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Regulatory mechanisms of O6-methylguanine methyltransferase expression in glioma cells

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Abstract

O6-methylguanine methyltransferase (MGMT), a pivotal DNA repair enzyme, has its dysregulation playing a substantial role in gliomagenesis, the development of therapeutic resistance, and patient prognosis. This narrative review is designed to offer an all-encompassing overview of the intricate regulatory mechanisms that govern MGMT

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expression in glioma cells. We systematically investigate the diverse levels of regulation that impact MGMT expression in glioma. These include epigenetic regulation, transcriptional control, post-translational modifications, and the influence exerted by the tumor microenvironment. Epigenetically, methylation of CpG islands within the MGMT promoter region represents a critical determinant for gene silencing. Conversely, histone modifications such as H3K4me3 augment MGMT expression. Transcriptionally, a complex network of transcription factors, which encompasses Sp1, p53, and NF-κB, along with signaling pathways like TGF-β, JAK/STAT, and PI3K/AKT, orchestrates MGMT expression in glioma cells. Furthermore, post-translational modifications of MGMT, such as phosphorylation and ubiquitination, are of pivotal importance in modulating its stability and enzymatic activity. The tumor microenvironment, with factors such as oxidative stress, hypoxia, and immune responses, also exerts a significant influence on MGMT expression. This narrative review delves deeper into the relationship between MGMT expression and drug resistance, especially resistance to alkylating chemotherapy agents, and accentuates the significance of evaluating MGMT expression for personalized glioma therapy. By elucidating these regulatory mechanisms, this review endeavors to enhance our understanding of MGMT's role in glioma biology and to provide insights for future therapeutic strategies aimed at surmounting current treatment challenges.

Keywords
Drug resistance, glioma, O6-methylguanine methyltransferase, regulatory mechanisms

Introduction

In the fields of cell biology and oncology, the study of DNA repair mechanisms has emerged as one of the most critical avenues for elucidating tumorigenesis, progression, and drug resistance.[1–3] Among these mechanisms, MGMT has garnered significant attention as a key enzyme within the DNA repair system. MGMT dysfunction is closely linked to tumorigenesis, directly impacts tumor response to specific chemotherapies, and influences patient prognosis.[4,5]

Alkylating agents—chemical carcinogens, environmental toxins, or chemotherapeutic drugs—can induce alkylation damage to DNA molecules. In this process, alkylating agents react with the cyclic nitrogen (N) and extracyclic oxygen (O) atoms of DNA bases, forming various covalent adducts,[6] such as O6-methylguanine (O6-meG). This process, known as alkylation, can lead to highly mutagenic and cytotoxic effects if left unrepaired. And direct demethylation of O via MGMT represents one of the most critical repair pathways for methylation-induced injury.[6,7] The MGMT gene is located in the 10q26 region of human chromosome 10 and encodes a single-chain polypeptide consisting of 207 amino acids. The protein structure features an active site located at cysteine position 145, which is integral to its biological function. During DNA damage repair, MGMT transfers methyl groups from DNA to its own cysteine residues through this active site to facilitate the

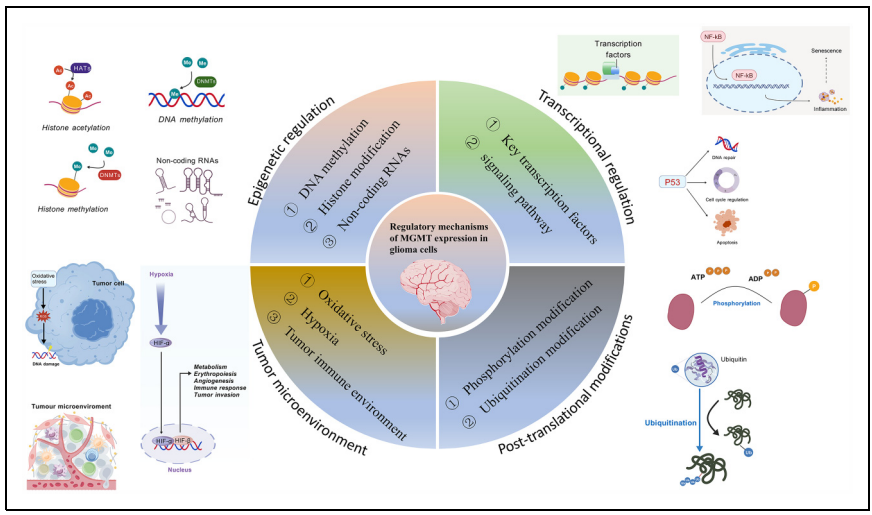

Figure 1. Regulatory mechanisms of MGMT expression in glioma cells.

dealkylation reaction. This transfer is a key function of MGMT, effectively removing alkyl groups from DNA and thereby preventing mutations and cellular damage.[8]

Given the biological functions of MGMT, it plays a dual role in gliomagenesis and cancer development. On one hand, deletion or low expression of MGMT leads to the accumulation of DNA alkylation damage, which subsequently promotes tumorigenesis. On the other hand, elevated MGMT expression is the main cause of drug resistance in glioma cells. Alkylating agents, such as temozolomide (TMZ), induce DNA alkylation damage to kill tumor cells; however, MGMT can repair this damage, thereby counteracting the cytotoxic effects of the drug.[9] Therefore, MGMT is not only crucial in the process of cancer development and progression, but its expression level also directly affects the prognosis and treatment response of glioma patients. The promoter methylation status of the MGMT gene has emerged as a significant prognostic marker for response to alkylating agent therapy and patient survival across a variety of cancers, including glioma.[10–12]

Recent studies have revealed that MGMT expression is regulated through diverse mechanisms, including epigenetic modifications (such as promoter methylation and histone modifications), transcriptional regulation by key factors such as p53 and NF-κB, post-translational modifications (PTMs) that influence MGMT protein stability, and the impact of the tumor microenvironment (such as hypoxia and oxidative stress). While a critical gap remains, the integrated analysis of its multi-level regulatory networks in glioma cells. Previous reviews have focused on isolated aspects of MGMT regulation. This narrative review systematically synthesizes the latest advancements in MGMT regulation, with a focus on integrating epigenetic, transcriptional, post-translational, and microenvironmental mechanisms (Figure 1). Targeting these levels of regulation may provide new strategies for glioma treatment, thus assessing MGMT status remains critical for

personalized treatment decisions. By addressing these interconnected layers, we aim to clarify how MGMT dysregulation promotes gliomagenesis and therapeutic resistance, thereby guiding the development of novel strategies to overcome treatment challenges. To ensure a comprehensive review, we conducted a literature search in PubMed and Web of Science using the following keywords: "MGMT AND glioma," "MGMT AND epigenetics," "MGMT AND transcriptional regulation," "MGMT AND post-translational modifications," "MGMT AND tumor microenvironment," and "MGMT AND gliomagenesis." The majority of citations are included from 2000 to 2024, with a preference for peer-reviewed original research and review articles, as well as seminal studies in the field of glioma research. Ultimately, only high-impact and representative studies were included in this review.

