Modulating glioblastoma chemotherapy response: Evaluating long non-coding RNA effects on DNA damage response, glioma stem cell function, and hypoxic processes

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Abstract
Glioblastoma (GBM) is the most common and aggressive primary adult brain tumor, with an estimated annual incidence of 17 000 new cases in the United States. Current treatments for GBM include chemotherapy, surgical resection, radiation therapy, and antiangiogenic therapy. However, despite the various therapeutic options, the 5-year survival rate remains at a dismal 5%. Temozolomide (TMZ) is the first-line chemotherapy drug for GBM; however, poor TMZ response is one of the main contributors to the dismal prognosis. Long non-coding RNAs (lncRNAs) are nonprotein coding transcripts greater than 200 nucleotides that have been implicated to mediate various GBM pathologies, including chemoresistance. In this review, we aim to frame the TMZ response in GBM via exploration of the lncRNAs mediating three major mechanisms of TMZ resistance: (1) regulation of the DNA damage response, (2) maintenance of glioma stem cell identity, and (3) exploitation of hypoxia-associated responses.

Keywords
chemo-response | glioblastoma | long non-coding RNAs | Temozolomide.
LncRNAs are nonprotein coding transcripts longer than 200 nucleotides that have emerged as critical regulators of gene expression under normal physiology as well as disease states. Studies have implicated lncRNAs in contributing to GBM pathogenesis by regulating gene expression at the epigenetic, transcriptional, and posttranscriptional levels. For instance, TUG1 is one of many lncRNAs that interacts with chromatin-modifying proteins responsible for histone methylations of genes critical for GBM progression. At the posttranscriptional level, many lncRNAs behave as competing endogenous RNAs that act as molecular sponges for micro-RNAs (miRNAs). These interactions disinhibit the miRNA’s downstream pathways and proteins, such as the Notch pathway and the VEGFA axis that promote glioma cell proliferation and angiogenesis, respectively.

In the past few years, studies have explored the association between IncRNAs and TMZ response in GBM. The rising number of IncRNAs discovered may offer novel insights into GBM resistance to TMZ and represent potential therapeutic targets. Notably, while other reviews have discussed chevlo-response in the context of IncRNAs, most prior reviews have focused on collections of well-known IncRNAs associated with chemosensitivity. Furthermore, prior reviews have also primarily concentrated on common IncRNAs noted to have chemoresistance roles without extensive exploration of chemosensitivity of specific IncRNAs. In contrast, this review aims to frame the TMZ response in GBM via exploration of the IncRNAs mediating the three major mechanisms of TMZ resistance: (1) regulation of the DDR, (2) maintenance of glioma stem cell (GSC) identity, and (3) exploitation of hypoxia-associated responses (Table 1).

LncRNAs and the DDR

The DDR is a network of cellular processes that identifies and repairs DNA damage to prevent mutations and other genetic aberrations that threaten cell viability. Direct DNA-lesion reversal, MMR, base excision repair (BER), DNA DSB, and single-strand breaks (SSBs) repair are all components of the DDR. Dysregulated DDR pathways lead to the accumulation of DNA damage, evasion of cell cycle arrest, and chemoresistance. DSBs have an important role in mediating TMZ-induced apoptosis by triggering DNA damage kinase, ATM serine/threonine kinase, which then activates the p53-driven apoptosis pathway. In addition to O6-methylguanine, TMZ also forms N7-methyladenine and N7-methyladenine that are recognized and repaired by the BER pathway, specifically by N-methylpurine DNA glycosylase and DNA polymerase β. The abasic sites generated from the DNA glycosylase in the BER pathway are inherently unstable and readily convert into DNA SSBs; unrepaired SSBs can collapse the DNA replication pathway resulting in cell death (Figure 1B).

LncRNAs have been identified to utilize the DDR pathways as a method of modulating treatment resistance. SBF2-AS1, which is upregulated in TMZ-resistant GBM cells, functions as a ceRNA against miR-151a-3p to enhance X-ray repair cross-complementing 4 (XRCC4) protein levels, augmenting DSB repair in gliomas. Radiation-induced DNA damage can similarly activate lncRNAs. HMMR-AS1 was found to be elevated after irradiation along with increased expression of DDR proteins ATM, RAD51, and BMI1, ultimately promoting glioma cell growth. Below, we will describe in further detail on how lncRNAs regulate TMZ resistance through direct DNA-lesion reversal and MMR.

