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Review

DNA direct repair pathways in cancer

Roberto Gutierrez, Yesenia Thompson and Timothy R. O'Connor*

Department of Cancer Biology, Beckman Research Institute/City of Hope, Duarte, CA 91010 USA

* **Correspondence:** Email: toconnor@coh.org; Tel: +6262181220.

Abstract: DNA direct repair (DR) pathways are unique in DNA repair because those mechanisms restore the genetic information without any DNA synthesis and are thus error free. We review the DR mechanisms, consequences of the absence of those systems in cells, their occurrence in cancer, regulation of their genes and proteins in cancer cells, and the potential exploitation of inhibitors to enhance chemotherapy.

Keywords: DNA direct reversal repair; MGMT; ALKBH2; ALKBH3; DNA repair inhibitors

1. Introduction

Cellular DNA is constantly under attack by endogenous and exogenous chemicals that induce a diverse array of harmful lesions, referred to as adducts. Adducts compromise the welfare of cells because they trigger mutations, and block DNA and RNA polymerases, which can result in arrest of DNA/RNA synthesis, DNA strand breaks, block gene expression, or mutations. Fortunately, mammalian cells have developed a variety of DNA damage repair mechanisms that help preserve cellular function by removing DNA lesions. Most DNA damage repair pathways remove damaged lesions by breaking the phosphodiester backbone, excising the damaged base, and resynthesizing a segment of DNA using a complementary template and error-prone DNA polymerases. However, direct repair (DR) removes DNA, and RNA damage, without excision, and without resynthesis; thereby making this repair pathway error-free.

DR maintains genomic integrity by protecting DNA mainly from endogenous and exogenous forms of alkylation damage. Endogenous forms of alkylating agents are produced as byproducts of oxidative metabolism, and from the enzymatic cofactor, S-adenosylmethionine [1,2]. Exogenous alkylating agents are commonly found in food, and in the air as contaminants from tobacco smoke and fuel combustion [3,4]. Alkylating agents react with DNA and RNA to form a diverse pattern of

simple and complex lesions. The pattern of lesions depends on numerous factors such as: The substrate damage site, the chemical nature of the alkylating agent (substitution nucleophilic unimolecular [S_N1] vs. substitution nucleophilic bimolecular [S_N2]), the DNA structure (single-stranded [ss] vs. double-stranded [ds]), and the DNA sequence [5]. The most commonly occurring lesions caused by alkylating agents include N1-methylguanine (1meG), O^6-methylguanine (O^6meG), N7-methylguanine (7meG), N3-methylguanine (3meG), N3-methylcytosine (3meC), N1-methyladenine (1meA), and N3-methyladenine (3meA) (Figure 1) [6–8]. Alkylation-induced DNA lesions pose a great threat to human health because they can compromise the genome by generating DNA strand breaks, and inducing mutations that can lead to diseases, such as cancer [9–11].

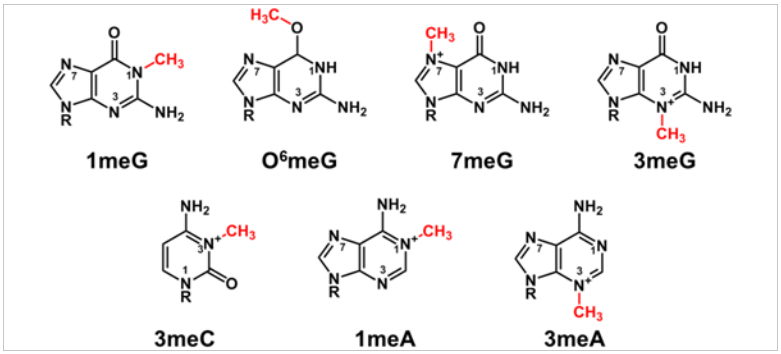

Figure 1. Major DNA lesions induced by methylating agents.

This review focuses on the pathways implicated in the direct reversal of alkylation-induced damage. Furthermore, we will highlight how DR mechanisms function in protecting cells from cancer development, and the therapeutic applications during cancer treatment.

