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REVIEW

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Epigenetic alterations in glioblastomas: Diagnostic, prognostic and therapeutic relevance

Liliana Montella[1] | Mariella Cuomo[2,3] | Nunzio Del Gaudio[4] | Michela Buonaiuto[2,3] | Davide Costabile[2,5] | Roberta Visconti[2,6] | Teodolinda Di Risi[2,7] | Roberta Vinciguerra[2] | Federica Trio[2] | Sara Ferraro[2] | Guglielmo Bove[4] | Gaetano Facchini[1] | Lucia Altucci[4,8] | Lorenzo Chiariotti[2,3] | Rosa Della Monica[2,3]

[1]ASL NA2 NORD, Oncology Operative Unit, "Santa Maria delle Grazie" Hospital, Pozzuoli, Italy

[2]CEINGE Biotecnologie Avanzate scarl, Naples, Italy

[3]Department of Molecular Medicine and Medical Biotechnologies, University of Naples "Federico II", Naples, Italy

[4]Department of Precision Medicine, University of Campania "Luigi Vanvitelli", Naples, Italy

[5]SEMM-European School of Molecular Medicine, University of Naples "Federico II", Naples, Italy

[6]Institute for the Experimental Endocrinology and Oncology, Italian National Council of Research, Naples, Italy

[7]Department of Public Health, University of Naples "Federico II", Naples, Italy

[8]BIOGEM, Ariano Irpino, Italy

Correspondence
Lucia Altucci, Department of Precision Medicine, University of Campania "Luigi Vanvitelli", Vico L. de Crecchio 7, 80138 Naples, Italy.
Email: lucia.altucci@unicampania.it

Lorenzo Chiariotti, Department of Molecular Medicine and Medical Biotechnologies, University of Naples "Federico II", via S. Pansini 5, 80131 Naples, Italy.
Email: chiariot@unina.it

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Abstract

Glioblastoma, the most common and heterogeneous tumor affecting brain parenchyma, is dismally characterized by a very poor prognosis. Thus, the search of new, more effective treatments is a vital need. Here, we will review the druggable epigenetic features of glioblastomas that are, indeed, currently explored in preclinical studies and in clinical trials for the development of more effective, personalized treatments. In detail, we will review the studies that have led to the identification of epigenetic signatures, IDH mutations, MGMT gene methylation, histone modification alterations, H3K27 mutations and epitranscriptome landscapes of glioblastomas, in each case discussing the corresponding targeted therapies and their potential efficacy. Finally, we will emphasize how recent technological improvements permit to routinely investigate many glioblastoma epigenetic biomarkers in clinical practice, further enforcing the hope that personalized drugs, targeting specific epigenetic features, could be in future a therapeutic option for selected patients.

KEYWORDS
DNA methylation, glioblastoma, histone modifications, molecular classification, targeted therapy

Abbreviations: BBB, blood brain barrier; GBM, glioblastoma multiforme; IDH, isocitrate dehydrogenase; MGMT, O6-methylguanine-DNA methyltransferase; MLPA, multiplex ligation probes amplification; MSP, methylation specific PCR; TMZ, temozolomide.

1 | INTRODUCTION

Gliomas and glioblastomas (GBMs) are the most common and heterogeneous tumors affecting brain parenchyma, representing 81% of the central nervous system (CNS) tumors. During brain tumorigenesis, epigenetic and genetic mutations are crucial oncogenic drivers involved in tumor progression and in the establishment of tumor identity. The relevance of epigenetic modifications, by means of DNA methylation and/or histone modifications, is strengthened by at least four main considerations: (1) they may represent a mechanism of tumor progression based on selective conservation of favorable stochastically acquired mutations at genes involved in epigenetic processes; (2) they may be a consequence of mutations of genes involved in the epigenetic control of transcriptional program; (3) epigenetic signatures may be evocative of specific cancer cell trajectories, thus representing excellent markers for molecular classification; (4) combined with analysis of genetic aberrations, the epigenetic analysis may greatly help to predict response to targeted therapy.

Currently, glioma molecular classification, based on genetic and epigenetic modifications, has substantially surpassed pathological classification as highlighted in the new WHO 2021 edition. Indeed, in this edition, several genetic and epigenetic biomarkers have been introduced to finely classify and subclassify gliomas. Among the new molecular parameters added to resolve doubtful cases, the presence of EGFR amplification, chromosome 7 amplification, loss of heterozygosity involving chromosome 10 and TERT promoter mutations have been associated with GBM diagnosis, although morphological features would often indicate a lower histological grading.[1,2] Thus, morphological features alone appear to be insufficient to settle GBM diagnosis. However, despite considerable quantities of molecular information for subclassification of gliomas, unfortunately, the transformations of these parameters in clinical application are still far away.

Importantly, in the last decade, the analysis of the epigenetic and epigenomic profiles, together with the study of genetic alterations, have greatly implemented the wealth of information that can help not only to understand the complex molecular mechanisms underlying glioma transformation but also to open up new therapeutic perspectives. Indeed, epigenome manipulation is assumed as one of the potential therapeutic opportunities in GBM treatment, a tumor whose current therapy is not decisive and the prognosis is very poor.[3,4]

Thus, in this review, we will discuss the epigenetic landscape of GBM, focusing on putative therapeutic targets. Additionally, we will describe effective epigenetic drugs explorable for the treatment of GBM. As the role of noncoding RNAs has been extensively and recently reviewed elsewhere,[5-7] we will focus, in particular, on the relevance of DNA and histone modifications in glioblastoma diagnosis, prognosis and therapy.

2 | EPIGENETIC SIGNATURES

Epigenetic signatures based on DNA methylation and chromatin organization allow characterizing with extreme precision different types of tumors. For the ~100 known CNS tumor entities the developing approach, based on the whole genome methylation analysis, allows to identify and subclassify different tumor types by refining histopathological diagnosis.[8] The epigenetic profile of brain tumors represents an identity card, notably, the tumor will retain some specific "signatures" of the cell of origin and will acquire the typical "signatures" that define the tumor specificity. Following these considerations, Capper et al 2018 developed a sophisticated classifier based on tumors methylome analysis discriminating among 82 different types of brain tumors, including both primary and metastatic. For wider accessibility, they designed a free online classifier tool (www.molecularneuropathology.org) requiring no further processing of data.[9]

The methylomic classifier is constantly updated, integrating ever-greater numbers of data to classify and subtype brain tumors, as well as to identify new tumor entities. Although this effort leads to a fine tumor definition, it may help to identify new epigenetic markers. This might be used as a potential therapeutic target leading to the usage of

TABLE 1 Glioblastoma methylation class IDH wt

	Includes histopathological GBM IDH wt and anaplastic astrocytoma (rare)
H3.3 G34 mutant	
Mesenchymal	Includes histopathological gliosarcoma. Recurrent genetic alterations are: • Amplification of chromosome 7 with or without EGFR amplifications • Loss of chromosome 10 • Loss of CDKN2A/B
MYCN	Includes histopathological GBM IDHwt. Recurrent genetic alterations are: • Amplification of MYCN • ID2 amplification gene
RTK I	Includes histopathological GBM IDH wt. Recurrent genetic alterations are: • Gain for chromosome 7 with or without EGFR amplification • Loss of CDKN2A/B • Amplification of PDGFRA gene
RTK II	Includes histopathological GBM IDH wt and, rarely gliosarcoma. Recurrent chromosomal alterations are: • Gain for chromosome 7 with or without EGFR amplification • Loss of CDKN2A/B • Gain for chromosomes 19 and 20
RTK III	Includes histopathological GBM IDH wt. Recurrent genetic alterations are: • Amplifications of EGFR • Loss of chromosome 10
Glioma IDH mutant	
Astrocytoma	Includes astrocytomas of WHO grades II and III
High grade astrocytoma	Includes: high grade astrocytoma and anaplastic astrocytoma, IDH mutant

personalized medicine, which unfortunately remains a long way off for brain tumors, especially GBMs.

In the last V edition of the WHO classification of brain tumors (published in March 2022), the study of epigenome has assumed an important role in discriminating the various tumor subtypes.

Of note, the DNA methylation classifier, according to the WHO 2021 classification of brain tumors, divided GBMs into two different groups: IDH wild-type and IDH mutated. In addition, the DNA methylation classifier subtypes GBMs into different entities, characterized by specific molecular and histological feature[9] (summarized in Table 1).

3 | IDH1-IDH2 MUTATIONS

The investigation of the "episignatures" plays a fundamental role in defining GBM tumor molecular subtype.[10] Mutations in Isocitrate Dehydrogenase 1 or 2 (IDH1 or IDH2), involved in epigenetic regulation, and the histone genes H3F3A or HIST1H3 B turned out to be crucial biomarkers for tumor classification, highlighting the central role of epigenetic alterations as drivers of tumor initiation, progression and aggressiveness.[11] Gliomas with mutations in the IDH1 or IDH2 genes show a different genome methylation profile compared to gliomas without these mutations[8] (Figure 1). Mutations in IDH1 or IDH2 gene are early lesions in cancer development and the prognosis associated with IDH mutated gliomas is better than IDH wild-type. To date, no more than 12 functional mutations affecting the IDH1 and IDH2 genes are known.[12] In 2008, a sequencing implementation of GBM tumor samples found IDH1 mutations at R132 position in 12% of the patients. The most frequent mutations found in GBM patients involved codon 132 of IDH1 gene resulting in the replacement of an Arginine with Histidine, Serine, Cysteine, Glycine or Leucine. Subsequent analyses, not only confirmed the IDH1 mutations in secondary GBMs, but also identified IDH1 and IDH2 in gliomas, myeloid leukemia (AML), melanoma and cholangiocarcinoma.[13] Most of these mutations are located in the catalytic domain of IDH gene resulting in a gain of new catalytic function able to convert α-Ketoglutarate into

