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Review

Elucidating the mechanisms of Temozolomide resistance in gliomas and the strategies to overcome the resistance.

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ABSTRACT

Temozolomide (TMZ) is a first-choice alkylating agent inducted as a gold standard therapy for glioblastoma multiforme (GBM) and astrocytoma. A majority of patients do not respond to TMZ during the course of their treatment. Activation of DNA repair pathways is the principal mechanism for this phenomenon that detaches TMZ-induced O-6-methylguanine adducts and restores genomic integrity. Current understanding in the domain of oncology adds several other novel mechanisms of resistance such as the involvement of miRNAs, drug efflux transporters, gap junction's activity, the advent of glioma stem cells as well as upregulation of cell survival autophagy. This review describes a multifaceted account of different mechanisms responsible for the intrinsic and acquired TMZ-resistance. Here, we summarize different strategies that intensify the TMZ effect such as MGMT inhibition, development of novel imidazotetrazine analog, and combination therapy; with an aim to incorporate a successful treatment and increased overall survival in GBM patients.

1. Introduction

Glioblastoma Multiforme (GBM) is the primary brain neoplasm of the central nervous system (CNS) with a high degree of lethality and have a low median survival of 15 months after the initial diagnosis [1]. CNS tumors have an incidence of <10 per 100,000 people, and the number has increased steadily *in the past decade* [2]. The centerpiece of GBM treatment is surgery, followed by radiation and adjuvant chemotherapy [3]. Delivery of therapeutic agents to the CNS is hampered by the blood-brain barrier (BBB), and accessibility to the desired targets is the main obstacle for the development of new drugs for GBM [4]. Temozolomide (TMZ) is a lipophilic agent, so it can easily penetrate the BBB and thereby serves as an effective agent for the treatment of glioma [5]. It alkylates the genomic DNA at the N^7 and O^6 sites of guanine and N^3 position of adenine, and induces nucleotide mismatch in subsequent replication cycles [6]. Nucleotide mismatch leads to the replacement of cytosine with *thymine,* opposite of methylated guanine. Mismatch repair identifies small *nucleotide* insertion/deletion and promotes the cell cycle arrest in the G2/ M phase, leading to cell death of GBM cells [7].

The main concern in GBM treatment is the development of TMZ resistance. TMZ and other conventional chemotherapeutic drugs exert their anti-tumor action in normal cells by damaging DNA and inciting programmed cell death (PCD) [8]. In contrast, DNA repair pathways are frequently impaired in TMZ-resistant tumor cells; therefore, resistant cancer cells recover from TMZ-induced lesions through alternative repair pathways [8]. DNA repair activity can affect the sensitivity to cytotoxic chemotherapeutic drugs. Accumulating evidence suggest that the hyperactivation of O^6-methylguanine-DNA methyltransferase (MGMT) is the most prominent factor that generates TMZ-resistance in GBM via removal of TMZ-induced alkylation from different nucleotides [9]. However, recent studies reveal that MGMT overexpression is not the only factor that causes TMZ-resistance in gliomas, several other pathways contribute to TMZ-resistance independent of MGMT [10].Towards this, hyperactivation of DNA repair pathways, increased drug efflux, high antiapoptotic potential or survival autophagy, and the advent of glioma stem cells (GSCs) complement to MGMT overexpression in TMZ-

Abbreviations: ABC, ATP binding cassettes; ATGs, autophagy-related genes; BBB, blood-brain barrier; BER, base excision repair; CNS, central nervous system; DSBs, double-strand breaks; FA pathway, fanconi anemia pathway; FANCD2, fanconi anemia complementation group D2; GBM, glioblastoma multiforme; GSCs, glioma stem cells; MGMT, O^6-methylguanine-DNA methyltransferase; MMR, mismatch repair; OS, overall survival; PARP1, poly [ADP-ribose] polymerase 1; PARPi, poly [ADP-ribose] polymerase 1 inhibitors; PCD, programmed cell death; SSEA1, stage-specific embryonic antigen-1; TMZ, temozolomide.

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resistance [10,11].

In the first part of the review, we discuss various mechanisms contributing to TMZ-resistance in glioma cells. In the second part, we discuss the cellular alterations which may modulate/increase TMZ efficacy and potential strategies to overcome its resistance.

2. Mechanism of action of TMZ

TMZ (trade name Temodar or Temodal) is an imidazotetrazine class of alkylating agent [12]. At physiological pH, TMZ is converted into 5-(3-methyl-triazeno) imidazole-4-carboxamide (MTIC) that further breaks down into methyldiazonium cation (Fig. 1). Subsequently, methyldiazonium cation transfers its methyl group to DNA, RNA, and cellular proteins [13]. The conversion of TMZ to its active form depends on pH because TMZ is usually stable at pH < 5.0, but above pH 7.0, it is rapidly converted into MTIC [14]. Methylation on the N^7 or O^6 position of guanine and N^3 of adenine is a cytotoxic lesion, which stimulates the mismatch of nucleotide bases during replication [15]. If MGMT mediated repair does not occur, MMR proteins identify mispairing in newly synthesized strand and thymine excision or DNA damage, followed by

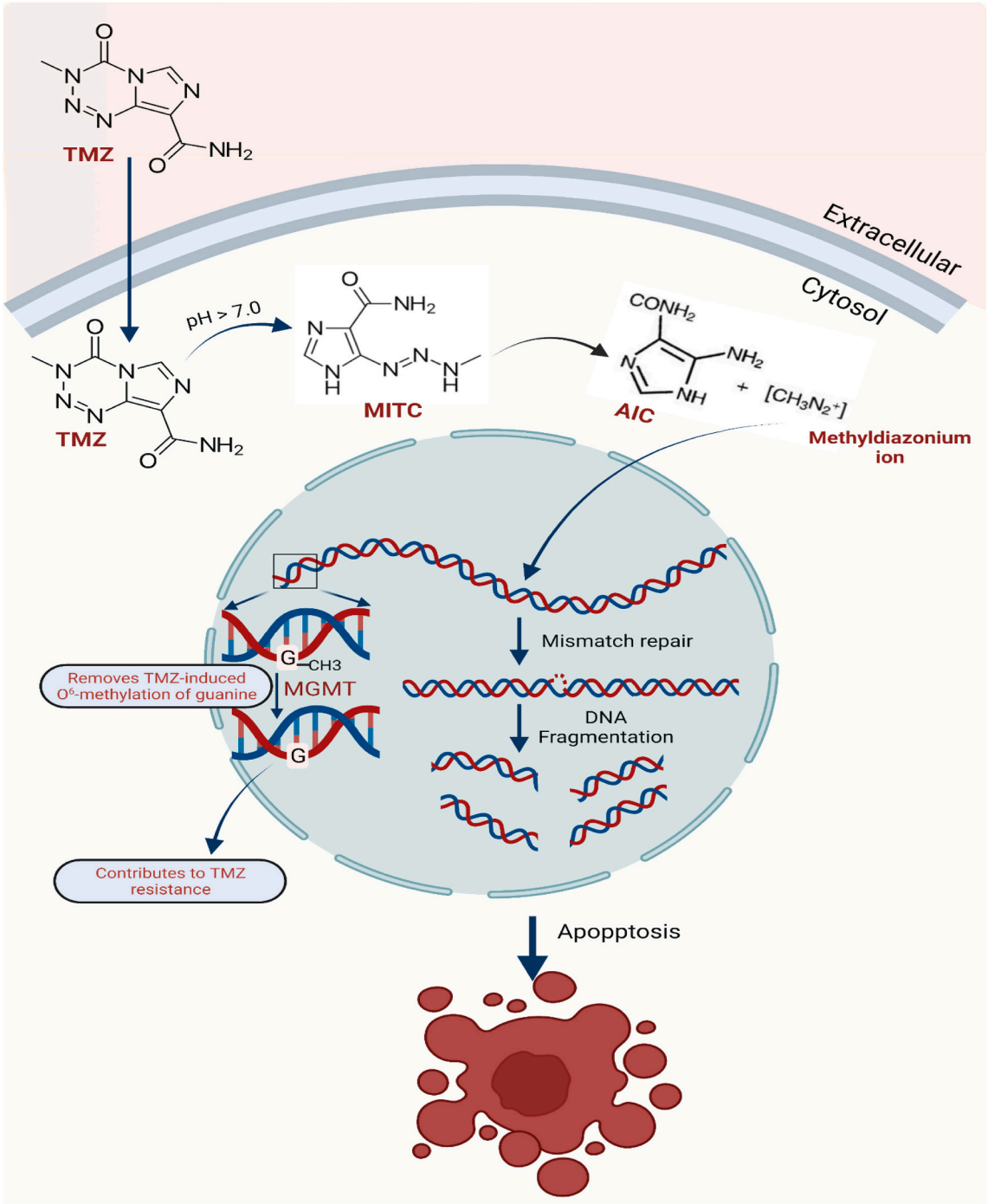

Fig. 1. TMZ mechanism of action. At cytosolic pH, TMZ is converted into 5-(3-methyl-triazeno) imidazole-4-carboxamide (MTIC) that generate methyldiazonium cation transfer their methyl group on N^7 and O^6 sites of guanine and the N^3 on adenine. The TMZ adduct imparts mutation in DNA fixed via O^6-methylguanine-DNA methyltransferase (MGMT), Mismatch Repair (MMR), and Base excision repair (BER). MMR generates DNA double strand breaks (DSBs) in TMZ sensitive cells and triggers PCD. Whereas overexpression of MGMT and other repair proteins remove TMZ adducts and generate TMZ resistant glioma cells.

cell cycle arrest, leading to PCD (Fig. 1) [10].