2. Direct repair

There are two major types of proteins that conduct DR in mammalian cells, O^6-methylguanine-DNA methyltransferase (MGMT) [12] and the AlkB homologs (ALKBH) family of α-ketoglutarate (α-KG)-Fe(II) dependent dioxygenases [13]. MGMT repairs most exocyclic O-linked alkyl-adducts by using a unique repair mechanism which renders the enzyme inactive in the process [14]. In contrast, ALKBH2 and 3 in the ALKBH family carry out numerous repair reactions, and can catalyze the removal of N-alkyl lesions present on cytosine, adenine, thymine, and guanine residues [15]. DR proteins are of significance because they protect cells from the cytotoxic and mutagenic potential of alkylating agents. Recently, a comprehensive analysis of all DNA damage repair pathways in multiple cancer types identified that DR genes, *e.g.*, *ALKBH3* and *MGMT*, are frequently altered, predominantly by epigenetic silencing [16], but cancer-related alteration in the expression of those genes in some cancers was predicted previously [11,17–19,20]. This suggests that altered DR genes can be used as prognostic markers for enhanced cancer risk. Furthermore, loss of DR function can sensitize cancer cells to alkylating chemotherapeutic agents [17,21–24]. Therefore, it is of clinical importance to identify inhibitors of DR enzymes to enhance treatment by synthetic lethality.

2.1. MGMT repair mechanism

The MGMT protein is evolutionary conserved across prokaryotes, archaea, and eukaryotes; most notably in the active site sequence [25]. While there is no significant sequence homology in the N-terminal domain among species, the C-terminal domain is absolutely conserved. The C-terminal contains an active-site cysteine motif (PCHR), the O^6meG binding channel, and a helix-turn-helix (HTH) DNA-binding motif [26]. The HTH motif allows MGMT to migrate along dsDNA, flipping bases into its active site until it detects weakened base pairing caused by alkyl damage [27]. The MGMT active site contains an evolutionary conserved cysteine residue (Cys145), which accepts an alkyl group from the DNA adduct, inactivating the enzyme in the process (Figure 2) [28].

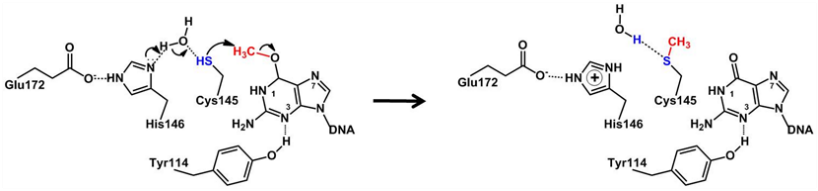

Figure 2. MGMT active site repair mechanism.

MGMT is of biological importance because it removes the most mutagenic lesions caused by alkylating agents. The preferred substrate of MGMT are O^6meG lesions, but it can also remove larger lesions such as O^6ethylG, as well as O^4meT, albeit at a much slower rate [29]. Loss of MGMT function makes cells susceptible to the mutagenic and cytotoxic effects of O^6meG lesions (Figure 3).

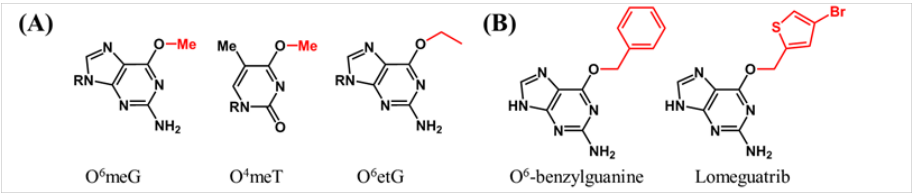

Figure 3. (A) MGMT substrates. R indicates deoxyribose position; (B) MGMT inhibitors.

Once alkylated, MGMT is inactivated; working a single time as a suicide enzyme [30]. That inactivation by protein alkylation causes a significant conformational change. The conformation change triggers a structural destabilization that results in rapid ubiquitination and degradation by the 26S proteasome system [31–33]. This single use mechanism means that cellular levels of MGMT are depleted as the reaction occurs, and continuous *de novo* synthesis of MGMT is required for continued repair [34]. Indeed, tissues and cells treated with alkylating agents show enhanced MGMT biosynthesis.