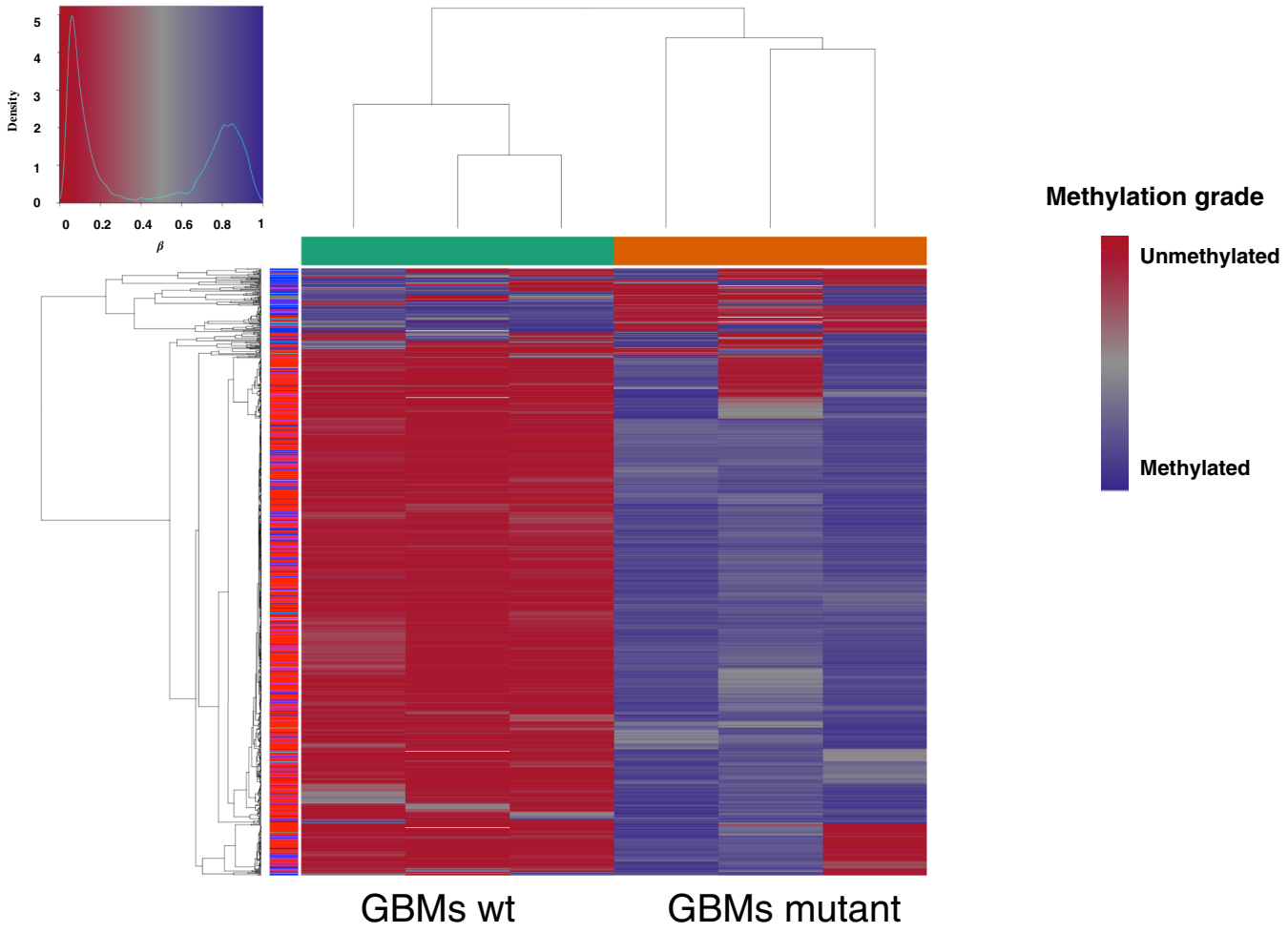

FIGURE 1 Heatmap showing different methylation patterns between GBM IDH-wt and GBM IDH mutant. Hierarchical clustering of 3 IDH1 wt tumors (orange) and 3 IDH1 mutant tumors (green), analyzed at CEINGE Biotecnologie Avanzate, based on methylation values at single CpG levels. The heatmap displays CpG sites with the highest variance across all samples. The scale color from red to purple indicates methylation value from 0 to 1, respectively. The top x-axis shows wt and mutated IDH tumors. The y-axis shows the CpG site based on CGI relation. Clustering was built by using Manhattan distance [Color figure can be viewed at wileyonlinelibrary.com]

2-Hydrossi-Chetoglutarate (2HG). 2HG acts as oncometabolite by inhibiting α-ketoglutarate-dependent enzymes. Among these enzymes, TET2 is an epigenetic modifier acting both demethylating DNA through the conversion of 5-methyl cytosine (5-mC) in 5-hydroxymethyl cytosine (5-hmC) and remodeling chromatin through the interaction with lysine-specific histone demethylase KDM2A[14] (Figure 2).

Different studies demonstrate that IDH-mutated tumors show a reduced proliferation rate compared to IDH1/2 wild type GBM and anaplastic astrocytomas (WHO grade III).[15] Several studies have indicated that IDH1/2 mutations are significantly associated with positive prognosis and increased chemo- and radio-sensitivity.[16,17] Mechanistically, the altered production of reactive oxygen species (ROS), due to the abnormal function of IDH mutant proteins, plays a central role in the reduction of cell viability induced by chemotherapy. Other studies demonstrated a potential link between IDH mutations and impaired repair of DNA.[18] Based on these observations, different preclinical studies supported the positive therapeutic effect of using DNA damaging drugs such as Procarbazine, Lomustine and Vincristine.[18] The overwhelming evidence that IDH mutations underlie tumor-defining epigenomic changes led to rapid development of IDH mutant inhibitor drugs that are currently in preclinical and clinical experimentation[18,19] (summarized in Table 2).

AG-221 is a selective orally administered inhibitor of IDH2 mutant that has been approved by the FDA for the treatment of patients with IDH2-mutated AML based on promising response rates and a satisfactory safety profile. The drug is now in phase I/II clinical trial for the treatment of different tumor types, including

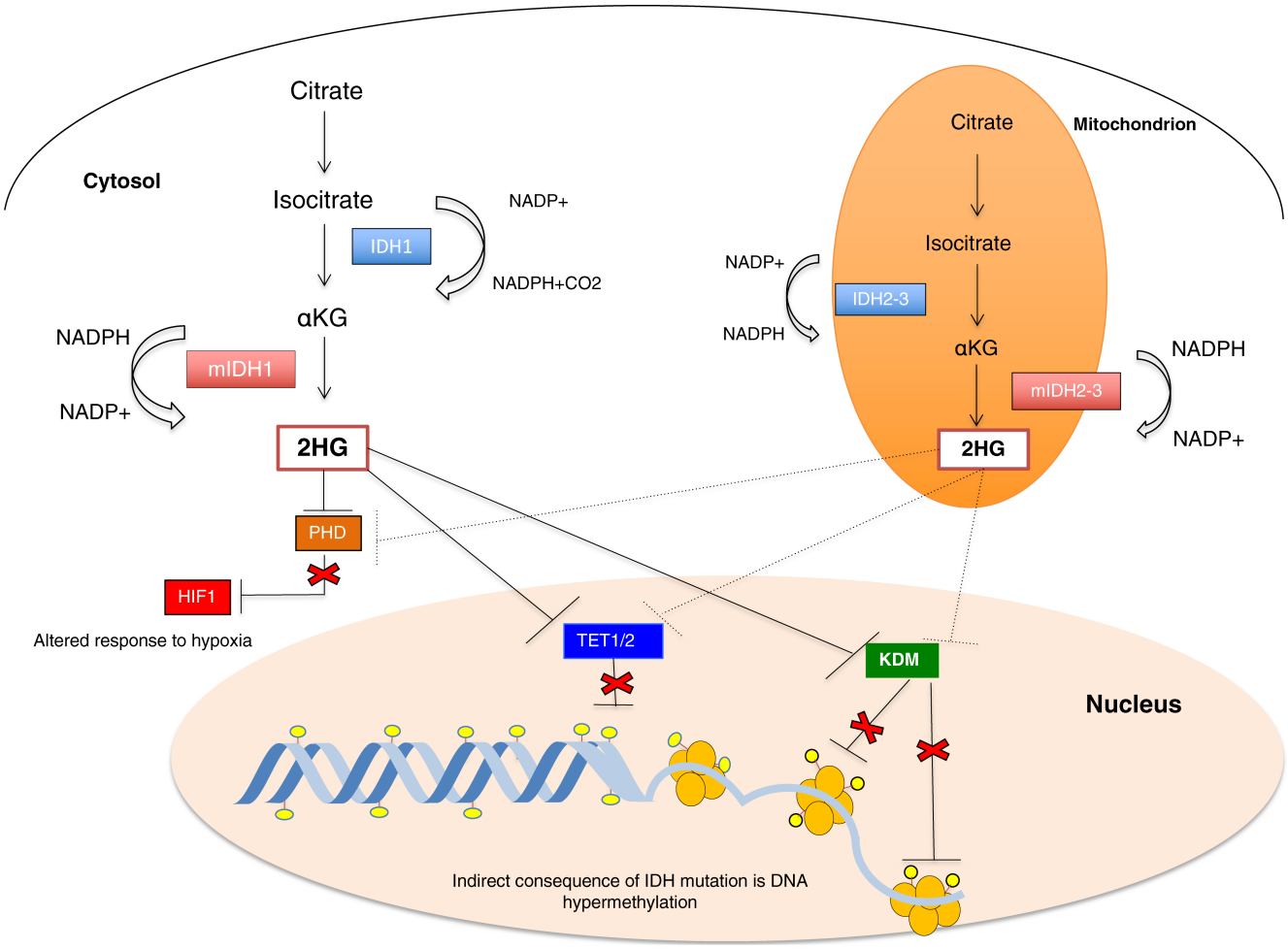

FIGURE 2 Overview of IDH enzyme role in cytosol, mitochondria and nucleus, highlighting its key interactors and consequences on epigenetic alterations. Throughout the figure, black arrows connect metabolites to each other, the curved arrows show redox reactions due to the catalytic activity of IDH1 and IDH2-3, while the flat end arrows represent the direct inhibitory effect of 2HG oncometabolite (red rectangle in cytosol and mitochondrion) on its interactors; the red cross indicates the suppression of enzyme activity. In the cytosol (white background), IDH1 role in altered response to hypoxia is represented; wild type IDH1 is shown in the light blue box, mutated IDH1 is shown in the light red box, while PHD and HIF1 in the orange and red box, respectively. In the mitochondrion (orange background), IDH2-3 activity in TCA (tricarboxylic acid cycle) is shown; mutated IDH2-3 is represented as a light red box, while wild type IDH2-3 as a blue box. In the nucleus (light orange background), a schematic representation of the effects of mutated IDH enzymes on DNA methylation and histone modifications; methyl groups are represented as yellow dots while histones as dark yellow spheres; key IDH interactors in the nucleus are represented as blue box (ten-eleven translocation, TET1/2) and green box (lysine demethylase, KDM) [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 2 Epigenetic drugs advanced in clinical phases for GBM treatments