3. Mechanisms of TMZ-resistance in GBM

3.1. Role of DNA repair mechanisms in the development of TMZ-resistance phenotype

3.1.1. Role of direct DNA repair through MGMT

MGMT enzyme removes cytotoxic O^6-methylguanine adducts from DNA and generates resistance against anti-tumor alkylating agents such

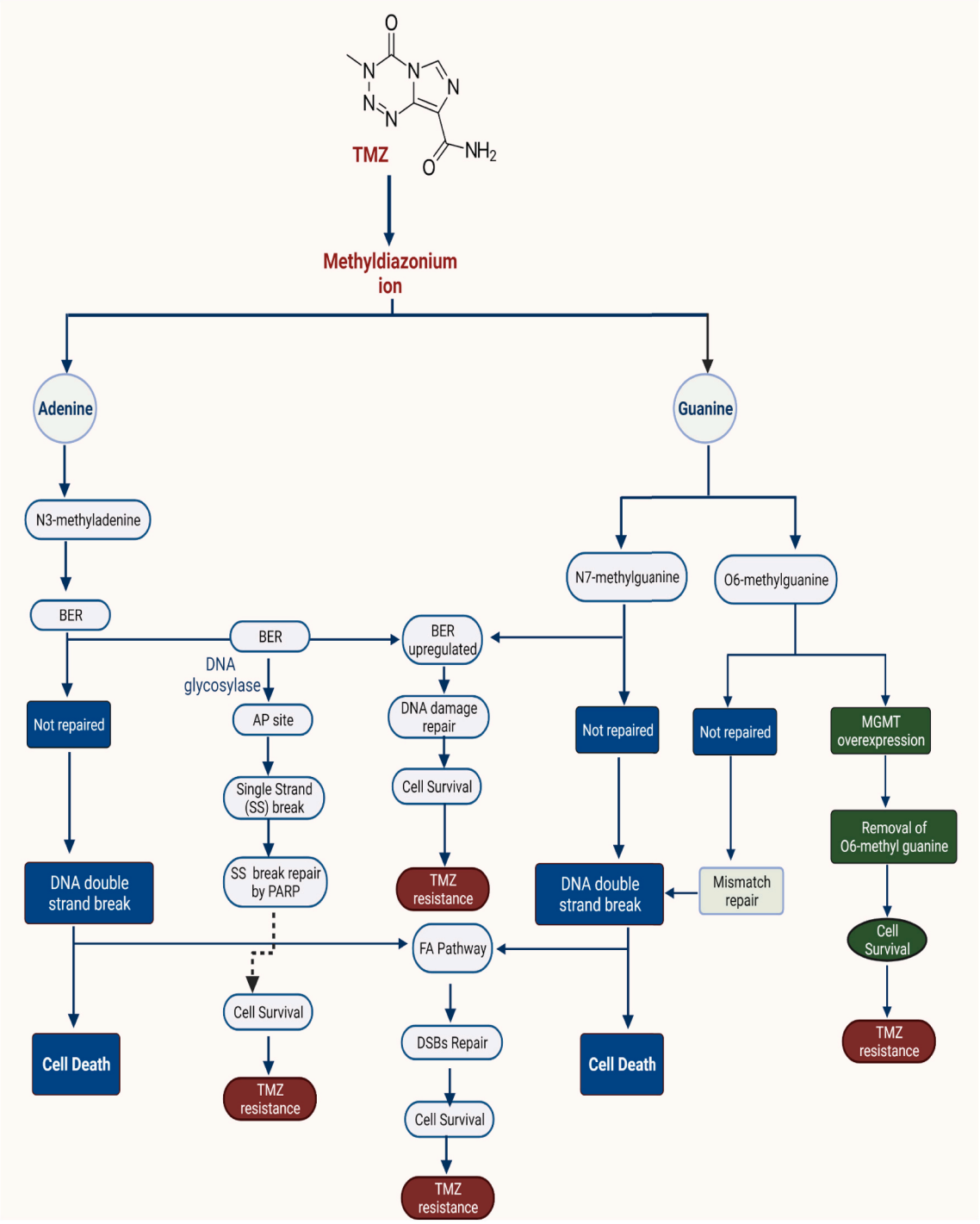

Fig. 2. DNA repair pathways and TMZ resistance: Methyldiazonium cation, the active product of TMZ methylates DNA on N^7 or O^6 site of guanine and N^3 site of adenine. MGMT remove methylation at O^6 site of guanine and promote cell survival. If O^6-methyl guanine is not repaired through MGMT, it leads to the activation of MMR and promote cell death. Whereas methylation of N^7 position of guanine and N^3 position of adenine are repaired through BER. During BER, ssDNA breaks are generated that are repaired directly through PARP via recruitment of DNA repair enzymes at damage site. Non-functional BER produces dsDNA break and cell death. In TMZ-resistant GBM, fanconi anemia pathway repair dsDNA break through recombination.

as TMZ and bis-chloroethyl nitrosourea (Fig. 2) [16]. Indeed, MGMT methylation status has a significant impact on histological typing and diagnosis, and is also a prognostic marker for the survival and therapy response in GBM patients [17]. MGMT expression is directly regulated by methylation of the CpG island at the MGMT gene promoter [18]. There are multiple CpG islands in the MGMT promoter that are located at -252 to -155 and -90 to +65 regions, and methylation of these CpG sites shows an inverse relationship with MGMT expression in GBM [19]. Growing evidence have shown a decrease or loss of MGMT methylation in primary and recurrent GBM with different grading [20].

Data from the orthotopic GBM xenograft model has revealed that TMZ responsiveness inversely correlates with cellular MGMT protein level and directly correlates with MGMT promoter methylation. Loss of MGMT promoter methylation is associated with intrinsic resistance to TMZ [21]. Primary GBM patients with MGMT promoter methylation had prolonged median survival (approximately five months) in comparison to MGMT promoter unmethylated patients [22]. Epigenetic silencing of the MGMT through methylation of gene promoter in adult GBM correlate with progression-free survival (PFS) and overall survival (OS) of patients [23]. Patients with MGMT promoter methylation respond better to TMZ therapy and have increased survival in both adults and pediatric GBM [24,25]. Moreover, Melguizo et al. reported that MGMT promoter methylation status might serve as a better predictive marker in treating patients with GBM [26]. Recently, it has been observed that in a subset of recurrent gliomas, genomic rearrangement leads to MGMT overexpression independent of promoter methylation [27]. However, further studies will be needed to determine the clinical utility of MGMT as a molecular biomarker in GBM patient's survival and prognosis.

3.1.1.1. Acquired TMZ-resistance in MGMT deficient cells. Approximately 50% GBMs are MGMT-deficient and are still resistant to TMZ; the underlying molecular mechanism of acquired resistance is not fully understood [28,29]. Towards this, a retrospective study reported four times higher non-synonymous mutations in the GBM with MGMT methylation. Furthermore, increased frequencies for mutations in the CDKN2A, PTEN, TP53, and PIK3CA genes were reported in GBM patients with MGMT methylation, compared to non-methylated GBM [29]. Dynein, cytoplasmic 2, heavy chain 1 (DYNC2H1 or DHC2) is overexpressed in MGMT-deficient recurrent GBMs and cells with MGMT promoter methylation, and it generates TMZ-resistance through nuclear localization of DNA repair proteins such as XPC and CBX5. Silencing of XPC, CBX5, or DHC2 enhances TMZ-induced cell death both in vitro and in vivo [30]. Recently Guo et al. identified nuclear proteins that promote acquired TMZ-resistance in MGMT-deficient human glioma cell lines [31]. In that study, in TMZ-treated U87 cells for one week, a total of 455 differentially expressed nuclear proteins were recognized; among them, 327 were highly expressed, and 128 have low expression. Furthermore, in silico analysis revealed that MSH6, MRE11, RPA1, RAD50, MSH2, RBX1, UBR5, CUL4A, CUL4B, DDB1, and DDB2 proteins are enriched in DNA repair pathways and formed protein-*protein interaction network* [31]. Epigenetic regulation of X-linked inhibitor of apoptosis (XIAP)-associated factor 1 (XAF1), a tumor suppressor protein, acts as the mediator of TMZ-resistance in MGMT methylated GBM. In fact, GBM patients with upregulated XAF1 expression had shorter OS, in comparison to patients with downregulated XAF1 expression [32].