Regulation of MGMT expression is still under discussion. In *E. coli*, there are two O^6meG DNA methyltransferases, the Ada and Ogt enzymes [35]. The expression of *ada* is inducible as part of the adaptive response to alkylating agents, whereas *ogt* expression is constitutive. Induction of MGMT to DNA damage exists in some mammalian cell lines [36–38], but the response is not universal. *MGMT* expression is inducible by glucocorticoids [39] that indicates more studies on factors controlling the expression of this important gene are required. Post-transcriptional regulation of

MGMT protein levels occurs through microRNA (miRNA) mediated degradation of MGMT mRNA. miRNA binding to RNA inhibits translation, and shuttles it towards degradation via the RNA-induced silencing complex (RISC). Four miRNAs have been identified to alter MGMT protein levels: miR-181d, miR-767-3p, miR-648, and miR-370-3p [40,41]. Expression levels of miR-181d and miR-370-3p are inversely correlated with those of the *MGMT* transcript, and are associated with a favorable response to temozolomide (TMZ) in glioblastomas [42,43]. These reports indicate that the expression status of miRNAs may be implicated in the development of TMZ resistance.

2.2. MGMT protects against O-linked alkyl adducts

Although the O^6meG adduct is generated to a lesser extent than other lesions, it has a severe biological impact by eliciting the mutagenic effects of alkylating agents. Alkylating agents that produce little O^6meG are considered weak carcinogens [44]. O^6meG mispairs with thymine during DNA replication by forming two hydrogen bonds (Figure 4), but the absence of MGMT can also result in cytotoxicity [45–47]. If not repaired by MGMT, O^6meG, is also subject to translesion synthesis (TLS) by low-fidelity DNA polymerases, resulting in G→A transition mutations [48–51], but the *in vivo* role of these enzymes in bypass is still not completely understood. Therefore, in the absence of MGMT, O^6meG lesions formed by endogenous and exogenous sources of alkylating agents contribute to mutations in the genome.

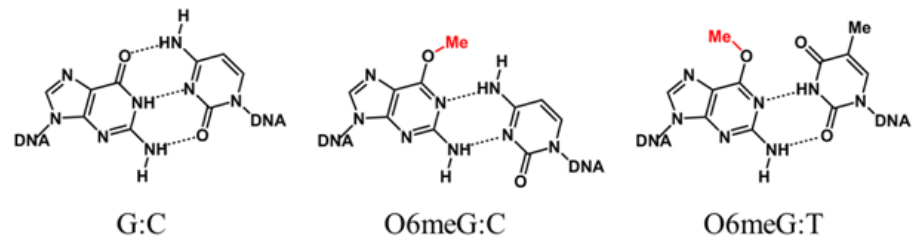

Figure 4. Mispairs formed by O^6meG lesions.

2.3. MGMT models

Exposure of various MGMT-deficient models to alkylating agents have validated the biological importance of MGMT. Early studies found that Mgmt-deficient cells show enhanced sensitivity to alkylating agents as compared to normal cells expressing *Mgmt* [52]. In murine models, Mgmt-deficiency increased cell death in proliferating tissues, and increased mutation frequency after exposure to either S_N1 or S_N2 alkylating agents [47,53–58]. In Chinese hamster ovary cells with an inducible MGMT cDNA expression construct, low levels of MGMT are linked to accumulation of O^6meG lesions and a 10-fold increase in *HPRT* mutation frequency, with G→A mutations dominating the mutation spectrum [59]. Induction of MGMT expression resulted in reduced mutation frequencies.

Furthermore, the cytotoxic potential of O^6meG is observed in the absence of MGMT and the presence of an intact mismatch repair (MMR) pathway. O^6meG:T mispairs are recognized by the MMR machinery resulting in the excision of thymine, leaving O^6meG behind. This initiates a "futile" cycle in which MMR machinery continuously binds to O^6meG:T mispairs through several rounds of repair, eventually resulting in the formation of double-strand breaks and cell death [60–63].

However, alkyl-induced mutation inactivation of MMR genes has been observed in recurrent GBM tumors, and contributes to resistance to alkylating agents, such as TMZ [64].

