REVIEW ARTICLE

Post-translational modifications in DNA damage repair: mechanisms underlying temozolomide resistance in glioblastoma

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Temozolomide (TMZ) resistance is one of the critical factors contributing to the poor prognosis of glioblastoma (GBM). As a first-line chemotherapeutic agent for GBM, TMZ exerts its cytotoxic effects through DNA alkylation. However, its therapeutic efficacy is significantly compromised by enhanced DNA damage repair (DDR) mechanisms in GBM cells. Although several DDR-targeting drugs have been developed, their clinical outcomes remain suboptimal. Post-translational modifications (PTMs) in GBM cells play a pivotal role in maintaining the genomic stability of DDR mechanisms, including methylguanine-DNA methyltransferase-mediated repair, DNA mismatch repair dysfunction, base excision repair, and double-strand break repair. This review focuses on elucidating the regulatory roles of PTMs in the intrinsic mechanisms underlying TMZ resistance in GBM. Furthermore, we explore the feasibility of enhancing TMZ-induced cytotoxicity by targeting PTM-related enzymatic to disrupt key steps in PTM-mediated DDR pathways. By integrating current preclinical insights and clinical challenges, this work highlights the potential of modulating PTM-driven networks as a novel therapeutic strategy to overcome TMZ resistance and improve treatment outcomes for GBM patients.

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INTRODUCTION

Overview of Glioblastoma

Glioblastoma (GBM) is characterized by high malignancy and poor prognosis. The aggressive invasiveness of GBM cells complicates the accurate identification of tumor-infiltrated regions during surgery, leading to residual tumor cells that contribute to disease progression and recurrence [1]. Consequently, adjuvant therapy with radiation and chemotherapy is essential for effective GBM treatment. A Phase III clinical trial conducted in 2005 demonstrated that temozolomide (TMZ) in combination with radiation therapy (the Stupp regimen) significantly improves patient outcomes compared to radiation alone, with a median survival of 14.6 months versus 12.1 months, respectively [2]. Due to its favorable therapeutic effects, TMZ has become the standard first-line treatment for GBM.

Mechanisms and Advances in Temozolomide Resistance

TMZ is a small, lipophilic alkylating agent and an imidazotetrazine derivative of dacarbazine [3]. Key attributes supporting its use as a first-line treatment include stability in acidic conditions, rapid absorption after oral administration, peak plasma concentrations within one hour, and effective blood-brain barrier penetration. TMZ's primary anticancer mechanism involves methylation of purine bases, resulting in N7-guanine (70%), O6-guanine (6%), and N3-adenine (9%) adducts, leading to DNA mismatch and repair failure, which causes G2/M phase arrest and programmed cell death in GBM cells [4].

Although TMZ has advanced GBM treatment, its palliative nature and the intrinsic chemoresistance of tumors limit efficacy, with 90% of recurrent GBM exhibiting resistance [4, 5]. The mechanisms underlying GBM resistance to TMZ are multifaceted, encompassing enhanced DNA damage repair (DDR) pathways, an immunosuppressive tumor microenvironment, drug efflux transporter activities [6]. While the complexity of TMZ resistance involves cross-talk between multiple systems, the augmented DDR machinery represents a predominant molecular determinant and the fundamental driver of GBM chemoresistance.

To counteract TMZ-induced cytotoxicity, GBM cells activate DNA repair mechanisms, including methylguanine-DNA methyltransferase (MGMT), DNA mismatch repair (MMR) dysfunction, base

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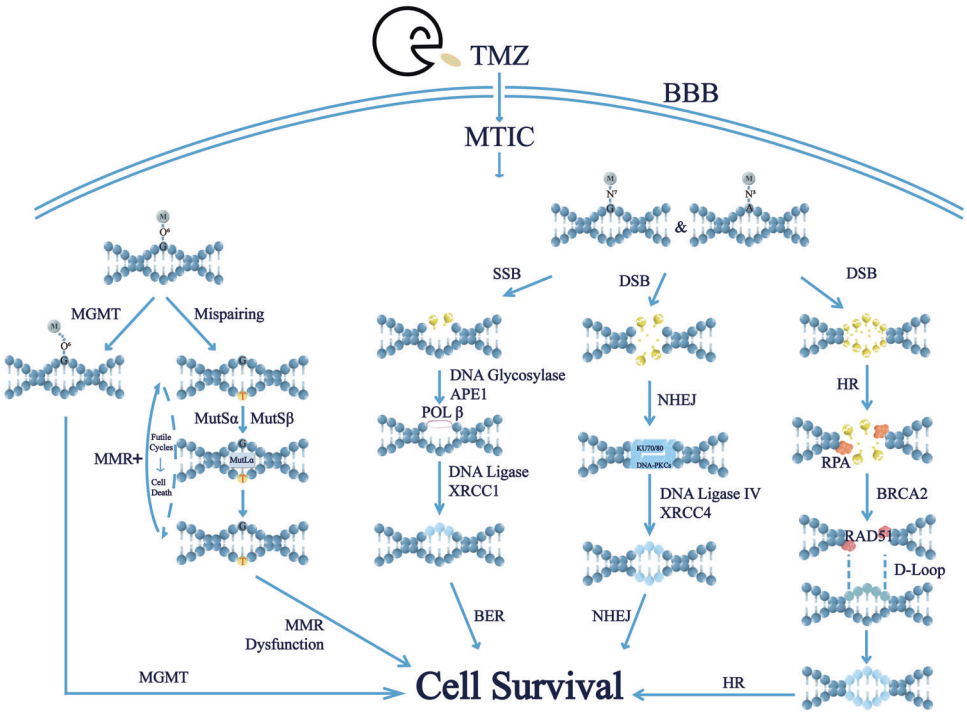

