

DNMTi in Overcoming Immunosuppression in Glioblastoma: Mechanisms and Therapeutic Potential

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Abstract. Glioblastoma (GBM) is the most aggressive primary malignancy of the central nervous system, characterized by poor prognosis and limited therapeutic options. Epigenetic dysregulation, particularly aberrant DNA methylation, plays a pivotal role in glioblastoma pathogenesis by modulating tumor suppressor genes and oncogenes. The corresponding epigenetic writers and erasers, DNA methyltransferases (DNMTs) and ten-eleven translocation (TET) enzymes, maintain methylation homeostasis, and alterations in their activity can cause abnormal gene expression that drive glioblastoma progression. DNMT1 preserves methylation patterns during DNA replication, whereas DNMT3A and DNMT3B establish de novo methylation. The glioma CpG island methylator phenotype (G-CIMP), an epigenetic subtype of glioblastoma, is frequently associated with mutations in isocitrate dehydrogenase 1 and 2 (IDH1/2), which impair TET-mediated demethylation and result in widespread hypermethylation. Moreover, altered methylation contributes to drug resistance, exemplified by temozolomide (TMZ) resistance mediated through SNHG12 hypomethylation. Currently, two DNMT inhibitors, 5-azacytidine and decitabine, are FDA-approved for certain cancers, while several novel agents, including zebularine, hydralazine and MA17, are under investigation. Although these emerging drugs show promise, their clinical utility remains limited by issues of stability, cytotoxicity and therapy resistance. Understanding the interplay between DNA methylation, tumor progression, and therapy resistance is important to develop new precision medicine strategies in glioblastoma.

Keywords: Glioblastoma, Epigenetics, DNA methylation, Drug resistance.

1. Introduction

Glioblastoma is the most aggressive primary malignancy of the central nervous system (CNS) and is classified as World Health Organization (WHO) Grade IV CNS glioma, with a median overall survival of approximately 9.2 months [1, 2]. Epigenetics is a rapidly evolving field that studies factors regulating gene expression without altering the underlying DNA sequence. These mechanisms include DNA methylation, histone modifications, and non-coding RNA expression. Because aberrant gene expression contributes to tumorigenesis, epigenetic modifications have emerged as important regulatory mechanisms in various cancers, including glioblastoma. Hypermethylation of some genes and global hypomethylation have been observed in glioblastoma, suggesting possible epigenetic involvement in glioblastoma progression. DNA methylation is tightly regulated by “writers”, the DNA methyltransferases (DNMTs) family, and “erasers”, the ten-eleven-translocation (TET) family. Due to the reversible nature of DNA methylation, it has been thought to have significant therapeutic potential. Several potential targets have been identified, but few have been developed and approved. One limitation of epigenetic drugs targeting DNA methylation level in glioblastoma is the drug resistance that reduces drug efficacy.

2. Enzyme Involved in DNA Methylation

DNA methyltransferase (DNMT) acts as epigenetic writers that add a methyl group to cytosine-phosphate-guanosine (CpG) residues on the DNA forming 5-methylcytosine (5mC). Since promoter regions of many genes contain a large amount of CpG residues, known as CpG islands, the expression of tumor-related genes can be activated or repressed by these epigenetic marks when they are recognized by epigenetic readers, such as methyl-CpG binding domain 2 (MBD2) [3]. Currently,

several DNMTs have been found and can be classified into several subtypes: DNMT1, DNMT2, DNMT3A, DNMT3B and DNA methyltransferase-like protein (DNMT3L).

DNMT1 is the first DNA methyltransferase discovered and its preference for hemi-methylated DNA (hmDNA) suggests that it is responsible for maintenance of DNA methylation during DNA replication [4]. It consists of multiple domains: N-terminal independently folded domain (NTD), bromo adjacent homology 1 and 2 (BAH1 and BAH2) domains, CXXC domain and replication foci targeting sequence (RFTS) domain. Accurate localization of DNMT1 to hmDNA requires an additional factor, ubiquitin-like, containing PHD and RING finger domain 1 (UHRF1). UHRF1 is an E3 ubiquitin ligase expressed during the G1/S phase and G2/M phase of the cell cycle. Its SET-and-RING-associated (SRA) domain specifically binds to hmDNA while the ubiquitin-like domain (UBL) binds to RFTS domain of DNMT1 and activates DNMT1. Although DNMT1 shows significant preference of hmDNA, studies have found that it can also establish de novo methylation as well. Overexpression of DNMT1 is frequently observed in gliomas and may lead to hypermethylation and inactivation of tumor suppressor genes [5]. Moreover, alteration in DNMT1 leads to global hypomethylation, while hypomethylation of certain oncogenes promoters, such as HEY1, can contribute to glioblastoma progression [5, 6].