LncRNA and MGMT

MGMT is a DNA methyltransferase that augments TMZ resistance by removing TMZ-induced alkyl groups and preventing the mispairing between O6-methylguanine and thymine (Figure 1B). As part of the direct DNA-lesion reversal process, MGMT expression is induced with alkylating agents and serves as an important prognostic marker.

Clinical trials have demonstrated that MGMT promoter CpG methylation is associated with significantly improved overall survival in primary and recurrent GBM patients.

However, even though clinical data on direct MGMT inactivators, such as O6-benzylguanine and O6-(4-bromoethyl) guanine (O6-BG), showed improved survival in patients simultaneously treated with TMZ, TMZ was associated with increased hematopoietic toxicity due to nonspecific targeting, indicating that additional, more precise therapies targeting MGMT are required. Because lncRNAs are highly cell type-specific, targeting lncRNAs as a method of regulating MGMT expressions may represent promising alternatives. Here, we summarize the data on lncRNA-mediated epigenetic regulation of MGMT and the effect on TMZ resistance in GBM.

**Lnc-TALC.**—TMZ-associated IncRNA in GBM recurrence (lnc-TALC) is a newly identified IncRNA highly expressed in patient-derived TMZ-resistant GBM tissues. Clinically, GBM patients with lower lnc-TALC expression exhibit a significantly better response to TMZ with a lower likelihood of tumor recurrence. To determine the association between lnc-TAC and TMZ-resistance, Wu et al. conducted clustered regularly interspaced short palindromic repeats (CRISPR)-induced lnc-TAC knockdown in TMZ-resistant human GBM cell lines, LN229R and U251R, and patient-derived GBM cells. The cells with decreased lnc-TALC had reduced MGMT mRNA and protein levels and increased TMZ-induced apoptosis. LN229R cells with lnc-TAC knockdown were then implanted in TMZ-treated mice; these mice showed significantly longer survival and smaller tumors when compared to mice with LN229R xenografts. This suggested that decreased lnc-TALC was associated with restored TMZ sensitivity in GBM cells. Additional in vitro experiments were conducted to delineate the mechanism linking higher lnc-TALC expression with TMZ resistance. A luciferase bioluminescent reporter assay confirmed that lnc-TALC functions as a ceRNA against miR-20b-3p to indirectly promote MGMT expression. Through sequestration of miR-20b-3p, lnc-TALC upregulates c-MET and its downstream target Stat3.

Protein coimmunoprecipitation analysis subsequently demonstrated that Stat3 binds to a histone acetyltransferase to increase the acetylation activity of the MGMT promoter, effectively increasing MGMT expression.
FOXD2-AS1.—FoxD2 adjacent opposite strand RNA 1 (FOXD2-AS1) is a lncRNA highly expressed in recurrent GBM tumors and is associated with decreased patient survival.45 A study showed that suppression of FOXD2-AS1 via a small interfering RNA (siRNA) is associated with decreased GBM cell viability in TMZ.16 While the specific mechanism is unclear, the study reported that FOXD2-AS1 may oppose TMZ cytotoxicity through MGMT upregulation. siRNA-induced suppression of FOXD2-AS1 (si-FOXD2-AS1) in human GBM cell lines, U251 and A172, is associated with hypermethylation of the MGMT promoter, resulting in decreased MGMT mRNA and protein levels. Furthermore,
### Table 1. Summary of Mechanisms on LncRNA and TMZ Response

