Eurasian Journal of Molecular and Biochemical Sciences

Dysregulation of m6A, m5C, and m1A Pathways in Gliomas Reveals EIF3A and TET1 as Candidate Biomarkers

Gaye Açcan[1], Turan Demircan[2*]

[1]*Institute of Health Sciences, Muğla Sıtkı Koçman University, Muğla, Turkey*
[*2]*Medical Biology Department, School of Medicine, Muğla Sıtkı Koçman University, Muğla, Turkey*

Cite: *Açcan G, Demircan T. Dysregulation of m6A, m5C, and m1A Pathways in Gliomas Reveals EIF3A and TET1 as Candidate BiomarkersEurasian Mol Biochem Sci 2025;4(2): 99-115*

Received: 26 August 2025, Accepted: 18 October 2025 *DOI: 10.5281/zenodo.17786737*

Abstract

Epitranscriptomic modifications such as N6-methyladenosine, 5-methylcytosine, and N1-methyladenosine have recently emerged as critical regulators of cancer biology. These pathways influence tumor initiation, progression, invasion, metastasis, and cellular differentiation. Understanding their contribution to glioblastoma aggressiveness may provide new avenues for therapeutic and prognostic applications. Here, we systematically analyzed m6A, m5C, and m1A pathway regulators in GBM and compared their dynamics with lower-grade gliomas using publicly available datasets. Bioinformatics approaches included mutation profiling, alteration frequency assessment, differential expression analysis, and correlation with overall survival. Our results revealed that m5C regulators exhibited higher mutation frequencies than m6A and m1A regulators in both glioma types. Moreover, a greater number of regulators were significantly associated with OS in LGG compared to GBM, suggesting tumor grade-specific prognostic relevance. Gene Ontology, KEGG, and Gene Set Enrichment Analysis further indicated that each pathway contributes to distinct biological processes and cellular signaling cascades. Receiver operating characteristic analysis identified several regulators with diagnostic and prognostic potential. Notably, EIF3A and TET1 showed strong biomarker potential, as their elevated expression negatively correlated with WHO tumor grade and distinguished between GBM and LGG. In summary, our study highlights the distinct roles of m6A, m5C, and m1A pathways in glioma biology and identifies EIF3A and TET1 as promising biomarkers with potential diagnostic and therapeutic implications. Targeting epitranscriptomic regulators may represent a novel strategy for glioma management.

Keywords: Epitranscriptomics, RNA Modifications, GBM, LGG, Bioinformatics Analysis

[*]**Correspondence**: Turan Demircan
Medical Biology Department,
School of Medicine, Muğla Sıtkı Koçman University, Muğla, Turkey
E-mail: turandemircan@gmail.com; turandemircan@mu.edu.tr,
Tel: +90 252-2114846; +90 505 679 4868

Introduction

Gliomas, malignant tumors originating from glial cells, are classified according to histopathological and molecular characteristics, which determine prognosis, treatment strategies, metastatic potential, and patient survival (1,2). Among them, glioblastoma multiforme (GBM) represents the

most prevalent and lethal form, corresponding to grade IV gliomas. GBM is characterized by remarkable heterogeneity, rapid proliferation, diffuse infiltration, and therapeutic resistance, yet it exhibits relatively low systemic metastatic potential due to the protective role of the blood–brain barrier (3,4). Despite multimodal treatment approaches including surgical resection, radiotherapy, and temozolomide-based chemotherapy, prognosis remains dismal, with median survival ranging between 8–15 months and five-year survival below 6% (5,6).

In contrast, low-grade gliomas (LGGs; grades II–III) are relatively indolent tumors that constitute approximately 15% of primary brain neoplasms. Although LGGs exhibit longer survival (median up to 7 years), they frequently progress to secondary GBM within 5–10 years of diagnosis, particularly in patients over 40 years or with large tumor diameters (>4–6 cm) (7,8). Molecular profiling has substantially refined glioma classification. The revised WHO 2021 criteria incorporate isocitrate dehydrogenase (IDH) mutation status, 1p/19q co-deletion, and additional biomarkers such as ATRX and TP53 mutations to distinguish molecular subtypes with distinct clinical outcomes (9). Common alterations in primary GBM include EGFR amplification, PTEN loss, chromosome 10q loss of heterozygosity, and CDKN2A/p16 deletion, whereas LGGs often exhibit TP53 and RB pathway deregulation along with PDGFA/PDGFRα overexpression (10,11).

Beyond genomic and transcriptomic changes, increasing attention has turned to post-transcriptional RNA modifications collectively termed the epitranscriptome, as an additional regulatory layer in glioma biology. High-throughput sequencing combined with immunoprecipitation has revealed that RNAs undergo more than 170 distinct reversible modifications, influencing RNA stability, translation, splicing, and localization (12,13). Among them, N6-methyladenosine (m6A) is the most abundant and best-studied, while N1-methyladenosine (m1A) and 5-methylcytosine (m5C) have also emerged as key modifications with roles in tumorigenesis (14,15). These modifications are dynamically regulated by “writers” (methyltransferases), “erasers” (demethylases), and “readers” (RNA-binding proteins), which collectively orchestrate essential cellular processes such as differentiation, proliferation, stress response, and oncogenic transformation (16,17). Importantly, dysregulation of RNA modification pathways has been increasingly linked to cancer initiation, progression, and therapeutic resistance, positioning epitranscriptomic regulators as promising biomarkers and therapeutic targets (18).

In this study, we systematically analyzed regulators of the m6A, m5C, and m1A pathways in GBM and LGG using transcriptomic and genomic data from The Cancer Genome Atlas (TCGA). By integrating mutation profiles, alteration frequencies, differential expression analyses, and survival correlations, we identified key regulators associated with glioma progression and patient outcomes. Functional enrichment analyses (GO, KEGG, GSEA) revealed that these pathways contribute to distinct biological processes and signaling cascades. Notably, we demonstrate that EIF3A and TET1 exhibit significant diagnostic and prognostic potential, with expression levels correlating with glioma grade and overall survival. These findings provide new insights into the epitranscriptomic landscape of gliomas and highlight novel biomarker candidates with potential relevance for clinical management.