DNMT3A and DNMT3B were discovered to be responsible for de novo methylation. They are crucial for the establishment of de novo DNA methylation during development and also play roles in maintaining DNA methylation in DNMT1-deficient mice [7]. DNMT3 enzymes contains a variable N-terminal domain, PWWP (Pro-Trp-Trp-Pro) domain, an ATRX-DNMT3-DNMT3L (ADD) domain and a catalytic methyltransferase domain [7]. Alternatively, DNMT3L is a truncated form of DNMT3, lacking catalytic activity. It participates in de novo DNA methylation during development by binding to and regulating DNMT3A and DNMT3B [7, 8]. The initiation of DNA methylation started by the binding of function DNMT3 heterotetrametric complex to DNA via target recognition domain (TRD) in the PWWP domain, but interaction between DNA and the catalytic methyltransferase domain is blocked by the ADD domain [8, 9]. The blockade can be removed when the ADD domain binds to specific histone marks, ensuring DNA methylation at the correct position [9].

Lastly, DNMT2 acts very differently from the other DNMTs. Although it shares a similar 3D structure with DNMT1 and DNMT3, it does not participate significantly in DNA methylation. Instead, it acts as an RNA methyltransferase to stabilize certain tRNAs [10]. Therefore, it is also known as tRNA aspartic acid methyltransferase 1 (TRDMT1). Moreover, it is also different from other RNA methyltransferase as it methylates tRNA via DNMT pathways [11]. Although DNMT2 is not involved in DNA methylation, DNMT2 knock out can worsen the outcome of glioblastoma treatment [12].

3. Epigenetic Modification in Glioblastoma

DNA methylation levels can affect the expression of tumor suppressor genes and oncogenes. For example, hypermethylation can lead to transcriptional suppression of tumor suppressor genes while hypomethylation can lead to transcriptional activation of oncogenes. Therefore, alterations in DNA methylation and its associated enzymes can often lead to glioblastoma.

Glioma CpG island methylator phenotype (G-CIMP) is an epigenetic subtype of glioblastoma, characterized by extensive DNA hypermethylation. It is associated with mutations in isocitrate dehydrogenase 1 and 2 (IDH1/2) and characterized by global hypermethylation that leads to altered gene expression [13, 14]. IDH1/2 are key metabolic enzymes that catalyze oxidative decarboxylation of isocitrate into α -ketoglutarate (α -KG). However, IDH1/2 mutations lead to the reduction of α -KG to oncometabolite, 2-hydroxyglutarate (2-HG) [15]. The accumulation of 2-HG inhibits ten-eleven-translocation (TET) enzymes, which are epigenetic erasers of DNA methylation, leading to global hypermethylation [15].

However, the mean DNA methylation level may not accurately indicate the molecular mechanism and prognosis of glioblastoma. This is because distinct CpG sites have been found to be hypermethylated or hypomethylated in glioblastoma which may counteract with each other. Instead, methylation of promoters of tumor-associated genes should be carefully monitored. Recent studies have used bioinformatics to identify several hypermethylated and hypomethylated genes and classify them based on their DNA methylation [16]. For example, studies have reported that ADCY2, NPY and SST are hypermethylated, while FOXA1, KCNC3 and CASP8 are hypomethylated [16]. NPY is a neurotransmitter in the central nervous system, while CASP8 is related to chemotherapy resistance. Notably, multiple DNA methylation events can occur simultaneously, which should be considered when predicting the glioblastoma progression and developing personalized treatments.

