



## Epigenetic Factors in Glioblastoma Multiforme: Understanding Molecular Mechanisms

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### ABSTRACT

Glioblastoma multiforme is the most common malignant brain tumor that arises with high morbidity, having a rather very poor prognosis with only 5.5% five-year survival. Such tumors exhibit aggressive behavior due to intrinsic heterogeneity, glioma stem cell dynamics, and resistance to conventional and emerging therapies. Epigenetic modifications are highlighted in recent studies to be of importance for DNA methylation and histone modifications in the tumorigenesis and progression of GBM. Additionally, some aberrant signaling pathways have been identified, including Hedgehog, Notch, and Wnt, which might act as both a driving force in the tumor microenvironment and a promising therapeutic target. Improved understanding of the molecular and cellular mechanisms of GBM has led to ongoing efforts toward personalized medicine and novel therapeutic strategies in a continuous quest to improve patient outcomes in this challenging malignancy. The present manuscript reviews the current knowledge of the epigenetic landscape, signaling networks, and resulting treatment implications associated with glioblastoma, hence underlining the urgent need for innovative therapeutic approaches tailored to specific patient profiles.

**Keywords:** Glioblastoma Multiforme, Epigenetic, Genetic

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### INTRODUCTION

Glioblastoma multiforme, constitute about 14.5% of all the neoplasms located within the central nervous system, and 48.6% of all malignant central nervous system tumors (1). Grade IV GBM has been recognized to be the most frequent primary malignant brain tumor, notoriously marked by dismal survival predictions, with a five-year survival rate of only 5.5%. A high mortality rate has been attributed to tumor heterogeneity, chemoresistance, and the infiltrative nature of the tumor-morphological properties that stand in the way of complete tumor removal. Low-grade gliomas, grades I-II, on the other hand, are fairly favorable tumors with an estimate for overall survival of approximately seven years, ultimately develop into high-grade gliomas, grades III-IV (2).

Epidemiology studies show that most of the patients with GBM are men, and it is in excess compared to females (1). Epidemiology studies show that most of the patients with GBM are men, and it is in excess compared to females. Along this line, imbalance in sexes could reflect certain biological or geographical propensity for GBM. Furthermore, the age distribution indicates that GBM occurs almost exclusively in adults and is also particularly common among the aged population. This information on demographics is important for developing measures of public health and assessing the availability of resources for treatment. Patients diagnosed with glioblastoma multiforme often present with neurodeficiency and headaches as the most common initial symptoms. These symptoms slowly invade its way to brain function which requests early diagnosis and

treatment. Understanding the clinical presentation aids healthcare providers in recognizing potential cases of GBM and initiating timely diagnostic procedures (2).

The treatment landscape for glioblastoma multiforme includes multimodality therapy, which typically combines safe surgical resection with adjuvant radiotherapy or concurrent chemoradiation followed by sequential chemotherapy. The complexity of treatment underlines the need for a thorough understanding of the epidemiological factors that influence patient outcomes, as well as adherence to treatment protocols across varying healthcare settings (3).

Despite advances in the treatment modalities, glioblastoma multiforme remains essentially incurable, carrying with it a median overall survival for patients of about 13 months (4). This grim prognosis continues to dictate a need for studying the genetic and environmental factors influencing survival. These factors, if adequately characterized through broad epidemiological studies, will not only provide a better understanding but also facilitate improvement in therapeutic strategies and management of affected patients.

Continuous studies on glioblastoma multiforme epidemiology are, therefore, necessary for the complete understanding of pathophysiology and the institution of effective treatments of the condition (5). Much is yet to be learned regarding identifying various risk factors in the disease, including genetic and environmental ones. This may lead to better-individualized management or treatment, hence the outcomes for patients with these conditions, which might optimally be improved through the gaps in knowledge that focused epidemiological studies could fill.

Therein, the genetic and epigenetic heterogeneity in GBM concerns changes in methylation patterns, among others (6). Various gene mutations have been involved in the pathogenesis of GBM. Although most glioblastomas spread to other organs by hematogenous and lymphatic routes, this neoplasm has relatively low metastatic potential outside of the CNS due to the presence of the BBB and lack of lymphatic vessels (7). Some

notable gene mutations include P16, TP53, and epidermal growth factor receptor that have been identified in GBM and may be related to the survival rates and prognosis of patients diagnosed with the disease (8).