Dysregulated expression of mismatch repair (MMR) complex has been reported in MGMT methylated TMZ-resistant GBM cells [33,34]. Inhibition of poly (ADP-ribose) polymerase protein inhibition (PARPi) overcomes TMZ-resistance in MGMT-deficient primary GBM cells, suggesting a role of BER in TMZ-resistance independent of MGMT [35]. Moreover, dysregulation of cytochrome P450 17A1 (CYP17A1) increases dehydroepiandrosterone (DHEA) secretion that hampers TMZ-induced PCD in MGMT-deficient GBM. DHEA induces specificity protein 1 (Sp1) phosphorylation and localization in TMZ-damaged DNA to attenuate further DNA damage by recruiting proliferating cell nuclear antigen (PCNA) [36]. Additionally, Sp1 expression is also associated with superoxide dismutase 2 (SOD2) expression that act as a ROS scavenger. Inhibition of Sp1 in TMZ-resistant MGMT-deficient clinical samples and cell lines restores TMZ sensitivity [37].

3.1.2. Role of indirect DNA repair or mismatch repair (MMR)

Mismatch repair plays a decisive part in the correction of nucleotide base mismatch generated during the process of DNA replication [38]. TMZ-induced O^6-methyl guanine: thymine mispairing is fixed by the DNA mismatch repair system through excision of the newly synthesized strand [39]. Functional MMR creates a DNA double-strand break leading to PCD via apoptosis (Fig. 2) [40]. The absence of an MMR system is another important mechanism that generates resistance in GBM. Loss of function in MMR genes such as; post-meiotic segregation increased 2 *(Saccharomyces cerevisiae)* (PMS2), mutL Homology 1 (MLH1), melanocyte-stimulating hormone 2 (MSH2), MSH3 and MSH6 contribute to TMZ-resistance [40–42]. Notably, it has been shown that knockdown of MLH1 or PMS2 confers TMZ-resistance in recurrent GBM [43]. Attenuated expression of MSH2, MSH3 and MSH6 impact TMZ-resistance and MMR activity offers a predictive marker for TMZ therapy response [41,42,44]. All these MMR genes are vulnerable to TMZ-induced mutations and alterations in these genes cause resistance to TMZ in GBM.

3.1.3. Role of base excision repair (BER)

Base excision repair includes correction of DNA damage generated through oxidation, deamination, single-strand breaks, and alkylation [45]. A defective BER is recognized as a mediator of TMZ-resistance in GBM (Fig. 2) [46]. N^7-methylguanine and N^3-methyladenine adducts are the substrate of DNA glycosylase, an enzyme of BER. In addition to MGMT, the DNA-glycosylase enzyme removes the N^7-methylguanine and N^3-methyladenine adducts [47]. Furthermore, significantly higher expression of DNA glycosylase has been reported in glioma tissues in comparison to non-neoplastic brains [48]. Alkylpurine-DNA-N-glycosylase or *N*-methylpurine DNA glycosylase enzymes remove the alkyl group from nucleotide bases that are added by TMZ [49]. Furthermore, alkylpurine-DNA-N-glycosylase confers TMZ-resistance in xenograft models of GBM, and its higher nuclear level is associated with poor survival of GBM patients [50]. The process of healing of the apurinic/apyrimidinic site (AP site) is commonly known as the abasic site after the removal of glycosylase [51]. AP endonuclease 1 (APE1) allows perusing the abasic sites to cut at the 5'-end of the DNA. Subsequently, the damage repair process is completed with the help of DNA ligase along with DNA polymerase [51].

Usually, enzyme Poly [ADP-ribose] polymerase 1 (PARP 1) repairs ssDNA break generated during BER; however, fragmented DNA accumulation in cells leads to apoptosis downstream of PARP1 cleavage [52,53]. Mechanistically, PARP1 activation utilizes NAD+ and modifying associated proteins auto as well as via transfer of poly-ADP-ribose (PAR) phenomenon known as PARylation [54]. PARP1 mediated PARylation assigns MGMT protein at TMZ induced O^6-methylguanine lesions in GBM [55]. *PARPi*-mediated inhibition of *PARP*-1 catalytic domain *prevents* its *PARylation* and then its release from the *DNA damage* site generating replicative stress and replication-associated dsDNA breaks [56]. Pharmacological inhibition of PARP restores TMZ sensitivity in glioma or GSCs by reducing the function of DNA repair proteins [55,57]. The above study shows; BER actively plays a beneficial role in the treatment of TMZ-resistant GBM. Interruption in BER activity is one of the attractive targets to generate TMZ-sensitivity and resistance in GBM cells [58].

3.1.4. Double-strand break repair by Fanconi Anemia (FA) pathway

The FA pathway restores inter-strand crosslinks (ICLs) that covalently link both strands of DNA and halt unwinding through DNA helicases; thus, hampers the replication [59]. The FA pathway consists of 22 FA proteins (FANCA to FANCW), and mutation in any of the FA genes

generates disorders such as failure of bone marrow and cancer [60]. DNA lesions activate ATR signaling that induces the FA pathway [61]. Homologous recombination assists FA pathways to repair ICLs and various DNA lesions. When DNA lesions-caused by alkylating agents are not repaired by DNA repair pathways such as MGMT, MMR, and BER, then DNA double-strand breaks (DSBs) are produced (Fig. 2) [62]. These DSBs are the substrate of non-homologous DNA end-joining or can be repaired through homologous recombination repair [63,64]. It has been reported that Brca2/Xrcc2-dependent homologous recombination repair is needed for protection against O^6-methylguanine adduct-induced DSBs [65].