In many cells, loss of MGMT expression occurs by hypermethylation of the MGMT promoter region [65,66]. In contrast to MGMT deficiencies, an overwhelming amount of evidence suggests that overexpression of MGMT in normal cells provides enhanced protection against alkylating agents [67]. For example, mice overexpressing *Mgmt* show a significant reduction in alkylation-induced thymic lymphomas, colon carcinogenesis, and liver tumor formation [68–73]. Furthermore, *Mgmt*-overexpression in cancer-prone mouse models show reduced spontaneous formation of hepatocellular carcinoma, and alkylation-induced lymphoma [57,70,74,75]. In other work, skin keratinocyte specific expression of *MGMT* in mouse demonstrated that following N-nitroso-N-methylurea exposure, tumor initiation and progression were reduced compared to control mice not overexpressing *MGMT* [76,77]. However, higher MGMT levels did not protect against 12-O-tetradecanoylphorbol-13-acetate-mediated tumor promotion [77]. Such findings highlight the biological significance of MGMT in normal cells.

2.4. ALKBH2 and ALKBH3 repair mechanism

The ALKBH family of α-KG-iron (II) dependent dioxygenases is composed of nine proteins in mammalian cells, ALKBH1-8 and Fat Mass and Obesity-associated gene (FTO). However, only ALKBH1-3 and FTO have been identified to possess DNA repair activity [78,79]. Since *in vivo* studies on ALKBH1 and FTO concerning their role in DNA repair are limited, we will focus attention on ALKBH2 and ALKBH3. The ALKBH2 and 3 proteins directly repair alkyl lesions by an iron and α-KG-dependent oxidative demethylation reaction to yield an undamaged base with the methyl group being released as formaldehyde (Figure 5) [80,81]. Although the ALKBH2 and ALKBH3 utilize the same repair mechanism, they exhibit different cellular localization, and are implicated in different protein complexes. For example, ALKBH2 is strictly localized in the nucleus, and mainly repairs lesions present on dsDNA by interacting with PCNA at the replication fork [80,82]. In contrast, ALKBH3 is found in both the cytoplasm and nucleus where it has a high affinity for ssDNA and RNA methylated substrates as compared to the ALKBH2 for those substrates [80,83]. In the nucleus, ALKBH3 co-localizes with the activating signaling cointegrator complex 3 (ASCC3) helicase enzyme, which unwinds dsDNA to promote lesion repair by ALKBH3; this association may expand the substrate range for ALKBH3 to include dsDNA [84,85].

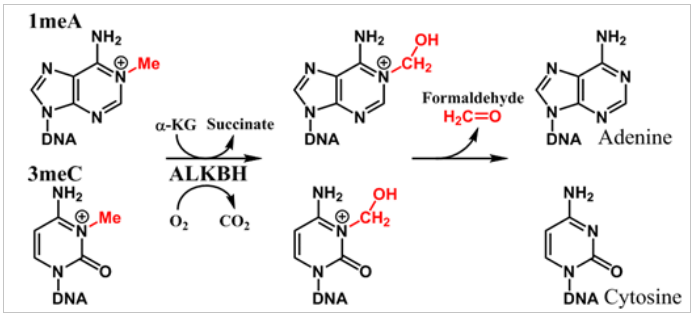

Figure 5. Repair of 1meA and 3meC by ALKBH proteins.

2.5. ALKBH proteins protect against N-linked alkyl adducts

Despite their differences, the ALKBH2 and 3 proteins repair similar N-alkyl lesions. The preferred substrates of ALKBH2 and ALKBH3 are 1meA and 3meC lesions present on DNA and/or RNA, but they can also remove other lesions such as 1meG, 3meT, 1etC, as well as ethenobase adducts such as 1,N6-ethenoadenine, and 3,N4-ethenocytosine [80,86–89]. Formation of 1meA and 3meC generally occurs in ssDNA most likely due to the protection conferred by base pairing in dsDNA. These lesions are considered highly cytotoxic due to their ability to block synthesis by DNA polymerase, thereby triggering apoptosis [90–92]. However, although toxic, 1meA lesions possess a low mutagenic potential, whereas 3meC lesions can induce C→T and C→A mutations, possibly due to adduct bypass by TLS DNA polymerases (Figure 6) [93,94]. Therefore, based on the enzymatic specificity, the role of the ALKBH2 and 3 repair proteins is to protect cells from the highly cytotoxic and mutagenic properties of N-alkyl lesions.