Fig. 1 DNA repair mechanisms in TMZ-resistant glioblastoma. MTIC: MTIC (5-(3-methyltriazen-1-yl) imidazole-4-carboxamide), the key bioactive metabolite of TMZ generated after traversing the BBB (blood-brain barrier). **MGMT:** MGMT, a 22 kDa protein found in both the cytoplasm and nucleus, transfers a methyl group from the O6 position of guanine to a cysteine residue in its active site, rendering itself inactive while protecting DNA [195]. **MMR Dysfunction:** The MMR system, involving MutSα (MSH2-MSH6) and MutSβ (MSH2-MSH3) complexes, detects mispairing, initiating futile repair and resulting in cell death. However, MMR deficiency permits the mispairing to persist, promoting tumor cell survival [3, 7]. **BER:** DNA glycosylases and APE1 recognize and excise the damage site, initiating BER. Pol β mediates the correct nucleobase insertion into the lesion, and DNA ligase, in conjunction with XRCC1, completes the final assembly [3]. **NHEJ:** Ku70/80 recognizes the DSB, promoting the binding of DNA-PKcs to the DNA ends and recruiting nucleotides to the site. The DNA ligase IV complex ultimately completes the repair by sealing the DNA ends [196]. **HR:** RPA binds to the DSB ends, preparing the DNA for repair. BRCA2 facilitates RAD51 recruitment, which forms a D-loop on the homologous sister chromatid. RAD51 mediates strand invasion and searches for a homologous sequence, followed by DNA synthesis to repair the break [197].

excision repair (BER), and double-strand break (DSB) repair via non-homologous end joining (NHEJ) and homologous recombination (HR) (Fig. 1) [7, 8].

Research on TMZ resistance in GBM primarily focuses on inhibiting MGMT expression or function. As early as 1996, studies showed that o6-benzylguanine, an MGMT inhibitor, could enhance TMZ sensitivity in GBM cells with high MGMT levels [9]. PARP inhibitors like olaparib inhibit MGMT PARylation, impair O6-methylguanine repair, and enhance TMZ sensitivity in MGMT+ GSCs [10]. Cordycepin reduces MGMT expression by down-regulating the Wnt/β-catenin pathway, thereby enhancing TMZ sensitivity [11]. The HDAC8 inhibitor NBM-BMX inhibits the β-catenin/c-Myc/SOX2 signaling pathway and upregulates WT-p53, suppressing MGMT-mediated DNA repair and increasing TMZ toxicity [12]. Parthenolide reduces MGMT expression through NF-κB pathway inhibition, decreasing TMZ resistance both in vitro and in vivo [13]. EPIC-0412, a small molecule inhibitor, targets the p21-E2F1 and ATF3-p-p65-MGMT axes, thereby improving TMZ efficacy [14]. Despite these interventions, there is limited evidence supporting the reversal of TMZ resistance or improvement in patient outcomes [15].

Methoxyamine and PARP inhibitors (e.g., olaparib, veliparib) effectively inhibit the activation of the BER system in GBM cells, thereby reducing TMZ resistance both in vitro and in vivo [5, 16]. Additionally, a positive correlation has been identified between BRD4 expression levels and key genes in the MMR pathway. BRD4 inhibitors can suppress the function of critical proteins in the MMR system [17]. However, studies aimed at enhancing GBM sensitivity to TMZ by targeting MMR and BER repair mechanisms remain limited. Further research is needed to better understand these pathways and develop new drugs and therapeutic strategies.

Post-translational modifications

Beyond direct targeting of DDR core components, emerging evidence implicates post-translational modifications (PTMs) — particularly phosphorylation, ubiquitination, SUMOylation, acetylation, and glycosylation — as critical regulatory layers that dynamically orchestrate DNA repair fidelity and therapeutic vulnerability, offering novel therapeutic strategies for targeting GBM progression and reversing chemoresistance [18]. Since PTMs are reversible and regulated by specific enzymes, they are dynamic and responsive to cellular changes [19]. Targeting key enzymes involved in PTMs—such as protein kinases, histone deacetylases, and proteasome inhibitors—holds promise for the development of new chemotherapeutic agents.

Numerous chemotherapy drugs targeting key enzymes involved in PTMs are available for the treatment of GBM. Imatinib, an Abelson tyrosine kinase inhibitor, was the first protein kinase inhibitor used clinically [20]. Since then, hundreds of protein kinase inhibitors have entered clinical trials, with 76 approved for clinical use [20]. Histone deacetylase inhibitors, including Vorinostat, Romidepsin, and benzamides, inhibit GBM cell proliferation in vitro, enhance the cytotoxic effects of radiotherapy and chemotherapy, and have shown promising results in phase II trials, particularly in GBM patients treated with Vorinostat [21–23]. Bortezomib, the first proteasome inhibitor approved for clinical use, effectively inhibits ubiquitin-mediated protein degradation [24]. Current research demonstrates its therapeutic efficacy in

Table 1. Chemotherapeutic agents targeting PTM enzymes in clinical trials for GBM treatment.

Drug names	Molecular targets	Clinical trial titles	Phase
Lapatinib	Tyrosine kinase inhibitor	Lapatinib Ditosylate Before Surgery in Treating Patients with Recurrent High-Grade Glioma (NCT02101905)	I
Sorafenib	multiple kinases inhibitor	Sorafenib, Valproic Acid, and Sildenafil in Treating Patients with Recurrent High-Grade Glioma (NCT01817751; Ref. [26])	II
Dasatinib; Afatinib	Tyrosine kinase inhibitor	Pilot Trial for Treatment of Recurrent Glioblastoma (NCT05432518)	I
Palbociclib	CDK4/6 kinase inhibitor	Pilot Trial for Treatment of Recurrent Glioblastoma (NCT05432518)	I
Ribociclib	CDK4/6 kinase inhibitor	Ribociclib (LEE011) in Preoperative Glioma and Meningioma Patients (NCT02933736)	I
Ribociclib	CDK4/6 kinase inhibitor	Study of Ribociclib and Everolimus in HGG and DIPG (NCT05843253)	II
Ibrutinib	Bruton's tyrosine inhibitor	Ibrutinib With Radiation and Temozolomide in Patients with Newly Diagnosed Glioblastoma (NCT03535350)	I
Ibrutinib	Bruton's tyrosine inhibitor	Chemo-immunotherapy Using Ibrutinib Plus Indoximod for Patients with Pediatric Brain Cancer (NCT05106296)	I
Bortezomib	Proteasome inhibitor	Bortezomib and Temozolomide in Recurrent Grade-4 Glioma Unmethylated MGMT Promoter (NCT03643549)	II
Vorinostat	Histone deacetylase inhibitors	Vorinostat, Isotretinoin and Temozolomide in Adults with Recurrent Glioblastoma Multiforme (NCT00555399)	II
Vorinostat	Histone deacetylase inhibitors	Vorinostat and Temozolomide in Treating Patients with Malignant Gliomas (NCT00268385)	I
Belinostat	Histone deacetylase inhibitors	MRSI to Predict Response to RT/ TMZ ± Belinostat in GBM (NCT02137759)	II
Panobinostat	Histone deacetylase inhibitors	A Study of Intra-tumoral Administered MTX110 in Patients with Recurrent Glioblastoma (NCT05324501)	I

glioblastoma-bearing mice, enhancing TMZ-induced cytotoxicity against GBM cells [25]. Thus, targeting specific key enzymes involved in PTMs to design corresponding chemotherapeutic agents holds significant clinical potential for improving the efficacy of GBM treatment. Currently, several related drugs are undergoing clinical trials aimed at exploiting this approach (Table 1) [26].