The FA gene expression correlates with glioma tumor grade, and

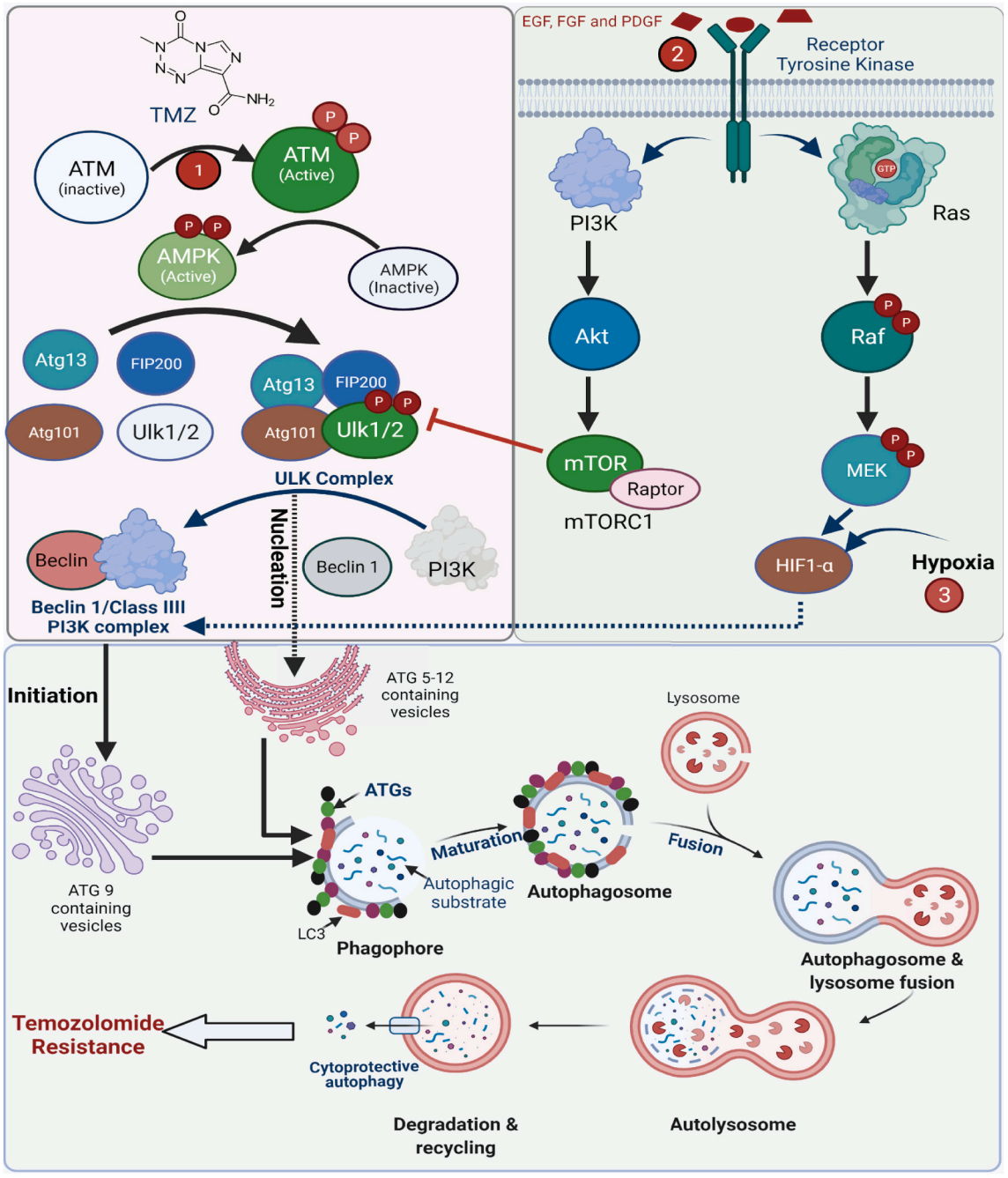

Fig. 3. Schematic process of autophagy in TMZ-resistant glioma cells. The autophagic pathway has several stages namely initiation, nucleation, elongation, and maturation or fusion with the lysosome. (1) TMZ treatment cause DNA damage that leads to activation of ATM/AMPK/ULK1 signaling that can induce acidic vesicular organelle formation, LC3 aggregation promotes interaction of autophagosome and lysosome, facilitating survival autophagy and contributes to glioma chemoresistance. (2) Binding of EGF, PDGF, and FGF to receptor tyrosine kinase (RTK) stimulates RAS/RAF/ERK and PI3K/AKT signaling pathways that activate downstream transcription factor and promote autophagy. TMZ treatment induces autophagy through ATGs and promotes phagophore formation. AKT activation inhibits autophagy through mTOR by inhibiting the ULK1 complex. (3) Low O_2 condition or hypoxia induces HIF1α that also promotes autophagy. Autophagosome biogenesis starts with the formation of initiation membrane from ER, golgi body, mitochondria, and other membrane sources. Nucleation of vesicle requires Class III PI3K /baclin 1 complex. Follows elongation and maturation of vesicles into autophagosomes requires ATGs (like ATG5-ATG12) and LC3 protein. LC3 protein play crucial role in the binding of autophagosomal membrane and identification of autophagy substrate. Then autophagosome fuses with lysosome and form autolysosome and lyse autophagic substrate. Cytoprotective autophagy promotes protein synthesis, energy generation, and increased cellular invasion of glioma cells.

suppression of the FA pathway sensitizes GBM to alkylating agents [66]. TMZ treatment increases FA complementation group D2 (FANCD2) mono-ubiquitination and FANCD2 nuclear foci, the hallmark of FA pathway, in a time or dose-dependent manner that provokes TMZ-resistance. [66]. Similarly, FANCD2 knockdown through siRNA sensitizes glioma cells to TMZ. Recent studies suggest that degradation of FANCD2 via celastrol elicits glioma's sensitivity towards DNA-crosslinking agent carboplatin [67]. In a similar study, a novel mechanism of FA pathway regulation through mTOR signaling in DNA damage and repair has been reported [68,69]. mTOR signaling pathway assists FANDC2 to stimulate DNA DSBs repair and impart chemoresistance [69]. In retrospective studies, it was noted that PARP inhibition stimulates FANDC2 mono-ubiquitination to basal level activation of the FA pathway in response to DSBs. Inhibition of DNA damage response process (FA-pathway, PARP, ATR, or ATM) enhances the sensitivity of GBM cells towards TMZ [70]. FA pathway proteins, FANCG, and FANCD1/BRCA2 are predominantly involved in the repair of DNA alkylating agents (TMZ or ACNU)-induced lesions [71]. However, siRNA-mediated silencing of *FANCD1/BRCA2* increases genomic instability and the sensitivity of human GBM A172 cells to TMZ [71]. The outcome of the above study underscores the significant role of the FA repair pathway in enhancing the complexity of cellular resistance of GBM towards DNA alkylating agents.

3.2. Survival autophagy in GBM cells generate TMZ-resistance

Autophagy is a conserved recycling system that occurs under stress conditions, including nutrient deprivation, hypoxia, damage in the DNA or organelles, and is controlled by series of proteins in which cells maintain homeostasis through self-degradation [72]. Autophagy serves as a double-edged sword in cancer therapy. Although autophagy acts as a tumor suppressor, conversely, it is a mechanism to adapt stress response in tumor cells that helps in the survival of cancer cells during cancer therapy [73]. Upregulated autophagy has a significant impact on motility, cell survival, chemoresistance in glioma cells, and maintenance of GSCs [74].

TMZ-induces autophagy via the ataxia–telangiectasia mutated (ATM) serine-threonine kinase/ AMP-activated protein kinase/ *Unc-51 like autophagy activating kinase-1* (ATM/AMPK/ULK1) pathway **(**Fig. 3**)**. This pathway can induce acidic vesicular organelle formation, LC3 aggregation that promotes the interaction of autophagosome with the lysosome, facilitating survival autophagy and thereby contributes to glioma chemoresistance [75]. Besides, receptor tyrosine kinase /rat sarcoma/phosphoinositide 3-kinase (RTK/Ras/PI3K) signaling regulates autophagy **(**Fig. 3**)** in the brain and is found to be altered in 89% of GBM patients [76]. Augmentation of survival autophagy further complicates GBM pathogenesis and response to therapy.

Genes linked with the regulation of autophagy are known as autophagy-related genes (ATGs) [77]. To date, 36 different types of ATGs, conserved from yeast to mammals, are reported. Among them, 18 Atg proteins are recruited to phagophore, which is involved in autophagosome formation [78,79]. *ULK1 complex* promotes autophagy initiation, and PI3K/beclin-1 complex (PI3K III C complex) supports autophagosome nucleation (Fig. 3) [80]. The *autophagy proteins ATG12–ATG5 are* needed for the conjugation *of LC3*-II or Atg 8 to *phosphatidyl-ethanolamine on an autophagic membrane to* regulate elongation and closure *autophagosomal membrane* [81]. Sequestosome 1/p62-like receptors act as adaptor proteins that bind to cargos, induces specific molecular targeting [77]. Membranous components of the endosome, endoplasmic reticulum, mitochondria, Golgi apparatus, and plasma membrane elongate autophagosomal membrane through ATG9 [72]. The phagophore membrane elongates gradually and closes to form a double-membrane bounded vesicle autophagosome, which eventually fuses with a lysosome to form an autolysosome [82]. Lysosomes have various acid hydrolases that degrade internalized autophagic cargo [72,82] (Fig. 3).

Recently it has been reported that DOC-2/DAB2-interacting protein (DAB2IP) deprived GBM become resistant to TMZ via ATG9B dysregulation [83]. DAB2IP negatively regulates ATG9B through Wnt/ β-catenin pathway and enhance TMZ sensitivity by suppressing TMZ-induced autophagy [83]. Furthermore, in glioma cells, vesicle-associated membrane protein 8 (VAMP8) significantly increases cell proliferation and TMZ-resistance by enhancing the expression of autophagy-related proteins and autophagosome numbers [84]. Knockdown of VAMP8 suppresses the growth of glioma cells through cell cycle arrest at G0/G1 phase [84]. Hence, autophagy is a key player of the cytoprotecting mechanism in unfavorable microenvironmental conditions of GBM.

3.3. Glioma stem cells (GSCs)

Subpopulation of cancer stem cells (CSCs) within GBM is another factor that aids the complexity of TMZ- resistance. GSCs have the ability to undergo self-renewal, indefinite proliferation, and multi-lineage differentiation [85]. GSCs are characterized by overexpression of various transcription factors, including octamer-binding transcription factor 4 (OCT4), sex-determining region Y-box 2 or SOX2, nestin, oligodendrocyte transcription factor, and inhibitor of DNA binding 1 (ID1). GSCs also overexpress several cell-surface proteins such as the cluster of differentiation 133 (CD133), CD44, CD15, neural cell adhesion molecule L1 (L1CAM), or A2B5 [86]. Accumulating evidence suggests a linkage between the emergence of GSCs to chemoresistance and tumor recurrence [87]. Now it is well established that TMZ treatment gradually increases GSCs pool [88].