Methods

Determination of Mutation Types and Alteration Frequencies Using the cBioportal Database: The relevant gene mutation frequencies were analyzed using the five

different publicly accessible glioma studies consisting of 3144 samples from 3234 patients. In order to form a comprehensive set of GBM and LGG datasets, Brain lower-grade glioma (TCGA, pan-cancer), Glioma (MSKCC, Clin Cancer Res 2019), Merged Cohort of LGG and GBM (TCGA, cell,2016), Anaplastic Oligodendroglioma and Anaplastic Oligoastrocytoma (MSKCC, Neuro Oncol 2017) and Glioblastoma multiforme (TCGA pan-cancer) datasets in cBioportal database (19,20) were selected. As described in a previous study (21), regulators in m6A, m5C, and m1A pathways were classified in 3 categories as writers, readers, and erasers. For the m6A pathway, VIRMA, METTL14, METTL3, METTL4, RBM15, RBM15B, WTAP are the writer, DGCR8, EIF3A, EIF3B, ELAVL1, HNRNPA2B1, HNRNPC, SFRS2, YDFDC1, YTHDC2, YTHDF1, YTHDC2, and YTHDF1 are the reader, and ALKBH5 and FTO are the eraser genes. In the m5C pathway, writers are DNMT1A, DNMT3A, DNMT3B, DNMT3L, NOP2, NSUN2, NSUN3, NSUN4, NSUN5, and TRDMT1, readers are MECP2, and UHRF1 and erasers are TET1, TET2, and TET3 genes. m1A pathway is comprised of HSD17B10, PRORP, TRMT10C, TRMT6, and TRMT61B writer, and ALKBH1 and ALKBH2 eraser regulators. Mutation types, alteration frequencies, and multiple genomic alterations were shown on histograms and heatmaps drawn through the cBioPortal as described elsewhere (22).

Retrieving TCGA-PanCancer Data for Downstream Analysis: The GBM (Glioblastoma Multiforme – TCGA, PanCancer Atlas) and LGG (Brain lower-grade glioma -TCGA, PanCancer Atlas) PanCancer data (23) utilized in this study were fetched by employing the RTCGA package in R environment. The latest version of the datasets, including 166 GBM samples and 530 LGG samples, were gathered for further analysis. RTCGA.rnaseq and RTCGA.clinical functions were implemented to retrieve the RNA-Seq and clinical data of the patients.

Principal Component Analysis of GBM and LGG Samples: In order to test the separation power of the epitranscriptomic pathways, gene expression level between GBM and LGG samples, Principal Component Analysis (PCA) was applied using the stats package in the R environment. The epitranscriptomic pathways were either used separately or together to depict the spatial distribution of the GBM and LGG datasets samples. Ggfortify package in R was exploited to visualize the results.

Overall Survival Analysis: Survival and Survminer R packages in the R environment were adopted for the overall survival analysis of GBM and LGG samples based on the Kaplan-Meier estimation model. High or low expression grouping for each gene in m6A, m5C, and 1mA pathways was determined considering the median expression value. For each gene, a p-value was calculated based on the correlation of expression values with survival time and status. The genes with a p-value<0.05 were illustrated on survival curves.

Comparison of mRNA Expression Levels Between GBM and LGG Samples: To find the statistically significant differences at mRNA levels of the m6A, m5C, and m1A pathway genes between GBM and LGG samples, wilcox test in rstatix package of R language was applied. $P < 0.05$ was considered statistically significant. Following the statistical analysis, mRNA levels of the genes compared between two datasets were visualized in a boxplot using the ggplot2 package in the R environment. Furthermore, the LIMMA package of R language was implemented to list the differentially expressed genes (DEGs) ($p<0.05$ and $|log2foldchange|>0.6$) between GBM and LGG datasets to evaluate the diagnostic potential of analyzed genes.

Transcriptomic Data of Altered and Unaltered Groups: GBM and LGG patients were grouped as altered and unaltered for each pathway. Patients with mutations in one or more genes in each transcriptomic pathway represent the altered group. The unaltered group consisted of the patient samples without the mutation in investigated pathways. A list of the genes with fold change and associated p-values comparing altered vs. unaltered groups was downloaded from cBioPortal.

Analysis of Over-representation and Gene Set Enrichment Data: Gene set enrichment analysis (GSEA) was performed in the R language utilizing the ClusterProfiler (24) package with the filtered genes after applying a p-value <0.05 cutoff to filter the transcriptomic dataset downloaded from cBioportal. The analyses were run to find all ontological terms by setting the nPerm as 10000, minGSSize as 20, maxGSSize 800, pvalueCutoff = 0.05, qvalueCutoff = 0.2, and pAdjustmethod was selected as false discovery rate (FDR).

Then, gene ontology (GO) over-representation and kyoto encyclopedia of genes and genomes (KEGG) analyses were executed using the slightly modified dataset utilized in GSEA by subsetting the DEGs with |Log Ratio |> 0.6. The clusterProfiler (24) package was employed in the R environment for the GO and KEGG analyses. The set parameters for the both analyses are as followings: pvalueCutoff = 0.05, pAdjustMethod = "fdr", qvalueCutoff = 0.2, minGSSize = 10, maxGSSize = 500. Pathways and GO terms with an adjusted p-value <0.05 were considered statistically significant. The obtained results were presented in dotplot and barplot figures using the ggplot2 package in the R environment.

ROC Curve Analysis: In order to test whether the genes highlighted as candidate biomarkers have diagnostic value for GBM and LGG, a receiver operating characteristic (ROC) curve was generated for each gene by using the sensitivity and specificity measures to detect the accuracy. ROC curve analysis was performed by utilizing the R language's pROC package. The area under the curve (AUC) was calculated to estimate sensitivity and specificity for distinguishing GBM from LGG. Regulators with AUC ≥ 0.70 were considered to have good discriminatory power.

Cell Culture and qRT-PCR: The procedure for cell culture and qRT-PCR was described elsewhere (25, 26). Human glioblastoma cell line U87-MG and the low-grade glioma cell line H4 were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were maintained in Dulbecco's Modified Eagle Medium (DMEM, Gibco, USA) supplemented with 10% fetal bovine serum (FBS, Gibco, USA), 1% penicillin–streptomycin (100 U/mL penicillin and 100 µg/mL streptomycin, Gibco, USA), and cultured at 37°C in a humidified atmosphere containing 5% CO_2. Culture medium was refreshed every 2–3 days, and cells were passaged at ~70–80% confluence using 0.25% trypsin–EDTA solution.

Total RNA was extracted from cultured U87 and H4 cells using the TRIzol reagent (Invitrogen, USA) according to the manufacturer's instructions. RNA purity and concentration were assessed with a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA), and integrity was verified by agarose gel electrophoresis. Subsequently, 1 µg of total RNA was reverse transcribed into complementary DNA (cDNA) using the PrimeScript RT Reagent Kit (Takara, Japan) with oligo(dT) primers. Quantitative PCR was performed on a StepOnePlus Real-Time PCR System (Applied Biosystems, USA) using SYBR Green Master Mix (Applied Biosystems, USA) in a total reaction volume of 20 µL. Cycling conditions were as follows: initial denaturation at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 55°C for 15 s, and 72°C for 60 s. Relative expression

levels were calculated by the $2^{-\Delta\Delta Ct}$ method, with GAPDH serving as the internal control.