Given the critical role of PTMs in cellular regulation and their link to tumor development, we will explore their relationship with TMZ resistance in GBM, focusing on key PTM types. This will provide insights into the molecular mechanisms of TMZ resistance in GBM and inform future therapeutic strategies.

RELATIONSHIP BETWEEN PHOSPHORYLATION AND TMZ RESISTANCE MECHANISM

Phosphorylation primarily occurs on serine, threonine, and tyrosine residues of target proteins [27]. Protein kinases and phosphatases are key enzymes in this process, responsible for phosphorylating and dephosphorylating proteins to regulate substrate phosphorylation levels [28]. Disruptions in the phosphorylation-dephosphorylation balance of tumor-associated genes are a major driver of tumorigenesis.

Phosphorylation in the MMR system

PCNA is a homotrimeric sliding clamp composed of three identical monomers (PCNA1, PCNA2, and PCNA3) that form a ring structure [29]. Several critical proteins, including MSH3, MSH6, MLH1, and EXO1, interact with PCNA in MMR [30]. The EGFR can phosphorylate the tyrosine residue Y211 of PCNA, inhibiting the activation of the MMR system [31]. MSH2 and MSH6 are phosphorylated by PKC and CK2, with MSH6 being more highly phosphorylated than MSH2. This phosphorylation may facilitate the transport of MSH2 and MSH6, promoting their accumulation in the nucleus and enabling MMR [32]. ATM/ATR, assisted by BRCA1, phosphorylates MLH1 at the S406 site, stabilizing MLH1 and promoting MMR [33]. In contrast, phosphorylation of MLH1 at S477 by CK2 inhibits MMR activation, likely by preventing MLH1 from binding to other key MMR proteins [34].

Phosphorylation in the BER system

Upon DNA damage induced by alkylating agents like TMZ, the ATM/Chk2 signaling pathway is activated. Chk2 then forms a complex with the BER scaffold protein XRCC1, promoting phosphorylation of T284 on XRCC1, which facilitates the recruitment of DNA glycosylases (MPG, UNG2) and downstream BER proteins (polβ, PARP1), thus enhancing BER [35, 36]. Mutations at the T284 site in XRCC1 lead to accumulation of BER intermediates, impairing DNA repair and increasing the cytotoxic effects of alkylating agents [37]. Further, XRCC1 can be phosphorylated by CK2, which stabilizes the XRCC1-Lig III complex and promotes XRCC1 nuclear accumulation, supporting BER [38]. Knockdown of histone demethylase KDM6B increases phosphorylation of Chk1 at S345 and S296, activating its downstream pathways and promoting XRCC1-mediated DNA repair. This suggests KDM6B could be a biomarker for TMZ-resistant GBM [39]. Phosphorylation at Y263 and S269 of DNA glycosylase NEIL1 is critical for its DNA binding and enzymatic activity; mutations at these sites impair BER function [40]. CDK5 and PKC mediate phosphorylation of NEIL2, with PKC phosphorylation inhibiting NEIL2's function in BER [41]. AID is phosphorylated at S38, promoting its binding to the key BER protein APE1 and regulating BER [42]. CDK2 phosphorylates T553 of Polλ, preventing its ubiquitination and degradation, thereby maintaining its stability [43]. APE1, a core endonuclease in the BER pathway, is phosphorylated at multiple sites by various kinases. Phosphorylation of T233 by CDK5 inhibits APE1's endonuclease activity, impairing BER and leading to DNA damage accumulation and neuronal death [44]. However, the role of APE1 phosphorylation remains debated. Some studies suggest that CK2-mediated phosphorylation inhibits APE1's activity, while others report no effect [45, 46]. Additionally, PKC-mediated phosphorylation of APE1 has been proposed to enhance its redox function, though this has not been observed in earlier studies [45, 47]. Further investigation is required to clarify the impact of these

phosphorylation modifications on APE1, which may provide insights into their role in the BER pathway in TMZ-resistant GBM cells.

Phosphorylation in the DSBs repair system

DNA-PKcs, the largest serine/threonine kinase in the PIKK family, is the most abundant PIKK in human cells and is crucial for NHEJ repair [48]. DNA-PKcs undergoes autophosphorylation, which inactivates its enzymatic activity and dissociates it from the DNA-binding factor Ku, a key step in NHEJ [49]. Autophosphorylation occurs at multiple residues in the M-HEAT domain, collectively known as the ABCDE clusters, including T2609, S2612, T2620, S2624, T2638, and T2647 [50, 51]. Mutations in these residues inhibit autophosphorylation and the dissociation of DNA-PKcs from Ku, impeding NHEJ [52]. In GBM cells, FGFR2-induced phosphorylation of Y240 on PTEN (pY240-PTEN) binds chromatin through Ki-67 and recruits RAD51, activating the HR repair system [53]. Isoflavones, as tyrosine kinase inhibitors, bind directly to DNA-PKcs and inhibit its phosphorylation, suppressing HR repair in GBM cells with high DNA-PKcs expression, thereby promoting DNA damage [54]. EXO1, a key exonuclease in HR, can be phosphorylated by CDK1/CDK2 at multiple sites (S639, T732, S815, and T824) in response to DNA damage, activating EXO1 [55]. After completing its function, ATR phosphorylates EXO1, leading to its degradation [56]. HPRT1 enhances TMZ metabolism in GBM cells, activates AMPK, and promotes phosphorylation of T52 of RRM1, activating RNR and increasing dNTPs production, facilitating HR repair of TMZ-induced DNA damage. This is a key mechanism of intrinsic and acquired resistance to TMZ in GBM [57]. Inhibiting HPRT1, using drugs like 6-mercaptopurine, blocks AMPK activation and enhances TMZ cytotoxicity in GBM cells [57]. In GBM cells, the small molecule protein AQB upregulates UBXN1 and inhibits NF-κB phosphorylation [58]. Synthetic AQB analogs, such as EPIC-1027, disrupt NF-κB phosphorylation, interrupting the EGFRvIII/MUC1-C feedback loop, inhibiting DSB repair activation, and increasing TMZ sensitivity [59, 60]. Additionally, as a DNA damage sensor, ATM promotes DNA repair by phosphorylating key proteins such as P53 (S46, S15), DNA-PKcs, Chk2, and BRCA1 in the early stages of DNA damage [61, 62].