Consistent exposure of glioma cells with *TMZ* chemotherapy is capable of interconversion of *non*-GSCs into new GSCs. Newly emerged GSCs possess several markers associated *with* normal stem cells like CD133, SSEA1, Oct4, SOX2, and nestin [88]. It has been reported that Wnt signaling pathway impart stemness in GBM [89]. The Wnt signaling inhibitor LGK974 along with TMZ decrease aldehyde dehydrogenase 3A1 (ALDH3A1) expression and markers of stem cells like-CD133, nestin, and Sox2 in GBM cells [90]. A phenolic compound 3,4-Dihydroquinolin-1(2*H*)-yl)(*p*-tolyl)methyl)phenol (THTMP) modulates genes of Wnt, Hedgehog, and notch pathways and decrease stemness of GSCs [91]. Additionally, cyclin-dependent kinases (CDKs) inhibitors induce apoptosis in GSCs, resistant to TMZ [92]. Studies have shown that the population of GSCs impart chemoresistance and can be a potential target for tumor regression.

3.3.1. Glioma stem cells markers

3.3.1.1. CD 133. GSCs are characterized by CD133 (Prominin 1) expression, an apical plasma membrane protein, and these GSCs contribute to TMZ-resistance [93]. CD133 expressing cells are known as glioma initiating cells, can form a tumor in vitro as well as in vivo in mice xenografts [93,94]. However, CD133 is a controversial marker because approximately 40% of human-derived GBM specimens did not express $CD133^{+}$ on the plasma membrane of tumor cells. Several studies reveal that $CD133^{-}$ cells can also generate tumors in immunocompromised animal models [85,95]. In addition to CD133, the assessment of several other markers, including nestin, integrin-α6, OCT-4, and SOX-2, is needed to identify GSCs. These studies showed that CD133 expression in glioma is mainly bioenergetic stress-dependent and partially cell cycle-dependent [96,97]. The majority of GBM cells have poor $CD133^{+}$ expression, but others have no or very high $CD133^{+}$ expression [98]. A recent study showed the importance of CD133 as a marker of GSCs. This study reported that $CD133^{+}$ cells exhibit less responsiveness to TMZ, than $CD133^{-}$ cells [99]. Dysregulated expression of notch and sonic-hedgehog pathway-related genes has been reported in CD133+ cells. Inhibition of these pathways augments TMZ sensitivity [100]. Further studies are needed for the characterization of CD133 function and to improve our knowledge about its participation in tumor initiation,

aggressiveness, and maintenance.

3.3.1.2. Stage-specific embryonic antigen-1 (SSEA-1). Read et al. first identified SSEA-1 as a marker of CSCs using the *Patched* mutant mouse model of medulloblastoma [101]. SSEA-1 is a cluster of differentiation antigen also known as CD15 and expressed in general stem cells as well as in cancer stem cells [102]. The tumor formation ability of SSEA-1$^+$ expressing xenograft encouraged that it may be a potential marker of tumor-initiating cells (TICs) in GBM [103]. Further studies confirmed SSEA1+ as a marker of TICs in GBM since (1) SSEA-1$^+$ expressing GBM cells have significant tumorigenic activity than SSEA-1$^-$ cells; (2) It maintains cellular hierarchy; and (3) Cells expressing SSEA1+ have stem *cell*-like properties such as *differentiation capacities*, *self-renewal properties* and ability to proliferate that promote tumor initiation [104]. Characterization of SSEA-1 supports the need of a deeper understanding to find other reliable markers for the identification of GSCs/CSCs.

3.3.1.3. Integrin-α6. Receptors of the integrin family are heterodimeric transmembrane glycoprotein that mediates cell-to-cell and cell-to-extracellular matrix adhesion [105]. Alteration in integrin signaling empowers tumor cells to uncontrolled proliferation, invade through tissue boundaries and survive in foreign microenvironments [106]. Integrin-α6 is co-expressed with conventional markers of GSCs, and short hairpin RNA-mediated knockdown of integrin-α6 causes inhibition of proliferation, self-renewal, and tumorigenic potential of GSCs [107]. These findings suggest that elevated expression of integrin-α6 serves as an enrichment marker in GSCs and also acts as a potential target in anti-GBM therapy [107].

3.3.1.4. A2B5. A2B5 epitope belongs to the family of the sialoganglioside that exists on the plasma membrane of neural and glial cells [108]. A2B5 is synthesized by the ST8 alpha-*N*-acetyl-neuraminidase α-2,8-sialyltransferase 3 (ST8SIA3) enzyme [109]. GBM cells that express A2B5+ have stem cell-like properties [110]. Overexpression of the ST8SIA3 enzyme induces cellular proliferation, invasion, and xenograft of glioma cells [109]. It has also been shown that GSCs with CD133$^-$ also can form a tumor in the presence of glial progenitor marker A2B5+ [111]. When CD133$^-$/A2B5$^-$ and CD133$^-$/A2B5$^+$ cells were propagated into immune-compromised mice, these two subpopulations generate a neurosphere with CSCs like characteristics [112]. Whereas A2B5+ cells had a higher invasion ability than that of the A2B5$^-$ cells [112]. Human A2B5+ CD133- glioma tissues have lower expression of tumor-suppressing miRNA such as miR-218-5p; highly expressed miR-218-5p inhibits neurosphere formation by lowering stem cell characteristics and invasion of glioma cells [113]. In addition, PAR1 signaling inhibition potently suppressed growth and self-renewal of A2B5+ derived GBM [114].

3.3.1.5. Oct-4. POU domain, class 5, transcription factor 1 (POU5F1) gene encodes Oct-4, responsible for the self-renewal of embryonic stem cells. Oct-4 overexpression has been reported in glioma, and its expression increases with the advancing grade of gliomas [115,116]. Oct-4 has a remarkable role in the existence of undifferentiated cells in GBM [117]. It is reported that the hypoxic environment of tumor cells aids the expression of OCT-4 and other transcription factors [118]. Further studies suggested that Oct-4 is co-expressed with CD133 and nestin in the perivascular area of astrocytomas; however, its expression was not significantly associated with the survival outcome [119].

3.3.1.6. SOX 2/3. Sox2 is crucial for neural stem cells (NSC); its transcription is promoted by the Sox9 transcription factor via notch signaling. In a positive feedforward loop, SOX2 overexpression diminishes notch1 methylation [120]. This loop exacerbates the *poor prognosis* in glioma patient's cohort. Furthermore, the suppression of notch signaling reduces the migration of glioma cells across white-matter-tract tropism of GSCs extent [120]. Significantly *increased expression* of SOX2 escapes cell cycle cessation and develops TMZ-resistance. Inhibition of the mTOR signaling pathway abates SOX2/9 expression and revokes chemoresistance [121].

Downregulation of SOX2 through the Wnt/β-catenin pathway enhances TMZ-sensitivity via reduction of colony-forming potential of tumor cells and provokes cell death [122]. Additionally, SOX3 has a significant role in both general *neuronal growth* and tumorigenesis. SOX3 overexpresses in a subset of primary GBM samples and patient-derived GSCs. Furthermore, overexpression of SOX3 is accompanied by upregulation of the Hedgehog signaling pathway [123].

3.4. Role of microRNAs (miRNAs) in TMZ-resistance in GBM

miRNAs belong to the class of small single-stranded non-coding RNAs (~21-25 nucleotides) synthesized through RNA polymerase II/III and regulate posttranscriptional expression of multiple genes [124]. miRNAs interact with the specific sites in the 3'-untranslated region (UTR) of the target mRNA and exert their effects by cleaving mRNA or translational repression of the target protein [124]. Depending on the type, miRNAs act as both tumor suppressors and oncogenic [125,126]. Alteration or overexpression of oncogenic miRNAs and downregulation of tumor-suppressing miRNAs triggers signaling that enhances chemotherapy resistance in GBM [127]. miRNAs also play a role in cell cycle regulation, proliferation, angiogenesis, invasion, and cancer stem cell behavior [128] (see Fig. 4). miRNAs also act as effective signaling molecules between cancer cells and the surrounding cells that make up the tumor microenvironment (TME) [129].

GBM cells transfer miRNAs via different mechanisms such as circulating cell-free miRNAs, gap junction-mediated intercellular transfer of miRNAs, and through extracellular membrane vesicles [125]. These miRNAs generate chemoresistance in GBM mainly by inhibition of PCD, upregulation of DNA damage repair pathways or MGMT upregulation, regulating cellular signaling pathways, increasing drug efflux, mediating epithelial interstitial transition, and via generation of drug resistance [10,11].

Numerous miRNAs are differentially expressed in the GBM [130]. Srinivasan et al. identified a panel of 10 signature miRNAs that segregate GBM patients in low and high-risk group patients. Among these 10-signature miRNAs; three (miRNA-20a, miRNA-106a and miRNA-17-5p) were tumor-suppressing whereas the other seven (miRNA-31, miRNA-222, miRNA-148a, miRNA-221, miRNA-146b, miRNA-200b and miRNA-193a) were oncogenic and directly linked to patient prognosis [131].

