correlated with MGMT levels in various tissue types.

Several studies of gliomas as well as case reports of pituitary adenomas have shown a correlation between MGMT activity and MGMT levels, both by IHC as well as Western blotting (11-26, 59, 60, 66-69). Nagane *et al.* (13) and Pollack *et al.* (14) reported that low MGMT levels correlate with progression-free survival in TMZ-treated gliomas, primarily glioblastoma (GBM). Pollack *et al.* also found a significant correlation between MGMT expression and progression-free survival in treatment trials of 109 pediatric patients with malignant glioma. Patients whose tumors showed lower levels of MGMT and p53 expression frequently experienced progression-free survival, whereas those with tumoral overexpression of these markers had a 0% progression-free outcome (14). Chinot *et al.* also observed a relationship between tumoral MGMT expression and outcome following TMZ and radiotherapy (16). Specifically, in 25 patients with GBM, low MGMT expression was associated with a significantly longer progression-free survival than was higher MGMT expression (16). Thus, immunoexpression of MGMT was associated with poor response, whereas tumors lacking reactivity showed marked tumor shrinkage and clinical improvements (16). It is apparent that lower MGMT expression is associated with better outcomes following TMZ therapy. This makes MGMT expression a biomarker distinguishing patients who would benefit from TMZ treatment from those who would not.

Aside from MGMT expression, the effects of *MGMT* promoter methylation upon efficacy of TMZ treatment have been studied. Wick *et al.* reported upon the relationship using methylation-specific PCR; no significant prognostic benefit of *MGMT* promoter methylation was noted (15). On the other hand, Hegi *et al.* found *MGMT* promoter methylation to be a favorable prognostic factor in patients with GBM treated with TMZ, lowering the risk of death by 55% in comparison to patients without *MGMT* promoter methylation. Their study clearly suggested that promoter methylation is a clinically valuable biomarker (11). Preusser *et al.* also studied MGMT expression and MGMT promoter methylation as a clinical biomarker in a series of 164 GBM. Although simple immunoexpression of MGMT was not found to be a reliable

prognostic factor with respect to TMZ treatment, *MGMT* promoter methylation had a favorable effect upon survival (19). These findings are in keeping with several recent reports of correlations between *MGMT* promoter methylation status and response to TMZ therapy (70-76). Thus, it may be useful to monitor *MGMT* promoter methylation as a biomarker by way of plasma levels of methylated DNA. Weaver *et al.* found that in glioma patients the amount of methylated DNA in plasma correlated significantly with the actual amount of methylated DNA in tissue, providing a clinically practical approach for the assessment of methylated *MGMT* as a predictor of TMZ response therapy (18).

Recently, successful use of TMZ therapy was reported in several case reports of aggressive pituitary adenomas lacking *MGMT* expression, while TMZ therapy was seen to fail in aggressive adenomas expressing abundant *MGMT* immunoreactivity (66-69). These immunoreactivity observations suggest that TMZ therapy plays a role in patients with aggressive pituitary tumors showing a low level or lack of *MGMT* expression (66-69).

The relationship between *MGMT* expression, promoter methylation and success of TMZ therapy has also been studied in skin and colorectal cancers. In metastatic melanoma patients, Rietschel *et al.* studied the effects of extended-dose TMZ treatment and found a correlation between *MGMT* immunoexpression and promoter methylation in 49 cases but saw no significant correlation between either one and clinical response to TMZ (65). This same study also found promoter methylation greater than 25% to be related to treatment response.

Microarray analysis of 26 human melanoma-derived cell lines showed a significant correlation between temozolomide sensitivity and *MGMT* expression, leading to the conclusion by the investigators that melanoma resistance to TMZ therapy is mainly conferred by *MGMT* expression (77). Additionally, in patients with neuroendocrine tumors, response to TMZ treatment was strongly predicted by *MGMT* deficiency as detected by its promoter methylation status (72). Whether *MGMT* and response to TMZ treatment are associated in other tumors requires further investigation.

Future Directions Involving MGMT

The future role of *MGMT* in the treatment of cancer patients appears to be substantial. Several lines of evidence support a role for *MGMT* as a tumor suppressor (1, 5, 6, 9, 11-27). In murine models, increased *MGMT* levels were found to protect against the development of tumors, including both colorectal and skin cancer. It may well be that *MGMT* levels can be manipulated in such a way as to prevent or abrogate tumorigenesis (34, 78,79).

As well, *MGMT* depletion has been proposed in tumors as a means to improve response to TMZ therapy (1, 4, 31).

Pseudo-substrates of *MGMT* such as Lomeguatrib (*O*⁶-(4-bromophenyl)guanine) and *O*⁶-BG have been shown to deplete *MGMT* levels in tumors with high-level *MGMT* expression, thus bolstering the therapeutic effect of alkylating agents (4, 31). Studies have been undertaken to test the efficacy of such dual drug therapies. Khan *et al.* tested tumor response to TMZ and Lomeguatrib administered 5 days per month in patients with stage IV colorectal cancer. None of the patients responded well to the treatment, even after 49 cycles of Lomeguatrib and TMZ, thus suggesting that metastatic colorectal cancer is resistant to TMZ (31). There was, however, a 92% depletion of *MGMT* protein within two hours of the oral dose of Lomeguatrib, indicating that even low levels of *MGMT* expression were prohibiting the actions of this drug (31). Several *MGMT* inhibitors such as cisplatin (a platinum compound found to down regulate *MGMT* mRNA expression), topoisomerase inhibitors and BF may be used in treatment trials of tumors found to be susceptible to TMZ, such as gliomas (4).

Finally, *MGMT* may be an effective agent to reduce harmful effects of chemotherapy (1, 36). For example, Milsom and Williams found that chemoselection of *MGMT* variants resistant to pseudosubstrates of *MGMT* administered in association with alkylating agents may decrease myelotoxicity. In their study, chemoselection was targeted at vectors inserted into hematopoietic stem cells (36). Although two other studies applying the same concept have not been effective, there is still hope that the process may reduce chemotherapeutic toxicity (79, 80). Gerson found hemopoietic cells transduced with *MGMT* mutants resistant to *O*⁶-BG to be protected from the effects of BCNU, the result being less myelosuppression (1).

Although our knowledge regarding *MGMT* has greatly increased since the discovery of its gene sequence in 1988 (22), its role in protecting cells from mutagenesis and in the development and progression of tumors remains to be fully understood. The same is true of its significance in diagnosis, treatment and prognosis.

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