4. DNA Methylation and Drug Resistance

Tumor cells arise from mutations that lead to uncontrolled cell division of undifferentiated cells. It has been reported that various mutations could lead to the development of glioblastoma which contributes to its heterogeneity. Due to different pathologies, tumor cells may not have the specific target site for drugs to act on and survive which give rise to drug resistance. Moreover, tumor cells with high mutation rate can acquire resistance during cancer therapy, preventing the drug from working and causing cancer recurrence. Despite genetic mutations, epigenetic alterations can also lead to the development of drug resistance.

One of the epigenetic pathways causing drug resistance is the Wnt pathways. It is important in early development and has frequently been observed to be elevated in cancers. Elevated activity of the Wnt signaling pathway can also lead to drug resistance in cancer. Studies have indicated that elements of the Wnt signaling pathway are either hypermethylated or hypomethylated. For example, Fzd-10 is hypermethylated while elevated Wnt5a, beta-catenin and Wnt3a are partially caused by hypomethylation [17]. Moreover, glioblastoma can acquire resistance to cancer treatment via DNA methylation. Hypomethylation at the promoter of small nucleolar RNA host gene 12 (SNHG12) has been observed in TMZ-resistance glioblastoma. SNHG12 is highly expressed in the TMZ-resistance glioblastoma and knockdown of SNHG12 has successfully overcome the TMZ resistance [18]. SNHG12 overexpression inhibits the expression of multiple microRNAs, in particular miR-129-5p which targets and inhibits the MAPK1 and E2F7. Hypomethylation is probably caused by downregulation of DNMT1, making the SNHG12 promoter more accessible to the Sp1 transcription factor. Thus, TMZ-resistance glioblastoma likely acquires resistance via SNHG12-mediated activation of the MAPK/ERK pathway caused by downregulation of DNMT1.

Glioblastoma is a solid tumor which may form an immunosuppressive tumor microenvironment (TME). TME suppresses the immune cells from attacking the tumor cells, limiting the effectiveness of immunotherapy which works by stimulating immune cells. For example, CD8 T cells which recognize and breakdown tumor cells are often suppressed in TMEs. Mutations that trigger the expression of inhibitory immune checkpoints such as CTLA-4 and PD-L1 allow tumor cells to suppress T cell activation and proliferation, leading to T cell exhaustion. Moreover, some tumors are resistant to immunotherapy due to its low T cell infiltration. These cold tumors lack the essential T cells in the TME to kill the tumor cells. Recent studies reported that DNA methylation contributes to the glioblastoma immunosuppressive nature. YTHDF2, a m6A reader protein crucial for maintaining RNA stability, has been found elevated in low-grade glioma. Elevated YTHDF2 is strongly correlated with immune checkpoint molecules and various immunosuppressive cells negatively correlated with activating immune cells [19]. Studies have found that DNA hypomethylation triggers the expression of YY1, transcription factor of YTHDF2, is responsible for the elevated YTHDF2 observed in glioblastoma and DNMT inhibition can reverse YTHDF2 expression [19]. Moreover, silencing of YTHDF2 via siRNA in combination with immune checkpoint inhibitors have been proven to overcome immunosuppression in hepatocellular carcinoma [20].

5. Current Epigenetic Drugs

5.1. DNMT Inhibition

DNA methylation is reversible, making the development of epigenetic drugs a promising approach. One class of these drugs is DNMT inhibitors (DNMTis). Currently, two DNMTis, 5-azacytidine (5-aza) and decitabine, have been approved by the US Food and Drug Administration (FDA) for the treatment of acute myeloid leukemia and myelodysplastic syndrome [21]. Both are cytidine analogs modified at the 5-position of the pyrimidine ring [21]. Upon administration, they are transported into cells and converted into active triphosphate forms that mimic nucleotides. Due to their characteristics, 5-aza preferentially incorporates into RNA, whereas decitabine incorporates into DNA. When incorporated during the S phase of the cell cycle, the period when DNA methylation mainly occurs, they disrupt interactions between DNA and DNMTs via their modified pyrimidine moiety, thereby preventing the establishment of DNA methylation [22].

