Targeted Modulation of MGMT: Clinical Implications

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Abstract O^6-Methylguanine DNA methyltransferase (MGMT) has been studied for >20 years as a gene that is associated with the mutagenicity and cytotoxicity induced by either methylating carcinogens or alkylating (methylating and chloroethylating) therapeutic agents. Pioneering studies of alkylating agents identified alkylated guanine at the O^6 position, the substrate of MGMT, as a potentially promutagenic and lethal toxic DNA lesion. MGMT plays a prominent role in DNA adduct repair that limits the mutagenic and cytotoxic effect of alkylating agents. Because of its role in cancer etiology and chemotherapy resistance, MGMT is of particular interest. In this article, the clinical effect of MGMT expression and targeted modulation of MGMT will be summarized.

Although the exact physiologic function of O^6-methylguanine DNA methyltransferase (MGMT) remains to be fully defined, MGMT is known to specifically remove the methyl/alkyl group from the O^6-position of guanine and restore the guanine to its normal form without causing DNA strand breaks (Fig. 1). MGMT-mediated repair is unique compared with other DNA repair pathways: (*a*) it acts alone without relying on any other proteins or cofactors; (*b*) it transfers the alkyl group to an internal cysteine residue in the protein, acting as both a transferase and an acceptor of the alkyl-group; (*c*) it inactivates itself after receiving the alkyl-group from guanine, and thus, it is a suicidal protein; (*d*) it repairs in a stoichiometric fashion. Because one molecule of MGMT removes one alkyl molecule, an excess of DNA adducts at the O^6-position could completely deplete MGMT. These properties make MGMT an important drug resistance factor and an ideal target for biochemical modulation of drug resistance (1, 2). A direct relationship between MGMT activity and resistance to alkylating therapeutic agents has been well documented (3, 4).

MGMT expression protects from alkylating agent cytotoxicities. The clinical effectiveness of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) and related methylating compounds procarbazine, streptozotocin, or temozolomide is attributed, in part, to the potentially cytotoxic DNA lesions O^6-chloroethylguanine, formed by BCNU, and O^6-methylguanine (O^6-meG) formed by the methylating agents. It has been known for several decades that O^6-chloroethylguanine lesions induce cell death by forming G-C interstrand cross-links that are able to inhibit DNA replication and transcription, eventually leading to DNA double-strand breaks and apoptosis. O^6-meG is a promutagenic and carcinogenic DNA lesion that becomes cytotoxic after persistent O^6-meG adducts are recognized by the DNA mismatch repair (MMR) pathway (5). The mispairing of O^6-meG with thymine during DNA replication initiates futile cycles of DNA MMR to generate DNA single- and double-strand breaks that activate apoptotic cell death (refs. 6, 7; Fig. 1B).

MGMT expression in tumors. MGMT is ubiquitously expressed in normal human tissues (8) but is overexpressed in all types of human tumors, including colon cancer, glioma, lung cancer, breast cancer, leukemia, lymphomas, and myeloma. Although tumor cells have many mechanisms of resistance to alkylating agents, the expression of MGMT in tumors has a protective effect against cell death induced by alkylating agents, showing a strong correlation between MGMT activity and tumor drug resistance. MGMT expressing tumor cells are 4- to 10-fold more resistant to BCNU, temozolomide, and related compounds (9, 10).

Recent Advances

Silencing of MGMT. Tumor tissues have long been noted to be heterogenous with respect to MGMT expression with a broad range of expression between tumors, a lack of MGMT in patches of tumors, or a deficiency in MGMT in entire tumors. It is possible that epigenetic inactivation of the MGMT gene contributes to this variation. Several studies have reported transcriptional silencing of this gene in up to 30% of certain primary human tumors because of hypermethylation of the CpG island in its promoter region (11–14). Loss of MGMT has been shown to be associated with, and to precede the appearance of, G to A point mutations in the K-ras gene during lung, brain, and colorectal tumorigenesis. MGMT silencing is also often observed in tumors in which a number of other genes are silenced by methylation. The silenced gene lists are not consistent between tumors or tumor types (15, 16). Thus, the epigenetic alteration of MGMT behaves like a carcinogenic marker. MGMT silencing is observed in normal tissues, in premalignant lesions or in peritumor tissue, suggesting that it could be an early abnormality in premalignant processes (13, 17).