This review is guided by the Scale for the Assessment of narrative review articles (SANRA).[13]

The role of epigenetic regulatory mechanisms in MGMT expression

Epigenetic regulation is a key determinant of MGMT gene expression, particularly in tumor cells, as it alters the transcriptional activity of genes by influencing the modification status of DNA and chromatin. Research has demonstrated that epigenetic mechanisms—including DNA methylation, histone modification, and ncRNAs—play crucial roles in regulating MGMT expression.

Regulation of DNA methylation

DNA methylation is a prevalent epigenetic modification in eukaryotes that influences gene expression and cellular function by adding methyl groups to specific sites within the DNA sequence, without altering the original sequence. This modification primarily regulates the transcriptional activity of genes by methylating cytosine-guanine dinucleotide (CpG) islands. CpG islands are regions characterized by high CpG density, typically located in the regulatory regions of promoters, and they are generally unmethylated in non-cancerous tissues. In the MGMT gene, methylation of the CpG islands in the promoter region plays a particularly significant role, directly leading to gene silencing and subsequently reducing MGMT expression. This mechanism is especially prevalent in glioblastoma (GBM).[14,15] The role of DNA methylation extends beyond the static regulation of MGMT transcription and has substantial clinical implications. In 2000, Esteller and his colleagues conducted a study and found that the methylation status of the MGMT promoter is closely related to the efficacy of treatment with alkylating agents such as TMZ. Patients with methylated MGMT promoters were typically more sensitive to these drugs, as MGMT silencing reduced DNA damage repair and increased the susceptibility of tumor cells to therapy. This article has become a foundational text for studying the transcriptional regulation of MGMT in the field of glioma.[16] Additionally, Everhard et al. investigated the methylation levels of CpG sites associated with MGMT expression

in 54 GBM samples using pyrophosphate sequencing. They found that methylation of key CpG sites in the promoter region of MGMT effectively represses its transcription.[17] As methylation levels increase, the MGMT gene becomes progressively silenced, leading to enhanced sensitivity of tumor cells to chemotherapy. Consequently, the methylation status of the MGMT promoter region has emerged as an important reference point for predicting tumor chemotherapy sensitivity and developing individualized treatment plans. Notably, DNA methylation is not an isolated epigenetic regulatory mechanism.

Histone modification

Histones are basic proteins found in chromosomes that bind to DNA, with histone octamers wrapping around DNA to form nucleosomes, the functional units of chromatin.[18] Histone modification refers to the covalent chemical modification of specific residues at the amino terminus of histones. Post-translational modifications of histones typically occur at the N-terminal tail and represent an important epigenetic mechanism; these modifications can include phosphorylation, ADP-ribosylation, methylation, or acetylation, among others.[19] Histone modification is a key mechanism influencing MGMT gene expression by regulating chromatin structure.

Similar to DNA methylation, post-translational modifications of histones are catalyzed by specific enzymes, including acetyltransferases, deacetylases, methyltransferases, and demethylases. Unlike DNA methylation, histone methylation can result in mono-, di-, or trimethylation of a single lysine residue, such as H3K4. The presence of multiple types of modifications on a single histone molecule contributes to the combinatorial complexity known as the "histone code."[20] Histone methylation is diverse, with different sites and degrees of methylation producing opposing regulatory effects. In glioma cells, histone methylation at specific sites, such as H3K9me3, H3K27me3, and H3K36me2 in the MGMT promoter region, is frequently associated with gene repression.[21,22] These repressive marks contribute to heterochromatin formation and gene silencing by recruiting repressive chromatin complexes, including polycomb repressive complexes PRC1 and PRC2, which promote chromatin condensation and inhibit the binding of transcription factors and RNA polymerase. Conversely, trimethylation of histone H3 lysine 4 (H3K4me3) acts as an activation mark. When present at the MGMT promoter, H3K4me3 facilitates transcription and increases MGMT expression, which is particularly relevant in gliomas that exhibit resistance to alkylating agents, as elevated MGMT levels confer protection against DNA damage.[23] These distinct methylation sites hold potential as biomarkers for glioma diagnostic grading and prognosis, although further investigation is needed to fully elucidate their clinical value.

In addition to methylation, acetylation modifications also play a crucial role in the regulation of MGMT expression. Acetylation primarily occurs at the N-terminal lysine residues of histones H3 and H4 and is regulated by histone acetyltransferases (HATs) and deacetylases (HDACs). These acetylation modifications neutralize the positive charge of histones, attenuating their interaction with DNA. This process loosens chromatin structure, facilitating the binding of transcription factors and RNA polymerase.[24,25] In the context of MGMT regulation, the acetylation status of histones in the promoter region

may modulate MGMT's transcriptional activity by influencing the recruitment of transcription factors and the openness of chromatin structure. In certain glioma models, pharmacological inhibition of HDACs has been shown to influence MGMT expression. For example, levetiracetam modulates HDAC levels, leading to the silencing of MGMT and enhancing the effectiveness of TMZ treatment.[26] This suggests that histone deacetylation may play a crucial role in the silencing of MGMT in glioma cells. However, it is worth noting that direct research on the effects of histone acetylation modifications on MGMT expression in glioma remains relatively limited. Despite this, the potential regulatory role of histone acetylation in MGMT expression should not be underestimated and warrants further investigation.

Regulation of ncRNAs

NcRNAs play a crucial role in the regulation of MGMT. These RNA molecules do not encode proteins; instead, they finely regulate MGMT expression and function at the post-transcriptional and epigenetic levels through various mechanisms. Consequently, they influence the cell's capacity for DNA damage repair and its sensitivity to therapeutic agents.