5.2. TMZ Resistance

Alter gene expressions caused by aberrant DNA methylation can give rise to drug resistance. Notably, DNMTi can be incorporated into existing cancer therapies to overcome drug resistance and improve therapeutic outcomes. TMZ is one of the most commonly used chemotherapies for glioblastoma. It is an alkylating agent that introduces cytotoxic methylation into DNA to induce tumor cell death [23]. However, some tumor cells can evade this cytotoxicity by recruiting DNA repair enzymes, MGMT, to remove these deleterious methylation marks [24]. Several strategies have been proposed to overcome such resistance, including the use of epigenetic drugs such as 5-aza. Studies have demonstrated that inhibition of DNMT3B by 5-aza can reverse epigenetic silencing of the tumor suppressor genes, Tumor Suppressor Candidate 3 (TUSC3) and enhance TMZ sensitivity in MGMT-expressing glioblastoma [25]. The studies also underscore the role of the PI3K/Akt pathway in the development of TMZ resistance.

6. Future Epigenetic Drugs

6.1. Zebularine

Although the development of DNMTis has expanded treatment options, current drugs are limited by low chemical stability and short half-lives, which requires high doses that increase cytotoxicity. Beyond 5-aza and decitabine, numerous DNMTis have been developed, but most failed to achieve comparable therapeutic outcomes. Zebularine, a cytidine analog, has demonstrated in vitro efficacy with improved chemical stability, which prolongs its half-lives and enables oral administration. Moreover, zebularine can induce immunogenic cell death which releases antigen to enhance anti-tumor immunity [26]. It was discovered that administration of zebularine can stimulate cGAS-STING DNA-sensing pathways to enhance antigen presentation, phagocytosis, and etc. [26]. Thus, zebularine showed great potential in overcoming immunosuppression, but more studies would be needed.

6.2. Hydralazine

Hydralazine, a potent vasodilator commonly used to treat hypertension, has recently been identified as a DNMTi [27]. High doses of hydralazine can reduce the expression of DNMT1, DNMT3A, and DNMT3B and induce apoptosis. Compared with 5-aza and decitabine, hydralazine exhibits much lower cytotoxicity. However, its demethylating activity is limited to other approved DNMTis, and its vasodilating effects necessitate careful dosing [27]. Moreover, hydralazine has not yet been evaluated in glioblastoma. Despite these limitations, its low toxicity suggests potential therapeutic in glioblastoma treatment.

6.3. MA17

MA17 is a novel phthalimido-alkanamide derivative that acts as both a DNMTi and a radiosensitizer. Preclinical studies have shown that it sensitizes glioblastoma cells to radiotherapy [28] likely through inhibition of DNA repair processes or reduction of post-radiotherapy repopulation. Significant downregulation of DNMT1, DNMT3A and DNMT3B has been observed; however, the specific contribution of DNMT inhibition to radiosensitization is not yet understood. Although MA17 significantly improves radiosensitivity in glioblastoma, it faces several clinical challenges. Its novelty raises the possibility of unexpected cytotoxicity, and optimal administration routes and dosing concentration have not been identified [28]. Once these challenges are resolved and the underlying molecular and epigenetic mechanisms are fully understood, MA17 could be an important therapeutic option for glioblastoma, particularly in cases where surgical removal has high risk.

7. Conclusion

Recent studies have revealed substantial epigenetic involvement in glioblastoma. DNA methylation, a key epigenetic modification, is critical in glioblastoma initiation, progression and immunosuppression. Dysregulation of DNMTs and their associated pathways results in global hypomethylation and locus-specific hypermethylation, thereby affecting tumor suppressors, oncogenes, and therapy resistance networks. Therefore, epigenetic drugs that reverse unfavorable methylation marks have been studied extensively. DNMTis have been applied in cancer treatment and demonstrate significant potential in overcoming therapeutic resistance. Despite limitations in stability and side effects, emerging epigenetic drugs exhibit certain advantages over complicated epigenetic mechanisms underlying glioblastoma and integrate this knowledge with existing therapeutic approaches. Moreover, establishing precise epigenetic profiles could enable personalized treatment strategies to address cancer heterogeneity.