	Molecular target	Stage of clinical trial	Results
HDACi drugs			
Vorinostat	Pan-HDACi	Phase II	Modest
Romidepsin	Class I/II HDACi	Phase I/II	No effect
Panobinostat (in combination with bevacizumab)	Pan-HDACi	Phase II	No effect
Valproic acid (in combination with TMZ)	Class I HDACi	Phase II	Modest
IDH mutant inhibitors			
AG-221	IDH2 mutant inhibitor	Phase I/II	Ongoing
AG-120	IDH1 mutant inhibitor	Phase I	Ongoing
AG-881	Pan-IDHs mutant inhibitor	Phase I	Ongoing
DS-1001b	IDH1 mutant inhibitor	Phase II	Ongoing

IDH2-mutated gliomas. The clinical study is available on http://www.clinicaltrial.gov (NCT02273739).

AG-120 is a selective IDH1 mutant inhibitor. In a phase I clinical trial, AG-120 showed a safety profile when used in monotherapy for solid cancers in advanced state (http://www.clinicaltrials.gov; NCT02073994).[18]

AG-881 (Vorasidenib) is a pan IDH mutant inhibitor. A phase I, randomized, controlled, multicenter trial recruiting patients with low-grade glioma to determine 2HG levels in tumor tissue after presurgical treatment with AG-120/AG-881 will end in 2024. (http://www.clinicaltrials.gov; NCT03343197).[19]

Targeting IDH mutants in gliomas presents several challenges including difficulties to cross the blood-brain barrier (BBB). In addition, the neovascularization of GBM shows a very tortuous architecture rendering pharmacological penetration extremely difficult. **IDH305** was optimized to penetrate BBB. The pharmacodynamic effects of IDH305 were evaluated in eight patients (NCT0238186), in which IDH305 was able to reduce the levels of 2HG. Surprisingly, in three out of eight patients the 2HG levels remained suppressed for more than 1 month.

DS-1001b is an IDH1/2 mutant inhibitor with high BBB permeability. In xenograft models of GBM, the treatment with this inhibitor impaired tumor growth. Currently, two clinical studies based on DS-1001b are ongoing (NCT04458272 and NCT030300066).[20]

Mutations of IDH1/2 in tumors remain an important finding in biomedical research. The production of 2HG has a significant impact on tumors and it can modify the entire epigenome, influencing metabolism and cell proliferation. However, the positive prognostic value given by IDH mutations has greatly slowed clinical experimentation with targeted drugs, as these tumors are more sensitive to classical treatment based on radio and chemotherapy.[18]

4 | MGMT

Methylation of O6-methylguanine-DNA methyltransferase (MGMT) has been extensively investigated as a biomarker for prediction of pharmacological response to temozolomide in patients affected by GBM.[21,22] MGMT gene is located on chromosome 10q26.3.[23-25] The expression of MGMT gene is mainly regulated by epigenetic mechanisms. Several studies demonstrate that loss of MGMT expression is due to methylation of the CpG island located in the MGMT promoter and only in rare cases to gene deletion, mutation or rearrangement.[23] The methylation of MGMT promoter has been accepted as a valid prognostic positive marker in response to temozolomide (TMZ) in terms of overall survival (OS) and Progression-Free Survival (PFS).[24,26-28] The positive clinical benefit of MGMT promoter methylation is implicated by the function of MGMT protein, an enzyme involved in DNA damage repair. Loss of MGMT expression is a valid help to induce, by using TMZ, DNA damage in GBM with consequent cellular death.[23] Conversely, MGMT expression nullifies the function of TMZ by removing the alkyl groups from the O6 position of guanine and restabilizing the correct sequence of DNA (Figure 3). MGMT has been also found predominantly implicated in TMZ resistance in recurrent GBM. However, approximately 15% of patients with MGMT hypomethylation benefit from TMZ therapy.[29] Nonetheless, despite presenting methylation at MGMT, many cases of GBM recurrence do not respond to TMZ because of chemo-resistance.[30] Several studies have demonstrated that GBM progression and recurrence were associated with the resistance of cancer stem cells to TMZ treatment. Specifically, it has been demonstrated that, even after effective therapeutic treatment, minimal residual stem cells may be activated to enter a new stage of differentiation and proliferation thus promoting GBM recurrence. Therefore, GBM stem cells are considered one of the causes of TMZ resistance, which enables them to survive during chemotherapy.

Additional studies have explored the potential MGMT enzymatic inhibition in unmethylated GBMs. For example, the cationic porphyrin5,10,15,20-tetrakis(diisopropyl-guanidine)-21H, 23H-porphine (DIGPor) selectively binds DNA-containing O6-methylguanine (O6MeG), inhibiting the MGMT enzymatic function.[31] However, the therapeutic efficacy of these inhibitors remains under investigation.

It is currently evident that the surgical resection followed by radio and chemotherapy appears to be inadequate for the long-term treatment of GBM. Intriguingly, some tumors, despite having unmethylated

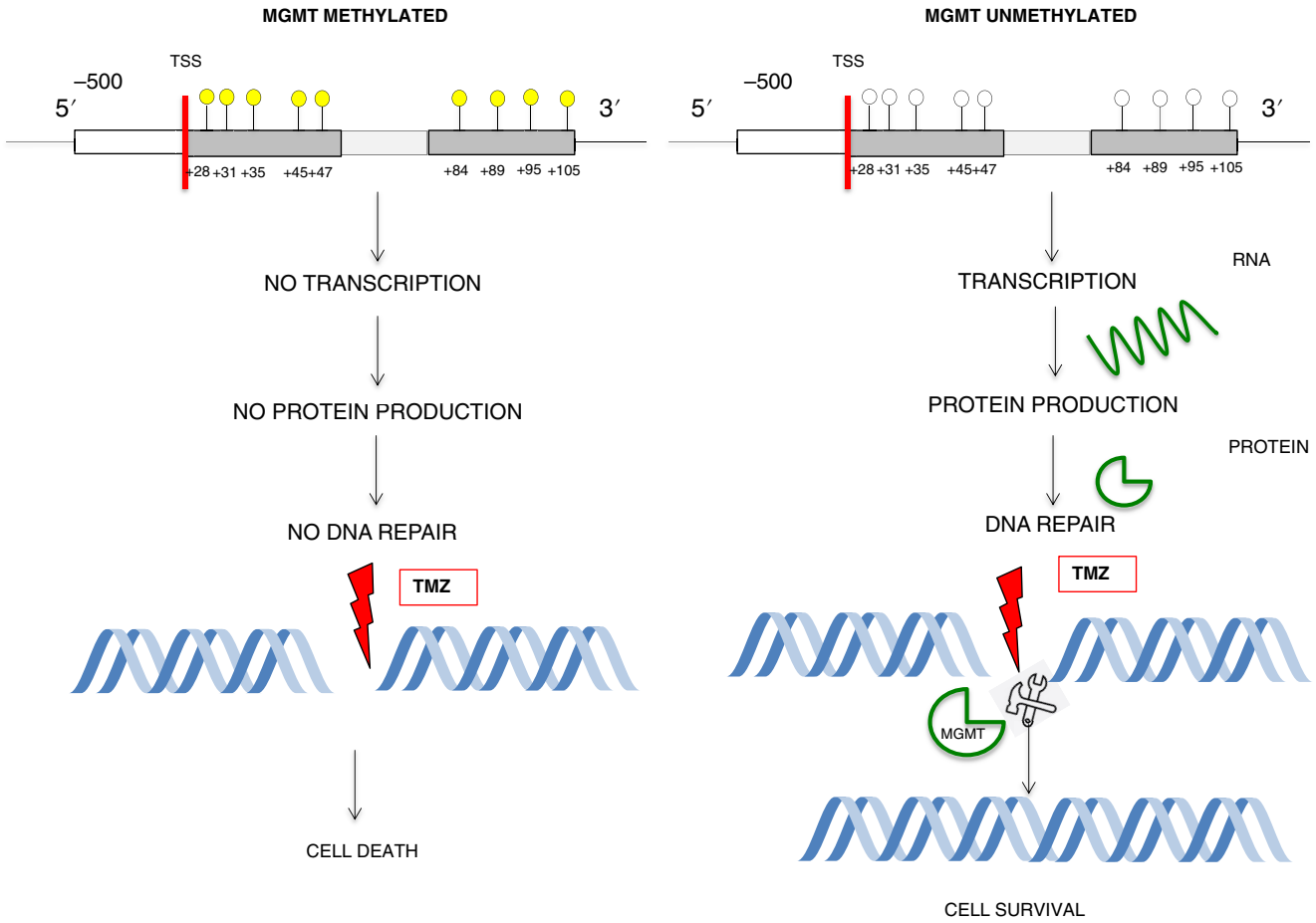

FIGURE 3 Schematic representation of the MGMT gene and the methylation-related downstream effects. The MGMT gene is represented in its methylated (left) and unmethylated (right) status. The 5′ and 3′ ends are shown. Methylated CpGs (yellow circles) and unmethylated CpGs (white circles) are represented; CpG position are related to the transcription start site (TSS, marked with a red line). The effects of MGMT methylation status are represented. Point arrows indicate the consequences of each event. The methylated status of MGMT leads to cell death thanks to the promoted activity of Temozolomide (TMZ, red thunderbolt). The unmethylated status of MGMT leads to the production of MGMT protein (in green) which repairs DNA, thus hampering TMZ activity and leading to cell survival [Color figure can be viewed at wileyonlinelibrary.com]