| Mechanism | LncRNA | Expression Level in TMZ Resistant GBM | Sample Used | Summary of Mechanism on TMZ Response | ceRNA | References |
|-----------|--------|--------------------------------------|-------------|--------------------------------------|-------|------------|
| I. DNA damage response | | | | | | |
| MGMT | Lnc-TALC | Upregulated | Patient samples and LN229, U251 | Increases MGMT expression through regulating c-met and stat3 | miR-20b-3p | 15 |
| FOXD2-AS1 | | Upregulated | U251 and A172 | Increases MGMT expression | N/a | 16 |
| | | Upregulated | U87, U251, LN229, A172 | Increases CPEB4 (unrelated to MGMT) | miR-98-5p | 17 |
| UCA1 | | Upregulated | Patient samples and A172, T98G, SHG44 | Increases MGMT Expression | miR-182-5p | 18 |
| MMR | XIST | Upregulated | Patient samples and U251, U373, LN229, U118, LN229 | Increases MSH6 indirectly through SP1, leading to abnormal MMR activity | miR-29c | 19 |
| DSB | SBF2-AS1 | Upregulated | U87, LN229, A172, T98, U251 | Enhances DSB repair by increasing XRCC4 | miR-151a-3p | 20 |
| II. Glioma stem cells | TP73-AS1 | Upregulated | G26 and G7 glioma stem cell lines | Highly upregulated in GSC and may mediate its effect through regulating ALDH1A1 | N/A | 21 |
| | SOX2OT | Upregulated | Patient samples and U87, U251 | Knockdown decreases SOX2 expression through methylating its promoter | let7g-5p | 22 |
| | NEAT1 | Upregulated | Patient samples and U87, U251 | Highly upregulated in GSCs and activates MAP3K1 | N/A | 23 |
| | | Upregulated | Patient samples | Activated by HMGB1 as part of the Wnt Pathway | | 24 |
| | Linc00174 | Upregulated | Patient samples and LN229, SHG44, U118, U251, U87 | Maintains SOX9 expressions to enhance GSC-like behavior | miR-138-5p | 25 |
| | BC200 | Upregulated | Patient samples, T98G, U87 | Overexpression promoted GSC behaviors and TMZ resistance | miR218-5p | 26 |
| III. Hypoxia | MALAT1 | Upregulated | U251, U87 | Knockdown decreases ZEB1 | N/A | 27 |
| | | Upregulated | U251 | Increases GSK3β, MGMT (unrelated to EMT) | miR-101 | 28 |
| | | Upregulated | Patient samples, U87, U251 | Increases thymidylate synthase (unrelated to EMT) | miR-203 | 29 |
| | RP11-838N2.4 | Downregulated | Patient samples and U87, U251 | Knockdown is associated with downregulation of EphA8 | miR-10a | 30 |
| | H19 | Upregulated | U251, M059J | Stimulates the Wnt/beta-catenin pathway | N/A | 31 |
| | | U251, LN229 | Stimulates the NF-kB pathway | N/A | 32 |
| Metabolism | HOTAIR | Upregulated | Patient samples and U87, A172 | Knockdown suppresses HK2 and increases TMZ-induced apoptosis | miR-125 | 33 |
| | | Patient samples and serum | Increases EVA1 expression indirectly by inhibiting miR-256b-3p | miR-526b-3p | 34 |
the si-FOX2-AS1 GBM cells showed decreased proliferation and increased cell death when treated with TMZ. This short and straightforward study indicated that FOX2-AS1 downregulation promotes TMZ sensitivity in GBM cell lines by decreasing MGMT expression. 16

FOX2-AS1 also drives TMZ resistance as part of the FOX2-AS1/miR-98-5p/CPEB4 axis unrelated to MGMT regulation. A separate study found that FOX2-AS1 knockdown inhibited cell proliferation and promoted TMZ-induced apoptosis. RNA pull-down assay indicated that FOX2-AS1 serves as a ceRNA to adsorb miR-98-5p. Computational analysis showed that miR-98-5p and cytoplasmic polyadenylation element binding (CPEB4) shared binding sites. An oncogenic RNA binding protein, CPEB4 is associated with glioma migration, growth, and vascularization. The current study showed that expression of CPEB4 was inhibited by knockdown of FOX2-AS1 or upregulation of miR-98-5p, leading to decreased apoptosis and increased survival in vitro.17

UCA1.—A recently published paper by Cheng et al. demonstrated that the urothelial cancer-associated 1 (UCA1) lncRNA modulates TMZ response through MGMT. Identified as an oncogene, UCA1 expression was directly correlated to glioma grade and MGMT expression. Transfection of GBM cells, A172 and SHG44, with si-UCA1 showed decreased glioma cell viability with increased cell apoptosis. The si-UCA1 cells also exhibited decreased MGMT protein level. In vivo experiments showed that the tumors with smallest volume and weight were from TMZ-treated mice implanted with si-UCA1 GBM cells. The authors also identified miR-182-5p as a target of UCA1, forming the UCA1/miR-182-5p/MGMT axis that modulates MGMT expression and TMZ sensitivity. 18