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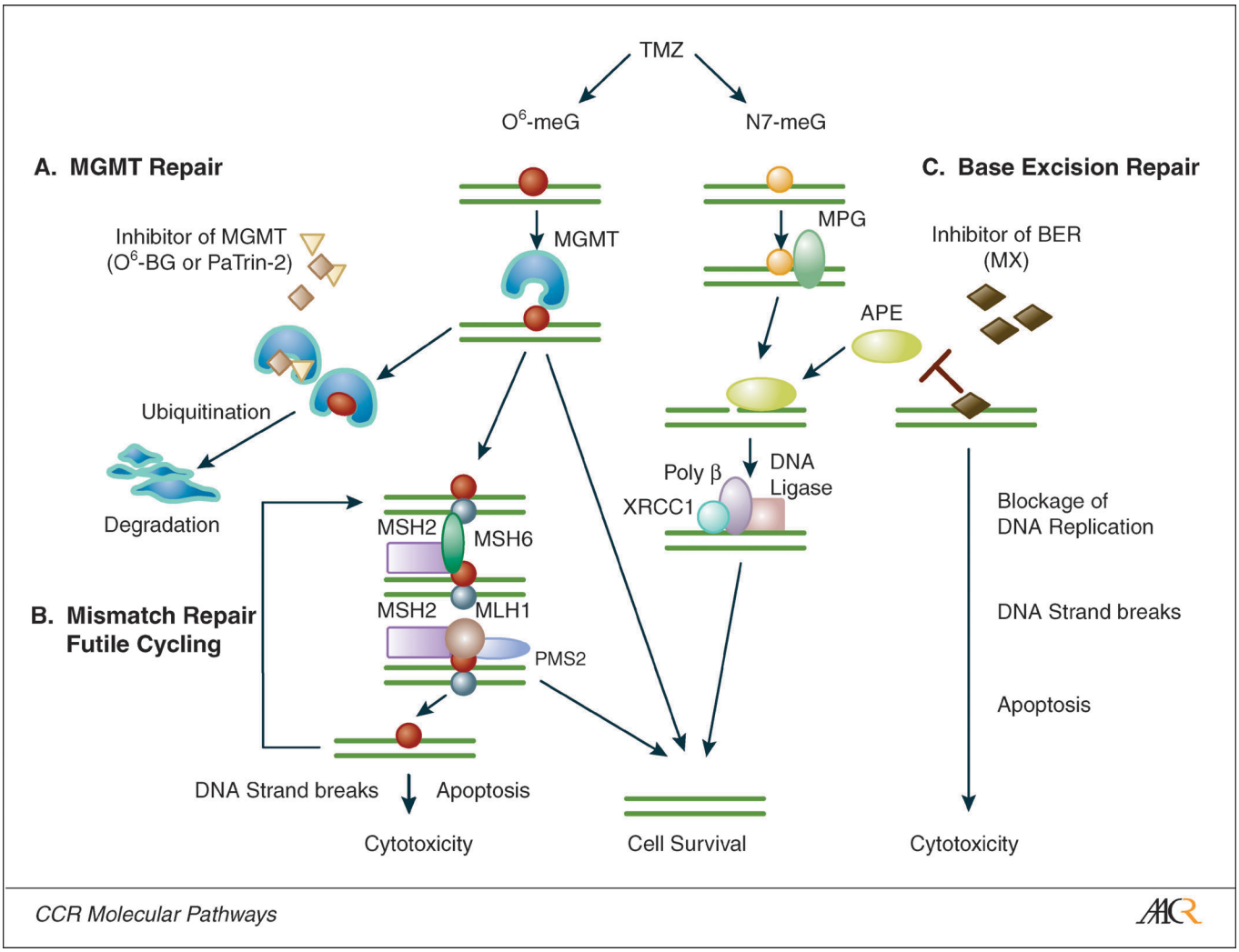