Phosphorylation in other DNA damage repair mechanisms

The cellular localization of YAP/TAZ is primarily regulated by their phosphorylation status. For instance, phosphorylation of S127 in YAP (S89 in TAZ) causes their accumulation in the cytoplasm [63]. Additionally, phosphorylation of S381 in YAP (S311 in TAZ) affects their protein stability [64, 65]. Knockout of the upstream gene Syx increases the phosphorylation of YAP/TAZ co-transcriptional activators, leading to cytoplasmic retention and reduced activity, thereby enhancing TMZ efficacy in TMZ-resistant GBM cells [66]. Overexpression of GNA13 downregulates the PRKACA subunit of PKA, inhibiting phosphorylation of RELA and MGMT, which increases GBM cell sensitivity to TMZ [67]. In GBM LN18 cells, TMZ treatment elevates the expression of the serine/threonine pseudokinase TRIB1, which mediates phosphorylation of ERK and Akt, activating the MEK/ERK and Akt/PI3K pathways, promoting cell proliferation and TMZ resistance [68]. Furthermore, CDKL5, a serine/threonine kinase, mediates Akt phosphorylation in GBM cells, activating the Akt/PI3K pathway and promoting proliferation, migration, and TMZ resistance [69]. Finally, phosphorylation modifications contribute to TMZ resistance by promoting changes in the GBM microenvironment. GBM cells release exosomes containing lnc-TALC, which are transferred to microglial cells. In these cells, lnc-TALC binds to ENO1 and promotes P53 phosphorylation [70]. This triggers the secretion of complement C5/C5a, inducing M2 polarization in microglial cells, reshaping the GBM microenvironment, and reducing tumor sensitivity to TMZ chemotherapy [70, 71].

RELATIONSHIP BETWEEN UBIQUITINATION AND TMZ RESISTANCE MECHANISM

Ubiquitination is a key post-translational modification, involving the covalent attachment of one or more ubiquitin molecules to target proteins. This process is facilitated by E1 activating enzymes, E2 conjugating enzymes, and E3 ligases [72]. Ubiquitination can be classified into different types based on the lysine or methionine residues involved in the chain formation, including K6, K11, K27, K29, K33, K48, K63, and M1-linked ubiquitination [73]. K48 and K63 are the most studied types, with K48 typically indicating proteasomal degradation of the target protein through the UPS, and K63 being involved in regulating kinase activity, signal transduction, and endocytosis [74, 75]. Dysregulated ubiquitination can activate or deactivate key oncogenic pathways, such as those involving p27, p53, and NF-κB, contributing to cancer development [76].

Ubiquitination in the MGMT system

Early studies indicated that MGMT serves as a substrate for ubiquitination under the influence of inactivating agents such as O6-benzylguanine or carmustine, leading to proteolytic degradation that enhances the efficacy of alkylating agents in cancer treatment [77]. Notably, the ubiquitin-mediated proteolysis of MGMT is more pronounced in the U87 glioma cell line, suggesting that promoting MGMT ubiquitination and subsequent degradation could be a promising therapeutic strategy to improve TMZ sensitivity in GBM cells [78]. Further research identified UBE2B, an E2 ubiquitin-conjugating enzyme, which works with the E3 ligase RAD18 in BCNU-induced MGMT ubiquitination [79]. Additionally, studies have shown that the knockout of UBE2B leads to MGMT inactivation and accumulation within tumor cells, causing cellular toxicity [79]. In melanoma, MGMT undergoes ubiquitination and proteolysis mediated by the E3 ligase TRIM72, with TRIM72 overexpression enhancing the cytotoxic effects of alkylating agents [80]. However, further studies are needed to clarify the specific types of MGMT ubiquitination and their functional roles.

Ubiquitination in the MMR system

MSH2/MSH6 acts as a key heterodimer in regulating MMR, with studies showing that the knockout of MSH6 leads to a 50% reduction in MSH2 protein levels, suggesting a correlation between the two proteins. Further investigations revealed that when MSH6 levels decrease, MSH2 undergoes ubiquitination-mediated proteolysis via the NOT4 ligase, indicating that the stability of the MutSα MMR repair heterodimer is influenced by subunit interactions and ubiquitination [81]. The histone deacetylase HDAC6 plays a critical role in cellular responses to external stimuli; its DAC1 domain functions as an E3 ubiquitin ligase, mediating MSH2 ubiquitination and subsequent degradation [82]. Additionally, OTUB1 inhibits MSH2 ubiquitination by blocking the ubiquitin transfer activity of E2 enzymes, thus maintaining MSH2 stability [83]. In summary, ubiquitination regulates the stability of MSH2 within the MutSα MMR repair heterodimer, which impacts the MMR system. MSH2 ubiquitination represents a potential therapeutic target for enhancing TMZ resistance in GBM cells.

Ubiquitination in the BER system

DNA glycosylase OGG1 undergoes ubiquitination by the E3 ligase CHIP, which mediates its degradation and translocation from the nucleus to the nucleoplasm, inhibiting its function in the BER pathway [84]. CHIP also facilitates the ubiquitination of several key BER proteins, including XRCC1, Lig III, and Pol β, thereby controlling cellular levels of BER enzymes and ensuring proper BER function [85]. Similarly, the E3 ligase Mule mediates the ubiquitination of the DNA glycosylase MutYH, resulting in its degradation and mislocalization, which impairs the BER repair process [86]. APE1, a crucial AP endonuclease in BER, is

ubiquitinated by the E3 ligase UBR3 [87]. In UBR3-deficient MEFs, APE1 degradation is disrupted, leading to increased APE1 levels [87]. Additionally, MDM2 has been shown to ubiquitinate APE1, with its RING domain being essential for this modification [88]. Beyond mediating protein degradation, ubiquitination can also directly regulate protein function. 5-hydroxymethylcytosine activates the E3 ligase UHRF2, which catalyzes K33-linked ubiquitination of XRCC1. This modification does not lead to XRCC1 degradation but instead promotes its interaction with the ubiquitin-binding domain of RAD23B, facilitating the recruitment of TDG to the BER complex and ensuring the stable operation of the BER system [89].