MiRNAs and lncRNAs are the primary members of ncRNAs that regulate MGMT, miRNAs are a class of ncRNAs approximately 20–25 nucleotides in length. Research has demonstrated that various specific miRNAs can directly bind to the mRNAs of MGMT, inhibiting the translation process and thereby decreasing MGMT protein levels.[27,28] For example, miRNA-4539 has been shown to specifically bind to the 3' untranslated region (3'-UTR) of MGMT, leading to mRNA degradation or translational repression. This mechanism down-regulates MGMT protein expression at the post-transcriptional level, which, in turn, affects the sensitivity of tumor cells to alkylating agents. Such direct targeting enables miRNAs to rapidly respond to changes in the intracellular environment and adjust the activity level of MGMT.[29]

lncRNAs are a class of RNA molecules that exceed 200 nucleotides in length and are involved in the regulation of gene expression through diverse mechanisms, including epigenetic, transcriptional, and post-transcriptional regulation. lncRNAs exhibit more complex and diverse mechanisms in the regulation of MGMT. For example, lncRNA HOTAIR has been shown to regulate PRC2 and EZH2, key methyltransferases responsible for the methylation of histone H3 at lysine 27 (H3K27). This process induces methylation of histone H3K27, leading to the formation of a heterochromatic state, which subsequently affects gene expression and silencing.[30] Similarly, lncRNAs may influence the methylation status and expression levels of MGMT genes through a parallel mechanism. These lncRNAs can function as competing endogenous RNAs (ceRNAs) that compete with miRNAs for the same binding sites, thereby mitigating the inhibitory effects of miRNAs on target mRNAs—a mechanism known as the "sponge" effect.[31] Certain lncRNAs may contain miRNA response elements (MREs) similar to those found in MGMT mRNAs, which protect MGMT mRNAs from degradation by binding to miRNAs, thus upregulating MGMT expression. In addition, lncRNAs can influence the epigenetic status of MGMT by recruiting chromatin remodeling and modification

complexes to the MGMT locus, including those involved in DNA methylation and histone modification, thereby regulating MGMT expression at the transcriptional level. This epigenetic regulatory mechanism enables lncRNAs to finely modulate MGMT function across a broader spatial and temporal range. The regulation through the ceRNA network has been observed in various cancer contexts, underscoring its significance in tumor biology.[32,33]

In addition to the aforementioned mechanisms, ncRNAs may indirectly influence MGMT expression through interactions with other regulatory factors. For example, lncRNAs can associate with transcription factors to form complexes that collaboratively regulate the transcription of MGMT genes. Furthermore, ncRNAs may participate in processes such as mRNA stability regulation and protein translation regulation, thereby indirectly affecting the protein levels of MGMT.

Interactions between histone modification and DNA methylation

Histone modifications and DNA methylation often synergize to regulate MGMT expression in glioma cells. For instance, the recruitment of DNA methyltransferases (DNMTs) to the MGMT promoter frequently depends on the presence of inhibitory histone marks, such as H3K9me3. In contrast, histone acetylation at the MGMT promoter inhibits DNA methylation, thereby maintaining MGMT expression. This interplay between histone modifications and DNA methylation is critical for understanding the epigenetic regulation of MGMT in gliomas and may offer a promising therapeutic target. Strategies aimed at modifying histone marks to control MGMT expression could hold significant potential for improving glioma treatment outcomes.

The role of regulatory mechanisms at the transcriptional level in MGMT expression

In glioma cells, the regulation of MGMT expression is a complex and nuanced process, with transcriptional-level mechanisms playing a central role. This regulation involves the direct action of multiple key transcription factors and is further modulated by various signaling pathways. Together, these factors form an intricate and comprehensive network that governs MGMT expression in glioma cells.

Key transcription factors

Key transcription factors regulate MGMT at the transcriptional level, promoting or repressing its expression depending on the cellular environment. The Sp1 transcription factor is one of the most significant regulators of MGMT, as it has been shown to bind directly to the promoter region of the MGMT gene, thereby enhancing its transcription. As a ubiquitous zinc finger protein, Sp1 not only participates in various biological processes, including cell proliferation, differentiation, and apoptosis, but also modulates the transcription of target genes through its specific binding to DNA

sequences, particularly the GC box. In the regulation of MGMT, Sp1 enhances MGMT mRNA synthesis and protein expression by directly binding to the GC box in the MGMT promoter region, recruiting coactivators (such as p300/CBP) and RNA polymerase II, and forming a complex that promotes transcription. This mechanism has been extensively studied in various cancer types, such as glioma, lung cancer, and ovarian cancer, where high expression of Sp1 is often associated with the hyperactivation of MGMT and increased chemoresistance.[34,35]

Hypoxia is a characteristic microenvironmental feature of solid tumors, including gliomas, where hypoxia-inducible factor-1α (HIF-1α), a key regulator of the hypoxic response, is stably expressed. HIF-1α activates a range of downstream genes that promote angiogenesis, glycolysis, and cell survival. However, in glioma cells, HIF-1α demonstrates a distinct regulatory effect on MGMT expression. Studies have shown that HIF-1α may downregulate MGMT expression through both direct and indirect mechanisms. On one hand, HIF-1α reduces MGMT transcription by interacting with other transcription factors or cofactors. On the other hand, it influences MGMT protein stability, affecting its synthesis and degradation processes, ultimately leading to lower MGMT expression levels. This downregulation enhances the sensitivity of glioma cells to alkylating agent-based chemotherapies under hypoxic conditions, providing new insights and potential strategies for glioma treatment.[36,37]

Signaling pathway

The regulation of MGMT at the transcriptional level is influenced by a variety of signaling pathways that respond to both intrinsic cellular states and extrinsic stimuli, contributing to the complexity of MGMT expression in tumor cells. Among these pathways, the p53 and NF-κB pathways play crucial roles; however, other pathways, such as TGF-β, JAK/STAT, and PI3K/AKT, also significantly contribute to the transcriptional regulation of MGMT.

p53 is a classic tumor suppressor gene that plays a key role in the DNA damage response, cell cycle regulation, and apoptosis, and it is mutated in more than 50% of patients with malignancies.[38] When cells are exposed to DNA damage, the p53 signaling pathway is activated, leading to cell cycle arrest and the initiation of DNA repair mechanisms. These processes may require the involvement of MGMT to repair specific types of DNA damage, thereby indirectly promoting the expression or activity of MGMT in tumor cells. However, p53 has been shown to downregulate MGMT expression, particularly in response to DNA-damaging agents.[39,40] Furthermore, p53 has also been reported to downregulate MGMT expression in studies involving tumors including glioma, breast, colorectal, and other malignancies.[41,42] This downregulation may represent a mechanism by which cells reduce their DNA repair capacity, thereby favoring apoptosis over survival in the face of significant damage, especially damage induced by alkylating agents. Analyses indicate that the inhibition of MGMT by p53 may occur indirectly, possibly through interactions with other transcription factors or repressors that regulate MGMT expression, highlighting the intricate balance between DNA repair and cell death during p53 signaling.

Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-κB) is a protein complex that plays a critical role in cell signaling, regulating transcribed DNA, cytokine production, and cell survival.[43] The NF-κB signaling pathway can respond to a wide range of stimuli and is essential for regulating various physiological processes, including immune responses, inflammatory responses, cell growth, and apoptosis. Activation of the NF-κB signaling pathway promotes the proliferation and survival of tumor cells.[44] When NF-κB is activated, it upregulates a series of genes associated with cell proliferation and survival, including Cyclin D1 and c-Myc. The overexpression of these genes can lead to the uncontrolled proliferation of tumor cells and enable their escape from the apoptotic program. In the context of MGMT expression regulation, activated NF-κB can directly bind to specific sequences within the promoter region of the MGMT gene, thereby promoting its transcriptional upregulation.[45] Furthermore, NF-κB serves as a key regulator of the inflammatory response; in tumor cells, a sustained inflammatory response can facilitate tumor growth and invasion. Activation of the NF-κB signaling pathway enhances the expression of various inflammation-related genes, such as IL-1β and TNF-α. These inflammatory factors not only promote tumor cell proliferation and survival but also indirectly influence MGMT expression through a complex network of signal transduction. For instance, inflammatory factors may regulate transcriptional or post-translational modifications of MGMT by activating other signaling pathways, such as MAPK and PI3K.[46,47] Research has demonstrated that NF-κB promotes the expression of MGMT under certain circumstances, thereby aiding tumor cells in resisting alkylating chemotherapy by enhancing DNA repair. This pro-survival function of NF-κB positions it as a critical factor in the development of chemotherapy resistance, particularly in gliomas, where MGMT serves as a primary determinant of resistance to the commonly used alkylating agent TMZ. Consequently, the activation of NF-κB may act as a protective mechanism that enables tumor cells to maintain the capacity to repair potentially lethal DNA lesions, thus contributing to their survival during treatment.[48] In addition to p53 and NF-κB, other signaling pathways significantly contribute to the transcriptional regulation of MGMT. The transforming growth factor β (TGF-β) pathway, recognized for its dual role in cancer progression and suppression, has also been implicated in the control of MGMT expression. TGF-β signaling modifies the epigenetic landscape of the MGMT promoter and influences its methylation status, which in turn regulates gene expression. The methylation of the MGMT promoter serves as a crucial determinant of MGMT silencing in glioma, and TGF-β-mediated alterations in this epigenetic mark impact tumor responses to alkylating agents.[49] Similarly, the JAK/STAT pathway, which transmits cytokine and growth factor signals, has been implicated in the regulation of DNA repair genes, including MGMT. Although studies of the JAK/STAT pathway have predominantly focused on inflammatory responses, cell proliferation, and immune regulation, some research has suggested that it may also regulate MGMT expression through interactions with other signaling pathways.[50] The PI3K/AKT pathway, frequently dysregulated in cancer, plays a critical role in promoting MGMT expression. Activation of this pathway supports tumor cell proliferation and survival, in part by upregulating DNA repair mechanisms such as MGMT. The regulation of MGMT by PI3K/AKT may occur through downstream targets that modulate the binding of transcription

factors to the MGMT promoter, thereby further enhancing the tumors' ability to repair DNA damage.[51]

Post-translational modifications and MGMT protein stability

Post-translational modification (PTM) encompasses a series of chemical modifications that proteins undergo following translation and synthesis. These modifications can significantly impact the structure, function, stability, and interactions of proteins, thereby playing a crucial role in biological processes such as cell signaling and gene expression regulation. MGMT, an important DNA repair enzyme, is profoundly influenced by the regulation of its expression, which is also significantly affected by post-translational modifications.

Phosphorylation modification of MGMT proteins

Phosphorylation is a crucial PTM that can significantly influence the function of MGMT. This modification involves the addition of a phosphate group to specific serine, threonine, or tyrosine residues and can either enhance or inhibit MGMT's ability to repair the O6-MeG lesion in DNA. O6-MeG is a highly mutagenic lesion that, if left unrepaired, can lead to mispairing during DNA replication and subsequent point mutations.[52]

Protein degradation is a crucial mechanism for maintaining protein homeostasis, and phosphorylation often serves as a signaling tag for this degradation process.[53] For MGMT, the phosphorylation of specific amino acid residues may trigger its ubiquitination, which is subsequently recognized and degraded by the proteasome.[54] This process ensures the regulation of intracellular MGMT levels. If MGMT is not degraded after repairing DNA damage, its persistent activity may lead to excessive repair of less severe DNA lesions, thereby interfering with critical cell signaling pathways, such as apoptosis.[55]

The balance between MGMT phosphorylation and degradation is critical for tumor progression and treatment response. Phosphorylation-dependent degradation functions as a regulatory checkpoint to ensure that MGMT levels remain within a controlled range. When MGMT levels are tightly regulated, cells can respond apoptotically to extensive DNA damage, particularly in the presence of therapeutic agents that induce O6-MeG lesions. Conversely, alterations in this regulatory mechanism, such as mutations that render MGMT unphosphorylatable or prevent recognition by ubiquitin ligases, can result in overexpression of MGMT, thereby increasing tumor resistance to therapy.

Ubiquitination modification of MGMT proteins

Corresponding to phosphorylation, ubiquitination serves as the primary pathway by which tagged MGMT proteins undergo degradation. Ubiquitination is a post-translational modification that involves the attachment of a ubiquitin moiety to a protein molecule, and it is typically associated with protein degradation and clearance.[56] The degradation of MGMT through ubiquitination modulates its function in repairing O6-alkylguanine lesions. When MGMT is ubiquitinated and subsequently degraded, the number of

available MGMT molecules in the cell decreases, leading to a diminished capacity for DNA repair. For tumor cells exposed to alkylating chemotherapeutic agents, this reduction in MGMT levels means they are less capable of withstanding drug-induced DNA damage, thereby increasing the efficacy of the chemotherapeutic agents and enhancing tumor cell mortality. Conversely, if the ubiquitination process of MGMT is obstructed, resulting in abnormal accumulation within the cell, tumor cells will exhibit stronger DNA repair capabilities and will more efficiently resist chemotherapeutic drug-induced damage, ultimately improving post-treatment survival. Furthermore, it has been demonstrated that MGMT, when inactivated by alkylation, can serve as a substrate for ubiquitin coupling and subsequent proteasomal degradation.[57,58]

In addition, the intricate interaction between phosphorylation and ubiquitination provides an additional layer of cellular regulation. In certain cases, the phosphorylation state of a protein can function as a "signal" recognized by specific E3 ubiquitin ligases, thereby triggering the ubiquitination process—a mechanism referred to as "phosphorylation-dependent ubiquitination." In the context of MGMT, phosphorylation may modulate the ubiquitination process by influencing its conformation or by interacting with other regulatory factors, thus controlling its degradation rate.[59] Notably, this phosphorylation-dependent ubiquitination may serve as a feedback mechanism to ensure that MGMT is removed in a timely manner when it is no longer needed, thereby avoiding unnecessary resource expenditure and mitigating potential negative impacts once MGMT has fulfilled its role in DNA repair.