Higher levels of exosomal miRNA-1238 have been detected in glioma patient's serum compared to healthy individuals. Furthermore, miRNA-1238 levels inversely correlate with TMZ-sensitivity to glioma cells. Loss of miRNA-1238 increases TMZ-sensitivity in GBM cells by directly targeting the caveolin 1/ epidermal growth factor receptor (CAV1/EGFR) signaling pathway [132]. Circulating miRNA-128 is a potential diagnostic marker for TMZ sensitivity in GBM [133]. miRNA-128-3p expression is very low in gliomas; and its overexpression in glioma cells downregulates the expression of EMT proteins (c-met, PDGFRα, Notch1, and slug) and enhances the therapeutic benefits of TMZ [134]. Circulating miRNAs may provide support in the differentiation of primary GBM from low-grade gliomas and found a constant drop in the level of miRNA-497 and miRNA-125b in serum as to tumor stages to the lesser extent in GBM than lower grade glioma [135]. To the best of our knowledge, there is no reliable biomarker in GBM for liquid biopsy; circulating miRNAs have great potential to be developed into one.

It has been shown that transfer of miRNA-5096 from glioma cell to astrocytes occurs through gap junction and reprogram the astrocytes for their pro-invasive effect in a Cx43-dependent manner [136]. Exosomal or gap junction-mediated transfer of miRNA-9 confers chemoresistance in GBM. Whereas; gap junction mediated intercellular transfer of anti-miRNA-9 in resistant cells helps to overcome TMZ-resistance by

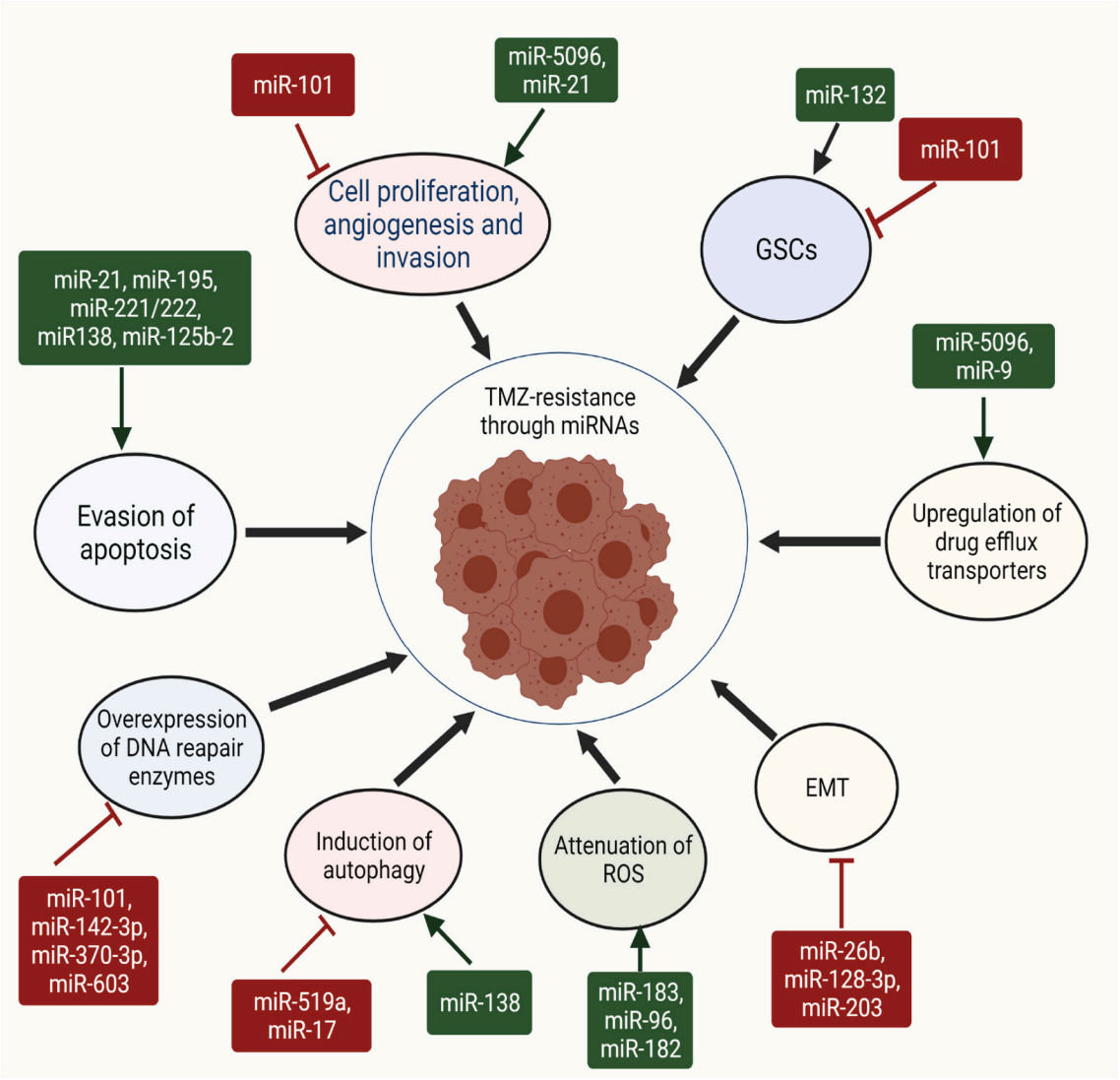

Fig. 4. A summary diagram represents role of miRNAs involved in TMZ-resistance in GBM. Red arrows represent tumor suppressor miRNAs and green arrows represent oncogenic miRNAs.

reducing the expression of MDR transporter and enhancing PCD [137]. A few studies have explored the role of gap junction-mediated intercellular miRNA transfer in chemoresistance in GBM, so further investigations are needed that improve our knowledge and may reveal unique therapeutic opportunities.

Several miRNAs acting as oncogenic miRNAs have been identified; miRNA-21 overexpression inhibits TMZ-induced apoptosis by reducing caspase-3 activity and Bax/Bcl-2 ratio [138]. Inhibition of miRNA-21 sensitizes glioma cells to TMZ by increasing the Bax /Bcl-2 ratio [139]. The overexpression of miRNA-21, miRNA-455-3p, miRNA-195, and miRNA-10a decreases chemosensitivity of GBM cells, whereas the inhibition of miRNA-195 enhances TMZ-induced cell death [140]. Dysfunctional cells are eliminated either via apoptosis or through autophagy; expectedly, oncogenic miRNA-21 and miRNA-221/222 target both the processes, autophagy, and apoptosis. miRNA-21 targets different components of p53, TGF-β, and the genes, responsible for mitochondria-mediated apoptotic pathways [141]. Whereas, downregulation of miRNA221/222 enhances TMZ efficacy and promote apoptosis independent of p53 status [142]. Oncogenic miRNA-335 targets disheveled-associated activator of morphogenesis 1 (Daam1) post-transcriptionally, Daam1 silencing reverse the oncogenic effects of miRNA-335 [143].

Furthermore, higher expression of miR-132 induces TMZ-resistance by promoting CSCs-like phenotype through downregulation of tumor suppressor candidate 3 (TUSC3) in GBM cells [144]. Higher miRNA-365 expression level has been reported in GSCs and TMZ-resistant glioma cells. In addition, long non-coding RNA (lncRNA) plasmacytoma variant translocation 1 (PVT1) regulates the stemness and TMZ efficacy of glioma cells through miR-365/ELF4/ SOX2 axis [145].

Many miRNAs are expressed at a lower rate in chemoresistant glioma and are functionally classified as tumor-suppressing miRNAs. An earlier study reported that downregulation of miRNA-101 significantly enhances TMZ-resistance and results in poor outcomes in GBM patients. Conversely, upregulation of miRNA-101 overcomes TMZ-resistance in glioma cells through downregulation of GSK3β and repression of MGMT through its promoter methylation [146]. Lower expression of miRNA-142-3p, miRNA-370-3p, miRNA-603, and miRNA-181d have been demonstrated in TMZ-resistant glioma, and their levels are inversely related to MGMT expression [147–149]. MiR-142-3p and miRNA-603 post-transcriptionally inhibit MGMT expression whereas transfection of miRNA-142-3p sensitizes GBM cells to TMZ [148,149]. Furthermore, delivery or overexpression of miRNA-370-3p enhances TMZ sensitivity of tumor cells by reducing the auto repairability of neoplasm DNA [147]. Recently it has been demonstrated that miRNA-130a-3p inhibits

cellular proliferation, invasion, and TMZ-resistance in GBM cells via targeting specificity protein 1 (SP1) that promotes metastasis in GBM [150].