Ubiquitination in the DSBs repair system

Ubiquitination plays a critical role in the repair of DSBs, particularly in the recruitment of DNA repair factors to damage sites. This process mainly involves K63-linked ubiquitination mediated by the RNF8/RNF168 pathway, which targets histones and chromatin-binding proteins [90]. Phosphorylated L3MBTL2 associates with MDC1 and binds to the DNA damage site, where RNF8 mediates its ubiquitination, recruiting RNF168 to the site [91]. RNF168 then ubiquitinates histone H2A at K13 and K15, promoting the assembly of ubiquitin-binding domains and repair factors such as 53BP1, RAP80, RNF169, and RAD18 at the damage site [91–93]. Notably, 53BP1 is recruited upon recognizing the K15 ubiquitination of H2A, facilitating NHEJ [94]. RAP80, ubiquitinated at K63 or K6, recruits BRCA1 to the site [95, 96]. RNF169, through its MIU2 motif, is also ubiquitinated by RNF8/RNF168 and competitively inhibits the recruitment of other repair factors like RNF168 and 53BP1 [97, 98]. RAD18, ubiquitinated by RNF168, attracts HR-related factors such as RAD51C and the SMC5/SMC6 complex, promoting HR [99]. In summary, many DSB repair proteins are recruited to damage sites via ubiquitin-dependent mechanisms. However, further research is needed to fully elucidate the mechanisms and dynamic regulation of this recruitment process.

Ubiquitination recruits DNA repair factors and selects the repair mechanism, either NHEJ or HR. During G1, CDH1-mediated ubiquitination of CtIP promotes its degradation, inhibiting HR [100]. RNF138 activates CtIP via UBE2Ds, facilitating HR [101]. RNF8 and RNF138 also ubiquitinate Ku80, removing it from damage sites to suppress NHEJ and promote HR [102, 103]. UHRF1 enhances K63-linked ubiquitination of RIF1, promoting HR by dissociating 53BP1 from damage sites [104]. Deubiquitination also regulates DSB repair. USP52 deubiquitinates CtIP, enhancing its ATM-mediated phosphorylation and promoting HR [105]. UCHL3, activated by ATM phosphorylation, deubiquitinates RAD51, enhancing RAD51 recruitment and its interaction with BRCA2 to facilitate HR [106]. UCHL3 is thus a key therapeutic target for enhancing chemotherapy sensitivity in tumor cells [106]. Furthermore, the polymerase Pol κ, which is upregulated in GBM cells following TMZ treatment, contributes to TMZ resistance in sensitive GBM cells [107]. Inactivating Pol kappa increases the ubiquitination and proteasomal degradation of Rad17 induced by TMZ, inhibiting ATR-CHK1 signaling and impairing HR, ultimately increasing GBM cells' sensitivity to TMZ [107].

In summary, ubiquitination not only mediates substrate degradation but also facilitates protein translocation and activation. It influences the abundance of key proteins in DSB repair, modulates the distribution of repair factors, and affects complex assembly. Therefore, ubiquitination plays a critical role in DSB repair and serves as a key target to overcome TMZ resistance in GBM cells.

Ubiquitination in other DNA damage repair mechanisms

The SCF-type E3 ligase FBW7, an important tumor suppressor, reduces the expression of Aurora B, Mcl1, and Notch1 upon overexpression, causing GBM cells to arrest in the G2/M phase and significantly enhancing TMZ efficacy [108]. Ube2C and Ube2S, two other E2 ubiquitin-conjugating enzymes, are overexpressed in GBM and associated with poor prognosis and reduced chemotherapy response [109]. The aberrant E3 ligase MAEA promotes K48-linked ubiquitination of PHD3 at K159, leading to PHD3 degradation and stabilizing HIF-1α, thereby enhancing GBM TMZ resistance [110].

THE RELATIONSHIP BETWEEN SUMOYLATION AND TMZ RESISTANCE MECHANISMS

SUMOylation, or small ubiquitin-like modifier conjugation, is a key post-translational modification process. In mammals, there are three SUMO paralogs: SUMO-1, SUMO-2, and SUMO-3 [111]. SUMO-1 primarily maintains normal cellular functions, while SUMO-2/3 are involved in stress responses to environmental changes [111]. Recent proteomic studies have identified over 1,000 human proteins with more than 3000 SUMOylation sites. These SUMOylated proteins are directly or indirectly involved in processes such as apoptosis, inflammation, immune regulation, DDR, angiogenesis, migration, DNA replication, cell division, and cell cycle regulation, all of which contribute to tumorigenesis [112].

SUMOylation in the BER system

UNG2 undergoes both SUMOylation and ubiquitination. Overexpression of SUMO-1 increases UNG2 SUMOylation while decreasing its ubiquitination, thereby stabilizing the protein [113]. TDG is SUMOylated at K330, which reduces its affinity for DNA substrates, promoting the release of aberrant bases and the formation of AP sites [114]. SUMOylation also enhances APE1's stimulatory effect on TDG, facilitating TDG dissociation from AP sites and promoting BER activity [115]. Additionally, SUMOylation at lysine 341 of TDG inhibits CBP-mediated acetylation, preventing its interaction with APE1 [116]. PARP-1, which is pivotal in BER, is SUMOylated at K203 and K486 by PIASy. This modification prevents PARP-1 acetylation and degradation via RNF4, modulating its role in BER [117]. FEN-1 is regulated by SUMOylation at lysine 168, promoting its ubiquitination by PRP19 and subsequent degradation [118]. Moreover, although XRCC1 undergoes SUMOylation, its functional implications remain unclear. Further research is needed to clarify the effects of SUMOylation on other key BER proteins.