Results and Discussion

Epitranscriptomic RNA modifications are increasingly recognized as critical regulators of cancer biology. Among them, m6A is the most extensively studied and has been implicated in nearly all aspects of RNA metabolism, including splicing, translation, and degradation (27). More recently, m5C and m1A have also been identified as important epitranscriptomic modifications contributing to cellular homeostasis, developmental processes, and tumor progression (28). However, their integrated contribution to glioma biology, particularly the distinction between LGG and GBM, remains insufficiently explored.

Gliomas represent a particularly suitable context for such analyses, as they account for approximately 81% of all malignant brain tumors and exhibit striking heterogeneity in terms of prognosis, therapeutic response, and molecular features (2). GBM, the most aggressive grade IV glioma, has a median survival of less than 15 months despite maximal therapy, while LGGs, encompassing grades II–III, often progress to secondary GBM within 5–10 years (8). The poor clinical outcomes highlight the urgent need to identify novel biomarkers and therapeutic targets.

Our study investigated the landscape of genetic alterations, transcriptomic expression, functional enrichment, and clinical significance of m6A, m5C, and m1A regulators in GBM and LGG. Particular attention was given to identifying candidate biomarkers with diagnostic and prognostic potential.

Mutation Profiles of m6A, m5C, and m1A Regulators in Gliomas: Using five publicly available glioma datasets from cBioPortal, we analyzed the genetic alteration patterns of 43 regulators across m6A, m5C, and m1A pathways. In line with prior reports in pan-cancer analyses (29,30), we also observed high alteration frequencies in regulators of m5C, m6A, and m1A (Figure 1).

In the m6A pathway (Figure 1a), amplification, deep deletion, and point mutations were predominant. Genes such as EIF3B, ELAVL1, and EIF3A exhibited the highest variation, while YTHDC2 and ALKBH5 were rarely altered. Importantly, EIF3A, a translation initiation factor, showed deep deletions in a subset of cases, suggesting potential tumor suppressive functions, which are consistent with recent work linking EIF3A loss to impaired DNA damage responses in gliomas (31).

Among m5C regulators, NOP2, DNMT3A, and DNMT1 showed the highest alteration burden, whereas TET3 and NSUN4 were rarely affected (Figure 1b). Notably, mutations were frequent in the TET family genes, consistent with their roles as DNA/RNA demethylases (32). Additionally, NSUN5 loss has been associated with ribosomal stress and glioma progression (33). DNA hypomethylation, characterized by a genome-wide reduction of 5-mC in gene-coding regions and satellite repeats, was the first identified epigenetic abnormality in tumor cells. This hypomethylation can promote genomic instability, leading to mitotic recombination, copy-number alterations, chromosomal rearrangements, and loss of genomic imprinting (34). The contribution of this process to tumor progression may explain the high alteration frequency of DNMT3A and DNMT1 observed in our dataset. Furthermore, the dysregulation of demethylation pathways is also critically involved in cancer. For instance, TET1 has been implicated in various solid tumors (e.g., breast and bladder cancer), while TET2 is frequently mutated in hematological malignancies. Emerging evidence also suggests a role for TET3 in certain leukemias

and solid tumors, such as glioblastoma and colorectal cancer (reviewed in 35).