Through the ubiquitination of MGMT and its interplay with phosphorylation, these mechanisms collectively establish a complex network for precise cellular control over MGMT levels. This regulatory system not only ensures that cells can respond swiftly and repair effectively in the face of DNA damage but also identifies potential targets for the treatment of tumor cells. By gaining a deeper understanding of these regulatory mechanisms, researchers aim to develop more precise and effective anticancer strategies to address the increasingly complex challenges of tumor therapy.[60]

In many cancers, the stability and activity of MGMT determine the resistance of cells to chemotherapeutic agents. Enhancing stability by reducing ubiquitination or modulating activity through phosphorylation can improve tumor cell survival. Conversely, the induction of PTMs that promote MGMT degradation can sensitize tumors to alkylating agents. Thus, understanding the PTMs of MGMT opens avenues for targeted therapies. Modulating these pathways can aid in designing inhibitors that reduce MGMT levels or inhibit its activity, thereby enhancing the response of GBM and other cancers to alkylating agents such as TMZ. Therefore, strategies that disrupt MGMT repair pathways by interfering with PTMs could serve as effective adjuncts to cancer therapy.

Tumor microenvironmental regulation of MGMT expression

The regulation of MGMT expression in glioma cells is influenced not only by genetic and epigenetic factors but also by the complex dynamics of the tumor microenvironment (TME). Various environmental factors within the TME, such as oxidative stress, hypoxia, nutrient deprivation, and immune responses, can significantly impact MGMT expression.

Collectively, these factors shape the adaptive responses of glioma cells, often modulating DNA repair mechanisms and thereby conferring resistance to chemotherapy and promoting tumor survival.

Effects of oxidative stress on MGMT expression

Oxidative stress is a common phenomenon in tumor cells, referring to the imbalance of oxygen concentration in the TME due to dysregulated cellular metabolism and chronic inflammation. This condition results in elevated levels of reactive oxygen species (ROS), which can cause DNA damage.[61] In response to increased DNA damage and heightened repair demands, the expression of MGMT, a critical DNA repair enzyme, may be upregulated. For instance, it has been observed that oxidative stress caused by chromium toxicity affects the balance of the antioxidant system in vivo, leading to deregulation of histone acetylation and methylation, as well as the methylation of MGMT.[62] Furthermore, studies have indicated that oxidative stress can increase MGMT expression in various toxin exposure models.[63] Additionally, oxidative stress may indirectly regulate MGMT expression by influencing epigenetic modifications, such as DNA methylation. Thus, the dual role of oxidative stress underscores the complexity of MGMT regulation: while short-term oxidative stress may activate protective mechanisms, chronic stress can inhibit repair pathways, contributing to tumor progression.

Effect of hypoxia on MGMT expression

Hypoxia is another critical feature of the TME, particularly in solid tumors, which often exhibit hypoxic regions due to increased oxygen demand arising from uneven vascular distribution and rapid cellular proliferation. Hypoxia can significantly regulate gene expression through hypoxia-inducible factors (HIFs). Among these, HIF-1α and HIF-2α are key transcription factors activated under hypoxic conditions that regulate a wide range of genes involved in angiogenesis, metabolism, and cell survival.[64] Under hypoxic conditions, the expression level of HIF-1α increases, enabling cells to adapt to the low-oxygen environment by modulating the expression of a series of downstream genes.

In regulating MGMT expression, HIF-1α, under hypoxic conditions, represses MGMT gene expression by binding to its promoter region. This inhibition may be achieved by altering the epigenetic regulatory mechanisms of the MGMT gene, such as its DNA methylation status, or through direct inhibition of transcription. Research has shown that hypoxia-induced HIF-1α activity is closely associated with decreased MGMT expression, resulting in increased sensitivity of tumor cells to alkylating agents, thereby rendering tumor cells in hypoxic regions more susceptible to DNA damage.[65] However, the hypoxic environment can also diminish the DNA damage repair capacity of tumor cells, which, in turn, promotes genomic instability and tumor progression.[66]

In addition, HIF-1α indirectly regulates MGMT expression through interactions with other signaling pathways and related effectors. For example, HIF-1α may modulate other

repair pathways in response to DNA damage by activating response genes such as p53; the activation of these pathways synergizes with the inhibitory effect of MGMT.[67] Furthermore, bone morphogenetic proteins (BMPs), particularly BMP2, enhance the responsiveness of hypoxia-resistant GBM to chemotherapy through the downregulation of the HIF-1α/MGMT axis.[36] Thus, HIF-1α plays a complex and dual role in regulating MGMT expression and glioma responsiveness to chemotherapy, enhancing the chemosensitivity of tumor cells while simultaneously promoting tumor progression by triggering increased genetic instability.

Potential role of the tumor immune environment in the regulation of MGMT

The tumor immune environment plays a crucial role in tumorigenesis, development, and treatment. Immune cells, such as T cells and macrophages, can influence the TME by secreting cytokines and directly killing tumor cells. The potential role of the tumor immune environment in regulating MGMT is a complex and underexplored area, with much remaining unknown about the mechanisms by which MGMT is regulated within this context. However, several studies have highlighted the significance of MGMT in tumor development and therapy, as well as the impact of the tumor immune environment on therapeutic outcomes. This provides a foundational understanding for elucidating the potential role of the tumor immune environment in MGMT regulation.

Tumor-associated macrophages (TAMs), as one of the predominant immune cell types within the TME, regulate the expression of MGMT through the release of pro-inflammatory cytokines, such as TNF-α and IL-1β. Recent studies have demonstrated that TAMs can significantly enhance MGMT expression, thereby improving the DNA repair capacity of tumor cells and facilitating their evasion of chemotherapy-induced cytotoxicity.[68] This mechanism is likely associated with the diverse growth factors secreted by TAMs, which can upregulate MGMT transcription by activating specific signaling pathways, notably the NF-κB pathway.[69]

Alternatively, certain immunomodulatory factors may enhance MGMT expression. Interferon-gamma (IFN-γ) is a pivotal cytokine in antitumor immunity; it inhibits the proliferation of transformed cells and augments the antitumor effects of other interferons.[70] Although direct evidence linking interferon-γ to the modulation of MGMT expression or activity is lacking, the potential interplay between the two in cellular function and disease states warrants further exploration. Interferon-γ may indirectly influence MGMT expression or activity by regulating the function of immune cells. For instance, interferon-γ activates macrophages and other immune cells, which in turn may impact MGMT expression levels through the secretion of cytokines or other signaling molecules. Additionally, epigenetic modifications play a critical role in regulating gene expression and cellular functions, potentially serving as a mechanism for interaction between IFN-γ and MGMT in glioma cells. Immune checkpoint molecules, such as PD-1 and CTLA-4, also play an essential role in tumor immune escape. Glioma cells express these checkpoint molecules to inhibit T cell activity, which may indirectly impact MGMT expression and function. Notably, some studies have demonstrated a significant association between PD-1 promoter methylation and MGMT methylation, highlighting a potential link between

immune regulation and epigenetic modifications. However, research in this area remains nascent, and further experimental validation is needed to elucidate these mechanisms in glioma cells.[71]

Relationship between MGMT and tumor therapy

As a pivotal enzyme in the DNA repair system, the expression level of MGMT in tumor cells significantly influences the efficacy of chemotherapeutic agents, particularly regarding resistance to alkylating agents such as TMZ. MGMT is directly involved in repairing DNA damage induced by alkylating agents through the removal of the alkyl group at the O6 position of guanine, thereby protecting cells from the cytotoxic effects of these chemotherapeutic agents.