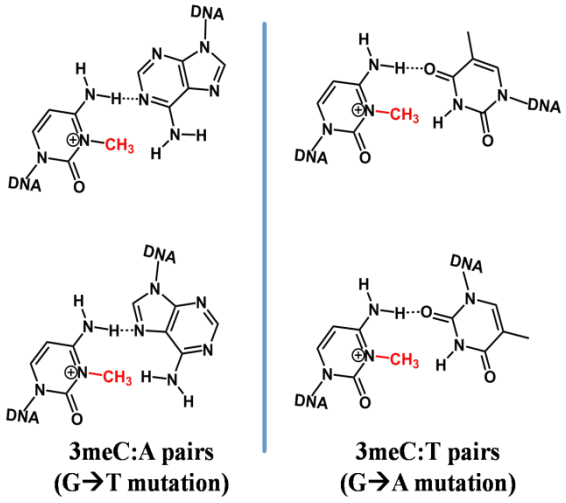

Figure 6. Mispairs formed by 3meC to form mutations.

2.6. Alkbh$^{-/-}$ models

Murine models deficient in *Alkbh2* or *Alkbh3* do not exhibit any overt phenotypic differences when compared to their wild-type counterparts. However, over time *Alkbh2*$^{-/-}$ mice show an age-related accumulation of 1meA lesions in the liver, whereas *Alkbh3*$^{-/-}$ do not show this phenotype, which indicates a preference of Alkbh2 for 1meA lesions [95,96]. In addition, using mouse embryonic fibroblast (MEFs) isolated from *Alkbh2*$^{-/-}$ and *Alkbh3*$^{-/-}$mice, researchers found that both mutant MEFs were sensitive to methyl methanesulfonate treatments, but only *Alkbh2*-deficiency provided protection against genomic mutations. Most notably, loss of *Alkbh2* in MEFs was associated with C→A and C→T mutations following methyl methanesulfonate treatments. Furthermore, *Alkbh2*-deficient MEFs showed an apparent increase in T→A mutations following methyl methanesulfonate exposure [97].

Loss of both *Alkbh2* and *Alkbh3* in mice does not result in any obvious phenotypic aberrations, and the mice are both fertile and live to normal ages [95–97]. Nonetheless, *Alkbh2*$^{-/-}$*Alkbh3*$^{-/-}$

double-mutant mice are susceptible to alkylation-induced tumor development; suggesting that both enzymes are required for alkylation resistance. In addition, this study constructed an *Aag*$^{-/-}$*Alkbh2*$^{-/-}$*Alkbh3*$^{-/-}$ (Note: *Aag* in the NIH database is *Mpg*) triple knockout mouse to determine the interaction between Aag-mediated base excision repair and DR in protecting against inflammation. Using this model, researchers found an accumulation of toxic and mutagenic εA and 1,N^2-εG lesion relative to Aag deficient mice, which suggests substrate redundancy between Aag and Alkbh proteins [96]. Based on these findings, monitoring the expression status of *ALKBH2* and *3* in cancer patients may prove useful when alkylating agent chemotherapeutics are used, given that loss of both enzymes could enhance secondary tumor development. However, the role that ALKBH2 and ALKBH3 play in cancer etiology is unclear.

3. DNA direct reversal repair and cancer

3.1. MGMT and cancer

Reduced levels of DR proteins contribute to elevated cancer risk, progression, and are important determinants of therapeutic response [98]. MGMT, the most frequent DR protein with altered levels, is decreased in 11% of cancer types [16]. In fact, gene silencing through promoter methylation is the dominant alteration of the *MGMT* gene, consisting of 92.4% of total alteration [16]. Methylation of the CpG islands on the *MGMT* promoter shields transcription factor binding sites from transcription machinery, resulting in reduced gene expression [65,66]. However, the mechanism that controls MGMT promoter silencing remains unclear.