LncRNA and MMR

MMR is an important postreplication mechanism that recognizes and repairs nucleotide mismatches. In TMZ-treated cells, MMR is activated to excise the mismatched thymine. Because O6-methylguanine remains on the template strand, this leads to futile cycles of thymine mispairing and excising that eventually results in DNA DSBs and cell death.46

Deficiencies in MMR render tumors resistant to TMZ, regardless of the MGMT methylation status.7 Aberrations in MMR proteins, such as melanocyte-stimulating hormone 2, MSH6, and mutL homolog 1, or defective DSB repairs can lead to suppressed apoptotic response and increased survival.46–49 Furthermore, while MSH6 mutations were not observed in pre-TMZ treated GBM tissues, 26% of recurrent tumors post-TMZ treatment had decreased MSH6 expression, suggesting that loss of MSH6 function is associated with tumor recurrence during TMZ treatment.46 Currently, the relationship between lncRNAs and MMR is considerably understudied and deserves additional extensive investigations. Thus far, only the lncRNA X-inactive specific transcripts (XIST) has been identified to alter the TMZ response in GBM through modulating the MMR.
**XIST.**—*XIST* is a well-characterized IncRNA in various cancers, including GBM. Paired analysis between patient-derived GBM and peritumoral brain tissues has revealed *XIST* to be significantly higher in the former. More importantly, *XIST* is directly associated with larger tumor size and overall shorter survival period. Human GBM cells transfected with a siRNA targeting *XIST* displayed diminished proliferation rate and increased sensitivity to TMZ as demonstrated by the BrdU and viability assay, respectively. RNA immunoprecipitation further showed that *XIST* affects the TMZ response by directly binding *miR-29c* and inhibiting its expression. This interaction results in upregulation of specificity protein 1 (SP1), a transcription factor that causes aberrant expression of the MMR-associated protein, MSH6. Taken together, the result suggests GBM cells with elevated *XIST* may exhibit decreased TMZ sensitivity due to the abnormal MSH6 expression.

**LncRNAs and GSCs**

GBM harbors a subset of self-renewing population of cells termed GSCs that have been hypothesized to play a key role in GBM pathogenesis. As TMZ preferentially targets proliferating glioma cells, the relatively quiescent GSCs are favored to survive and increase the likelihood of GBM recurrence. Notably, xenograft mouse models of human GBM cells have demonstrated that post-TMZ administration, the majority of residual, treatment-resistant tumor cells were fluorescently labeled GSCs with the ability to reinitiate GBM. In addition, CD133+ GSCs exhibit higher levels of MGMT mRNA and lower expressions of autophagy- and apoptotic-related proteins that allow them to evade conventional therapies (Figure 2).

Previous literature has illustrated that irregular expression of specific lncRNAs are closely related to the malignant phenotypes of GSCs. For instance, lncRNAs *XIST*, *H19*, and *MIAT* interact with transcription factors that promote and maintain GSCs in vitro. High throughput microarray profiling studies in GSCs have further identified differential expressions of specific lncRNAs to be associated with GSC behaviors, such as neurosphere formation and therapy resistance. GSC-associated lncRNAs represent an innovative strategy for targeting GSCs. One study has identified 1545 unique lncRNAs found to regulate GSCs’ differentiation status; the lncRNAs identified are highly specific, with the ability to promote differentiation in GSCs and decrease the tumor cells’ tumorigenicity. Studies described below have investigated how different lncRNAs affect the TMZ response through regulating GSC-like behaviors.

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**Figure 2.** Diagram of the role of IncRNA in TMZ resistance through GSCs. As TMZ preferentially targets rapidly dividing glioma cells, the relatively quiescent glioma stem cells are more resistant to TMZ and are associated with GBM recurrence. LncRNAs, including *TP73-AS1* and *SOX2OT*, have been found to have a role in the promotion and maintenance of GSCs.
**TP73-AS1**

In GBM, IncRNA P73 antisense RNA 1T (TP73-AS1) expression level is elevated in patient-derived GSCs and correlates directly with early mortality. Mazor et al. used CRISPR to downregulate TP73-AS1 in two established human GSC lines and noted that the cells exhibited decreased viability under TMZ treatment. Further elucidating the mechanism underlying TP73-AS1-associated TMZ resistance, transcriptome analysis of the TP73-AS1-attenuated GSCs revealed perturbations of neuronal differentiation and metabolic reprogramming pathways. One of the transcripts found to be particularly downregulated was aldehyde dehydrogenase 1 family member A1 (ALDH1A1). ALDH1A1 is recognized as a marker of GSCs and a promoter of clonogenicity; its expression is also elevated in recurrent, TMZ-resistant GBMs. Moreover, treating GSCs with a pharmacological ALDH1A1 inhibitor significantly increased their sensitivity to TMZ, suggesting that TP73-AS1 may mediate its effects on TMZ resistance through regulating ALDH1A1.