MGMT expression and alkylating agent efficacy

Alkylating agents are a class of highly reactive compounds, and their damage to DNA represents the primary mechanism of their antitumor effects. When these agents enter the cell, they initiate an alkylation reaction with the bases or phosphate groups in the DNA molecule, introducing alkyl groups primarily at the N7 and O6 positions of guanine to form DNA adducts. These adducts can mispair with thymine during DNA replication, leading to the activation of the mismatch repair (MMR) system. The MMR system is a DNA repair pathway specialized to correct replication errors that escape DNA polymerase proofreading activity. The repair process involves two stages:(a) Recognition phase: MMR proteins identify base-base mismatches and insertion-deletion loops (IDLs) in newly synthesized DNA.(b) Repair phase: MMR executes a repair program that excises a segment of the nascent DNA strand containing the erroneous base(s) and fills the resulting strand gap with the correct nucleotide sequence using the parental strand as a template.[72] This process requires the intervention of exonucleases (notably Exo1). MMR proteins recognize and attempt to resolve O6-meG:T and O6-meG:C mismatches; however, because the modified base resides in the template strand while MMR targets the newly synthesized strand, this repair event leads to degradation of the pyrimidine-containing strand and subsequent reinsertion of C or T opposite O6-meG. Repeated futile cycles of repair generate single-strand gaps in the nascent DNA, which convert to double-strand breaks (DSBs) during subsequent S-phase progression. DNA damage arising from failed processing of O6-meG:T and O6-meG:C mismatches activates a signaling cascade that induces G2 cell cycle arrest at the second cell doubling, followed by apoptosis, mitotic catastrophe, or senescence-like states, ultimately triggering cancer cell death.[54,73]MGMT plays a crucial role in safeguarding genomic integrity by removing alkyl adducts from the O6 position of guanine in DNA following exposure to cytotoxic alkylating agents. It achieves this by transferring the alkyl group to its own cysteine residue, effectively neutralizing the lesion. By repairing O6-alkylguanine DNA adducts, MGMT serves as a protective mechanism against the mutagenic and carcinogenic consequences of alkylation-induced DNA damage. However, when MGMT is overexpressed, it effectively repairs O6-MeG lesions before they can be recognized by the MMR system.

This repair process attenuates drug-induced DNA damage and prevents the onset of apoptosis, thereby enabling tumor cells to survive and proliferate following chemotherapy.[12]

Common alkylating agents, such as TMZ and carmustine, induce tumor cell death primarily through DNA damage. However, MGMT can reverse this damage and prevent apoptosis, making its high expression strongly associated with chemoresistance, while tumor cells exhibiting low or absent MGMT are generally more sensitive to these agents.[16,74,75] For instance, the analysis of phase III study on validating the therapeutic efficacy of TMZ for GBM showed that patients carrying methylated MGMT promoters benefited from treatment with TMZ + RT (median 21.7 months vs. 15.3 months), whereas patients with unmethylated MGMT promoters had minimal and statistically non-significant benefit (median 12.7 months vs. 11.8 months).[12] In clinical practice, this phenomenon necessitates the stratification of patients based on their MGMT status to predict therapeutic response accurately. Hypermethylation of the MGMT promoter inhibits the expression of the MGMT gene, resulting in improved responses to TMZ among tumor-bearing patients, as the absence of MGMT enables the alkylating agent to exert its full cytotoxic potential. Although MGMT is predominantly discussed in the context of GBM, its role in chemotherapy efficacy also extends to various other cancer types. For instance, in a phase II clinical trial evaluating the efficacy of dacarbazine in metastatic colorectal patients observed a significantly higher disease control rate (44.0% vs. 6%, $P = 0.012$) in the MGMT-hypermethylated group, and a trend toward better progression-free survival(PFS) [HR = 0.66; 95% confidence interval (CI) 0.40–1.10; $P = 0.0982$)] was also found in the MGMT-hypermethylated cases.[76] In another phase II study, TMZ in combination with irinotecan achieved a 24% objective remission rate with a favorable safety profile in patients with irinotecan-sensitive metastatic colorectal cancer with MGMT methylation.[77] Based on the above, it can be seen that in colorectal cancer, MGMT and methylation status are closely related to alkylating agent therapeutic effects, which is similar to its function in brain tumors. Furthermore, in additional tumor types, including non-small cell lung cancer (NSCLC) and lymphoma, elevated expression of MGMT has been correlated with a poor response to chemotherapy.[78–80] Consequently, the evaluation of MGMT expression levels is becoming increasingly important for individualized tumor therapy.

MGMT expression and therapeutic strategies

Given the critical role of MGMT in tumor treatment, its expression is frequently utilized to inform therapeutic strategies. In gliomas, patients whose tumors demonstrate low MGMT expression or promoter hypermethylation are more likely to benefit from TMZ-based chemotherapy, whereas those with high MGMT expression are less likely to respond favorably. Consequently, testing for MGMT promoter methylation has become a standard component of clinical practice in certain cancers, offering guidance in determining whether to proceed with TMZ or to consider alternative treatment options.