Low *MGMT* expression due to promoter silencing could also promote tumorigenesis by allowing O^{6}meG-induced mutagenesis in oncogenes and tumor-suppressor genes. Loss of MGMT is associated with point mutations in *KRAS*, observed in colon cancer and gastric cancer, and in *p53* of non-small cell lung cancer and astrocytic tumors [99–103]. In addition, MGMT promoter methylation is frequently observed in many cancer types such as glioma, lymphoma, breast, and retinoblastoma [104,105]. However, tumors with low MGMT activity manifest enhanced sensitivity towards chemotherapeutic alkylating agents. Therefore, detection of *MGMT* promoter methylation status is clinically relevant because that status can serve as a predictor for a positive therapeutic response to alkylating agents [24]. For example, patients with glioblastoma whose tumors had *MGMT* promoter hypermethylation showed a better response to TMZ, and improved survival as compared to patients with no *MGMT* promoter methylation [106]. In contrast, high MGMT activity is often associated with aggressive malignant tumors and drug resistance [107]. Breast and ovarian tumors with high MGMT activity are linked to rapid disease progression, and with high variation in MGMT activity in cancer cells [108–110]. In addition, cancer cells that express high levels of MGMT are resistant to treatment with alkylating agents. One therapeutic approach in treating MGMT-positive tumors is to deplete tumor cells of MGMT activity using inhibitors, but the value of using MGMT inhibitors therapeutically is still being evaluated.

3.2. ALKBH and cancer

The contribution that the ALKBH proteins play during the carcinogenesis is currently under debate. Given that *ALKBH2* and *ALKBH3* are often overexpressed in certain cancers, such as

non-small cell lung carcinoma and prostate adenocarcinoma [19,111], it is of clinical significance to understand the role these proteins play in cancer development and progression. ALKBH2 and ALKBH3 have been suggested to function as tumor suppressors [112]. Indeed, downregulation of *ALKBH2* contributes to the development and progression of various cancers such as gastric cancer [18]. Furthermore, a comprehensive analysis of The Cancer Genome Atlas revealed that methylation-driven transcriptional silencing of the *ALKBH3* gene occurs in 8% of cancers; with promoter silencing of *ALKBH3* observed in many breast cancers [16,113]. Those results are consistent with a loss of ALKBH2 or ALKBH3 activity increasing cancer risk.

In various cancers, *ALKBH2* or *ALKBH3* are overexpressed, most notably *ALKBH3* [76,77,81–83]. For example, increased levels of ALKBH3 are often found in prostate and non-small cell lung cancers, and increased levels of ALKBH3 in pancreatic adenocarcinoma is correlated with poor prognosis and higher pathological stage [20,84,111]. However, loss of *ALKBH2* or *ALKBH3* expression in cancer cells renders them sensitive to anticancer drugs. Such is seen in urothelial carcinoma where loss of *ALKBH3* induced cell cycle arrest and reduced tumor cell survival [114]. Also, loss of *ALKBH3* in pancreatic adenocarcinoma in xenograft mouse models resulted in reduced tumor proliferation and induced apoptosis [20]. Whether overexpression of *ALKBH2* or *3* helps drive tumor development, or if it is merely a response to alkylating agents remains to be determined. These findings suggest that inhibition of ALKBH2 or ALKBH3 function can serve as a potential approach to sensitize cancer cells to chemotherapeutic drugs.

4. DNA direct reversal repair and therapeutic applications

Alkylating agents were the first form of chemotherapeutics developed for the treatment of leukemia and lymphomas [115]. Currently, several methylating agents are used as anticancer drugs principally based on their ability to generate large amounts of 1meA and O^6meG lesions, or similar derivatives on genomic DNA. There are two major forms of alkylating agents used in therapy: Monofunctional and bifunctional (Figure 7). Methylating agents contain a single reactive group that interacts covalently with nucleophilic reactive centers in DNA; these chemicals are the most commonly used alkylating agents during chemotherapy [116]. Dacarbazine and procarbazine are S_N1 methylating agents currently used for the treatment Hodgkin's lymphoma and TMZ used in glioblastoma treatment. The chloroethylating nitrosoureas, nimustine, carmustine, and lomustine are used for the treatment of brain tumors [5]. Bifunctional alkylating agents contain two reactive groups that can form interstrand crosslinks. These agents include mechlorethamine, cyclophosphamide, and melphalan that are used for the treatment of leukemia, lymphoma, multiple myeloma, ovarian cancer, and solid tumors [5].