**SOX2OT**

LncRNA SOX2 overlapping transcript (SOX2OT) is mapped to the human chromosome 3q26.3 and harbors the SRY-Box Transcription Factor 2 (SOX2) gene in its intronic region. Analysis of patient-derived GBM samples has shown SOX2OT level to positively correlate with both advanced glioma grade and reduced median survival time. In vitro studies showed that GBM cells U87 and U251 transfected with SOX2OT siRNA lentiviral vectors (si-SOX2OT) displayed increased TMZ-mediated apoptosis. As IncRNAs can exert cellular functions through regulating proximal genes, the authors predicted SOX2OT modulates the TMZ response in GBM through upregulating SOX2. This was confirmed in si-SOX2OT U87 and U251 cells demonstrating significantly decreased SOX2 mRNA and protein levels. By de-differentiating tumor cells to GSCs, SOX2 is one of the four core transcription factors essential for GBM propagation. Downregulation of SOX2 interferes with GSCs’ ability to self-renew and consequently, to form gliomas. Importantly, SOX2 has previously been established to take part in mediating TMZ resistance in GBM. Mechanistically, SOX2OT maintains SOX2 expression by interacting directly with α-ketoglutarate-dependent dioxygenase alkB homolog 5, an RNA demethylase that binds to the SOX2 promoter and is similarly upregulated in TMZ-resistant GBMs.

**NEAT1**

The IncRNA nuclear paraspeckle assembly transcript 1 (NEAT1) is overexpressed in GBM tissues and is a marker of poor survival in primary GBM patients. NEAT1 is upregulated in GSCs and plays an important role in augmenting their proliferation rate and resistance to chemotherapy. Induced by high-mobility group box 1 in GBM cells treated with TMZ, NEAT1 has been shown to promote GSC formation through the Wnt/β-catenin pathway. In addition, a recent study showed that GSCs transfected with short hairpin RNA (shRNA) against NEAT1 (sh-NEAT1) are associated with significantly reduced viability and proliferation when exposed to TMZ in vitro. Using a bioluminescence assay, the authors identified let7g-5p as a downstream target of NEAT1. A prior study had demonstrated let7g-5p as a tumor-suppressive miRNA associated with impaired GSC behavior. Bioinformatic analysis predicted that let7g-5p shares a binding site with mitogen-activated protein kinase 1 (MAP3K1), a protein previously shown to correlate directly with shortened patient survival and increased TMZ resistance in GBM cells. Subsequent mRNA results showed that MAP3K1 expression was significantly increased in GBM cells transfected with let7g-5p shRNA. Through transcriptional and protein analyses, the authors concluded that NEAT1 suppresses let7g-5p to activate MAP3K1 and ultimately inhibit the cytotoxic effects of TMZ.

**LINC00174**

As a relatively newly discovered IncRNA, linc00174 is markedly upregulated in human GBM tissues and its expression level is inversely correlated with patient survival rate. Linc00174 has been reported to strengthen TMZ resistance in GBM cells by directly targeting miR-138-5p, a tumor suppressive miRNA shown to arrest GBM cell cycle. By inhibiting miR-138-5p, linc00174 increases SRY-Box Transcription Factor 9 (SOX9), a downstream target of miR-138-5p. In gliomas, SOX9 has been shown to maintain the self-renewal ability of GSCs. GBM cells transfected with shRNA-mediated linc00174 knockdown are associated with increased miR-138-5p and decreased SOX9 levels. In vitro, the transfected cells showed enriched apoptotic markers in addition to reduced viability and colony formation under TMZ exposure. The results indicated that inhibiting linc00174 improves TMZ-induced apoptosis by discouraging stem cell-like behaviors through regulating miR-138-5p and SOX9.