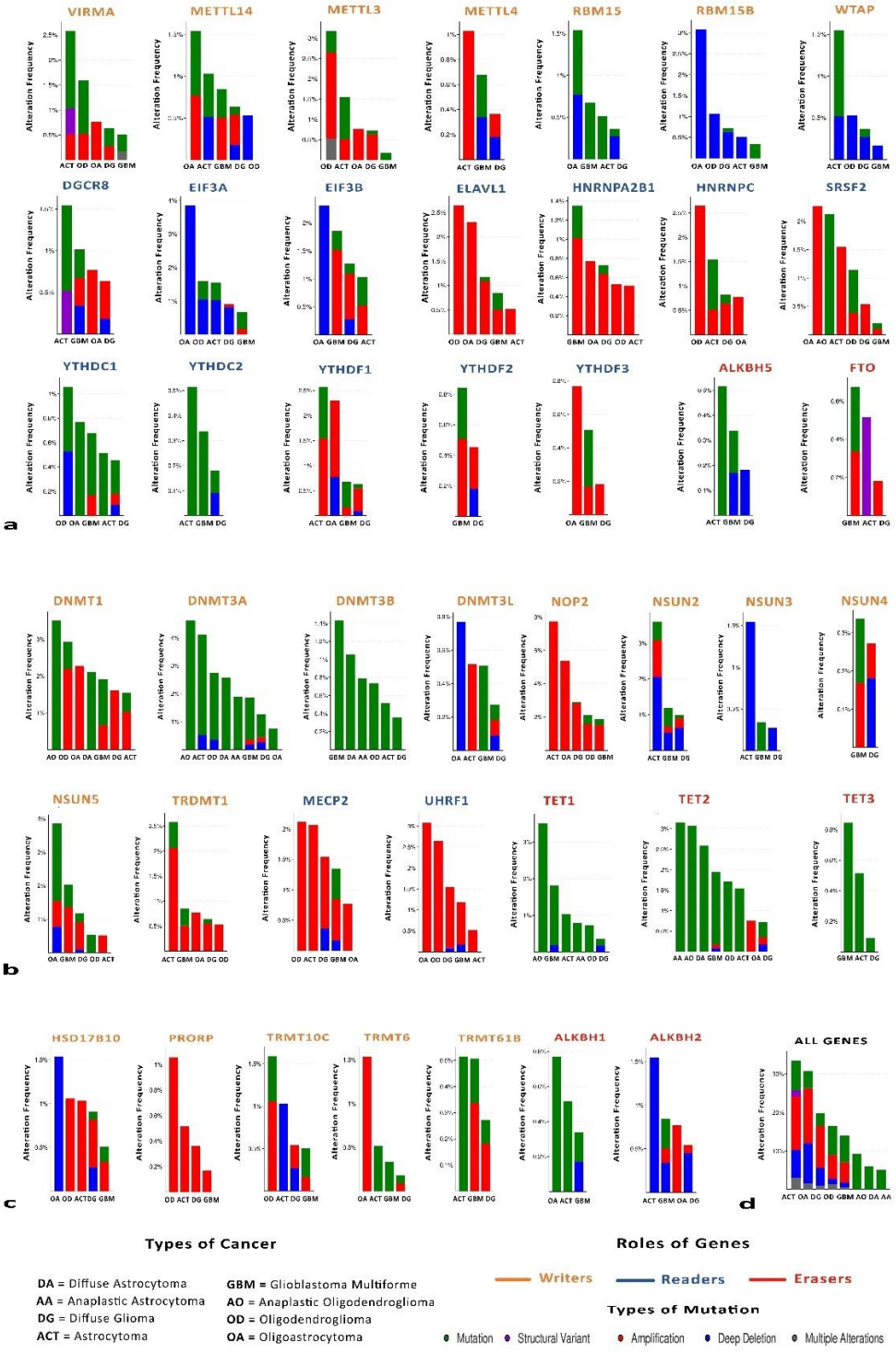

Figure 1. Alteration frequencies of m6A, m5C, and m1A regulators were determined in 5 public acces studies utilizing cBioportal database. (a) m6A pathway regulators, (b) m5C pathway regulators, (c) m1A pathway regulators, (d) All m6A, m5C and m1A pathway regulators. Regulator names on the diagrams were written in orange, blue, and red, according to their characteristics of writer, reader, and eraser, respectively. Subtypes of LGG and GBM cases are abbreviated as DA (Diffuse Astrocytoma), AA (Anaplastic Astrocytoma), DG (Diffuse Glioma), ACT (Astrocytoma), GBM (Glioblastoma Multiforme), AO (Anaplastic Oligodendroglioma), OD (Oligodendroglioma), OA (oligoastrocytoma). Mutation types such as, mutation, structural variant, amplification, deep deletion, and multiple alteration were indicated with green, purple, red, blue, and gray colors, respectively.

For m1A regulators, mutations were uncommon, with HSD17B10 and TRMT6 showing modest alterations (Figure 1c). Based on our finding of TRMT6 amplification in GBM, we propose a model where TRMT6 promotes glioma progression by activating the PI3K–AKT signaling pathway, a key driver of cell survival and proliferation in many cancers. This hypothesis is mechanistically plausible, as a previous study demonstrated that inhibiting TRMT6 suppresses the proliferation, migration, and invasion of glioma cells, an effect linked to the regulation of the PI3K-AKT pathway (36).

Interestingly, LGGs exhibited higher overall mutation frequencies than GBM, particularly in grade II tumors (Figure 1d), suggesting that epitranscriptomic pathway alterations may represent early molecular events in gliomagenesis (37). This observation aligns with the idea that LGGs accumulate regulatory pathway alterations that may later contribute to malignant transformation into GBM (38).

Transcriptomic Alterations and Functional Enrichment: Beyond DNA-level alterations, integration of RNA expression revealed additional insights (Figure 2). Strikingly, DNMT3L, a catalytically inactive regulator of DNA methylation, showed a dramatic increase in alteration frequency when mRNA expression was considered (~85% in GBM). Although DNMT3L cannot methylate DNA directly, it enhances the activity of DNMT3A/3B and has been associated with hypermethylation of tumor suppressor genes in other cancers (39). Intriguingly, we observed lower DNMT3L expression in GBM compared to LGG (Figure 2), which contrasts with its reported oncogenic role in other malignancies. Given its additional functions in transcription factor interactions and neural differentiation (40), DNMT3L may play a glioma-associated role requiring further mechanistic investigation.

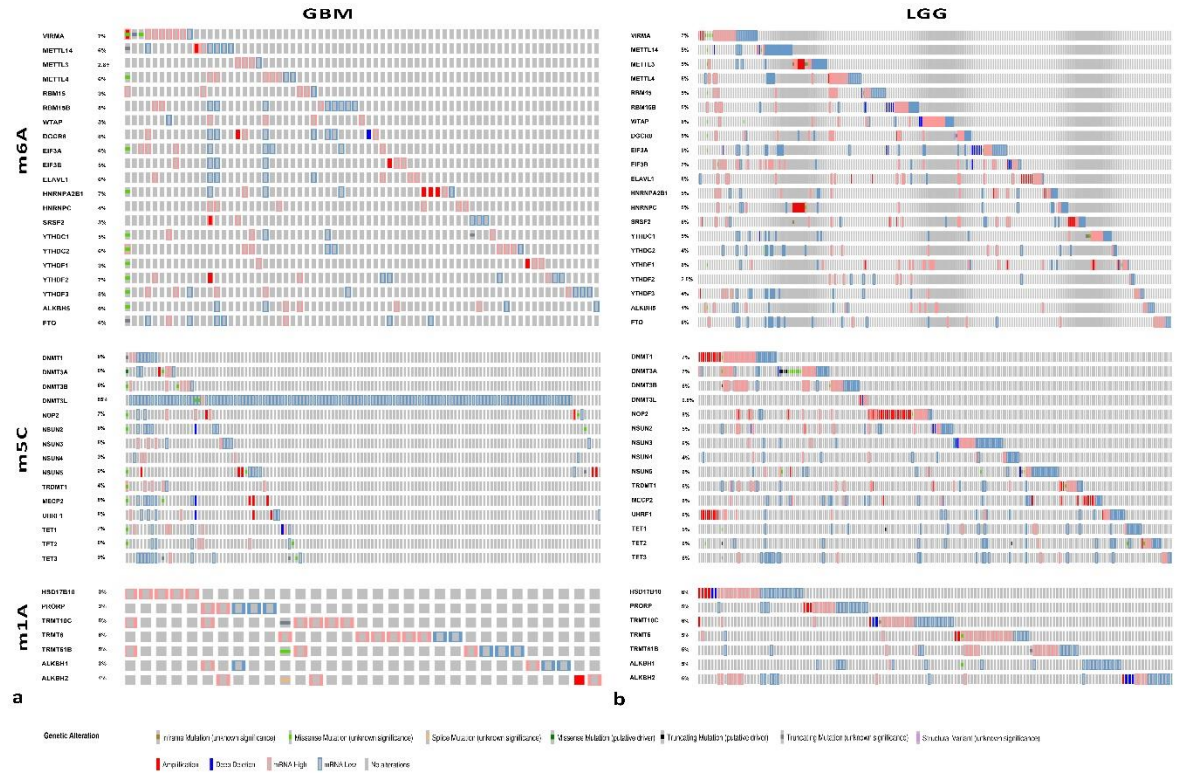

Figure 2. Oncoprint view of alteration frequency and genetic alterations, performed in the cBioportal database using only GBM and LGG PanCancer studies, including mRNA expression levels. (a) Alteration frequencies of m6A, m5C, and m1A regulators, respectively, from top to bottom, in GBM cases. (b) Alteration frequencies of m6A, m5C, and m1A regulators, respectively, from top to bottom, in LGG cases. Besides other genetic alterations, light blue bars indicate low mRNA expression level, and light pink bars indicate high mRNA expression level.