Our understanding of how cancer cells react to different chemotherapeutic agents is becoming better understood. Altered DNA damage repair pathways are often targeted with anti-cancer agents to enhance a positive tumor response through synthetic lethality. Cancer cells lacking DR pathways can be targeted with alkylating agents. However, many cancers overexpress DR enzymes rendering them resistant to alkylating agents. Therefore, using inhibitors to inactivate MGMT or ALKBH proteins in tumors is a useful strategy to increase the response to alkylating agents.

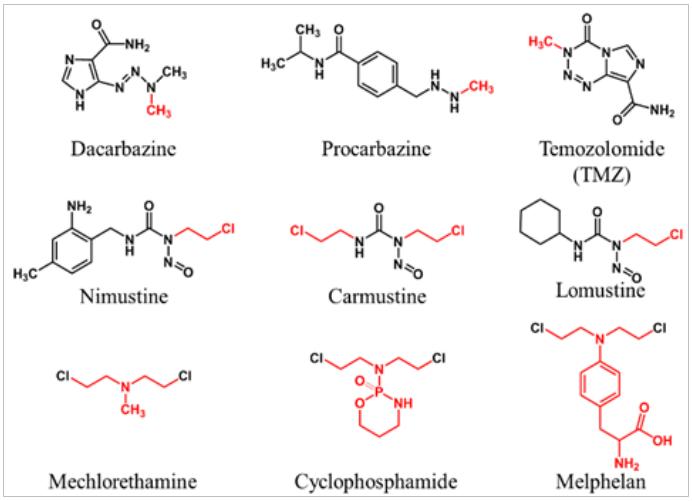

Figure 7. Selected alkylating agents used therapeutically. The species modifying DNA are indicated by red in the structures.

4.1. MGMT inhibitors

MGMT inhibitors are pseudosubstrates that mimic the structure of O^6meG (Figure 3B), and take advantage of its "suicide" mechanism by covalently binding to the enzyme active site rendering it inactive. In cancer cells, MGMT inhibitors are used to deplete MGMT-positive cancer cells of active enzyme, which enhances the efficacy of alkylating agents. To date, numerous MGMT inhibitors have been synthesized, such as O^6-benzylguanine (O^6BG), O^6-(4-bromothienyl)guanine (lomeguatrib), and O^6-[3-(aminomethyl)benzyl]guanine, and extensively studied in clinical trials in combination with DNA alkylating agents [117,118].

The first developed, and the most extensively studied MGMT inhibitor is O^6BG, which is 2,000-times more effective at inactivating MGMT as compared to the O^6meG lesion [119–121]. Various *in vitro* and *in vivo* studies have established that inactivation of MGMT with O^6BG enhances sensitivity to alkylation treatment, and inhibited tumor growth [122]. Clinical trial studies have shown that O^6BG sensitizes gliomas, melanomas, gastric adenocarcinomas, and medulloblastomas to the cell killing effects of TMZ and carmustine [123–127]. In addition, phase I trials using lomeguatrib in combination with TMZ effectively deplete patients of MGMT activity, and increase O^6meG adducts in various cancer forms [128,129]. However, contrasting clinical trials studies have reported that the use of MGMT inhibitors in combination with anticancer alkylating agents has no impact on clinical outcome [122,130,131]. In addition, numerous clinical trials, and animal studies have determined that O^6BG and lomeguatrib increases toxicity associated with alkylating therapy in non-tumor cells, especially in the bone marrow, resulting in myelosuppression [132–134]. Efforts to reduce off-target effects using O^6BG substrates conjugated to tumor cell metabolites like glucose or folate are currently being tested, but have not been used in clinical trials [135,136]. Another inhibitor of MGMT that also induces autophagy is lipoic acid [137]. It remains to be seen whether other MGMT inhibitors enhance efficacy against tumors, or merely increase non-tumor cell toxicity during treatment with alkylating agents.