**BC200**

In GBM tissues, Brain Cytoplasmic 200 (BC200) expression is significantly higher than in normal tissues. In addition, BC200 was positively correlated with p53-mutation, indicating BC200 association with poor prognosis in patients. Knockdown of BC200 via siRNA suppressed common stem cell markers, including SOX2, and the self-renewal capacity of GBM cells. In contrast, GBM cells transfected with BC200 overexpression vector demonstrated increased cell wound healing migration, invasiveness, and colony-forming ability. The cells also exhibited decreased TMZ resistance via viability assay. Western blot analysis showed increased MGMT, multidrug resistance protein, and ABC transporter. To further assess the effect of BC200 on miRNA expression, bioinformatic analysis, and online database identified miR-218-5p level to be negatively associated with BC200. GBM cells with miR-218-5p inhibited showed higher TMZ resistance, stem cell markers, and colony-forming abilities. In vivo studies supported the in vitro experiments, demonstrating TMZ-treated mice...
implanted with siBC200 GBM cells had significantly reduced tumor size and prolonged overall survival.26

LncRNAs and Hypoxic Processes

The brain tumor microenvironment is composed of a heterogeneous population of cancerous as well as nonneoplastic cells, including the surrounding tissue stroma and associated vascular supply. Tumor stromal cells secrete various angiogenesis-promoting factors where vascular endothelial growth factor is the most potent and its high expression level indicates poor outcome.27 As a result of poorly functional blood vessels, tumors exhibit extensive hypoxia and limited response to systemic chemotherapeutics. The presence of hypoxia upregulates gene expressions that promote cell survival and chemoresistance.71 Furthermore, the hypoxic niche hosts GSCs that stimulate endothelial cell growth and proliferation associated with neoangiogenesis (Figure 3).73

Composed of several subunits (ie, HIF-1α, HIF-2α), hypoxia-inducible factor (HIF) is the main transcriptional regulator of cellular response to hypoxia. HIF overexpression promotes cancer angiogenesis, drug resistance, and certain IncRNAs.8 LncRNA AWPPH level correlates directly with HIF-1α and is associated with metastatic GBM patients. Silencing HIF-1α attenuated the enhanced glioma invasion and migration seen with elevated AWPPH.74 Consequently, the hypoxic microenvironment mediates two important processes implicated in the GBM TMZ resistance, the epithelial to mesenchymal transition (EMT) and metabolic alterations.

LncRNA and EMT

The EMT is a process that transforms tumor cells into a more mesenchymal phenotype with enhanced migratory and invasive properties. The hypoxic environment promotes EMT through triggering the HIF1α/ZEB1 signaling axis.75 Cells undergoing EMT display decreased expression level of epithelial genes (ie, E-cadherin, ZO-1) and increased expression of mesenchymal genes (ie, N-cadherin and vimentin). These alterations lead to changes in cell morphology and promote stem cell-like features.76 Though EMT is most often associated with GBM invasion, it has been increasingly recognized for its contribution to chemoresistance.

Several studies have shown that the relationship between specific EMT proteins and IncRNAs regulates the chemotherapy response in different cancers. For example, H19 and MALAT1 have been shown to modulate the EMT, while HOTAIR is shown here to inhibit HK2 activity in the HIF-1α pathway.

Figure 3. Diagram of the role of HOTAIR and other IncRNAs in TMZ resistance and hypoxia. Hypoxia encourages TMZ resistance through induction of EMT and activation of the HIFα pathway. H19 and MALAT1 have been shown to modulate the EMT, while HOTAIR is shown here to inhibit HK2 activity in the HIF-1α pathway.
in colorectal cancer, lncRNA SLC25A25-AS1 increases the chemo-response through regulating EMT-associated proteins. Similarly, lncRNA-ATB inhibits the EMT proteins, ZEB1 and ZEB2, to increase trastuzumab resistance in breast cancer cells. Although the association between EMT and chemo-response necessitates more extensive investigation, understanding their relation to specific lncRNAs provides additional therapeutic avenues for patients with GBM.