Gene Ontology (GO) and KEGG enrichment analyses demonstrated pathway-specific associations (Figure 3,4). In GBM, altered m5C regulators enriched DNA replication, mitotic cell cycle, and repair pathways, hallmarks of aggressive tumor biology (Figure 3a,4a). Altered m1A regulators enriched ribosome biogenesis and translation initiation, supporting reports that TRMT6/61A drives glioma proliferation via PI3K–AKT signaling (36). By contrast, unaltered groups enriched synaptic signaling, neurotransmitter transport, and axon guidance—processes typically downregulated during malignant transformation. In LGG, altered groups across pathways were enriched for morphogenesis, embryonic development, and cell differentiation, while unaltered groups displayed enrichment in synaptic activity and neurotransmission (Figure 3b,4b). This is consistent with the observation that lower-grade gliomas retain partial neuronal-like transcriptional programs, which are progressively lost as they advance to GBM (41). Unaltered regulators consistently associated with synaptic and neuronal signaling, supporting the concept that epitranscriptomic dysregulation underlies the erosion of neuronal identity during glioma progression.

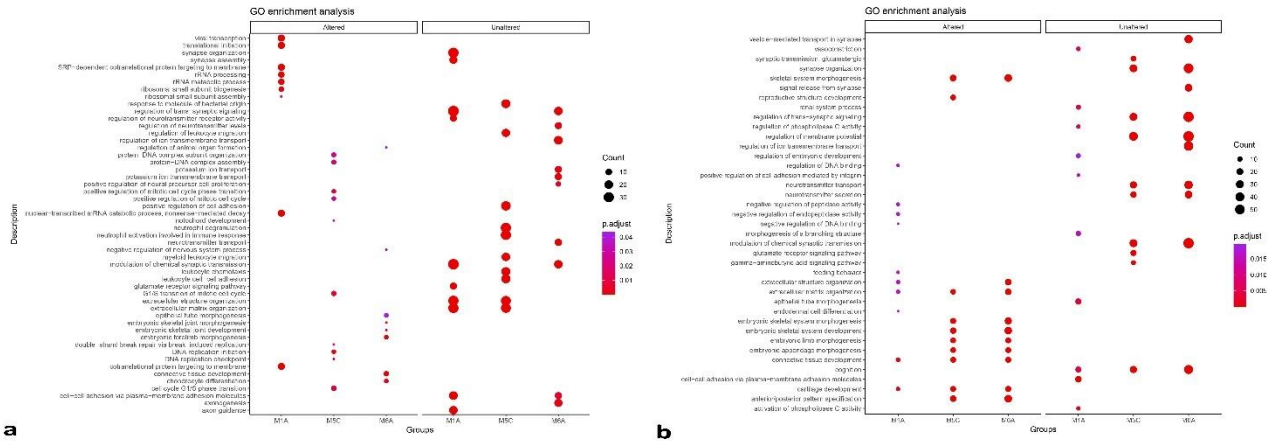

Figure 3. The transcriptomic data collected from cBioportal for m6A, m5C and m1A pathways were divided into altered and unaltered groups, and Gene Ontology (GO) enrichment analysis was performed via clusterprofiler. (a) GO analysis for GBM samples, (b) GO analysis for LGG samples. The size of the bubble was determined according to the number of genes enriched at terms, and the color was determined according to the pAdjust values. pAdjust< 0.05 was considered as significant.

Taken together, these findings underscore that m6A, m5C, and m1A regulators modulate divergent biological programs. While altered regulators favor proliferative and immune-related signatures, unaltered regulators align with preserved neuronal functions—highlighting their potential role in glioma aggressiveness.

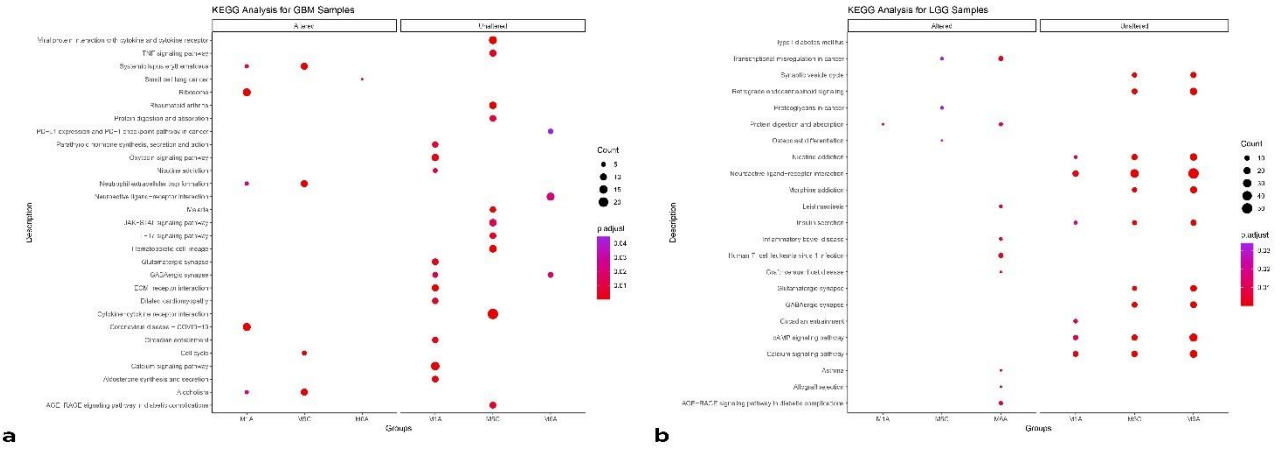

Figure 4. Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis was performed to identify downstream pathways in the R environment using transcriptome data. (a) KEGG analysis for GBM samples, (b) KEGG analysis for LGG samples. The size of the bubble was determined according to the number of genes enriched at terms, and the color was determined according to the pAdjust values. pAdjust < 0.05 was considered as significant.

Diagnostic and Prognostic Potential of Regulators: Principal component analysis (PCA) showed only partial separation of GBM and LGG when using the combined regulator set (Figure S1). However, expression profiles of the m1A pathway achieved superior discriminatory capacity (Figure S1d), suggesting that m1A regulators, though less frequently mutated, may provide robust transcriptional signatures distinguishing glioma grades. This mirrors emerging literature on m1A's role in ribosomal dynamics and stress responses (42).

Differential expression analysis revealed that WTAP, DNMT3A, DNMT3B, and NSUN5 were upregulated in GBM, while EIF3A and TET1 were consistently higher in LGG (Figure 5).