Fig. 1. MGMT and other DNA repair mechanisms deal with DNA damage produced by the methylating therapeutic drug, temozolomide, in human cells. Temozolomide and related drugs cause potentially cytotoxic DNA lesions such as O^6-methylguanine (O^6-meG, *red circle*) and N^7-methylguanine (N7-meG, *yellow circle*). *A,* MGMT (O^6-meG DNA methyltransferase) removes the O^6-alkylguanine DNA adduct through covalent transfer of the alkyl group to the conserved active-site cysteine and restores the guanine to normal. After receiving a methyl-group from O^6-meG, MGMT is inactivated, and subject to ubiquitin-mediated degradation. A similar suicidal enzyme reaction occurs when MGMT transfers and accepts an alkyl-group from O^6-benzylguanine (O^6-BG) or O^6-(4-bromothenyl)guanine (PaTrin-2), two therapeutic strategies. *B,* if an O^6-meG DNA adduct escapes MGMT repair, it would form a base pair with thymine (*blue circle*) during DNA replication. The mismatched base pair of the persistent O^6-meG with thymine is recognized by the mismatch repair pathway, resulting in futile cycles of repair leading to cell death. *C,* N7-meG DNA adducts (>70% of total DNA adducts formed by temozolomide) are efficiently repaired by the base excision repair (BER) pathway, and normally they contribute little to the cytotoxicity of temozolomide. Methoxyamine binds to AP sites produced by methylpurine glycosylase (MPG), the first step in BER processing. Methoxyamine-bound AP sites are refractory to AP endonuclease (APE, *green circle*) cleavage, resulting in the blockage of the BER pathway. This leads to strand breaks, disrupted replication, and increased cytotoxicity of temozolomide.

Recently, several clinical studies have shown that glioma patients with MGMT promoter methylation responded better to treatment with radiotherapy and either BCNU or temozolomide, and had improved survival compared with chemotherapy alone (18, 19). These observations suggest that the absence of MGMT expression is a positive survival predictive marker in patients with glioma. It would be appealing to consider a prospective clinical trial in which MGMT expression variables were used for personalized drug selection, electing alkylating agents for those tumors with low or absent MGMT expression, and other forms of treatment or inhibition of MGMT (vide infra) for those with MGMT expression. Tumor MGMT promoter methylation is being evaluated as a predictive marker. Because of the difficulty in obtaining these samples, however, measurement of circulating tumor or blood cell MGMT promoter methylation might be an effective surrogate for the status of tumor MGMT.

Depletion of MGMT. MGMT is an ideal target for modulation of its activity because it is a suicidal protein. O^6-Benzylguanine (O^6-BG) was developed based on its restricted mechanism of action (20). O^6-BG reacts with MGMT by covalent transfer of the benzyl group to the active site-cysteine and causes an irreversible inactivation of the enzyme (Fig. 1A). At therapeutic levels, O^6-BG is not toxic alone, but efficiently renders the tumor cells 2- to 14-fold more sensitive to alkylating agents in the *in vitro* and *in vivo* settings. This establishes the potential therapeutic effect of O^6-BG as an enhancer of these drugs. Recent clinical trials established the maximal tolerated dose of O^6-BG to achieve biochemical inhibition of MGMT to be 120 mg/m^2, and the maximal

doses of BCNU and temozolomide were found to be 40 and 470 mg/m^2, respectively, given with this dose of O^6-BG (21, 22). Phase 2 trials are currently under way. In addition to O^6-BG, another alternative inactivator has been described [O(6)-(4-bromothenyl)guanine, PaTrin-2], which shows potential advantages over O^6-BG in terms of higher reactivity against wild-type MGMT and oral formulation. A PaTrin-2-temozolomide combination is under clinical development in England (23, 24).

Translational Implications

Transfer of MGMT gene into bone marrow cells. Alkylating agents represent effective antitumor drugs. Unfortunately, these compounds are highly toxic to both malignant and normal cells. Bone marrow cells are especially sensitive to damage by alkylating agents because of reduced MGMT activity compared with tumor tissues. Thus, toxicity to the bone marrow is dose-limiting in cancer patients. MGMT has emerged as a lead target gene for the protection of bone marrow cells against the cytotoxic effects of many chemotherapeutic alkylating agents. Most gene transfer studies have evaluated two mutant forms of MGMT (G156A and P140K) that are resistant to O^6-BG-induced inactivation up to 60-fold to >500-fold, respectively (25, 26). Retroviral and lentiviral gene transfer of mutant MGMT into hematopoietic stem cells followed by transplantation has shown a very high degree of stem cell selection and reconstitution under both ablative and myeloablative conditions in mouse and dog models. Importantly, gene transfer of mutant MGMT into hematopoietic stem cells confers significant levels of drug resistance to the combination of O^6-BG and either temozolomide or BCNU. By this approach, tumor-bearing mice tolerated dose escalation of O^6-BG and BCNU with considerable moderation of myelosuppression, resulting in improved tumor response (27). Furthermore, because MGMT overexpression protects from leukemogenesis, it is possible that this approach would lessen the incidence of secondary leukemias after alkylating agent therapy (28). Clinical studies with gene transfer of mutant MGMT in CD34 cells collected from patients are ongoing to determine the gene transfer modeling and clinical applications (29). Potential applications include stem cell selection and protection in patients undergoing dose escalation of temozolomide for gliomas or other tumors and, at lower doses, as a selection strategy for genetically altered stem cells in other nonmalignant diseases.