MGMT, well respond to chemotherapy, while some others, harboring methylated MGMT, does not.[32]

5 | ROLE OF HISTONE MODIFICATIONS IN GBM

Looking at the extremely wide epigenomic panorama, several histone modifications participate in determining gene expression and eventually cell fate. For instance, the lysine acetylation mark (Ac), added or removed by histone acetyltransferases (HAT) and histone deacetylases (HDAC) respectively, holds broadly known functions in winding and unwinding the chromatin structure, resulting in the regulation of gene expression.[33,34] Since HDACs are constitutively expressed in various cancer types, including GBM,[35,36] pharmacological approaches exploiting HDAC inhibitors (HDACi) have been carried out. Early in vitro studies showed that the HDAC inhibitors Romidepsin and DWP0016 decreased GBM cells viability and caused a significant increase in cell death markers through the activation of p53/p21 pathway.[37,38] In the same way, Chiao et al used the suberoylanilide hydroxamic acid (SAHA) pan-HDACi in glioblastoma stem cells (GSCs), achieving a reduction of viability by apoptosis activation as well as induction of autophagy and cell differentiation.[39,40]

Further, HDAC inhibition by Panobinostat, Vorinostat (SAHA) and Romidepsin was demonstrated to affect GBM glucose metabolism and to reduce the global ATP levels, reverting the Warburg effect, both in vitro and in vivo.[41]

As HDACi can pass the BBB, their use was proposed for GBM clinical investigations. However, in clinical trials, Vorinostat and Romidepsin demonstrated modest or no therapeutic effects.[42,43]

Interestingly, combination therapies employing HDACi and chemotherapeutic agents are currently being investigated in clinical trials for GBM treatments. Specifically, Valproic acid used in combination with TMZ has been tested in phase II clinical trials showing satisfying results (Figure 4A and Table 2).[44] Beside acetylation, methylation is another modification frequently found on histone tails.[34] The

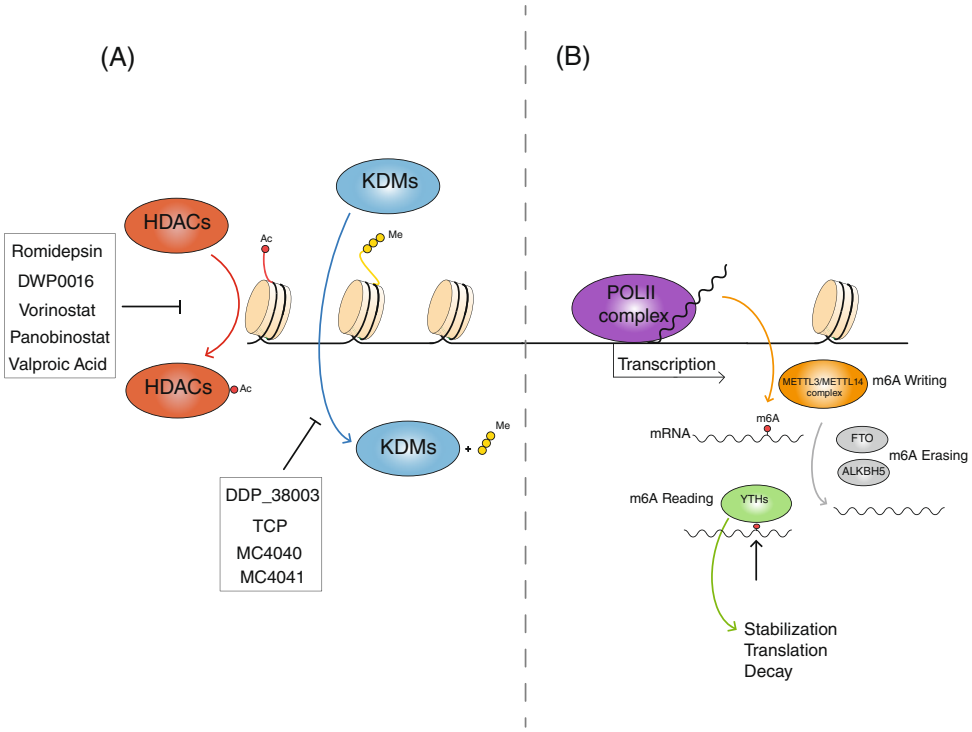

FIGURE 4 Schematic representation of epigenetic and epitranscriptomic regulation. Chromatin structure highlighting the activity of histone modifiers (A) as well as the dynamic control of the m6A mark (B) are depicted and separated by the dotted line. (A) Inhibitors impacting HDACs and KDMs enzymatic activity are reported. (B) Regulation of m6A dynamics process is shown: the writing complex methylates RNAs cotranscriptionally, erasers remove the methylation from RNA targets, readers decode the modification into a specific process leading to the regulation of RNA fate [Color figure can be viewed at wileyonlinelibrary.com]

insertion of methyl groups in these sites can trigger (H3K4, H3K36 and H3K79) or inhibit (H3K9, H3K27 and H4K20) gene expression.[45] Methylation dynamics are regulated by the activity of histone methylases (HMTs) and demethylases (KDMs) and have a strong impact on cancer onset and progression.[46] For instance, it has been reported that knockdown of the lysine-specific demethylase 1 (LSD1), which regulates the deposition of the H3K4me3 mark on histones, increased H3K4 methylation at the MYC promoter region, thus enhancing MYC expression and consequently GBM tumorigenesis.[47]

Interestingly, the recent use of the specific LSD1 inhibitor DDP_38003 showed to affect GSCs viability by impairing the induction of activating transcription factor 4 (ATF4) that triggers cellular stress responses inducing GBM growth arrest.[48] Similarly, the Jumonji domain-containing protein D3 (JMJD3, alias KDM6B) demethylase has been shown to regulate differentiation in patient-derived GSCs by reducing the repressive histone mark H3K27 tri-methylation at the genes' loci.[48]

JMJD3 has been found downregulated in GBM cells and its depletion or its gene reactivation, by reverting the JMJD3's promoter hypermethylation, could decrease GBM progression and invasiveness via p53 inhibition.[49,50] Unfortunately, although demethylase inhibitors have shown interesting preclinical results, they have not been advanced yet into clinical trials.

In the context of GBM methylation, HMTs also play a major role. For example, silencing of the Enhancer of zeste homolog 2 (EZH2), which participates in the Polycomb repressive complex 2 (PRC2) and catalyzes the H3K27me3 modification, affects GBM progression both in vitro and in vivo.[51] On top of that, EZH2 has been reported to inhibit the expression of PTEN by increasing the H3K27me3 deposition at its promoter level.[52] This resulted in the activation of the AKT/mTOR axis and stimulation of GBM progression.[53] Since EZH2 is overexpressed in GBM[54] and has been reported to regulate the resistance of GBM cells to TMZ,[55] efforts to generate EZH2 inhibitors have been made. Interestingly, in a recent paper, Stazi e al. have tested two EZH2 inhibitor compounds that decreased vascular endothelial growth factor (VEGF) expression and GBM cells migration, while increasing the levels of anti-inflammatory cytokines.[56] Despite the great efforts, only a few drugs targeting methylations have reached clinical trials and more investigation is needed to improve their efficiency. Nonetheless, treatment with HDACi in GBM cells exhibited to reduce LSD1 levels and increase the deposition of methylation. Additionally, cotreatment of GBM cells and GSCs with Vorinostat and the LSD1 inhibitor Tranylcypromine (TCP) led to synergistic tumor cell death by inducing apoptosis in vitro and GBM xenograft mice.[57,58] The crosstalk between different histone modifications opens up new combination therapeutic opportunities in clinics, targeting both acetylation and methylation in GBM tumors at the same time (Figure 4A and Table 2). Altogether, these findings indicate that the role of epigenetic marks and epi-factors involved in GBM remains poorly understood. Thus, further research will help to finely dissect the molecular mechanisms behind the epigenetic changes in GBM and discover novel Epi-targets for future clinical applications.

6 | EPIGENETIC ALTERATIONS CAUSED BY H3K27 MUTATION

Somatic mutations in histone 3 (H3) characterize a specific high-grade glioma that, according to the new WHO classification, is identified as diffuse midline glial lesion.[32] This tumor is more common in pediatric

patients but, rarely, also occurs in adults. As described in the previous paragraph, the heterozygous (gain of function) H3K27M mutation in either the H3F3A or HIST1H3B genes suppresses EZH2, a catalytic subunit of polycomb repressive complex 2 (PRC2) which trimethylates the histone H3 at lysine 27. Thus, H3K27M mutations lead to a global epigenetic dysregulation, caused by the loss of H3K27me3 and consequent transcriptional repression.[59] Hotspot mutations in H3F3A and H3K27M, as well as for IDH1/2, define clearly distinct epigenomic and biological subgroups of GBMs.[60]

The epigenetic alterations caused by the onco-histone H3K27M mutation can be specifically targeted by different therapeutic approaches. Preclinical studies aimed at restoring H3K27 repressive mark by inhibiting the KDM6 demethylase with GSK-JA. In vitro, GSK-JA treatment was effective in decreasing cell viability.[61] Furthermore, the drug had greater effect in cells with the H3 mutation than in H3 wild type cells, promising tumor specific effects.[62] However, the clinical use of GSK-JA has been scarce because it does not efficiently penetrate BBB.