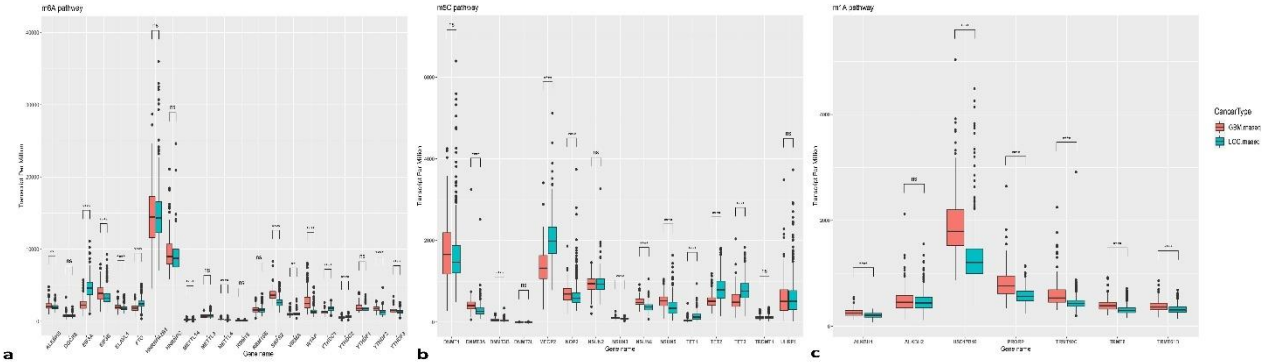

Figure 5. RNA-seq data obtained from the RTCGA package were used to compare the relative expression leves of m6A, m5C and m1A pathway regulators, between GBM and LGG samples. (a) for m6A pathway regulators, (b) for m5C pathway regulators, (c) for m1A regulators. Pink bars represent GBM samples and blue bars represent LGG samples. Wilcox test was carried out to determine the expression levels differences between GBM and LGG samples. ns: p-value > 0.05; *: p-value < 0.05; **: p-value < 0.01; ***: p-value < 0.001; ****: p-value < 0.0001.

Survival analysis further emphasized the prognostic importance of these regulators (Figure S2-3). In GBM, high expression of VIRMA, METTL3/4, EIF3A, TET2/3, and TRMT6 correlated with improved outcomes, whereas high expression of DNMT3A, DNMT3B, and NSUN5 predicted poor survival, in agreement with recent findings linking NSUN5 loss of function to worse outcomes (33). In LGG, a larger number of regulators showed significant prognostic associations, suggesting that epitranscriptomic pathways exert stronger prognostic influence in early-stage gliomas compared to advanced GBM. Importantly, across both tumor types, EIF3A and

TET1 consistently emerged as robust biomarkers (Figure S2-3, Figure 6).

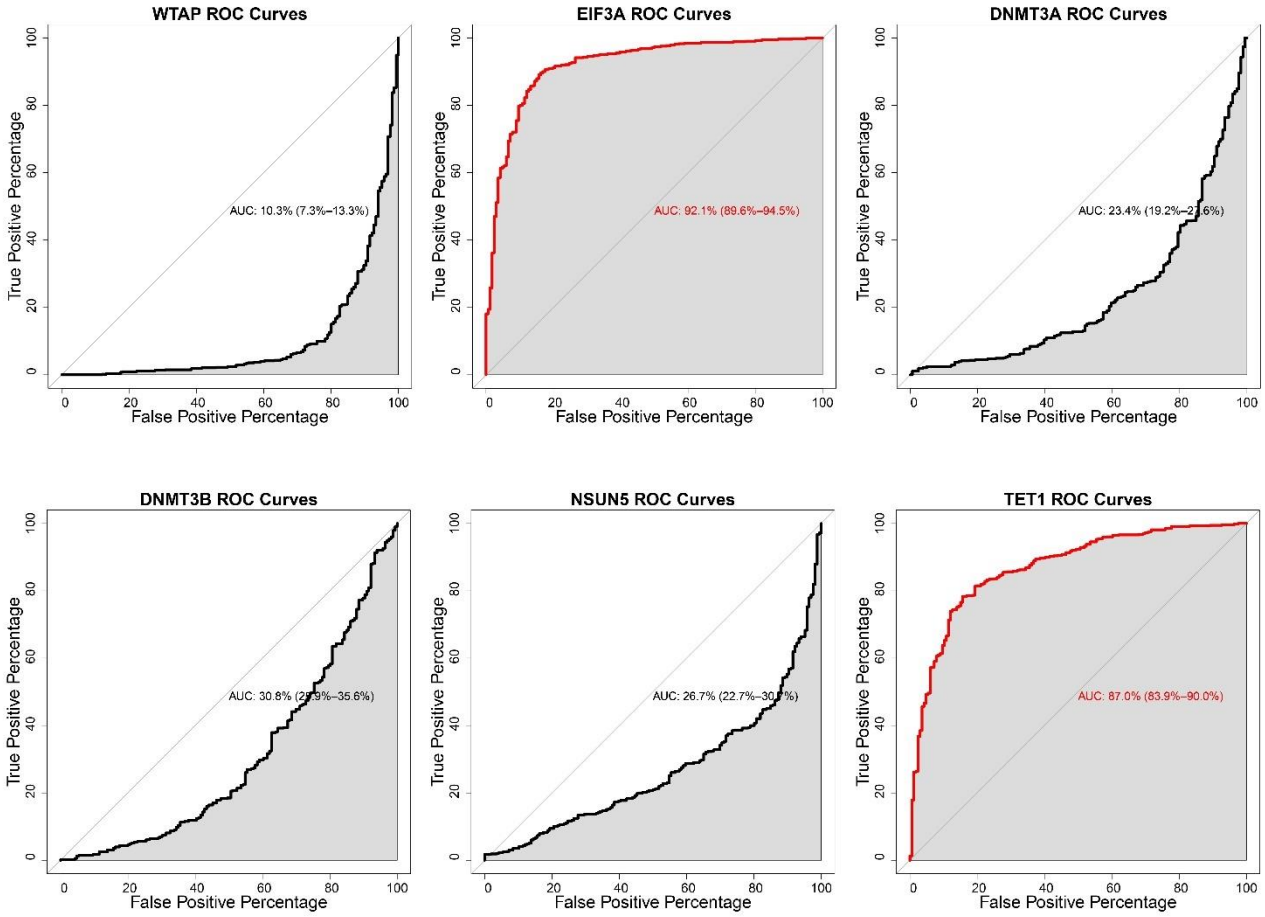

Figure 6. ROC curves of 6 genes (WTAP, EIF3A, DNMT3A, DNMT3B, NSUN5 and TET1) wich were observed genes that differentially expressed an area under the curve (AUC) values. The red curves exhibited AUC values above 70% and black curves exhibited AUC values under 70%. Confidence interval (CI) was applied as 95%.