In instances of elevated MGMT expression, strategies to overcome drug resistance become essential. One approach involves the use of MGMT inhibitors, such as O6-benzylguanine (O6-BG), to sensitize tumor cells to alkylating agents.[81] These

inhibitors function by depleting MGMT levels, allowing DNA damage to accumulate and enhancing the efficacy of alkylating agents.[58,81] However, only two inhibitors, O6-BG and O6-4bromophenylguanine (O6-4-BTG), are currently undergoing clinical trials.[82–84] In clinical trials, these agents have not yet led to improvements in therapeutic outcomes (in terms of overall survival) for glioma and melanoma treatment.[85–87] A primary reason for this is that while these drugs themselves are non-toxic, they also inactivate MGMT in normal tissues, thereby exacerbating the toxic side effects of alkylating agents and necessitating dose reductions. Thus, there is a critical need to develop strategies for selectively targeting MGMT inactivators. One straightforward yet technically complex approach involves local administration of inhibitors. This was demonstrated in a pilot trial for GBM patients, where an Ommaya reservoir was implanted into the tumor cavity to directly inject O6-BG into the brain prior to systemic TMZ. No systemic or neuronal toxicity was observed with intracranial O6-BG administration, indicating feasibility and tolerability.[88] Additionally, O6-BG or O6-4-BTG-induced MGMT depletion in normal cells sensitizes host tissues to chemotherapy, leading to severe myelosuppression. To mitigate this toxicity, leveraging high MGMT expression in hematopoietic stem cells via autologous bone marrow transplantation represents a viable strategy, which has gained significant attention in preclinical and in vitro studies,[89,90] may be a new direction for future research.

New insights and treatments

As research on MGMT and its association with chemotherapy resistance intensifies, new therapeutic approaches and strategies are emerging. Numerous studies are currently focusing on alternating administration of TMZ using various regimens (7 days on/7 days off (7/14 d), 21 days on/7 days off (21/28 d), and continuous or metronomic administration (28/28 d)). Relevant clinical trials have demonstrated that these alternating administration regimens for the treatment of relapsed or progressive GBM are superior to standard regimens in terms of PFS and overall survival (OS), without a significant increase in adverse events.[91] This improvement is likely due to the fact that dose-intensive or rhythmic TMZ treatment promotes the accumulation of MGMT methylation and enhances sensitivity to TMZ.[92] Furthermore, it has been shown that when glioma stem cells (GSCs) are treated with SP600125, a specific inhibitor of c-Jun NH2-terminal kinases (JNKs), MGMT expression in tumor cells is suppressed, demonstrating a synergistic effect in combination with TMZ.[93] However, JNK inhibition was ineffective in GSCs lacking MGMT expression.[94]

The JNK pathway may serve as a therapeutic target to overcome tumor-associated chemotherapy resistance and represents a novel approach to treating MGMT-expressing gliomas. Additionally, gene therapy techniques aimed at inactivating MGMT have emerged as innovative strategies to combat TMZ resistance in tumor therapy, exemplified by the synergistic interaction between human interferon-β (IFN-β) and TMZ, which downregulates MGMT expression at relevant concentrations.[95] p53 is a transcription factor that activates a variety of genes in response to cellular injury, and Harris et al. demonstrated that adenoviral vectors carrying p53 downregulated the expression of

the DNA repair gene MGMT in human fibroblasts.[39] Furthermore, gene editing technologies, such as CRISPR-Cas9, are anticipated to provide new research tools for the regulation of MGMT, enhancing our understanding of its specific role in different tumor types.[96]

Clinical significance and future perspectives

Prospects for clinical applications of MGMT expression regulation

Regulation of MGMT expression has demonstrated significant promise in clinical applications, particularly in predicting resistance to tumor therapy. MGMT promoter methylation has emerged as a valuable predictive biomarker for the efficacy of TMZ treatment in patients with GBM and guiding the development of individualized treatment regimens. In patients with promoter methylation, MGMT expression levels are reduced, and DNA repair capabilities are diminished, thereby increasing susceptibility to alkylating agents and resulting in enhanced therapeutic response and prolonged survival.[12,16] Furthermore, genomic rearrangements of MGMT may lead to its overexpression independent of promoter methylation status, thereby contributing to drug resistance.[97] This highlights the necessity for further research to develop more effective therapeutic strategies. In summary, in-depth exploration of MGMT expression regulatory mechanisms not only aids in optimizing chemotherapy regimens and enhancing treatment efficacy but also helps alleviate financial burdens and physical side effects for patients. In a 2024 study, researchers from Fudan University constructed tissue microarrays using 70 pairs of pancreatic neuroendocrine tumor (PanNET) tissues and adjacent normal tissues. Their findings revealed that MGMT is overexpressed in PanNETs and correlates with shortened PFS. Mechanistically, high MGMT expression promotes tumor cell growth and reduces sensitivity to TMZ. MEN1 plays a critical role in regulating TMZ chemosensitivity via the β-Catenin-MGMT axis.[98] These results highlight that MGMT expression levels serve as a key biomarker for personalized treatment strategies in cancers beyond glioma, including PanNETs. However, the variability in tumor types, tissue heterogeneity, and treatment responses suggests that the application of MGMT methylation as a universal biomarker in these tumors requires further validation.[74]

Applications in personalized medicine

With the increasing emphasis on precision medicine and individualized treatment, the application of MGMT expression regulation in glioma management has garnered significant attention. By assessing a glioma patient's MGMT expression level or promoter methylation status and integrating this information with other molecular markers—such as tumor grade, pathological subtype, and genetic mutations—clinicians can design more tailored chemotherapy regimens, thereby enhancing therapeutic efficacy and improving patient survival outcomes. Furthermore, the regulation of MGMT expression can be strategically combined with other therapeutic modalities, such as radiotherapy and immunotherapy, to create comprehensive treatment plans that optimize therapeutic results. For instance, in glioma patients with high MGMT expression, the use of MGMT inhibitors or other targeted

agents is expected to counteract therapy resistance and enhance the efficacy of alkylating agents like TMZ. Additionally, the evaluation of MGMT methylation and expression status may help predict patient responses to emerging therapies, including immune checkpoint inhibitors, offering valuable insights for treatment stratification. As research advances into the mechanisms regulating MGMT expression specifically in gliomas, this knowledge is likely to become an integral component of individualized treatment strategies, improving outcomes for patients with these challenging tumors.[99]

Moreover, the application of high-throughput genomics technologies, such as next-generation sequencing (NGS), enables a comprehensive dissection of the regulatory networks within tumors. This approach not only determines the expression status of MGMT but also elucidates other molecular drivers of chemotherapy resistance. Future individualized therapies may target upstream regulators of MGMT or synergize with additional pathways that enhance tumor repair mechanisms.

The next frontier in individualized therapy lies in integrating machine learning algorithms and predictive modeling to establish decision-making frameworks that consider MGMT status alongside a multitude of other genetic, epigenetic, and proteomic factors. This approach will enhance patient stratification and yield more accurate, data-driven treatment recommendations.

Future research directions and challenges

Despite significant progress in elucidating the regulatory mechanisms of MGMT expression, several challenges and opportunities remain:

Understanding complex regulatory networks: While promoter methylation is the most well-studied mechanism regulating MGMT expression, other regulatory layers—including transcription factors (e.g. p53 and NF-κB), microRNAs, and post-translational modifications (e.g. ubiquitylation)—remain underexplored. A more comprehensive understanding of these networks will uncover new targets for therapeutic intervention.