Epigenetic alterations are one of the hallmarks of the development and progression of GBM. Epigenetics is defined as heritable changes in gene expression occurring in the absence of alterations to the DNA sequence itself, and such modifications form critical markers for human cancers. Abnormal epigenetic mechanisms include changes in histone modifications, changes in the expression of non-coding RNAs, DNA methylation, and chromatin remodeling long known to be well-documented contributors to tumorigenesis. Most of the studies so far have focused on DNA methylation alterations in GBM, and indeed genome-wide hypomethylation occurs, while there are gene-specific hypomethylations and hypermethylation (9-11).

Till now, no standardized treatment exists to deal effectively with GBM, and the effects of most treatments so far are limited. Hence, the underlying molecular mechanisms leading to recurrence and aggressive behavior in GBM need to be understood (12). The targeting of the epigenome has been one of the promising therapeutic approaches in the treatment of cancer. Moreover, it has been deeply investigated that the epigenetic landscape in GBM displays a number of epigenetic alterations that have already been related to the biological behavior of the tumor; some are even considered potential therapeutic targets (13, 14). This review will delve into the epigenetics of GBM and explore potential therapeutic targets, as well as ongoing research into effective drug treatments for GBM.

### **Epigenetic Alterations in Glioblastoma DNA Methylation**

Among the best-studied epigenetic modifications, DNA methylation predominantly occurs in mammals at the cytosine in CpG dinucleotides, giving rise to 5-methylcytosine (5mC). The methylation events catalyzed by DNA methyltransferases (DNMTs) may have very dramatic effects on gene expression. DNA

methylation profiles distinctly differ between normal and tumor cells in gliomas. Hypermethylation and hypomethylation of CpG islands may serve as biomarkers for the GBM diagnosis (15, 16).

Hypermethylation of gene promoters in tumor cells is a common feature that constitutes the major mechanism for transcriptional inactivation of such TSGs as P14ARF, P16INK4a, and MGMT. Restitution of p16INK4a expression has been associated with inhibited cell proliferation in GBM, while hypermethylation of its MGMT promoter is considered an important biomarker for treatment responses due to the increased sensitivity to TMZ (17, 18).

Moreover, recent discoveries have underlined the different methylation patterns of pediatric versus adult GBM, suggesting the need for tailored prognostic indicators in pediatric cases. DNA methylation changes represent major epigenetic signs of glioma development and progression, further supporting their potential as targets for therapeutic strategies.

### Histone Modification

Histone modifications are one of the important epigenetic changes that generally affect gene expression without actually changing the DNA sequence (19). These include methylation, acetylation, phosphorylation, and ubiquitination, which in turn take place mainly at the N-terminal tails of histone proteins. Aberrant histone modifications bring about disturbances in transcription and are thereby major contributors to glioma development (20, 21).

Notably, a number of HDACs and methyltransferases are under keen investigation in gliomas. Overexpression of these enzymes is associated with influencing tumor behavior, hence serving as targets for therapeutic intervention, especially in combination with chemotherapy (21). New studies have pointed to the possible involvement of targeting histone methylation pathways in inhibiting the growth of GBM cells and driving tumor cells into a state of senescence (22).

Some histone mutations might act as regulators for post-transcriptional modifications; for example, mutations in the H3F3A gene are very common in pediatric GBM (23).

Accordingly, such histone modifications could serve as useful biomarkers of this disease. Targeting pathways of histone modification might provide new therapeutic strategies in the management of glioma (24).

### Chromatin Remodeling

Chromatin remodeling complexes use the energy of ATP hydrolysis to change the structure of chromatin, providing a regulatory mechanism for critical cellular processes like DNA repair, transcription, and the cell cycle. Proper nucleosome positioning and transcriptional control disrupted by mutations in chromatin remodeling proteins have been implicated in diseases, including cancer (25).

Recent studies have pointed out that chromatin remodeling among GBM cells is significant in drug resistance. The manipulation of chromatin dynamics thus could represent a promising avenue in therapeutic interventions. For example, it has been evidenced that upregulated chromatin remodeling factors correlate with glioma progression, pointing toward their targetable value in novel treatments. Understanding the mechanistic role of such factors in the development of glioma will definitely enhance therapeutic strategies and improve clinical outcomes for patients (26, 27).

### Signaling Pathways in Glioblastoma and Their Epigenetic Regulation

#### Notch Signaling Pathway in Glioblastoma

The Notch signaling pathway has been reported to be involved in various cellular and developmental processes such as neurogenesis and gliogenesis, cell migration, differentiation, fate determination, apoptosis, maintenance of stem cells, self-renewal, and homeostasis (28). The four main receptor types of the pathway, Notch 1 through Notch 4, are located on recipient cells, whose ligands are provided by the Delta-like and Jagged families, specifically DII1–4 and Jagged 1 and 2, respectively, on the signal-providing cells (29). These receptors are highly distributed within the adult brain: Notch 1 is expressed in neurons, astrocytes, precursor cells, ependymal cells, and the endothelial cells; Notch 2 and 3 highly in precursor cells, while Notch 4 is specifically expressed in the

endothelium (30). The ligands DII1 and DII3 show a high expression in neuronal progenitors and intermediate neurons, while DII4 within the endothelial cells. Notch pathway activation occurs via trans-interactions between receptors and their ligands on neighboring cells; cis-interactions inhibit the pathway (28).