EIF3A and TET1 as Candidate Biomarkers: Among all regulators, EIF3A and TET1 stood out for their dual diagnostic and prognostic potential. EIF3A is a core subunit of the eukaryotic initiation factor 3 complex that facilitates ribosome assembly and translation initiation. High EIF3A expression correlated with favorable outcomes in GBM and LGG, and its expression inversely correlated with tumor grade. Previous studies report that EIF3A can sensitize cancer cells to chemotherapy and suppress malignant progression (43), whereas its downregulation is associated with advanced tumor stages (44). Our data reinforce these findings, suggesting that EIF3A acts as a tumor-suppressive factor in gliomas. Our findings of high EIF3A expression correlating with improved survival echo reports that EIF3A enhances DNA repair and suppresses glioma progression (31). TET1, a member of the ten-eleven translocation family, catalyzes hydroxylation of 5-methylcytosine to 5-hydroxymethylcytosine and plays a critical role in DNA demethylation. High TET1 expression correlated with prolonged survival and was downregulated in GBM relative to LGG. Previous studies have established TET1 as a tumor suppressor in hematological and solid tumors, including gliomas, where it regulates autophagy and gene expression programs (45). Our findings confirm its protective role and extend its potential as a biomarker for distinguishing glioma grades. Loss of TET1/5hmC has been linked to GBM aggressiveness (46). We observed TET1

downregulation in GBM relative to LGG, consistent with its role as a tumor suppressor. ROC curve analysis confirmed their diagnostic value, with EIF3A (AUC = 0.92) and TET1 (AUC = 0.87) showing high discriminatory accuracy between GBM and LGG. Mutation analysis revealed EIF3A harbored missense variants in both glioma types, while TET1 displayed more frequent mutations in GBM, including truncating variants, suggesting possible mechanisms for functional impairment.

Validation of Candidate Regulators by qPCR: To validate the transcriptomic findings, we performed qPCR on selected regulators (WTAP, DNMT3A, DNMT3B, NSUN5, DNMT3L, EIF3A, and TET1) in GBM and LGG cell line samples (Figure 7). Consistent with the in silico analyses, qPCR demonstrated that WTAP, DNMT3A, DNMT3B, NSUN5, DNMT3L, and EIF3A were expressed at significantly higher levels in GBM compared to LGG ($p < 0.05$). These regulators are well-established oncogenic drivers in diverse cancers, and their overexpression has been linked to enhanced proliferation, DNA methylation imbalance, and resistance to apoptosis. The concordance between bioinformatics and qPCR results further highlights their robust involvement in GBM biology.

In contrast, EIF3A and TET1 showed higher expression in LGG compared to GBM, consistent with our prognostic analyses. Elevated EIF3A in LGG may reflect a protective role in maintaining translational fidelity and DNA repair capacity. Similarly, higher TET1 expression in LGG supports its role as a tumor suppressor that preserves 5-hydroxymethylcytosine balance and limits malignant progression (46). Their downregulation in GBM highlights a potential molecular switch during the transition from LGG to high-grade disease.

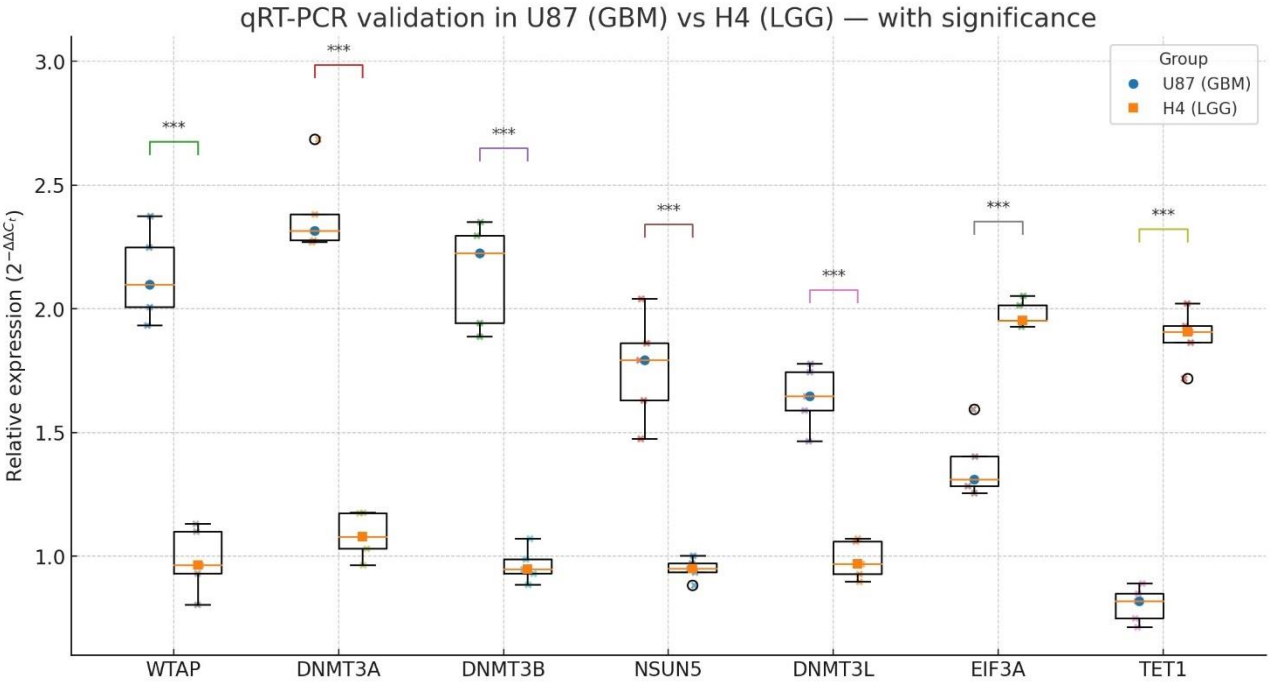

Figure 7. Validation of candidate RNA modification regulators by qRT-PCR. Expression levels of WTAP, DNMT3A, DNMT3B, NSUN5, DNMT3L, EIF3A, and TET1 were quantified in U87 (GBM) and H4 (LGG) cell lines using qRT-PCR. Data are shown as mean ± SD of three independent experiments; $p < 0.05$ for all tested genes.

Together, these qPCR validations strengthen the reliability of our integrative approach, confirming that WTAP, DNMT3A, DNMT3B, NSUN5, DNMT3L, and EIF3A are upregulated in aggressive GBM, whereas EIF3A and TET1 serve as potential suppressive regulators enriched in LGG. These

findings not only reinforce the clinical relevance of the bioinformatics analysis but also suggest that combined evaluation of these regulators may improve the molecular stratification and prognostic assessment of glioma patients.

Broader Implications and Future Directions: Collectively, our results demonstrate that epitranscriptomic regulators are not only markers of glioma progression but also potential drivers of tumor biology. While m5C regulators carried the highest mutation burden and m1A regulators exhibited the strongest transcriptional discrimination, EIF3A and TET1 emerged as the most consistent biomarkers across analyses. The broader implication is that epitranscriptomic dysregulation contributes to the shift from neuronal-like signaling to proliferative and immune-activated states, facilitating glioma aggressiveness. Future directions include validating EIF3A and TET1 in independent clinical cohorts, dissecting their mechanistic roles using in vitro and in vivo glioma models, and exploring whether therapeutic modulation of these regulators can enhance response to standard therapies such as temozolomide and radiotherapy.