SUMOylation in the DSBs repair system

Knockout of SUMO-1 and SUMO-2 in GBM cells impairs DNA synthesis and DSB repair, suggesting SUMOylation protects GBM cells from chemotherapy or radiotherapy induced damage [119]. SUMOylation of 53BP1 is crucial for its accumulation at DNA damage sites [120]. The absence of Nup153 leads to dissociation of the SUMO protease SENP1 from the NPC, inhibiting 53BP1 SUMOylation [120]. Artificially tethering SENP1 to the NPC in Nup153-deficient cells restores 53BP1 SUMOylation and facilitates NHEJ [120]. Phosphorylated ARF mediates SUMOylation of PTEN with SUMO-1, allowing SUMOylated PTEN to be recruited to DNA damage sites via the SUMO interaction motif of BRCA1 [121]. PTEN then dephosphorylates 53BP1, promoting its dissociation and activating HR repair [121]. Inhibition of PTEN SUMOylation enhances tumor cell sensitivity to chemotherapeutic agents [121]. SUMOylation of Sp1 at K16 leads to its ubiquitin-dependent degradation via RNF4, which removes Sp1 and 53BP1 from DNA damage sites, promoting HR [122]. A similar modification occurs in CtIP, where SUMOylation at K578, mediated by PIAS4, leads to its ubiquitin-dependent degradation via RNF4, promoting HR [123]. PIAS4 also mediates SUMOylation of TIP60 at K430 with SUMO-2, preventing its binding to DNA-PKcs [124]. Mutations at this site increase DNA-PKcs phosphorylation, inhibiting HR [124]. TIP60 thus represents a key target for enhancing GBM cell sensitivity to TMZ [124]. Additionally, SUMOylation of ZMYM2, mediated by PIAS4, is critical for its

enrichment at DNA damage sites, opposing 53BP1 accumulation and promoting HR-related protein recruitment [125].

Research shows that the three SUMO isoforms have distinct roles in DSB repair. SUMO-1 is involved in both HR and NHEJ, while SUMO-2 and -3 are mainly associated with NHEJ [126]. Additionally, the deSUMOylating enzyme SENP2 regulates MDC1 deSUMOylation, preventing its RNF4-VCP-mediated degradation and promoting NHEJ [127]. Despite significant progress, unresolved questions remain, particularly regarding the mechanisms governing protein accumulation at DNA damage sites and the balance between SUMOylation and deSUMOylation.

SUMOylation in other DNA damage repair mechanisms

Studies have shown that MGMT can generate two SUMOylated products with SUMO-1 in vitro, facilitated by the SUMO ligase Ubc-9, which may influence MGMT protein stability [78]. The K26 site of the oncogene YB-1 is crucial for SUMOylation [128]. Although this modification does not alter YB-1's expression or stability, further studies have shown that the level of YB-1 SUMOylation affects its binding to PCNA, thereby disrupting the MutSα-PCNA interaction in MMR [128]. As an E3 SUMO ligase, NUSAP1 promotes ATR SUMOylation, preventing ATR degradation and stabilizing it, thereby increasing GBM cell resistance to TMZ and other chemotherapy agents [129]. SUMO-1 can bind to lysine residues 27, 76, and 112 on Olig2. SUMOylated Olig2 enhances its binding to the Cdkn1a gene, preventing p53 from binding to the Cdkn1a promoter, thereby inhibiting p53-mediated cell cycle arrest and apoptosis, and increasing TMZ resistance [130]. SUMOylation of the C-terminal K330 site in DNA glycosylase TDG is essential for its enzymatic activity [114]. This modification alters TDG's conformation, reducing its affinity for AP sites and impeding its DNA binding activity [114].

THE RELATIONSHIP BETWEEN ACETYLATION AND TMZ RESISTANCE MECHANISMS

Acetylation is a PTM where an acetyl group is transferred to lysine residues or the N-terminus of proteins by acetyltransferases. It is a prevalent PTM in the proteome, playing a crucial role in cellular homeostasis, with over 35,000 acetylation sites identified in humans to date [131]. Lysine acetylation is primarily regulated by two enzyme classes: lysine acetyltransferases (KATs/HATs) and lysine deacetylases (KDACs/HDACs) [132, 133]. Abnormal expression or mutation of these enzymes is implicated in various cancers. For example, KAT2A mediates acetylation of c-MYC at K323, stabilizing c-MYC [134]. KAT2A also recruits c-MYC to RNA polymerase III, promoting the transcription of c-MYC target genes [135]. Overexpression of HDAC1, 2, and 3 is observed in many cancers, promoting tumor cell proliferation, invasion, and migration [136, 137]. HDAC6 regulates the acetylation of α-tubulin and cortactin, influencing cell migration, chemotaxis, angiogenesis, and cancer metastasis [138].

Acetylation in the MMR system

The histone acetyltransferase CBP promotes MLH1 acetylation, preventing its ubiquitin-dependent degradation and stabilizing MLH1, thereby facilitating the formation of the MutSα-MutLα complex [139]. In contrast, HDAC6 induces deacetylation of MLH1, inhibiting MutSα-MutLα complex formation [140]. The K73 site of MSH2 is acetylated by HBO1 and deacetylated by HDAC10, with HDAC10-mediated deacetylation being crucial for MSH2's role in MMR [141]. However, other studies suggest that HDAC6-mediated deacetylation of MSH2 promotes its ubiquitination, reducing its levels and inhibiting MMR [82]. In conclusion, the balance between acetylation and deacetylation plays a crucial role in regulating MMR proteins, warranting further investigation to clarify specific mechanisms.

Acetylation in the BER system

TDG is acetylated at K94, 95, and 98 by p300, inhibiting APE1 recruitment [142]. p300 also acetylates OGG1 at K338 and 341, enhancing its glycosylase activity, reducing affinity for AP site products, and promoting APE1 recruitment [143]. Acetylation of NEIL2 by p300 at K49 and K153 alters its activities, with K49 acetylation inhibiting base excision and AP nuclease functions [144]. Furthermore, p300 and ERα acetylate MPG, facilitating its binding to alkylation-induced DNA damage [145]. Multiple lysine residues of APE1, including K6 and K7, are acetylated by p300, promoting interaction with YB-1, which activates multidrug resistance gene MDR1 [145]. Acetylation at residues K27, K31, K32, and K35 may influence APE1's nuclease activity and its roles in BER and RNA metabolism [146]. The autophagy adapter p62 undergoes acetylation by hMOF and deacetylation by SIRT7; acetylated p62 accumulates at DNA damage sites, interacting with APE1 to activate its nuclease activity and initiate BER [147].