Development of novel therapeutic strategies: Current approaches, such as gene therapy, drug therapy, and epigenetic modifications, still exhibit numerous shortcomings and limitations; therefore, there is a pressing need to develop more efficient, less toxic, and stable regulatory approaches. Novel therapeutic strategies based on the regulatory mechanisms of MGMT hold significant potential. For instance, advances in drug design and delivery technologies could enhance the effectiveness of these inhibitors through the development of tumor-targeted delivery systems or the use of prodrugs that are activated exclusively in the TME.

Overcoming tumor heterogeneity: A major challenge in targeting MGMT is the heterogeneity of MGMT expression both within and between tumors. Even within a single tumor, regions exhibiting high and low MGMT expression may coexist, leading to partial resistance. Future studies should focus on identifying markers of intratumor heterogeneity and developing therapies that effectively target these mixed populations.

Clinical translation: Translating research findings into clinical applications is the ultimate goal. In the future, more clinical trials are necessary to validate the efficacy and safety of novel therapeutic strategies and to facilitate their widespread implementation in clinical practice.

Discussion

Compared with other review articles, our innovative points are as follows: Holistic and comprehensive analysis: 1. Most of the existing review articles usually focus on one or several aspects of MGMT regulation in glioma. In contrast, our review comprehensively integrates all the major regulatory levels-epigenetic, transcriptional, post-translational, and the influence of the tumor microenvironment. By taking these aspects into consideration comprehensively, we have obtained a more comprehensive understanding of how the expression of MGMT is regulated in glioma cells. 2. Emphasis on the tumor microenvironment: There has been relatively little discussion on the regulatory role of the tumor microenvironment in the expression of MGMT in gliomas. Our review focuses on how factors such as oxidative stress, hypoxia, and immune responses within the tumor microenvironment affect the expression of MGMT. 3. We link molecular regulation with therapeutic strategies by placing significant emphasis on clinical applications.

Despite significant progress, the following key challenges remain for MGMT regulation in gliomas:

(1)Epigenetic regulation

Although MGMT promoter methylation is a well-established biomarker, there is a lack of standardized protocols for methylation detection methods. Current clinical practices primarily utilize methylation-specific PCR (MSP) and pyrosequencing. However, MSP only interrogates localized CpG sites and cannot reflect the full promoter methylation status, while pyrosequencing, though more precise, incurs higher costs.[100] Heterogeneity in threshold settings across different platforms further compromises result comparability, necessitating the urgent development of standardized workflows.

Additionally, epigenetic drugs demonstrate promising efficacy in overcoming temozolomide chemoresistance and counteracting glioblastoma recurrence,[101] representing a potential novel therapy for eradicating these lethal tumors. However, GSCs may evade drug effects through dynamic epigenetic reprogramming, and the underlying complex signaling pathways remain poorly understood.

(2)Dynamic post-translational modifications

PTMs serve as critical regulators of MGMT protein stability and function. However, how spatiotemporal dynamics of PTMs—such as modification fluctuations following radiotherapy or temozolomide treatment—impact MGMT activity and drug resistance remains to be elucidated through single-cell or time-course analyses. Implementation of these approaches is hindered by high costs and standardization challenges for clinical translation. Additionally, MGMT protein may be regulated by multiple modifying enzymes (e.g. kinases, deacetylases), yet the specific enzymes and their functional pathways have not been systematically characterized.

(3)Impact of the tumor microenvironment

TME establishes a complex regulatory network for MGMT expression. Hypoxia exerts bidirectional effects on MGMT—either upregulating or downregulating its expression—complicating the interpretation of treatment responses.[102] Furthermore, the spatiotemporal heterogeneity of the TME renders single-targeted strategies (e.g. anti-angiogenic drugs) insufficient for comprehensively suppressing MGMT expression, necessitating the development of multi-targeted combination therapies. Potential co-regulatory relationships exist between immune checkpoint molecules like PD-L1 and MGMT expression,[103] and dissecting these interactions is critical for optimizing the combination of immune checkpoint inhibitors with alkylating agents.

To address the above challenges, the following research directions are proposed for prioritized attention:

Multi-omics Integrative Analysis: Integrate whole-genome methylation, ATAC-seq (assay for transposase-accessible chromatin with sequencing), and transcriptomic data to systematically dissect the epigenetic regulatory network of MGMT and identify critical cis/trans-acting elements.

Single-Cell Technology Applications: Utilize single-cell mass spectrometry or spatial transcriptomics to resolve cell-specific differences in PTMs within tumor heterogeneity, thereby guiding personalized treatment strategies.

Multi-targeted combination therapy strategies: Addressing TME heterogeneity, design combinatorial regimens incorporating anti-angiogenic drugs (e.g. bevacizumab) and MGMT inhibitors (e.g. O6-BG) to simultaneously suppress angiogenesis and DNA repair pathways. Additionally, explore synergistic mechanisms between immune checkpoint inhibitors (e.g. anti-PD-L1) and alkylating agents.

Targeting post-translational modifications: Develop small-molecule inhibitors or stabilizers targeting enzymes involved in MGMT phosphorylation or ubiquitination.

Microenvironment-directed therapies: Combine immune checkpoint inhibitors or hypoxia-targeted agents with TMZ to overcome MGMT-related chemoresistance.

Additionally, breakthrough applications of artificial intelligence (AI) in medical research are profoundly transforming precision medicine practices. Deep learning-based predictive models can efficiently analyze multi-omics data to accurately predict MGMT promoter methylation status and OS in GBM patients.[104] A multicenter AI study also confirmed that deep learning models using MRI images can reliably and accurately determine the survival time and status of GBM patients after radiotherapy.[105] These technological innovations not only accelerate the translational process from mechanistic research to clinical application but also provide intelligent solutions for glioma treatment through real-time dynamic monitoring of tumor heterogeneity.

The in-depth understanding of MGMT regulation holds significant clinical value: MGMT promoter methylation has emerged as a key biomarker guiding TMZ therapy. Further elucidation of epigenetic and post-translational regulatory mechanisms will provide a basis for novel therapeutic targets and combinatorial strategies. Additionally, leveraging liquid biopsy and multi-omics technologies to monitor MGMT expression and modification status enables real-time assessment of treatment response and resistance mechanisms.

Conclusion

Understanding the regulatory mechanisms of MGMT in glioma cells serves as the cornerstone for advancing precision oncology. Continuous exploration of the intricate regulatory network of MGMT will drive the development of innovative therapeutic approaches. By addressing unresolved challenges and harnessing emerging technologies, researchers can enhance treatment precision and overcome MGMT-mediated drug resistance, ultimately improving clinical outcomes and patient prognoses in glioma management.

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