Also, panobinostat, a pan-histone deacetylase inhibitor that partially rescues H3K27M-induced global hypomethylation by counteracting PRC2 inhibition, has been tested. In detail, poly-acetylation of the H3 N-terminal tail by panobinostat can "detoxify" K27M-induced inhibition of PRC2 and rescue the H3K27 hypomethylation phenotype.[63] It has also been demonstrated that panobinostat induced an increase in global H3 acetylation as well as in H3K27 methylation, these events being associated with reduction in oncogene expression.[64] Promisingly, in vitro *assays* have shown synergic effects between panobinostat and GSKJA in reducing proliferation of H3K27M mutant cells.[65]

Another therapeutic strategy is based on the use of BET inhibitors that reduce H3K27 acetylation, in turn resulting in the upregulation of differentiation markers.[66]

Based on positive data obtained in in vitro and in preclinical studies, several clinical trials using epigenetic drugs have been designed. Among the many still ongoing, especially worth of notice is the phase I NCT02717455 clinical trial, based on use of panobinostat in 53 children with diffuse intrinsic pontine glioma, whose results are eagerly awaited.

7 | THE EPITRANSCRIPTOME LANDSCAPE OF GBM

An increasing amount of evidence is highlighting the role of RNA modifications in cancer cell proliferation and tumorigenesis.[67]

Specifically, epitranscriptomic marks can affect several stages of RNA metabolism, including maturation, stability and degradation of oncogenes and oncogenic-related transcripts.[68] On top of that, the dynamic nature of these modifications, supported by the function of writer, eraser and reader proteins, that "add, remove and read" the modification respectively, allows them to regulate multiple tumorigenic pathways at the same time (Figure 4B). Pseudouridine, a modification that influences RNA translation[69] has been found to strongly affect GBM survival, growth and migration.[70] In addition, Cui et al demonstrated that increased expression of pseudouridine synthase 7 (PUS7) in glioma stem cells (GSCs) enhances the translation of the tyrosine kinase 2 (TYK2) tRNA and inhibits GBM tumorigenesis through the TYK2-STAT1 axis.[71] On the contrary, downregulation of adenosine-to-inosine (A-I) RNA modification, catalyzed by ADAR proteins, can reduce the levels of edited miR-376a* and increase GBM cells proliferation.[72]

5-methyl cytosine (5mC) is an additional RNA modification, catalyzed by Nop2/Sun RNA methyltransferase (NSUN6), that regulates RNA stability.[73] It has been shown that increased levels of NSUN6, and consequently of 5mC, ameliorated the response of GBM cells to the alkylating agent TMZ, suggesting a possible role of this mark in regulating drug resistance.[74] Within Epi-modifications, the N6-methyladenosine (m6A) surely carves a niche for itself, as it is the most abundant mark on eukaryotic mRNAs and by far the most studied.[75] Accordingly, the role of the m6A mark and its effectors in GBM has been explored. In 2017, it was reported that the m6A demethylase α-ketoglutarate-dependent dioxygenase alkB homolog 5 (ALKBH5) is overexpressed in GSCs and governs the stability of the forkhead box protein M1 (FOXM1) mRNA, therefore driving GBM progression.[76]

Interestingly, small molecules inhibiting ALKBH5 were recently developed and might offer a promising therapeutic alternative for the treatment of GBM in the future.[77] In addition, selective inhibition of the fat mass and obesity-associated (FTO) demethylase by using the ethyl ester form of meclofenamic acid (MA2) has shown to dramatically reduce cancer growth both in vitro and in vivo (Figure 4B).[78] Intriguingly, a recent publication showed that the MA2 compound was able to enhance the sensitivity of GBM cells to TMZ treatment, thus opening up new perspectives for advancing the combinatorial approach to clinical trials for GBM.[79]

On the contrary, knockdown of methyltransferases like 3 and 14 (METTL3, METTL14), the catalytic components of the m6A writing complex, by reducing m6A global cell levels, strikingly enhanced GSCs proliferation and tumorigenesis.[77] Additionally, elevated m6A levels are also required for GBM differentiation.[78] These findings strongly highlight that m6A plays a crucial role in GBM development. Therefore, considering this evidence, therapeutic routes targeting m6A erasers and enhancing m6A global levels in GBM cells have been followed. This led to the production of several efficient pharmacological inhibitors tested in vitro, however, at present, they still need further improvement to enter clinical trials.[79,80]

In recent years, a comparison of methyl RNA-immunoprecipitation followed by sequencing (meRIP-seq) data between normal stem cells (NSCs) and GSCs identified dependency on GSCs for the m6A reader YT521-B homology domain family 2 (YTHDF2).[81] YTHDF2 was associated with either stabilization or destabilization of several mRNAs such as MYC or UBXN1, resulting in enhanced GBM tumor progression.[81,82] Conversely, the heterogeneous nuclear ribonucleoprotein C (HNRNPC), an additional m6A reader, was correlated to GBM, with a favorable prognosis.[83] However, further investigations are required to better elucidate the

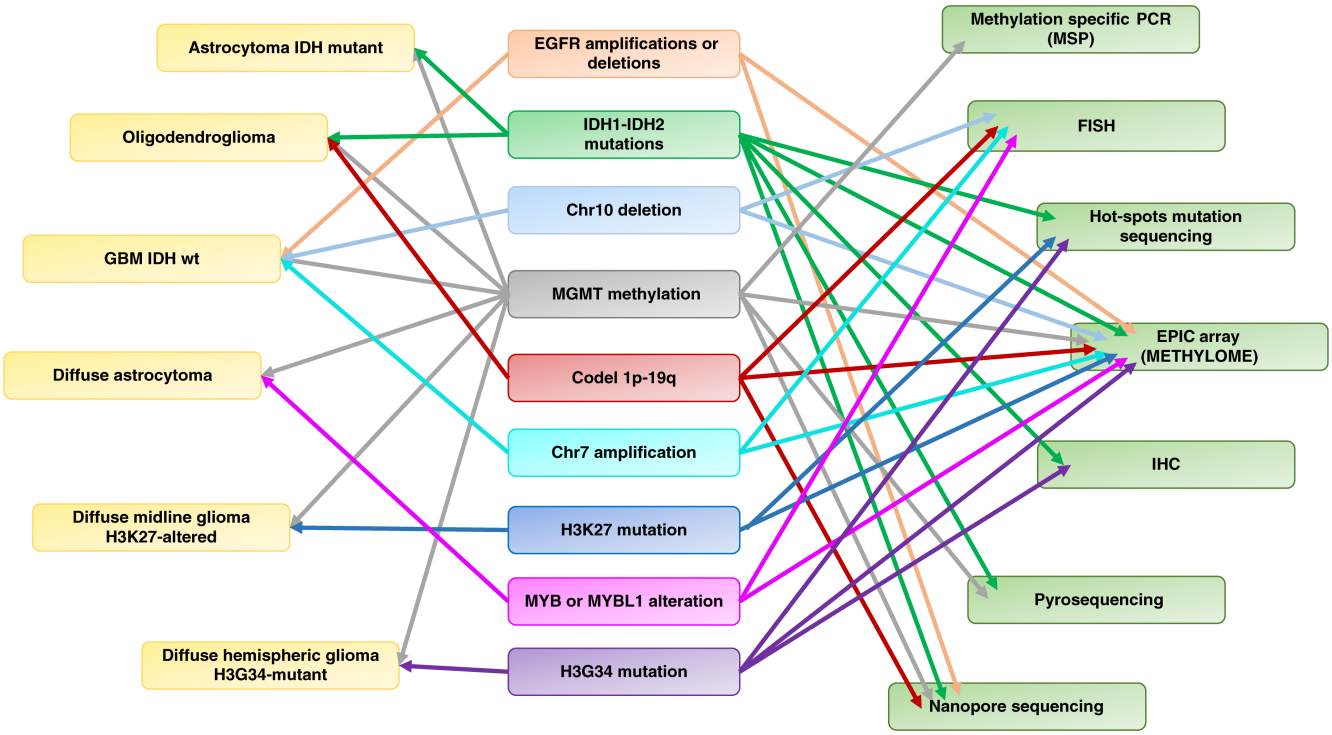

FIGURE 5 A schematic overview of the most relevant epigenetic and genetic mutations investigated to narrow brain tumor diagnosis and of the better techniques for investigating them. In the middle, in boxes of different colors, the epigenetic and genetic mutations investigated for narrowing brain tumor diagnosis. On the left, the brain tumor subtypes whose diagnosis is permitted by the genetic and epigenetic mutations shown in the middle: the boxes indicating mutations are connected to the boxes indicating tumor subtypes in which they are found by matching color arrows. On the right, the most relevant techniques that can be utilized for investigating the genetic and epigenetic modifications shown in the middle. Again, the boxes indicating mutations relevant for diagnosis are connected to the boxes indicating the techniques utilized for their investigation by matching color arrows [Color figure can be viewed at wileyonlinelibrary.com]

involvement of m6A regulators in GBM. Finally, a positive feedback loop involving METTL3, YTHDF1 and ADAR1 regulating RNA dynamics was discovered to play an oncogenic role in GBM survival, suggesting an additional layer of drug interventions.[84] Altogether, recent findings are highlighting that RNA modifications are gaining a pivotal role in regulating the hallmarks of cancer.[85] Their strong impact in the oncogenesis of GBM calls for further research, to elucidate the mechanisms of action of these m6A-effectors as well as synthesizing new molecules, activating or inhibiting specific Epitran-targets. Additionally, more investigations are needed to expand the spectrum of m6A regulators as possible GBM druggable targets. The advent of novel drugs regulating mRNA marks is bound to dramatically change the way we look at GBM cancer therapy today.