The above study illustrates the crucial role of dysregulated miRNAs expression in regulating the chemosensitivity of glioma cells but also sensitizes them to targeted therapies. Further studies need to be performed on larger cohorts to identify miRNAs exhibiting higher sensitivity and specificity as a promising diagnostic biomarker for chemoresistant GBM. Additionally, the combination of conventional chemotherapy along with miRNA-targeted therapy has the potential to intensify therapeutic benefits, especially in TMZ-resistant GBM patients.

Here (Table 1), we illustrate the role of different miRNAs that promote resistance and sensitize the cells to TMZ therapy response.

Table 1
List of different miRNAs that regulates TMZ therapy response.

MicroRNAs (miRNAs)	Expression status	Role in TMZ therapy response
miRNA-21	Upregulated	Inhibits TMZ-induced apoptosis via reduction of Bax/Bcl-2 ratio and caspase 3 activity. miRNA-21 inhibition sensitizes resistant cells to TMZ [138,193].
miRNA-101	Downregulated	Reverse TMZ-resistance by GSK3β inhibition [146].
miRNA-1238	Upregulated	Loss of miRNA-1238 increases TMZ sensitivity by targeting CAV1/EGFR signaling pathway [132].
miRNA-128-3p	Downregulated	Regulate epithelial to mesenchymal transition [134].
miRNA195, miR-455-3p and miRNA-10a	Upregulated	Acquired TMZ-resistance in MGMT deficient cell lines and knockdown shows cell killing effect [140].
miRNA-9	Upregulated	Silencing of miRNA-9 downregulated the expression of MDR transpoter and promote apoptosis [194].
miRNA-181b	Downregulated	Reduces neurosphere formation and chemoresistance [195].
miRNA-125b-2	Upregulated	Inhibition increased expression of proapoptotic protein Bax in GSCs [196].
miRNA-145	Downregulated	Decreases the level of OCT-4 and SOX-2 and alone suppressed tumorigenesis with stemness [197].
miRNA-211	Downregulated	MMP-9 mediated regulation of miR-211 lead to the activation of the intrinsic mitochondrial/Caspase-9/3 mediated apoptosis in both glioma and GSCs [198].
miRNA-17		miRNA-17 incorporation modulates autophagy by regulating expression of ATG7 and LC3B gene [199].
miRNA-9	Upregulated	Involved in overexpression of drug efflux transporter, P-glycoprotein. Delivary of Anti-miRNA-9 reverse chemoresistance in GBM cells [200].
miRNA-183/96/182	Upregulated	Overexpression of miRNAs induced ROS mediated apoptosis [201].
miRNA-221/222	Upregulated	Downregulation or knockdown of miRNA-221/222 sensitize glioma cells to TMZ by regulating apoptosis [142].
miRNA-132	Upregulated	Generate GSCs and TMZ-resistance by downregulating TUSC3 in glioma [144].
miRNA-142-3p/370-3p/603/181d	Downregulated	Promote TMZ-resistance by enhancing MGMT mediated direct repair [147–149].
miRNA-519a	Downregulated	miRNA-519a have inverse relation to chemosensitivity of GBM cells and enhance PCD by targeting STAT3/Bcl-2/Beclin-1 pathway [202].
miRNA-26a	Upregulated	miRNA-26a reduces the expression of AP-2α that have tumor suppressive role. Inhibitor of miRNA-26a reduces tumor stemness and TMZ resistance [203].

3.5. Drug efflux transporters

Efflux of cytotoxic drugs or anticancer agents is a prominent way of multidrug resistance generation in cancerous cells. Transporters of ATP-binding cassette (ABC) superfamily mainly participate in this mechanism [151]. ABC transporters are ATP-driven efflux pumps that pump multiple molecules across the plasma membrane [151]. Overexpression of ABCB1, ABCE1, or MDR1 transporter protein associated with TMZ-resistance has been reported in gliomas [33]. Modulation of ABCB1 and ABCG2 through leucine-rich repeats and immunoglobulin-like domains 1 (LRIG1) improves glioma sensitivity towards TMZ [152]. Repression of ABCE1 increased the efficacy of TMZ on glioma cells both in vitro and in vivo [153]. The above studies suggest that p-glycoprotein and ABC transporters may serve as potential targets that can help in TMZ potency boost up [154].

3.6. Other molecular alterations in TMZ resistant glioma

The new WHO update on classification of CNS tumor (2016) is based on molecular alterations such as isocitrate dehydrogenase (IDH) 1 and 2 genes mutation, loss of ATRX, mutant TP53/p53, and 1p/19q co-deletion [155–157]. These all are molecular biomarkers beyond tumor morphology for the refinement of CNS tumor grading. In addition, *tumor* cells can have pre-existing *genetic* alterations that confer complexity in GBM treatment and resistance to TMZ [158]. The emerging molecular targets that aid resistance in GBM and have potential value in the diagnosis and treatment of GBM patients are summarised below (Table 2).

3.6.1. Role of IDH mutation in tumor grading and TMZ response

IDH converts Isocitrate into α-Ketoglutarate (αKG), NADP into NADPH, and releases CO_2. IDH1 and 2 are key enzymes linking cellular metabolism to epigenetic regulation and redox states [159]. IDH1 and IDH2 genes are present on chromosomes 2q33 and 15q26.1 respectively [160]. Somatic mutations are the most frequently present in IDH1 gene (75-80%) as compared to the IDH2 gene (20%) in glioma [161]. According to WHO reports, IDH mutation was recognized in more than 80% of grade II and III gliomas. IDH mutation is also present in grade IV GBM, but frequently in secondary GBM (73%) but significantly less in primary GBM (3.7%) [162].

Earlier studies had revealed that that the patients with IDH1 or IDH2

Table 2
Molecular alterations in TMZ resistant glioma.

Gene	Model of study	Role in TMZ-resistance
Isocitrate dehydrogenases (IDHs)	U-87 GBM cell line	Overexpression of IDH1 generates resistance in glioma cells and mutant IDH1 enhanced cells sensitivity to TMZ [164].
TP53/p53	PRT-HU2, U-87MG and T98G	MGMT expression was negatively regulated by p53 in GBM. p53 reactivation may be an effective strategy to overcome TMZ resistance [204].
TERT promoter mutation	U373-MG and U87-MG	Lower activity of telomerase is directly linked with TMZ sensitivity of glioma [205].
EGFR	U87MG and U251	EGFR/EGFRvIII overexpression generates GSCs, tumorigenesis, tumor-recurrence and resistance to chemotherapy. Upregulation of HER2/3 and RTK pathway limiting effect of anti-EGFR therapies [206].
ATRX	LN 229	Upregulation of ATRX expression intensify DNA repair through fortify PARP1 protein. ATRX prompt PARP1 stabilization through FADD suppression via H3K27 methylation and generate TMZ-resistance [207].

mutant glioma had significantly higher OS than the patients with wild-type IDH [163]. However, in vivo and in vitro studies show that over-expression of IDH1 confer TMZ-resistance in glioma cells [164]. Whereas IDH1 mutation induces chemosensitivity, cell cycle arrest at the G1 phase, and inhibits cellular proliferation and invasion [164]. mIDH patients treated with TMZ + RT or RT alone exhibit better OS than wtIDH [165]. Additionally, the introduction of mIDH1/2 or silencing of mtIDH1/2 sensitizes cancer cells to chemotherapy or RT and improves therapy response [159]. A recent study showed that IDH1/2 is an independent prognostic factor, but the combination of IDH1/2 mutation along with MGMT promoter methylation show increased OS in patients with malignant glioma on TMZ treatment [166]. IDH maintains cellular redox status by regulating the balance of NADPH/NAPD+. wtIDH produces NADPH that protects cells from ROS-induced by TMZ, whereas mIDH is associated with an elevated level of ROS [167]. Therefore, IDH mutation is a significant marker of TMZ response in secondary GBM.