References

- [1] N. F. Brown et al., “Survival Outcomes and Prognostic Factors in Glioblastoma,” *Cancers (Basel)*, vol. 14, no. 13, p. 3161, June 2022, doi: 10.3390/cancers14133161.
- [2] D. N. Louis et al., “The 2021 WHO Classification of Tumors of the Central Nervous System: a summary,” *Neuro Oncol*, vol. 23, no. 8, pp. 1231–1251, Aug. 2021, doi: 10.1093/neuonc/noab106.
- [3] K. H. Wood and Z. Zhou, “Emerging Molecular and Biological Functions of MBD2, a Reader of DNA Methylation,” *Front. Genet.*, vol. 7, May 2016, doi: 10.3389/fgene.2016.00093.
- [4] M. Okuwaki and A. Verreault, “Maintenance DNA Methylation of Nucleosome Core Particles,” *Journal of Biological Chemistry*, vol. 279, no. 4, pp. 2904–2912, Jan. 2004, doi: 10.1074/jbc.M310111200.
- [5] G. Rajendran, K. Shanmuganandam, A. Bendre, D. Mujumdar, A. Goel, and A. Shiras, “Epigenetic regulation of DNA methyltransferases: DNMT1 and DNMT3B in gliomas,” *J Neurooncol*, vol. 104, no. 2, pp. 483–494, Sept. 2011, doi: 10.1007/s11060-010-0520-2.
- [6] A. J. Tsung et al., “Methylation regulates HEY1 expression in glioblastoma,” *Oncotarget*, vol. 8, no. 27, pp. 44398–44409, July 2017, doi: 10.18632/oncotarget.17897.
- [7] F. Chédin, “The DNMT3 Family of Mammalian De Novo DNA Methyltransferases,” in *Progress in Molecular Biology and Translational Science*, vol. 101, Elsevier, 2011, pp. 255–285. doi: 10.1016/B978-0-12-387685-0.00007-X.
- [8] Z.-M. Zhang et al., “Structural basis for DNMT3A-mediated de novo DNA methylation,” *Nature*, vol. 554, no. 7692, pp. 387–391, Feb. 2018, doi: 10.1038/nature25477.
- [9] S. Tajima, I. Suetake, K. Takeshita, A. Nakagawa, and H. Kimura, “Domain Structure of the Dnmt1, Dnmt3a, and Dnmt3b DNA Methyltransferases,” *Adv Exp Med Biol*, vol. 1389, pp. 45–68, 2022, doi: 10.1007/978-3-031-11454-0_3.
- [10] A. Jeltsch et al., “Mechanism and biological role of Dnmt2 in Nucleic Acid Methylation,” *RNA Biology*, vol. 14, no. 9, pp. 1108–1123, Sept. 2017, doi: 10.1080/15476286.2016.1191737.