8 | TECHNICAL APPROACHES TO INVESTIGATE EPIGENETIC BIOMARKERS IN GBM IN CLINICAL PRACTICE

The molecular analysis carried out on brain tumors includes to date both genetic and epigenetic aspects. Of note, molecular characterization of brain tumors has been profoundly perfected in the last decade in consequence of the many recent technological improvements.[86-88] A lot of techniques are utilized in preclinical studies, for investigating brain tumor development and progression, and in clinical routine, for narrowing brain tumor diagnosis. Here, we will focus only on some techniques routinely utilized for the genetic and epigenetic characterization of brain tumors that have had a strong impact on brain tumor diagnostics (Figure 5). In general, in clinical practice, the choice of the technology to be used is always the result of the evaluation of the cost–benefit ratio. Investigation of IDH mutations can be evaluated by different approaches: IHC with specific antibody allow to identify a specific mutation R132H, the most common in gliomas. Sanger sequencing or Next Generation Sequencing (NGS) are more often used for detecting all possible mutations affecting IDH genes.[89]

There are different approaches to assess MGMT methylation status: the Methylation Specific PCR (MSP) remains considered the gold standard method to evaluate MGMT methylation status. This technique is based on discrimination of the methylated vs the unmethylated DNA sequence following the chemical treatment of the DNA with sodium bisulfite which converts the unmethylated Cytosine (C) in the Cytosine-Guanine (CG) context into Thymine (T). MSP approach does not provide quantitative evaluation of the methylation degree.[22,88,90] These limitations are overcome by pyrosequencing.[91] Also, methylation-sensitive Multiplex Ligation Probes Amplification (MLPA)-based kits are used to evaluate the methylation status of

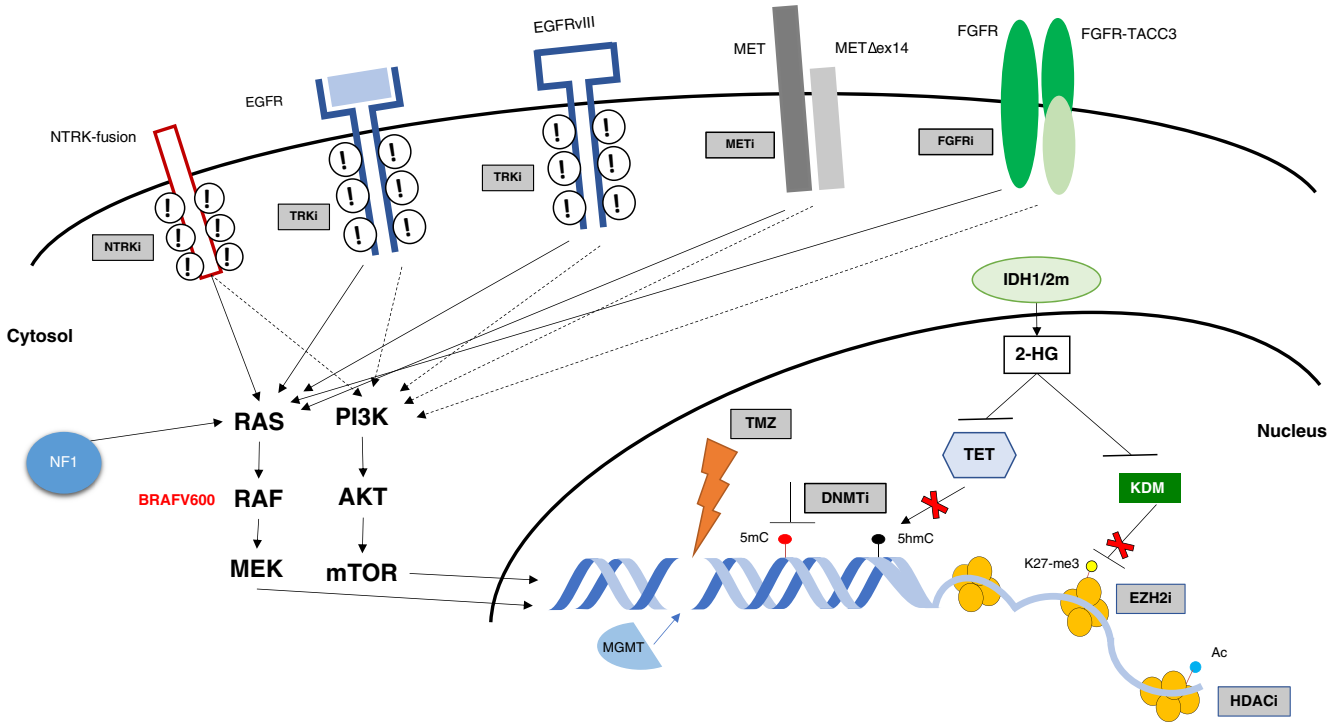

FIGURE 6 A schematic overview of the most important tumor-driving pathways and interactors in gliomas. The arrows link each oncogenic factor to the principal activated pathway. On the plasma membrane: NTRK fusion (red rectangle); EGFR (in blue) and its ligand EGF (light blue rectangle); EGFRvIII (in blue, without ligand); MET (in dark gray) and its corresponding ligand-independent form, METΔex14 (in light gray); FGFR (green oval) and FGFR-TACC3 fusion (green and light green ovals). In the cytosol: NF1 oncogenic form (light-blue circle); the MAP kinase pathway (nearby, the most oncogenic RAF mutation is written in red); the PI3K/AKT/mTOR pathway. Between cytosol and nucleus: the IDH pathway. In the nucleus: the DNA with 5-methyl-cytosine (5mC, red sphere) and 5-hydroxymethyl-cytosine (5hmC, black sphere); histones (yellow spheres) with some drug-targeted epigenetic modifications (acetylation as a blue sphere, K27 tri-methylation as a light-yellow sphere); MGMT enzyme (in blue) and temozolomide (orange thunderbolt). Blunt arrows indicate inhibition and red crosses indicate the removal of each modification. In the whole figure, drugs and inhibitors of oncogenic proteins are reported in the gray boxes [Color figure can be viewed at wileyonlinelibrary.com]

MGMT. This approach does not require bisulfite conversion and, in a single analytical session, it allows one to also assess the presence of mutation at IDH1 and IDH2.[92,93] However, MLPA may be expensive and requires a strong effort by the biologist. To evaluate the methylation status of target genes, it is possible to also perform Amplicon Bisulfite sequencing (ABS) based on NGS technology.[94] This technique allows one to study the methylation status of a target gene with high sequencing depth, but, also in this case, the costs of the technology are quite high and thus not routinely used in clinical practice.[25]

The use of Infinium methylation EPIC array, based on evaluation of 850 k CpG sites, is now rising among the forward-looking diagnostic laboratories. Using user-friendly bioinformatic tool, it is indeed easy to extrapolate the methylation status of each single CpG site, and the Copy Number Variations (CNVs), which provides critical genetic information such as loss or gain of chromosome segments or specific genes deletions or amplifications (including 1p-19q codeletion, EGFR amplification, loss of CDKN2A/B, etc).[9] Raw data may be processed by available brain tumor methylation classifiers leading to a very precise subtype classification, which may be an important implementation in clinical practice.

A promising approach for possible use in routine diagnostics is the sequencing by Nanopore device (Nanoporetech, Oxford, England). This approach provides long reads thus analyzing structural variants, point mutations, bisulfite-free methylation and epitranscriptome profiles, using a single device with affordable capital cost.[95] Intriguingly, this method has been also proposed for potential intraoperatorial diagnosis. This technology, if it will enter in clinical practice, in the next future, could be a valid help both for the surgeon that intraoperatively will be able to decide the extension of surgical resection, and for the oncologist who will quickly have all the information useful for correct management of patients. However, to date bioinformatic interpretations are sometime complex and the technology remains evolving to optimize sequencing in order to obtain timely information on the mutational status of individual genes.

9 | CONCLUSIONS

Epigenomic signatures in GBMs gained increasing importance in refining brain tumors classification criteria. However, if detailed molecular characterization may help to identify more and more tumor entities, on the other hand all this information risk confusing clinicians in the management of patients affected by GBM. Currently, despite the efforts made, progresses in the treatment of GBM have been very

slow compared to the impressive advances seen in molecular biology field. To date, glioblastoma management typically involves surgical resection, as radical as possible, followed by the STUPP protocol based on cycles of radium and temozolomide-based chemotherapy. The biological variability of GBM, due to heterogeneous cell populations involved in brain tumorigenesis, and the site of tumors, that makes access to drugs difficult for the presence of BBB, remain the main problems for GBM target therapy. This means that many drugs that demonstrate efficacy in vitro or on animal models are actually not very effective in clinical studies. On the other hand, however, the example of the two recently FDA-approved drugs, entrectenib and larotrectenib, for the treatment of glioblastomas bearing a specific genetic lesion (ie, NTRK gene rearrangements), does suggest that targeted therapy can be envisioned for selected patients. Thus, although to date the resolutive cure of GBM still seems to be a mirage, we think that the accumulation of all epigenetic and genetic information and the extreme subclassification that derives from those may be the right road to obtain niche therapeutic findings allowing the development of personalized therapies (Figure 6).

AUTHOR CONTRIBUTIONS

The work reported in the study has been performed by the authors, unless clearly specified in the text. Rosa Della Monica, Liliana Montella, Lucia Altucci and Lorenzo Chiariotti conceived the study. Rosa Della Monica, Liliana Montella, Nunzio Del Gaudio, Roberta Visconti, Guglielmo Bove, Gaetano Facchini, Michela Buonaiuto, Davide Costabile, Teodolinda Di Risi, Mariella Cuomo conceptualized the review. Liliana Montella, Lucia Altucci, Lorenzo Chiariotti, Nunzio Del Gaudio, Guglielmo Bove, Michela Buonaiuto and Rosa Della Monica wrote the original draft. Roberta Vinciguerra, Roberta Visconti, Sara Ferraro, Federica Trio, Mariella Cuomo, Gaetano Facchini, Davide Costabile and Rosa Della Monica performed figures. Lucia Altucci, Roberta Visconti and Lorenzo Chiariotti revised the manuscript. All authors reviewed and approved the final manuscript.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interests.