MALAT1. In GBM, lncRNA metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) is associated with poor TMZ response and survival in GBM patients. MALAT1 is a well-studied lncRNA known to be upregulated following TMZ-induced alkyl damage and recognized to increase chemoresistance through various mechanisms, including upregulating GSK3β, MGMT, and thymidylate synthase. A recent study described the direct association between MALAT1-mediated TMZ resistance and EMT. The investigators first transfected U87 and U251 GBM cells with MALAT1 siRNA (si-MALAT1) to generate MALAT1-knockdown cells. After being treated with TMZ, the si-MALAT1 cells showed reduced viability compared to the non-transfected cells. In addition, downregulation of ZEB1 was confirmed in the si-MALAT1 cells at the mRNA and protein levels. Furthermore, MALAT1-associated TMZ resistance was reversed in U87 and U251 with siRNA-induced ZEB1 knockdown, suggesting that MALAT1 enhances TMZ resistance via regulation of ZEB1. This data complements other studies that have indicated ZEB1 to facilitate TMZ resistance in GBM cells by elevating MGMT expression and accelerating double-stranded break repairs. Lastly, in mice implanted with patient-derived GBM cells, concurrent treatment of TMZ and a nanocomplex delivering si-MALAT1 has been shown to significantly prolong survival, solidifying MALAT1 as a lncRNA with promising therapeutic potential.

RP11-838N2.4. RP11-838N2.4 is a largely understudied lncRNA recognized for modulating the TMZ response in GBM. Reports of RP11-838N2.4 and primary GBM tissues have revealed lower expression of RP11-838N2.4 is associated with an increased risk of tumor recurrence and early mortality. Contrasting most other lncRNAs mentioned previously, RP11-838N2.4 is inversely correlated with TMZ resistance. This is demonstrated when transfection of TMZ-resistant U87 (U87R) and U251 (U251R) GBM cells with a RP11-838N2.4-overexpressed plasmid exhibited increased TMZ-mediated cell death. Furthermore, the authors determined that RP11-838N2.4 level is inversely associated with miR-10a, which negatively regulates its downstream target, ephrine tyrosine kinase receptor A8 (EphA8). Prior data has shown enhanced EphA8 reverses EMT and transforms glioma cells to the more cobble-stoned appearance of epithelial cells. In contrast, loss of EphA8 is associated with shorter survival time, and diminished TMZ response in GBM patients. Further mRNA and protein analysis indicated that RP11-838N2.4 reduces the expression of miR-10a and relieves its inhibitory effect on EphA8, resulting in enhanced TMZ cytotoxicity by decreasing the EMT process.

H19. LncRNA H19 is upregulated in human TMZ-resistant GBM tissues and is directly associated with decreased patient survival rate. Reports have shown H19 to support TMZ resistance in GBM through different mechanisms, including catalyzation of EMT-related pathways. Studies have delineated the relationship between H19-associated TMZ resistance and the Wnt/β-catenin and NF-κB processes, two signaling cascades known to induce both TMZ inefficiency and EMT. TMZ-resistant GBM cells transfected with H19 shRNA had significantly lower half maximal inhibitory concentration (IC_{50}) value for TMZ when compared to the control group. The H19-silenced cells also exhibited weaker signals of EMT-related and Wnt/β-catenin proteins. However, treatment with the Wnt/β-catenin activator, Licl, reversed the protein expressions and restored TMZ resistance in the H19-silenced cells. Similarly, H19 has also been implicated to promote TMZ resistance in GBM through the NF-κB pathway. U251 and LN229 GBM cells cotransfected with H19 siRNA (si-H19) and a NF-κB luciferase reporter showed decreased bioluminescence and mRNA levels of NF-κB downstream targets, suggesting an association between H19 and the NF-κB pathway proteins. The si-H19 cells also underwent apoptosis at a lower TMZ concentration. Lastly, exposure to a NF-κB inhibitor significantly impaired TMZ resistance in GBM cells transfected with a H19-overexpressing plasmid.

LncRNA and Metabolism

Hypoxia is one of the key characteristics of GBM and glioma cells shift to aerobic glycolysis as a main source of ATP, a phenomenon referred to as the Warburg effect. As one of the chief glycolytic enzymes and upregulated by HIF-1α, HK2 is a compelling metabolic target because of its strong presence in gliomas and its role in maintaining high glycolytic rates. Elevated expression of HK2 is also associated with TMZ-resistant GBM tissues and poor survival in patients. Importantly, HK2 inhibition has been shown to restore oxidative glucose metabolism and decrease the overall tumorigenesis in GBM cell lines. Currently, HOX antisense intergenic RNA (HOTAIR) is the only lncRNA found to mediate its effects through HK2 in GBM.