Acetylation in the DSBs repair system

Knockout of CBP/p300 proteins significantly decreases acetylation at histone H3 K18 and histone H4 K5, 8, 12, and 16, inhibiting the recruitment of key NHEJ proteins, KU70 and KU80, to DNA damage sites [148]. Additionally, the recruitment of BRM, a catalytic subunit of the SWI/SNF chromatin remodeling complex, is also impaired [148]. ATM phosphorylation of Sp1 promotes its interaction with p300, facilitating the accumulation of p300 at DNA damage sites and enhancing histone H3 and H4 acetylation [149]. Specifically, acetylation of H3K18 is associated with SWI/SNF and Ku70 recruitment for NHEJ repair [149]. SET/TAF-I β, which interacts with Ku70/80 to inhibit Ku70 acetylation, dissociates from the Ku complex upon DNA damage, releasing Ku70/80 and activating NHEJ repair [150]. Acetylation of Ku70 at K331 and 338 by CBP/p300 is critical for its binding to DNA, with acetylation at K317 facilitating DNA binding through a salt bridge with E330 [151]. MCL-1 can also form a complex with MOF and BID to regulate the acetylation of histone H4K16, thereby influencing the function of HR repair systems [152]. The dynamic balance between acetylation and deacetylation is crucial for the choice between NHEJ and HR. Acetylation of 53BP1 by CBP at K1626 and 1628 inhibits its recruitment to DNA damage sites, thus promoting HR repair [153]. HDAC2 works with CBP to maintain acetylation/deacetylation balance of 53BP1, regulating DSB repair pathway choice [153]. Furthermore, CBP/p300-mediated acetylation of RAD52 is counteracted by deacetylation by SIRT2/SIRT3, preventing premature dissociation of RAD52 and RAD51 from DNA damage sites and limiting HR repair. Maintaining this acetylation/deacetylation balance is critical for HR stability [154].

Acetylation in other DNA damage repair mechanisms

Numerous studies have linked HDACs to TMZ resistance in GBM cells. HDAC6, for instance, stabilizes EGFR, enhancing cell proliferation and spheroid formation, which increases resistance to TMZ-induced growth inhibition and apoptosis [155]. HDAC6 inhibitors can reduce MGMT expression in TMZ-resistant GBM cells, increasing TMZ sensitivity and inducing apoptosis [156]. Histone acetylation plays a key role in high MGMT expression in tumors [157]. High-throughput lncRNA sequencing of TMZ-resistant and sensitive GBM cells identified Lnc-TALC, an lncRNA that binds miR-20b-3p, activating the c-Met/Stat3/p300 pathway. This promotes acetylation of H3K9, H3K27, and H3K36 in the MGMT promoter, increasing MGMT expression and TMZ resistance [158]. DIP2A, a multifunctional protein, collaborates with the HDAC2-DMAP1 complex to deacetylate H3K9Ac, inhibiting MGMT transcription and enhancing TMZ sensitivity in GBM cells [159]. Fstl1, a glycoprotein overexpressed in TMZ-resistant GBM cells, inhibits DIP2A binding to the HDAC2-DMAP1 complex, preserving H3K9 acetylation and promoting MGMT expression, thus boosting resistance to TMZ [159]. Acetylation of H3K27 regulates

cis-regulatory elements to promote gene transcription, while HDAC complexes deacetylate core histones, reducing transcription. The RET finger protein (RFP), in complex with HDAC1, modulates histone modifications. Downregulation of RFP or disruption of the RFP/HDAC1 complex alters histone modifications, impacting cell division, cycle progression, and apoptosis, thereby enhancing TMZ efficacy in GBM treatment [160].

OTHER MODIFICATIONS AND THEIR RELATION TO TMZ RESISTANCE MECHANISMS

PARylation

PARylation plays a critical role in BER pathway, which is catalyzed by poly(ADP-ribose) polymerases (PARPs). PARP1 functions as a DNA damage sensor that is rapidly activated in response to DNA lesions. Upon activation, it catalyzes the formation of poly(ADP-ribose) (PAR) chains, which create a molecular scaffold for the recruitment of key proteins in BER (e.g., XRCC1, DNA ligase), thereby coordinating a highly complex biochemical repair response [161]. During the NHEJ repair process, PARP stimulates DNA-PKcs activity through PARylation [162]. A structural PARP1/DNA-PKcs/Ku molecular complex has been identified, in which PARP1 induces a major architectural rearrangement of the DNA-PKcs-mediated assembly and further recruits critical NHEJ repair proteins (e.g., POL β, XRCC1) [161, 163]. Samuele Lodovichi et al. demonstrated that inhibition of BRCA1 PARylation promotes EXO1- and BRCA2-dependent homologous recombination (HR) while destabilizing the RIF1-53BP1 oligomeric complex at DNA double-strand break (DSB) sites, thereby suppressing non-homologous end joining (NHEJ) [164]. Therefore, PARylation exerts multifaceted effects on DNA double-strand break (DSB) repair mechanisms. PARylation may play a critical role in maintaining the homeostatic balance between HR and NHEJ pathways. Furthermore, Shaofang Wu et al. discovered that PARP can mediate PARylation modification of MGMT, thereby enhancing its binding affinity to DNA [10].

Multiple studies have demonstrated that pharmacological inhibition of PARylation effectively disrupts various DDR pathways. Moreover, PARP inhibitors exhibit a “PARP trapping” effect - the formation of cytotoxic PARP-DNA complexes at damage sites that potentiate tumor cell lethality [165]. Consequently, targeting PARylation has emerged as a pivotal therapeutic strategy to overcome TMZ resistance in GBM.

Methylation

Methylation of NFAT5 at K668 is associated with drug resistance and prognosis in GBM patients. This modification inhibits NFAT5’s interaction with E3 ligase, preventing its degradation and promoting its nuclear accumulation and activation, thereby increasing MGMT expression [166]. DNA Pol β, a key enzyme in BER, undergoes methylation at various arginine residues by different arginine methyltransferases (PRMT1 and PRMT6), influencing its function. Methylation of Pol β at R137 by PRMT1 disrupts its binding to PCNA, impairing BER, while methylation at R83 and 152 by PRMT6 enhances its DNA binding affinity, facilitating BER [167].

Neddylation

Neddylation facilitates Ku ubiquitylation following DSBs, promoting the release of Ku and associated proteins from damage sites during NHEJ [168]. The neddylation inhibitor MLN4924 reduces MGMT levels and enhances TMZ toxicity in GBM cells, underscoring neddylation’s key role in TMZ resistance in GBM [169].

Crotonylation

In response to DSBs, GCN5 mediates K525 crotonylation of DNA-PKcs, promoting DNA-PK complex assembly and enhancing its DNA binding capacity [170]. This process is essential for tumor cells to repair DNA damage induced by radiotherapy or chemotherapy via the NHEJ pathway [171]. Crotonylation of MSH6 at K544 affects its interaction with Ku70, facilitating NHEJ while inhibiting HR, thereby regulating the balance between these repair pathways [172].