ORCID

Rosa Della Monica https://orcid.org/0000-0002-5933-5556

REFERENCES

1. Louis DN, Perry A, Wesseling P, et al. The 2021 WHO classification of tumors of the central nervous system: a summary. *Neuro Oncol*. 2021;23:1231-1235.
2. Montella L, Del Gaudio N, Bove G, et al. Looking beyond the glioblastoma mask: is genomics the right path? *Front Oncol*. 2022;12:926967.
3. Dawson MA. The cancer epigenome: concepts, challenges, and therapeutic opportunities. *Science*. 2017;355:1147-1152.
4. Roberti A, Valdes AF, Torrecillas R, Fraga MF, Fernandez AF. Epigenetics in cancer therapy and nanomedicine. *Clin Epigenetics*. 2019;11:81.
5. Zeng Z, Chen Y, Geng X, et al. NcRNAs: multi-angle participation in the regulation of glioma chemotherapy resistance. *Int J Oncol*. 2022;60:76.
6. Aalami AH, Abdeahad H, Shoghi A, Mesgari M, Amirabadi A, Sahebkar A. Brain tumors and circulating micrornas: a systematic review and diagnostic meta-analysis. *Expert Rev Mol Diagn*. 2022;22:201-211.
7. Sharma RK, Calderon C, Vivas-Mejia PE. Targeting non-coding RNA for glioblastoma therapy: the challenge of overcomes the blood-brain barrier. *Front Med Technol*. 2021;3:678593.
8. Sturm D, Orr BA, Toprak UH, et al. New brain tumor entities emerge from molecular classification of CNS-PNETs. *Cell*. 2016;164:1060-1072.
9. Capper D, Jones DTW, Sill M, et al. DNA methylation-based classification of central nervous system tumours. *Nature*. 2018;555:469-474.
10. Sill M, Plass C, Pfister SM, Lipka DB. Molecular tumor classification using DNA methylome analysis. *Hum Mol Genet*. 2020;29:R205-R213.
11. Tesileanu CMS, Vallentgoed WR, Sanson M, et al. Non-IDH1-R132H IDH1/2 mutations are associated with increased DNA methylation and improved survival in astrocytomas, compared to IDH1-R132H mutations. *Acta Neuropathol*. 2021;141:945-957.
12. Han S, Liu Y, Cai SJ, et al. IDH mutation in glioma: molecular mechanisms and potential therapeutic targets. *Br J Cancer*. 2020;122:1580-1589.
13. Bleeker FE, Lamba S, Leenstra S, et al. IDH1 mutations at residue p.R132 (IDH1[R132]) occur frequently in high-grade gliomas but not in other solid tumors. *Hum Mutat*. 2009;30:7-11.
14. Lamadema N, Burr S, Brewer AC. Dynamic regulation of epigenetic demethylation by oxygen availability and cellular redox. *Free Radic Biol Med*. 2019;131:282-298.
15. Davis MI, Gross S, Shen M, et al. Biochemical, cellular, and biophysical characterization of a potent inhibitor of mutant isocitrate dehydrogenase IDH1. *J Biol Chem*. 2014;289:13717-13725.
16. Malta TM, de Souza CF, Sabedot TS, et al. Glioma CpG Island methylator phenotype (G-CIMP): biological and clinical implications. *Neuro Oncol*. 2018;20:608-620.
17. Yin A, Shang Z, Etcheverry A, et al. Integrative analysis identifies an immune-relevant epigenetic signature for prognostication of non-G-CIMP glioblastomas. *Onco Targets Ther*. 2021;10:1902071.
18. Karpel-Massler G, Nguyen TTT, Shang E, Siegelin MD. Novel IDH1-targeted glioma therapies. *CNS Drugs*. 2019;33:1155-1166.
19. Mellinghoff IK, Wen PY, Taylor JW, et al. PL3.1 a phase 1, open-label, perioperative study of ivosidenib (AG-120) and vorasidenib (AG-881) in recurrent, IDH1-mutant, low-grade glioma: results from cohort 1. *Neuro Oncol*. 2019;21(Suppl 3):iii2.
20. Pirozzi CJ, Yan H. The implications of IDH mutations for cancer development and therapy. *Nat Rev Clin Oncol*. 2021;18:645-661.
21. Rao AM, Quddusi A, Shamim MS. The significance of MGMT methylation in glioblastoma multiforme prognosis. *J Pak Med Assoc*. 2018;68:1137-1139.
22. Esteller M, Garcia-Foncillas J, Andion E, et al. Inactivation of the DNA-repair gene MGMT and the clinical response of gliomas to alkylating agents. *N Engl J Med*. 2000;343:1350-1354.
23. Pandith AA, Qasim I, Zahoor W, et al. Concordant association validates MGMT methylation and protein expression as favorable prognostic factors in glioma patients on alkylating chemotherapy (Temozolomide). *Sci Rep*. 2018;8:6704.

24. Malmström A, Grønberg BH, Marosi C, et al. Nordic clinical brain tumour study group (NCBTSG). Temozolomide versus standard 6-week radiotherapy versus hypofractionated radiotherapy in patients older than 60 years with glioblastoma: the nordic randomised, phase 3 trial. *Lancet Oncol*. 2012;13:916-926.
25. Della Monica R, Cuomo M, Visconti R, et al. Evaluation of MGMT gene methylation in neuroendocrine neoplasms. *Oncol Res*. 2022;28: 837-845.
26. Sturm D, Witt H, Hovestadt V, et al. Hotspot mutations in H3F3A and IDH1 define distinct epigenetic and biological subgroups of glioblastoma. *Cancer Cell*. 2012;22:425-437.
27. Sharifzad F, Ghavami S, Verdi J, et al. Glioblastoma cancer stem cell biology: potential theranostic targets. *Drug Resist Updat*. 2019;42: 35-45.
28. Dullea A, Marignol L. MGMT testing allows for personalised therapy in the temozolomide era. *Tumour Biol*. 2016;37:87-96.
29. Hegi ME, Diserens AC, Gorlia T, et al. MGMT gene silencing and benefit from temozolomide in glioblastoma. *N Engl J Med*. 2005;352:997-1003.
30. Singh N, Miner A, Hennis L, Mittal S. Mechanisms of temozolomide resistance in glioblastoma—a comprehensive review. *Cancer Drug Resist*. 2021;4:17-43.
31. Marsoner T, Schmidt OP, Triemer T, Luedtke NW. DNA-targeted inhibition of MGMT. *Chembiochem*. 2017;18:894-898.
32. Mason M, Laperriere N, Wick W, et al. Glioblastoma in the elderly: making sense of the evidence. *Neurooncol Pract*. 2016;3:77-86.
33. Jenuwein T, Allis CD. Translating the histone code. *Science*. 2001; 293:1074-1080.
34. Allfrey VG, Faulkner R, Mirsky AE. Acetylation and methylation of histones and their possible role in the regulation of RNA synthesis. *Proc Natl Acad Sci U S A*. 1964;51:786-794.
35. Was H, Krol SK, Rotili D, et al. Histone deacetylase inhibitors exert anti-tumor effects on human adherent and stem-like glioma cells. *Clin Epigenetics*. 2019;11:11.
36. Yang R, Wu Y, Wang M, et al. HDAC9 promotes glioblastoma growth via TAZ-mediated EGFR pathway activation. *Oncotarget*. 2015;6: 7644-7656.
37. Sawa H, Murakami H, Kumagai M, et al. Histone deacetylase inhibitor, FK228, induces apoptosis and suppresses cell proliferation of human glioblastoma cells in vitro and in vivo. *Acta Neuropathol*. 2004;107: 523-531.
38. Jin H, Liang L, Liu L, Deng W, Liu J. HDAC inhibitor DWP0016 activates p53 transcription and acetylation to inhibit cell growth in U251 glioblastoma cells. *J Cell Biochem*. 2013;114:1498-1509.
39. Chiao MT, Cheng WY, Yang YC, Shen CC, Ko JL. Suberoylanilide hydroxamic acid (SAHA) causes tumor growth slowdown and triggers autophagy in glioblastoma stem cells. *Autophagy*. 2013;9:1509-1526.
40. Alvarez AA, Field M, Bushnev S, Longo MS, Sugaya K. The effects of histone deacetylase inhibitors on glioblastoma-derived stem cells. *J Mol Neurosci*. 2015;55:7-20.
41. Nguyen TTT, Zhang Y, Shang E, et al. HDAC inhibitors elicit metabolic reprogramming by targeting super-enhancers in glioblastoma models. *J Clin Invest*. 2020;130:3699-3716.
42. Galanis E, Jaeckle KA, Maurer MJ, et al. Phase II trial of vorinostat in recurrent glioblastoma multiforme: a north central cancer treatment group study. *J Clin Oncol*. 2009;27:2052-2058.
43. Iwamoto FM, Lamborn KR, Kuhn JG, et al. A phase I/II trial of the histone deacetylase inhibitor romidepsin for adults with recurrent malignant glioma: North American brain tumor consortium study 03-03. *Neuro Oncol*. 2011;13:509-516.
44. Barker CA, Bishop AJ, Chang M, Beal K, Chan TA. Valproic acid use during radiation therapy for glioblastoma associated with improved survival. *Int J Radiat Oncol Biol Phys*. 2013;86:504-509.
45. Berger SL. The complex language of chromatin regulation during transcription. *Nature*. 2007;447:407-412.
46. Zhang L, Lu Q, Chang C. Epigenetics in health and disease. *Adv Exp Med Biol*. 2020;1253:3-55.
47. Kozono D, Li J, Nitta M, et al. Dynamic epigenetic regulation of glioblastoma tumorigenicity through LSD1 modulation of MYC expression. *Proc Natl Acad Sci U S A*. 2015;112:E4055-E4064.
48. Faletti S, Osti D, Ceccacci E, et al. LSD1-directed therapy affects glioblastoma tumorigenicity by deregulating the protective ATF4-dependent integrated stress response. *Sci Transl Med*. 2021; 13(623):eabf7036.
49. Ene CI, Edwards L, Riddick G, et al. Histone demethylase Jumonji D3 (JMJD3) as a tumor suppressor by regulating p53 protein nuclear stabilization. *PLoS One*. 2012;7:e51407.
50. Sherry-Lynes MM, Sengupta S, Kulkarni S, Cochran BH. Regulation of the JMJD3 (KDM6B) histone demethylase in glioblastoma stem cells by STAT3. *PLoS One*. 2017;12:e0174775.
51. Suvà ML, Riggi N, Janiszewska M, et al. EZH2 is essential for glioblastoma cancer stem cell maintenance. *Cancer Res*. 2009;69:9211-9218.
52. Gan L, Xu M, Hua R, et al. The polycomb group protein EZH2 induces epithelial-mesenchymal transition and pluripotent phenotype of gastric cancer cells by binding to PTEN promoter. *J Hematol Oncol*. 2018;11:9.
53. Yang R, Wang M, Zhang G, et al. E2F7-EZH2 axis regulates PTEN/AKT/mTOR signalling and glioblastoma progression. *Br J Cancer*. 2020;123:1445-1455.
54. Chen YN, Hou SQ, Jiang R, Sun JL, Cheng CD, Qian ZR. EZH2 is a potential prognostic predictor of glioma. *J Cell Mol Med*. 2021;25: 925-936.
55. Han B, Meng X, Wu P, et al. ATRX/EZH2 complex epigenetically regulates FADD/PARP1 axis, contributing to TMZ resistance in glioma. *Theranostics*. 2020;10:3351-3365.
56. Stazi G, Taglieri L, Nicolai A, et al. Dissecting the role of novel EZH2 inhibitors in primary glioblastoma cell cultures: effects on proliferation, epithelial-mesenchymal transition, migration, and on the pro-inflammatory phenotype. *Clin Epigenetics*. 2019;11:173.
57. Singh MM, Manton CA, Bhat KP, et al. Inhibition of LSD1 sensitizes glioblastoma cells to histone deacetylase inhibitors. *Neuro Oncol*. 2011;13:894-903.
58. Singh MM, Johnson B, Venkatarayan A, et al. Preclinical activity of combined HDAC and KDM1A inhibition in glioblastoma. *Neuro Oncol*. 2015;17:1463-1473.
59. Wierzbicki K, Ravi K, Franson A, et al. Targeting and therapeutic monitoring of H3K27M-mutant glioma. *Curr Oncol Rep*. 2020;22:19.
60. Bender S, Tang Y, Lindroth AM, et al. Reduced H3K27me3 and DNA hypomethylation are major drivers of gene expression in K27M mutant pediatric high-grade gliomas. *Cancer Cell*. 2013;24:660-672.
61. Hashizume R, Andor N, Ihara Y, et al. Pharmacologic inhibition of histone demethylation as a therapy for pediatric brainstem glioma. *Nat Med*. 2014;20:1394-1396.
62. Katagi H, Louis N, Unruh D, et al. Radiosensitization by histone H3 demethylase inhibition in diffuse intrinsic pontine glioma. *Clin Cancer Res*. 2019;25:5572-5583.
63. Hashizume R. Epigenetic targeted therapy for diffuse intrinsic pontine glioma. *Neurol Med Chir (Tokyo)*. 2017;57:331-342.
64. Chan KM, Fang D, Gan H, et al. The histone H3.3K27M mutation in pediatric glioma reprograms H3K27 methylation and gene expression. *Genes Dev*. 2013;27:985-990.
65. Grasso CS, Tang Y, Truffaux N, et al. Functionally defined therapeutic targets in diffuse intrinsic pontine glioma. *Nat Med*. 2015;21:827.
66. Piunti A, Hashizume R, Morgan MA, et al. Therapeutic targeting of polycomb and BET bromodomain proteins in diffuse intrinsic pontine gliomas. *Nat Med*. 2017;23:493-500.
67. Gu C, Shi X, Dai C, et al. RNA m^6A modification in cancers: molecular mechanisms and potential clinical applications. *Innovation (Camb)*. 2020;1:100066.
68. Zaccara S, Ries RJ, Jaffrey SR. Reading, writing and erasing mRNA methylation. *Nat Rev Mol Cell Biol*. 2019;20:608-624.
69. Eyler DE, Franco MK, Batool Z, et al. Pseudouridinylation of mRNA coding sequences alters translation. *Proc Natl Acad Sci U S A*. 2019; 116:23068-23074.