In contrast to inhibiting MGMT activity, other studies have focused on reducing *MGMT* expression in cancer cells. Targeting pathways that are often overactive in cancer cells may restore

sensitivity towards alkylating agents. Various studies have revealed that inhibiting signaling pathways, such as Hedgehog/GLI1 and WNT/β-catenin, reduces *MGMT* expression and restores sensitivity towards alkylating agents [138,139]. In addition, another strategy to reduce *MGMT* expression is by promoting gene promoter methylation [21,140]. Efforts to reduce *MGMT* gene expression include using the histone deacetylase (HDAC) inhibitors valproic acid (VPA), and the DNA methylation inhibitors [141–145]. Other work showed that TMZ and VPA treatments of melanoma cells, overexpressing HDACs, substantially increase apoptosis and/or increase survival compared to controls [143]. VPA treatment in melanoma cells also reduces proteins involved in homologous recombination (RAD52) and the Fanconi anemia pathway (FANCD2) [143]. Although VPA reactivates capase-8 and increased caspase-3 levels, at least one report suggests little if no benefit for using radiation and TMZ along with VPA [146]. Nonetheless that assessment is still under study. Despite the numerous proposed methods to deplete *MGMT* gene expression in tumor cells, there are no viable clinical approaches developed to inhibit MGMT production. However, these methods have the potential to be used as a treatment option for MGMT-positive cancers, and help avoid the toxicities associated with O^6meG derivatives.

4.2. ALKBH inhibitors

Similar to *MGMT*, overexpression of *ALKBH2* promotes chemoresistance to alkylating agents [147]. Unlike MGMT, the potential inhibitors remain in the developmental stages, and there are currently no inhibitors of ALKBH proteins being tested in clinical trials [147]. Recent studies have suggested indirect methods for inhibiting ALKBH protein functions, which can be used as novel chemotherapeutic approaches to cancer treatment. One study proposed deregulating protein stability by targeting the OTU/USP7/USP9X deubiquitinase pathway, which acts as a master regulator of ALKBH2 and ALKBH3 protein stabilization. The OTU/USP7/USP9X deubiquitinase complex regulates ALKBH by K48-ubiquitination, a signaling peptide that marks proteins for proteasomal degradation [148,149]. In the absence of USP7 or USP9X, cells are sensitized to alkylation-mediated damage due to ALKBH protein destabilization. This suggests that small-molecule inhibitors of USP7 and USP9X can be used to sensitize cancer cells to chemotherapeutic drugs [150,151].

Limiting the required metabolic α-KG in cancer cells can serve as a therapeutic approach because it reduces ALKBH2 and ALKBH3 activity levels. A recent study found that the oncometabolite ᴅ-2-hydroxyglutarate (ᴅ-2-HG), which accumulates in *IDH*-mutant cancer cell lines, is an α-KG structural analog that acts as an inhibitor of ALKBH2 and 3 by blocking its demethylation activity. Therefore, *IDH*-mutant cancer cell lines have increased sensitivity to alkylating agents [152]. Another study found that glutamine deficiency inhibits ALKBH2 and 3 from repairing DNA alkylation damage. Glutamine is a precursor of α-KG and that study indicated reduction in α-KG levels using glutaminase inhibitors, in combination with alkylating agents, can improve drug efficacy [153]. Whether these methods will elicit a positive response in clinical trials has yet to be determined.

5. Conclusions

The DR systems in mammalian cells are more limited in their capacity to repair a variety of adducts as compared to base excision and nucleotide excision repair pathways. However, DR has

major roles in the elimination of adducts that can lead to mutations or cell death. The development of inhibitors of MGMT, ALKBH2, and ALKBH3 could help augment existing chemotherapies based on small DNA alkylating agents. Coupled with new technologies for drug delivery, targeting could be more specific to tumor cells, resulting in improved patient outcomes.

Acknowledgments

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Conflict of interest

The authors report no conflict of interest.

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