DNA repair through MGMT and MMR pathways influences cell sensitivity to methylating agents. The relationship between MGMT activity and methylating agent resistance is complex, because O^6-meG lesions not repaired by MGMT are processed by MMR (Fig. 1B). O^6-meG preferentially pairs with thymine during DNA replicative synthesis and this mispair is bound by the mut S complex of MMR and the newly synthesized strand is excised by the mut L heterodimer of MMR. Unfortunately, the opposing strand O^6-meG is then paired with thymine again, reinitiating a futile repair process. In the process, extended single-strand patches are present, overlapping double-strand breaks could occur, triggering apoptotic cell death as well as G to A point mutations. Thus, O^6-meG is a potent mutagenic and cytotoxic DNA adduct.

MMR-deficient tumors are very resistant to methylating agents. Furthermore, inactivation of MGMT by O^6-BG fails to potentiate temozolomide-toxicity in MMR-deficient tumor cells. These results indicate that even large numbers of O^6-meG DNA adducts are not cytotoxic in the absence of the MMR recognition, although they may be very mutagenic (30, 31). In addition, MGMT mutants acquired in MMR-deficient cell lines selected for resistance to O^6-BG and BCNU are resistant to inactivation and sensitization of alkylating agents by O^6-BG, indicating that MGMT mutations might emerge in human tumors after drug exposure leading to drug resistance (32, 33). These studies also indicate that the function of MMR is to prevent mutations and to sensitize genetically unstable cells to certain agents. When MMR is inactivated, however, cells exhibit a high mutation rate, and the relationship of drug resistance to methylating agents with MGMT becomes even more complicated. Thus, MMR-proficient tumors are resistant to methylating agents on the basis of MGMT activity, whereas MMR-deficient tumors are drug-resistant regardless of MGMT activity (34).

Inactivation of MGMT coupled with interruption of base excision repair enhances therapeutic efficacy of alkylating agents. Therapeutic methylating therapeutic agents, such as temozolomide, from O^6-meG (~6% of adducts formed), as well as 7-methylguanine (N^7-meG; 70% of adducts formed), and 3-methyladenine (N^3-mA; 9% of adducts formed). The latter adducts, N^7-mG and N^3-mA, are removed by the methylpurine glycosylase followed by AP endonuclease, the first two enzymes in the base excision repair (BER) pathway (Fig. 1C). Efficient BER repair minimizes the effect of these lesions in normal and tumor cells. Thus, these abundant *N*-methylated DNA adducts become highly cytotoxic only when BER is disrupted (35, 36). Most importantly, BER disruption is able to bypass other drug-resistant factors such as MMR defects and high MGMT activity.

Several previous studies have shown considerable efforts toward improving the therapeutic efficacy of methylating agents (37–40) by blocking BER using methoxyamine, which binds the aldehyde group at the AP site and prevents AP endonuclease from clipping the backbone and thereby blocking the BER pathway. When combined with temozolomide or BCNU, methoxyamine potentiated therapeutic efficacy in colon cancer and malignant glioma xenografts. Importantly, no additive systemic toxicity was noted in mice after receiving treatment with temozolomide or BCNU and methoxyamine. Combinations of O^6-BG, methoxyamine, and a lower dose temozolomide or BCNU (1/3 of effective doses of agent used alone) showed significant antitumor effects without toxic death. Thus, modulation of MGMT, but not BER, requires a MMR-proficient background. The simultaneous targeting of MGMT and BER is a promising strategy to improve the therapeutic efficacy of alkylating agents in both MMR-deficient and MMR-proficient tumors.

It has recently become clear that MGMT may have a more complex function in the cell. The protein is phosphorylated, ubiquitinated, and may function in transcription regulation, cell cycle control, and apoptotic signaling. Whether MGMT inhibition will enhance drug efficacy, be of predictive value in patient chemotherapy selection, and become a target for other anticancer agents remain fruitful areas of discovery.

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