4. Strategies to overcome TMZ-resistance in GBM

4.1. Inhibition of MGMT

As discussed earlier MGMT undo alkylation of DNA that has been triggered by TMZ and restore the normal DNA. MGMT-mediated repair of damaged DNA generates resistance to TMZ in gliomas [168]. In previous studies, several pathways have been identified that play essential roles in MGMT expression regulation. The Wnt/β-catenin signaling regulates MGMT expression, and cordycepin-mediated downregulation of Wnt/β catenin signaling subsequently suppresses MGMT expression, thereby augmenting chemosensitivity of glioma cells towards TMZ [169]. BMX, an HDAC8 inhibitor overcomes TMZ-resistance in GBM cells that have wild type p53 by enhancing cell death via downregulation of β catenin/c-Myc/SOX2 cellular signaling and MGMT inhibition [170]. In addition, nuclear factor 1A (NFIA) aids TMZ-resistance in GBM through nuclear factor-κB (NF-κB) signaling and contributes to the poor prognosis and recurrence of GBM patients [171]. Blocking of NF-κB has anti-glioma outcomes and reduces TMZ-resistance by decreasing MGMT gene expression [172]. Resveratrol overcomes TMZ-resistance by suppression of MGMT in glioma cell lines through NF-κB signaling [173]. These results indicate that NFIA-NF-κB axis might be a novel therapeutic approach to TMZ-resistant GBM. Dysregulation of IKBKE enhances resistance to TMZ through overexpression of MGMT in GBM [174]. *S*-nitroso-*N*-acetyl penicillamine (SNAP), a nitric oxide donor reverses TMZ-resistance by downregulating MGMT expression [175]. Furthermore, the resistance is also overcome by silencing of SATB1 by decreasing MGMT expression and overexpression of solute carrier family 22 member 18 (SLC22A18) in human GBM cells [176].

4.2. Modulation of BER

BER modulation *potentially enhanced* chemosensitivity and pathological *outcomes* in glioma [177].

4.2.1. PARP inhibition

Inhibition of PARP enhances the therapeutic benefits of TMZ in the occurrence of malignant gliomas, especially in tumors with DNA mismatch [178]. The combination of PARPi (PARP inhibitors) and TMZ shows synergistic anti-neoplastic outcomes in 8 out of 10 GSCs tested. Consequently, TMZ dose reduction is associated with PARPi susceptibility of cell lines. Notably, a *combination* of *PARPi* with *TMZ* may be considered as a beneficial treatment strategy for the reversal of chemoresistance in glioma [57]. Furthermore, PARP inhibition may be a promising therapy target in MMR-deficient resistant cells and restore TMZ-sensitivity in MSH6-inactivated glioma [179]. Mechanistically, this PARPi-induced synthetic phenotype was unconventional of BER blockage and not recapitulated by loss of PARP1 [179].

4.2.2. Inhibition of APE-1

APE1 or Ref-1 is a crucial mammalian apurinic/apyrimidinic (AP) endonuclease, impart resistance against TMZ in glioma by incising DNA at abasic sites [180]. Overexpression or anomalous subcellular dispersal of APE1 observed in different types of cancer corresponds to drug response, patient survival, and prognosis [181]. The activity of the APE-1 enzyme significantly contributes to TMZ-resistant gliomas [46,182]. Cut Like homeobox 1 (CUX1) triggers activity of APE1 enzyme and induce resistance to TMZ in GBM cells [183]. Knockdown/silencing of APE1 is strongly linked with decreased growth as well as invasion of resistant cells. APE1 suppression has a limited cytotoxic effect on TMZ-sensitive cell lines as compared to resistant cell lines. APE1 may be the potential target to alter the response of TMZ in resistant GBM cells. Besides chemoresistance, APE1 protein also participates in modifying radiation tolerance and generate radio-resistance in glioma cells [184]. The above studies suggest APE1 act as a potential drug target and have therapeutic benefits in glioma patients.

4.3. Combination therapy to overcome TMZ-resistance

Nowadays, the use of TMZ in combination with other anti-neoplastic agents/ therapeutics has become the primary strategy to treat resistant glioma [185]. The rationale of combination therapy is to use drugs that target key pathways through separate mechanisms, thereby decreasing drug-resistant cancer cells [186]. Importantly, when drugs are used in combination, each drug is used at its optimum dose because it may be toxic to patients with intolerable side effects [187]. *Combination* treatment with *TMZ has resulted in prolonging the survival of GBM patients* [188]. *Here, we enumerate different drugs that are used to improve the OS of GBM patients with TMZ resistant neoplasm (Table 3).*

4.4. Imidazotetrazine analogs

As discussed earlier, TMZ is an imidazotetrazine prodrug; however, resistance against TMZ limits efficacy and therapeutic benefits [189]. TMZ-resistance is mainly conferred by MGMT overexpression, which intensifies tolerance to O^6-methylguanine lesions and limits the clinical application of TMZ [190]. Therefore, the advancement in TMZ analogs is urgently needed to conquer TMZ-resistance. TMZ analogs C^8-imidazolyl and C^8-methylimidazole tetrazines induce anti-neoplastic actions in T98G glioma cell lines that hyperactivate MGMT and promote arrest of cell cycle progression, DNA breakage, and cell death [191]. C8 analogs have similar actions like TMZ and methylate DNA adducts, but unlike TMZ; MGMT cannot remove incorporated lesions [191].

Novel TMZ analog DP68 and DP86 are designed to conquer resistance in glioma cell lines and primary culture models [192]. The potency of these syntheses is unstrained from MGMT and MMR activities. DP68 cross-links DNA and induces cell cycle arrest with the convergence of cells in the S phase [192]. Besides, DP68 give rise to intense DNA damage response through phosphorylating ATM (a serine/ threonine kinase activated when double-strand DNA damage occurs), KAP1, Chk1, and Chk2 kinase (regulators of the cell cycle), and histone variant H2AX (phosphorylated on serine residue at the time of DNA double-strand break). Blocking expression of FANCD2 (Fanconi anemia group D2 protein) or ATR kinase action increased the anti-neoplastic outcomes of DP68 [192].

5. Conclusion

TMZ induces cell death through DNA damage and have been a mainstay drug to treat glioma patients. Resistance to TMZ in glioma involves multiple molecular pathways and constitute a multifaceted challenge. Elucidating the different mechanisms of TMZ-resistance by using Patient derived GBM xenograft in animal models and the tissue samples of TMZ resistant glioma can provide significant insight into underlying mechanisms of TMZ resistance. Sustained research on how

Table 3
Combination therapy used in TMZ resistant glioma treatment.

Drug	Model of Study	Mechanism of action
Metformin	T98G / *Invivo* mice model	Suppress the expression of GSCs marker CD90 and significantly reduce the growth of TMZ resistant tumor in mice model [208].
Rapamycin	Gli36, U87MG Xenograft of GBM6 and GBM10	Rapamycin inhibits mTOR signaling [209].
Isofuranodiene	U87, T98, U251	Induced cell cycle arrest and necrosis through increasing ROS level [210].
Psammaplin C	Primary human GBM cells (CV17, 010627, No3)	Potent inhibitor of carbonic anhydrase XII and helps to overcome P-glycoprotein mediated TMZ-resistance [211].
Chloroquine	U87MG and U373	Promotes TMZ induced autophagy, activation of caspase 3/ p53 dependent apoptosis and induces cell death by inhibiting mitochondrial autophagy via increasing ROS level [212–214].
Lovastatin	U87 and U251	Impairment of autophagic flux induces apoptosis [215].
Cordycepin	LN18, T98G, U87, U251 and rat C6 cells.	Enhance chemosensitivity to TMZ by upregulating AMPK signaling and slowdown the AKT Signaling [216].
Romidepsin	T98G, U-138MG, A-172 and U87MG Human glioma cell line	Inhibiting PI3K/AKT/mTOR signaling pathways [217].
Cannabinoids	U87MG, A172, SW1783, U373MG, T98G, SW1088, and LN405	Enhance Autophagy [218].
Tamoxifen	U87, U118	Inhibit PKC-pan and promote antiproliferative and apoptotic effects [219].
Levetiracetam	Neurospheres of GBM and peritumoral tissue	MGMT suppression, upregulating nuclear transport of HDAC4 and promote apoptosis [220].
Difluoromethyl-ornithine	U87G, U251MG and T98G GBM cell line	Cell cycle arrest at G2/M phase [221].
Afatinib	U87MG and U251	Down regulates cellular multiplication by repressing EGFRvIII/AKT/EGFRvIII/JAK2/ STAT3 and FAK (Focal adhesion kinase) pathways [206].
Hypericin	A172 and LA567	Inhibited tumor growth by inducing apoptosis [222].
BIX01294	LN18 and U251 glioma cell lines	Inhibitor of histone methyltransferase G9a, increased the TMZ sensitivity in glioma cell and GSCs [223].

these functions are regulated in TMZ-resistance can provide the therapeutic window to effectively manage the TMZ-resistant gliomas. Therefore, continued research in the area of functional regulation of TMZ resistance can be the basis of custom-tailored therapeutics to treat recalcitrant glioma and enhance patient's OS.

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Author's contributions

AS and MST conceptualized the review manuscript. AS, MST and AK wrote and evaluated the manuscript. CS evaluated the manuscript. All authors concur to the final version of the manuscript.

Declaration of Competing Interest

Authors declare no conflict of interest.

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