70. Miao FA, Chu K, Chen HR, et al. Increased DKC1 expression in glioma and its significance in tumor cell proliferation, migration and invasion. *Invest New Drugs*. 2019;37:1177-1186.
71. Cui Q, Yin K, Zhang X, et al. Targeting PUS7 suppresses tRNA pseudouridylation and glioblastoma tumorigenesis. *Nat Cancer*. 2021;2:932-949.
72. Choudhury Y, Tay FC, Lam DH, et al. Attenuated adenosine-to-inosine editing of microRNA-376a* promotes invasiveness of glioblastoma cells. *J Clin Invest*. 2012;122:4059-4076.
73. Bohnsack KE, Höbartner C, Bohnsack MT. Eukaryotic 5-methylcytosine (m^5C) RNA methyltransferases: mechanisms, cellular functions, and links to disease. *Genes (Basel)*. 2019;10:102.
74. Awah CU, Winter J, Mazdoom CM, Ogunwobi OO. NSUN6, an RNA methyltransferase of 5-mC controls glioblastoma response to temozolomide (TMZ) via NELFB and RPS6KB2 interaction. *Cancer Biol Ther*. 2021;22:587-597.
75. Jiang X, Liu B, Nie Z, et al. The role of m6A modification in the biological functions and diseases. *Signal Transduct Target Ther*. 2021;6:74.
76. Zhang S, Zhao BS, Zhou A, et al. m^6A demethylase ALKBH5 maintains tumorigenicity of glioblastoma stem-like cells by sustaining FOXM1 expression and cell proliferation program. *Cancer Cell*. 2017;31:591-606.
77. Sabnis RW. Novel small molecule RNA m6A demethylase AlkBH5 inhibitors for treating cancer. *ACS Med Chem Lett*. 2021;12:856-857.
78. Cui Q, Shi H, Ye P, et al. m^6A RNA methylation regulates the self-renewal and tumorigenesis of glioblastoma stem cells. *Cell Rep*. 2017;18:2622-2634.
79. Xiao L, Li X, Mu Z, et al. FTO inhibition enhances the antitumor effect of temozolomide by targeting MYC-miR-155/23a cluster-MXI1 feedback circuit in glioma. *Cancer Res*. 2020;80:3945-3958.
80. Huff S, Tiwari SK, Gonzalez GM, Wang Y, Rana TM. m^6A-RNA demethylase FTO inhibitors impair self-renewal in glioblastoma stem cells. *ACS Chem Biol*. 2021;16:324-333.
81. Dixit D, Prager BC, Gimple RC, et al. The RNA m6A reader YTHDF2 maintains oncogene expression and is a targetable dependency in glioblastoma stem cells. *Cancer Discov*. 2021;11:480-499.
82. Fang R, Chen X, Zhang S, et al. EGFR/SRC/ERK-stabilized YTHDF2 promotes cholesterol dysregulation and invasive growth of glioblastoma. *Nat Commun*. 2021;12:177.
83. Wang LC, Chen SH, Shen XL, et al. M6A RNA methylation regulator HNRNPC contributes to tumorigenesis and predicts prognosis in glioblastoma multiforme. *Front Oncol*. 2020;10:536875.
84. Tassinari V, Cesarini V, Tomaselli S, et al. ADAR1 is a new target of METTL3 and plays a pro-oncogenic role in glioblastoma by an editing-independent mechanism. *Genome Biol*. 2021;22:51.
85. Barbieri I, Kouzarides T. Role of RNA modifications in cancer. *Nat Rev Cancer*. 2020;20:303-322.
86. Kristensen BW, Priesterbach-Ackley LP, Petersen JK, Wesseling P. Molecular pathology of tumors of the central nervous system. *Ann Oncol*. 2019;30:1265-1278.
87. Aldape K, Zadeh G, Mansouri S, Reifenberger G, von Deimling A. Glioblastoma: pathology, molecular mechanisms and markers. *Acta Neuropathol*. 2015;129:829-848.
88. Della Monica R, Cuomo M, Buonaiuto M, et al. MGMT and whole genome DNA methylation impacts on diagnosis, prognosis and therapy of glioblastoma multiforme. *Int J Mol Sci*. 2022;23:7148.
89. Gondim DD, Gener MA, Curless KL, Cohen-Gadol AA, Hattab EM, Cheng L. Determining IDH-mutational status in gliomas using IDH1-R132H antibody and polymerase chain reaction. *Appl Immunohistochem Mol Morphol*. 2019;27:722-725.
90. Hench IB, Monica RD, Chiariotti L, et al. Fast routine assessment of MGMT promoter methylation. *Neurooncol Adv*. 2020;3:vdaa170.
91. Gurrieri L, De Carlo E, Gerratana L, et al. MGMT pyrosequencing-based cut-off methylation level and clinical outcome in patients with glioblastoma multiforme. *Future Oncol*. 2018;14:699-707.
92. Park CK, Kim J, Yim SY, et al. Usefulness of MS-MLPA for detection of MGMT promoter methylation in the evaluation of pseudoprogression in glioblastoma patients. *Neuro Oncol*. 2011;13:195-202.
93. Lee JY, Park CK, Park SH, Wang KC, Cho BK, Kim SK. MGMT promoter gene methylation in pediatric glioblastoma: analysis using MS-MLPA. *Childs Nerv Syst*. 2011;27:1877-1883.
94. Cuomo M, Borrelli L, Della Monica R, et al. DNA methylation profiles of Tph1A and BDNF in gut and brain of *L. rhamnosus*-treated zebrafish. *Biomolecules*. 2021;11:142.
95. Patel A, Belykh E, Miller EJ, et al. MinION rapid sequencing: review of potential applications in neurosurgery. *Surg Neurol Int*. 2018;9:157.

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