HOTAIR. HOTAIR is highly expressed in GBM tissues and serum-derived extracellular vesicles, serving as a negative prognostic factor. Moreover, HOTAIR was found to be upregulated in TMZ-resistant GBM tissues and U87 and A172 GBM cells. A recent investigation described increased TMZ sensitivity in U87 and A172 GBM cells transfected with shRNA targeting HOTAIR (sh-HOTAIR). Zhang et al. treated sh-HOTAIR U87 and A172 cells with TMZ and analyzed the gene expressions. They found that the cells not only exhibited upregulated miR-125 and decreased HK2 levels, but also had increased apoptotic response to TMZ. Lastly, depletion of HOTAIR also enhanced the therapeutic effects of TMZ in vivo. Mouse xenograft models of sh-HOTAIR U87 cells were more sensitive to TMZ, leading to significantly smaller tumor and longer survival. Furthermore, the sh-HOTAIR U87 cells had suppressed HK2 and enhanced apoptotic protein levels. Collectively, these
results indicate that HOTAIR regulates GBM TMZ response through the expression of miR-125 and HK2.33

**Targeting LncRNA**

Therapeutic regulation of lncRNAs represents an attractive approach for GBMs as they are highly tissue- and cell-type specific.19 To target non-coding RNAs, multiple approaches including siRNA, antisense oligonucleotides (ASOs), and the CRISPR/Cas9 system have been investigated.87 siRNAs are short double-stranded RNAs between 19 and 30 nucleotides. They induce gene silencing by recruiting the RNA-induced silencing complex to deplete the target gene posttranscriptionally.98 Although siRNAs can effectively target lncRNAs regardless of their intracellular locations, they are susceptible to nucleases and have lower bioavailability due to their large size and anionic charge. However, chemical modifications have improved their stability, specificity, and delivery.88,89 siRNA drugs have been approved for diseases such as adult hereditary amyloidogenic transthyretin.90

ASOs are single-stranded ASOs that are taken up freely by cells and form heteroduplexes with the complementary lncRNA. ASOs can inhibit or alter gene expression via steric hinderance, splicing alterations, or cleavage induction by endogenous RNaseH.91 ASOs have similarly undergone modifications of their backbone to improve their stability and delivery. Compared to siRNAs, ASOs are smaller, less immunogenic, and exhibit fewer off-target effects; the development of locked nucleic acids has also improved the potency significantly, though with increase hepatotoxicity.92,93 Moreover, certain ASOs have demonstrated lower RNase H cleavage and can only enter the central nervous system via intrathecal injections.94 Clinically, ASO-mediated therapies are currently used for treating diseases such as spinal muscular atrophy.95

The CRISPR/Cas9 system is composed of a single guide (sgRNA) and a Cas9 enzyme. The sgRNA guides the Cas9 nuclease to specific sites in the genome via complementary base pairing. The high efficiency and ease of programming makes CRISPR/Cas9 a robust tool to target lncRNAs.96 Furthermore, CRISPR/Cas9 can be utilized to target splice sites to produce intron retention or exon deletion, which can be used for genome-wide screening for essential lncRNAs.97 However, one major limitation is the risk of impacting adjacent genes, such as those that may overlap with the target lncRNA gene or those with DNA elements in the lncRNA locus that may regulate other genes.98 Unlike siRNAs and ASOs, CRISPR/Cas9 drugs have not yet been approved for treating diseases.

**Conclusion**

TMZ resistance is a significant barrier to improving the dire prognosis of GBM. From the current aspects of recent research, lncRNAs are being increasingly recognized for their diverse roles in modulating various GBM pathologies. To the best of our knowledge, this review is the first to focus on and describe, in detail, the lncRNAs specifically involved in TMZ response in GBM. Furthermore, this review concurrently explores the mechanisms commonly associated with GBM chemo-resistance in the context of lncRNAs. We divided lncRNA-mediated TMZ resistance into three large categories: (1) regulation of the DDR, (2) maintenance of GSC identity, and (3) association with tumor hypoxia. Naturally, there are other existing mechanisms that do not fit in the above categories included in Table 1.

LncRNAs undoubtedly command a much more critical role in GBM pathogenesis than previously thought. The complex network between lncRNAs and TMZ resistance are only beginning to transpire and would require considerably more experiments to thoroughly understand and appreciate. Nevertheless, the highly-specific differential lncRNA expressions between normal and glioma cells, in conjunction with rapidly advancing sequence-based nucleic acid therapies emphasize lncRNAs as promising targets for novel GBM therapies.
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