The altered expression of the Notch signaling pathway has been implicated in various cancers, such as those of the breast, cervix, colon, pancreas, skin, and brain. The expression of Notch receptors and components is also aberrantly expressed in brain tumors. However, most of the literature reports overexpression of Notch receptors in GBM. Overexpression of Notch 1 and 4 along with other signaling components of this pathway has been related to aggressive phenotypes of GBM with less than poor survival rate among patients (31, 32).

Epigenetic functions of the Notch signaling pathway in GBM have not been well explored. So far, studies have suggested that methylation patterns of the Hey1 protein, a member of the aforementioned pathway, can be associated with the pathogenesis of GBM (33). Treatment of HDAC inhibitors to GBM xenograft models has shown potential in inducing apoptosis within GBM cells by downregulating the expression of Hey1 (33). Further studies are needed for a better understanding of the epigenetic mechanisms operating within the Notch pathway in GBM, and these could also provide therapeutic targets.

### **Hedgehog Signaling Pathway in Glioblastoma**

Hedgehog (HH) signaling is often inappropriately activated in GBM to facilitate tumor proliferation and sustenance. This pathway, crucial in embryonic development, becomes reactivated in various cancers, including GBM (34, 35). The GLI family of transcription factors, which includes GLI1, GLI2, and GLI3, has an integral role in the HH signaling mechanism. In GBM, GLI proteins promote cell proliferation, survival, and stemness, which are critical for tumor progression (36, 37).

### **Mechanisms of Radioresistance**

GBM cells exhibit enhanced DNA repair capabilities, particularly through non-homologous end joining (NHEJ) and homologous recombination, which are influenced by GLI activation (38, 39). Radiation therapy generates reactive oxygen species (ROS) that damage DNA; however, GBM cells can adapt by upregulating antioxidant defenses, partly regulated by HH signaling, allowing them to survive radiation-induced damage (40, 41).

### **Cancer Stem Cells (CSCs)**

This is characterized by a subpopulation of CSCs that have self-renewal and differentiation capabilities. These CSCs are often more radio- and chemoresistant, in part through the activation of the HH pathway that promotes stemness-related genes like SOX2 and Nanog (42, 43). GLI proteins augment the expression of genes linked to stemness, thereby facilitating the persistence of cancer stem cells (CSCs) in glioblastoma (GBM) and their involvement in tumor recurrence following therapeutic interventions (44).

### **Tumor Microenvironment (TME)**

The tumor microenvironment (TME), characterized by hypoxic conditions and inflammatory signals, has the capacity to activate Hedgehog (HH) signaling, thereby facilitating tumor proliferation and resistance to therapeutic interventions. For example, hypoxia-inducible factor 1-alpha (HIF-1 $\alpha$ ) can augment HH signaling, which is associated with heightened aggressiveness of GBM (45, 46). Additionally, the relationship between GBM cells and immune cells, specifically macrophages, can significantly impact the TME. M2-polarized macrophages secrete factors that promote tumor growth and radioresistance, creating a supportive environment for GBM (47, 48).

### **Therapeutic Implications**

Given the importance of HH signaling in GBM, in addition of inhibitors against this pathway, such as GLI inhibitors, could improve the effectiveness of conventional treatments, including radiation. Treatment approaches combining HH inhibitors can, in principle,

overcome resistance mechanisms (49, 50). However, challenges such as toxicity and/or development of resistance against HH inhibitors need to be properly addressed in the clinics.

In summary, the aberrant activation of the Hedgehog signaling pathway and the role of GLI transcription factors are critical to the underlying mechanisms in GBM, especially with regard to tumor growth, radioresistance, and maintenance of cancer stem cells. A deeper understanding of these mechanisms will provide valuable insights into the development of targeted therapies that could lead to better treatment outcomes for patients with GBM (51, 52).

### **Wingless Signaling Pathway in Glioblastoma**

Wingless (WNT) is involved mainly in the process of embryonic development, cell proliferation, and migration. Besides, this pathway takes part in maintaining stem cells. Abnormalities in WNT signaling are mainly linked to different disorders of the CNS and tumors such as glioblastoma multiforme (53). Upon the binding of WNT ligands to Frizzled receptors on the cell membrane, this pathway becomes activated, resulting in the cytosolic accumulation and nuclear translocation of  $\beta$ -catenin. Here,  $\beta$ -catenin interacts with transcription factors to activate target genes involved in cell division and survival (54).