- [11] F. Lyko, “The DNA methyltransferase family: a versatile toolkit for epigenetic regulation,” *Nat Rev Genet*, vol. 19, no. 2, pp. 81–92, Feb. 2018, doi: 10.1038/nrg.2017.80.
- [12] D. Bloniarz, J. Adamczyk-Grochala, A. Lewinska, and M. Wnuk, “The lack of functional DNMT2/TRDMT1 gene modulates cancer cell responses during drug-induced senescence,” *Aging*, vol. 13, no. 12, pp. 15833–15874, June 2021, doi: 10.18632/aging.203203.
- [13] J. Dejaegher et al., “DNA methylation based glioblastoma subclassification is related to tumoral T-cell infiltration and patient survival,” *Neuro-Oncology*, vol. 23, no. 2, pp. 240–250, Feb. 2021, doi: 10.1093/neuonc/noaa247.
- [14] T. M. Malta et al., “Glioma CpG island methylator phenotype (G-CIMP): biological and clinical implications,” *Neuro-Oncology*, vol. 20, no. 5, pp. 608–620, Apr. 2018, doi: 10.1093/neuonc/nox183.
- [15] S. Han et al., “IDH mutation in glioma: molecular mechanisms and potential therapeutic targets,” *Br J Cancer*, vol. 122, no. 11, pp. 1580–1589, May 2020, doi: 10.1038/s41416-020-0814-x.
- [16] J. W. Park, K. Lee, E. E. Kim, S.-I. Kim, and S.-H. Park, “Brain Tumor Classification by Methylation Profile,” *Journal of Korean Medical Science*, vol. 38, no. 43, Sept. 2023, doi: 10.3346/jkms.2023.38.e356.
- [17] M. Tompa, B. Kajtar, B. Galik, A. Gyenesei, and B. Kalman, “DNA methylation and protein expression of Wnt pathway markers in progressive glioblastoma,” *Pathology - Research and Practice*, vol. 222, p. 153429, June 2021, doi: 10.1016/j.prp.2021.153429.
- [18] S. Lehtipuro, M. Nykter, and K. J. Granberg, “Modes of immunosuppression in glioblastoma microenvironment,” *Oncotarget*, vol. 10, no. 9, pp. 920–921, Jan. 2019, doi: 10.18632/oncotarget.26643.
- [19] X. Jiang et al., “DNA methylation-regulated YTHDF2 correlates with cell migration and immune cell infiltration in glioma,” *Aging*, vol. 14, no. 19, pp. 7774–7793, June 2022, doi: 10.18632/aging.204104.
- [20] Z. Guo, Q. Huang, Z. Cui, C. Yang, and L. Yang, “Targeting YTHDF2 with pH-responsive siRNA nanoparticles suppresses MYC m6A modification and restores antitumor immunity in hepatocellular carcinoma,” *J Nanobiotechnology*, vol. 23, no. 1, p. 469, July 2025, doi: 10.1186/s12951-025-03538-0.
- [21] A. Gnyszka, Z. Jastrzębski, and S. Flis, “DNA Methyltransferase Inhibitors and Their Emerging Role in Epigenetic Therapy of Cancer,” *Anticancer Research*, vol. 33, no. 8, pp. 2989–2996, Aug. 2013.
- [22] X. Yang, F. Lay, H. Han, and P. A. Jones, “Targeting DNA methylation for epigenetic therapy,” *Trends Pharmacol Sci*, vol. 31, no. 11, pp. 536–546, Nov. 2010, doi: 10.1016/j.tips.2010.08.001.
- [23] M. S. Tomar, A. Kumar, C. Srivastava, and A. Shrivastava, “Elucidating the mechanisms of Temozolomide resistance in gliomas and the strategies to overcome the resistance,” *Biochim Biophys Acta Rev Cancer*, vol. 1876, no. 2, p. 188616, Dec. 2021, doi: 10.1016/j.bbcan.2021.188616.
- [24] M. Weller et al., “MGMT promoter methylation in malignant gliomas: ready for personalized medicine?,” *Nat Rev Neurol*, vol. 6, no. 1, pp. 39–51, Jan. 2010, doi: 10.1038/nrneurol.2009.197.
- [25] W. Kan et al., “Downregulated the expression of DNMT3B by suppressing PI3K/Akt signaling pathway and enhances the chemosensitivity of glioblastoma to temozolomide.,” Oct. 03, 2023, Research Square. doi: 10.21203/rs.3.rs-3358904/v1.
- [26] Y. Zhang, H. Zhao, W. Deng, J. Lai, K. Sang, and Q. Chen, “Zebularine potentiates anti-tumor immunity by inducing tumor immunogenicity and improving antigen processing through cGAS-STING pathway,” *Commun Biol*, vol. 7, no. 1, p. 587, May 2024, doi: 10.1038/s42003-024-06271-w.
- [27] M. J. Ruiz-Magaña, R. Martínez-Aguilar, E. Lucendo, D. Campillo-Davo, K. Schulze-Osthoff, and C. Ruiz-Ruiz, “The antihypertensive drug hydralazine activates the intrinsic pathway of apoptosis and causes DNA damage in leukemic T cells,” *Oncotarget*, vol. 7, no. 16, p. 21875, Mar. 2016, doi: 10.18632/oncotarget.7871.
- [28] C. W. Wee et al., “Radiosensitization of Glioblastoma Cells by a Novel DNA Methyltransferase-inhibiting Phthalimido-Alkanamide Derivative,” *Anticancer Res*, vol. 39, no. 2, pp. 759–769, Feb. 2019, doi: 10.21873/anticancer.13173.