Lactylation

In recurrent GBM tissues and TMZ-resistant cells, increased lactylation of H3K9 at the LUC7L2 promoter enhances LUC7L2 expression [173]. This, in turn, leads to intron retention in MLH1, impairing MMR function and contributing to TMZ resistance in GBM [173]. Inhibition of lactate dehydrogenase A/B with stiripentol enhances the cytotoxic effect of TMZ on GBM both in vitro and in vivo [173].

O-GlcNAcylation

O-GlcNAcylation regulates DSBs repair and influences tumor sensitivity to radiotherapy and chemotherapy [174, 175]. O-GlcNAcylation of DNA-PKcs modulates its kinase activity, thereby regulating NHEJ [176]. Inhibition of RAD52 O-GlcNAcylation using O-GlcNAc transferase inhibitors suppresses homologous HR [177].

SUMMARY AND DISCUSSION

TMZ resistance remains a significant challenge in GBM treatment. The development of resistance is primarily linked to multiple DDR pathways, including MGMT, MMR, BER, and DSB repair mechanisms such as NHEJ and HR. Current research on TMZ resistance in GBM predominantly focuses on MGMT and the upstream regulatory pathways of BER, MMR, NHEJ, and HR. While considerable attention has been given to the MGMT promoter status, some studies indicate that its methylation levels remain relatively stable throughout TMZ treatment in GBM patients [178]. Thus, MGMT alone cannot fully explain TMZ resistance in GBM, necessitating further exploration of the mechanisms driving acquired resistance.

Studies have shown that GBM acquires resistance to alkylating agents, including TMZ, due to MMR dysfunction caused by reduced expression of MMR proteins such as MLH1, MSH2, and MSH6 [179, 180]. To overcome this, targeting MMR dysfunction, researchers have identified decitabine as a potential agent to enhance TMZ sensitivity by modulating MLH1 promoter methylation [181]. Multiple studies have demonstrated that mutations in MMR genes are critical drivers of tumorigenesis and may also contribute to chemotherapy resistance [182, 183]. Research by Hamzeh Kayhanian et al. revealed that the MMR genes MSH3 and MSH6 contain coding homopolymers, which are frequent mutational targets in MMR-deficient cancers [184]. In colorectal cancer, Casey G. et al. identified a high prevalence of mutations in MLH1, MSH2, or MSH6 through whole-genome sequencing of patient samples. The genetic heterogeneity of MMR genes is a critical factor underlying tumor chemoresistance. Takahashi M. et al. further discovered that most MLH1 mutations causing MMR dysfunction are localized around the putative ATP-binding pocket of the NH (2)-terminal domain or span the entire COOH-terminal domain [185], providing pivotal insights for developing targeted therapies against MLH1 mutations.

Research on the role of BER in TMZ resistance in GBM has primarily centered on PARPs, which are critical for initiating the BER pathway. PARP inhibitors have been shown to reduce PARP binding in the BER complex and impair O6-methylguanine repair, thereby enhancing TMZ sensitivity in GBM cells [10]. However, clinical trials of the PARP inhibitor Veliparib have failed to significantly improve patient outcomes [186], and have caused severe myelosuppressive effects when combined with radiotherapy [187]. Olaparib has shown potential in crossing the blood-brain barrier, but its therapeutic efficacy needs further clinical validation [188]. Other PARP inhibitors, such as Pamiparib and Niraparib, lack sufficient evidence of efficacy in GBM patients.

Moreover, research into other key steps in the BER process contributing to TMZ resistance in GBM remains limited. Disruption of XRCC1 complex formation and inhibition of APE1's recognition of damage sites are promising therapeutic targets for improving TMZ efficacy.

Knockdown or inhibition of genes involved in HR or NHEJ, such as RAD51, BRCA2, XLF, 53BP1, and APLF, enhances the cytotoxic effects of TMZ and radiotherapy on GBM [189–191]. However, due to the complexity of DNA repair in HR and NHEJ, current research often focuses on the expression or activation of key genes within these pathways. Further studies are needed to explore the regulatory mechanisms of crucial steps in the repair process, such as the formation of the Ku70/Ku80 heterodimer, assembly of the Rad51 complex, recruitment of repair proteins, and the balance between HR and NHEJ.

Different PTMs exert distinct effects on DNA repair processes: phosphorylation regulates protein or enzyme activity, ubiquitination promotes protein degradation or translocation, SUMOylation counters ubiquitin-dependent degradation to stabilize key proteins, and acetylation promotes gene transcription through histone modifications. Although each PTM has its unique functions, they interact and cooperate during the regulation of DDR, making it challenging to attribute a specific repair mechanism to a single modification. Furthermore, PTMs are reversible, requiring multiple enzymes whose activities can be influenced by factors such as hypoxia, radiation, and drug exposure. As a result, PTMs in GBM cells often exist in a dynamic equilibrium. TMZ-resistant GBM cells can adapt to TMZ-induced damage and other stimuli by modulating this equilibrium, allowing tumor cells to choose the most efficient repair pathways to maintain normal cellular functions. While drugs targeting PTMs are available, clinical trials have generally shown unsatisfactory results. For instance, a 2008 phase II trial found that imatinib was ineffective for recurrent gliomas [192]; a phase II study of bevacizumab and erlotinib following radiation and TMZ in MGMT-unmethylated GBM patients did not improve outcomes [193]; and a phase II trial of panobinostat, despite good patient tolerance, was terminated early due to insufficient efficacy [194]. Clinically, effective strategies to overcome TMZ resistance in GBM remain lacking. Therefore, in the further research, in addition to the intrinsic mechanism underlying TMZ resistance in GBM, we also need to conduct in-depth research by integrating the administration routes and regimens, tumor heterogeneity, and the tumor microenvironment.

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AUTHOR CONTRIBUTIONS

YKC, KKD, and SYZ wrote and edited the manuscript. STG, and XXH produced the figures and illustrations. HJW, FQZ, YJW, JFX, CW, CHL, JX, LW, QW, GC and GG gave intellectual input, JMZ, CGY, and JXJ conceived and organized the manuscript.

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COMPETING INTERESTS

The authors declare no competing interests.

CONSENT FOR PUBLICATION

All authors read this manuscript and approve for publication.

ADDITIONAL INFORMATION

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