Aberrant WNT signaling has a special importance in the context of cancer stem cells, although this pathway is seldom persistently activated in glioma (55). However, studies have demonstrated that WNT signaling indeed plays an important role in glioma stem cells and is linked to the most aggressive features of tumors and poor outcomes. Many reports have demonstrated the overexpression of components related to the WNT pathway in high-grade gliomas, and alterations in WNT/ $\beta$ -catenin signaling can contribute to chemoresistance (56).

Several inhibitors of WNT pathways, including WIF-1, are underexpressed in GBM, associating these with increased tumor invasion. In general, dysregulation of the WNT signaling pathway has been associated with aggressive glioma development and may offer potential targets for therapeutic intervention (57).

### **Norrin Effects in Glioblastoma via Notch and Wnt**

In this regard, the role of a protein encoded by the NDP gene, Norrin, has been observed in GBM. Data showed dual roles of Norrin regarding the regulation of GSCs, revealing both tumor-suppressive and tumor-promoting actions of this factor depending on ASCL1 expression levels, a proneural transcription factor (58).

Norrin triggers canonical Wnt signaling through the FZD4/LRP5 complex (59). It is upregulated in glioblastoma and directly correlates with better patient outcome. In ASCL1<sup>lo</sup> GSCs, Norrin suppresses proliferation. However, it increases Notch signaling in ASCL1<sup>hi</sup> GSCs, leading to increased tumorigenesis (60). This indicates that Norrin acts contextually, influenced by the GBM molecular subtype. This study demonstrated that Norrin controls cell proliferation, stemness, and differentiation in GSCs. Knockdown of Norrin increases proliferation in ASCL1<sup>lo</sup> GSCs but inhibits the growth of ASCL1<sup>hi</sup> GSCs (60).

### **Therapeutic Implications**

The findings would point toward a new therapeutic approach directed against the Norrin/FZD4 signaling axis in defined GBM subtypes and, therefore, a necessity for therapeutic strategies.

### **Combination Therapies**

The importance of combination in epigenetic therapies with classical cytotoxic drugs like TMZ is underlined. Proper timing and scheduling of such combinations will be important in efforts toward optimal clinical outcomes, thus enhancing treatment efficacy and overcoming resistance.

### **Targeting of Histone Modifications**

More recently, the possibility of targeting histone chaperone molecules has been explored, one of which is FACT-a complex involved in the reorganization of nucleosomes. In animal models where FACT targeting has been combined with TMZ, promising results have been obtained (61, 62).

## Challenges and Future Directions

**Mechanisms of Resistance:** While epigenetic therapies are outstanding for their potential, the inherent resistance mechanisms in GBM are much of a challenge. Most therapies experimented with have not met expected results and hence call for continued research into the underlying mechanisms of glioma biology.

**Emerging Therapeutics:** Phase testing of several preclinical and clinical molecules with epigenetic action. Few of them include IDH1/2 mutant inhibitors and modifications in histone that can modulate the tumor environment and restore sensitivity to standard chemotherapy.

## CONCLUSION

Glioblastoma multiforme (GBM), a highly aggressive and prevalent primary malignant brain tumor, poses significant challenges in both diagnosis and treatment. The profound impact of tumor heterogeneity, resistance to therapy, and infiltrative growth patterns results in a disheartening survival outlook, bringing the urgency for enhanced research and novel therapeutic strategies. The exploration of epigenetic alterations, particularly through DNA methylation and histone modifications, highlights crucial pathways for potential intervention, supporting the importance of personalized treatment approaches tailored to the tumor's molecular characteristics. Recent studies reveal the pivotal roles of various signaling pathways, including Hedgehog and Wnt, which significantly contribute to tumor growth, aggressiveness, and resistance mechanisms associated with GBM. The dual function of factors such as Norrin further underscores the complexity of GBM biology, indicating that context-dependent responses could inform treatment modalities. Thus, integrating epigenetic therapy with conventional approaches, while addressing resistance mechanisms, presents a promising avenue for enhancing therapeutic outcomes. In summation, ongoing investigation into the underlying biological mechanisms of GBM is critical to bridging the current knowledge gaps. By refining our understanding of tumor biology and individual patient profiles, we can better target

therapeutic strategies that yield improved survival rates and quality of life for patients battling this formidable disease.

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There is no conflict of interest.

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