Conclusion

This integrative analysis of glioma datasets demonstrates that m6A, m5C, and m1A regulators are differentially mutated, expressed, and prognostically significant in GBM and LGG. Despite the complexity of epitranscriptomic regulation, EIF3A and TET1 consistently emerged as strong diagnostic and prognostic biomarkers, with clear potential for translational application. These findings open avenues for the development of epitranscriptome-based stratification tools and therapeutic interventions in glioma management.

Funding: The authors did not receive support from any organization for the submitted work.

Declaration of Competing Interests: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contributions: Conceptualization: Turan Demircan, Formal Analysis: Gaye Açcan, Turan Demircan, Investigation: Gaye Açcan, Turan Demircan, Methodology: Gaye Açcan, Turan Demircan, Project Administration: Turan Demircan, Writing – Original Draft: Gaye Açcan, Turan Demircan, Writing – Review & Editing: Turan Demircan

ORCID:

First Author https://orcid.org/0000-0001-7750-4459

Second Author https://orcid.org/0000-0002-2424-9893

References

1. Louis DN, Ohgaki H, Wiestler OD, Cavenee WK.. World Health Organization Histological Classification of Tumours of the Central Nervous System. 4th ed.Lyon: International Agency for Research on Cancer; 2007
2. Louis, D. N., Perry, A., Wesseling, P., et al. (2021). The 2021 WHO classification of tumors of the central nervous system: a summary. Neuro-oncology, 23(8), 1231-1251.
3. Rabah, N., Ait Mohand, F. E., & Kravchenko-Balasha, N. (2023). Understanding glioblastoma signaling, heterogeneity, invasiveness, and drug delivery barriers. International Journal of Molecular Sciences, 24(18), 14256.
4. Wu, W., Klockow, J. L., Zhang, M., et al. (2021). Glioblastoma multiforme (GBM): An overview of current therapies and mechanisms of resistance. Pharmacological research, 171, 105780.
5. Sipos, D., Raposa, B. L., Freihat, O., Simon, M., Mekis, N., Cornacchione, P., & Kovács, Á. (2025). Glioblastoma: clinical presentation, multidisciplinary management, and Long-Term outcomes. Cancers, 17(1), 146.
6. Krenzlin, H., Jankovic, D., Dauth, A., et al. (2024). Multimodal treatment of glioblastoma with multiple lesions-a multi-center retrospective analysis. *Journal of Neuro-oncology*, *170*(3), 555-566.
7. Claus, E. B., Walsh, K. M., Wiencke, J.K., et al. (2015). Survival and low-grade glioma: the emergence of genetic information. Neurosurgical focus, 38(1), E6.
8. Saqib, M., Zahoor, A., Rahib, A., Shamim, A., & Mumtaz, H. (2024). Clinical and translational advances in primary brain tumor therapy with a focus on glioblastoma-A comprehensive review of the literature. World Neurosurgery: X, 24, 100399.
9. Antonelli, M., & Poliani, P. L. (2022). Adult type diffuse gliomas in the new 2021 WHO Classification. Pathologica, 114(6), 397.
10. Singh, S., Dey, D., Barik, D., Mohapatra, I., Kim, S., Sharma, M., ... & Singh, G. (2025). Glioblastoma at the crossroads: current understanding and future therapeutic horizons. Signal Transduction and Targeted Therapy, 10(1), 213.
11. Ohgaki, H., & Kleihues, P. (2007). Genetic pathways to primary and secondary glioblastoma. The American journal of pathology, 170(5), 1445-1453.
12. Mukherjee, P., Kurup, R. R., & Hundley, H. A. (2021). RNA immunoprecipitation to identify in vivo targets of RNA

editing and modifying enzymes. In Methods in enzymology (Vol. 658, pp. 137-160). Academic Press.
13. Liang, W., Lin, Z., Du, C., Qiu, D., & Zhang, Q. (2020). mRNA modification orchestrates cancer stem cell fate decisions. Molecular cancer, 19(1), 38.
14. Yue Y, Liu J, He C. RNA N6-methyladenosine methylation in post-transcriptional gene expression regulation. Genes Dev. 2015;29:1343–1355. doi: 10.1101/gad.262766.115
15. Cappannini, A., Ray, A., Purta, E., et al. (2024). MODOMICS: a database of RNA modifications and related information. 2023 update. Nucleic Acids Research, 52(D1), D239-D244.
16. Esteve-Puig, R., Bueno-Costa, A., & Esteller, M. (2020). Writers, readers and erasers of RNA modifications in cancer. Cancer letters, 474, 127-137.
17. Chen, D., Gu, X., Nurzat, Y., et al. (2024). Writers, readers, and erasers RNA modifications and drug resistance in cancer. Molecular cancer, 23(1), 178.
18. Zhao, Y., Chen, X., Zhang, X., & Liu, H. (2025). RNA epigenetic modifications as dynamic biomarkers in cancer: from mechanisms to clinical translation. Biomarker Research, 13(1), 81.
19. Gao, J., Aksoy, B. A., Dogrusoz, U., et al. (2013). Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Science signaling, 6(269), pl1-pl1.
20. Cerami, E., Gao, J., Dogrusoz, U., Gross, B.E., et al. (2012). The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer discovery, 2(5), 401-404.
21. Malvi, P., Wang, B., Shah, S., & Gupta, R. (2019). Dissecting the role of RNA modification regulatory proteins in melanoma. *Oncotarget*, *10*(38), 3745.
22. Demircan, T., Yavuz, M., & Akgül, S. (2021a). m6A pathway regulators are frequently mutated in breast invasive carcinoma and may play an important role in disease pathogenesis. OMICS: A Journal of Integrative Biology, 25(10), 660-678.
23. Ceccarelli, M., Barthel, F. P., Malta, T. M., et al. (2016). Molecular Profiling Reveals Biologically Discrete Subsets and Pathways of Progression in Diffuse Glioma. Cell, 164(3), 550–563.
24. Yu, G., Wang, L. G., Han, Y., & He, Q. Y. (2012). clusterProfiler: an R package for comparing biological themes among gene clusters. Omics: a journal of integrative biology, 16(5), 284-287.
25. Yavuz, M., & Demircan, T. (2023). The effect of hydroquinidine on proliferation and apoptosis of TMZ-sensitive and-resistant GBM Cells. Anti-Cancer Agents in Medicinal Chemistry-Anti-Cancer Agents), 23(8), 938-952.
26. Demircan, T., Yavuz, M., Kaya, E., Akgül, S., & Altuntaş, E. (2021b). Cellular and molecular comparison of glioblastoma multiform cell lines. Cureus, 13(6).
27. Zhang, S. Z., Liu, S. Y., Cheng, M. D., Zhang, Y. F., & Tian, J. W. (2025). The role of RNA methylation in glioma progression: mechanisms, diagnostic implications, and therapeutic value. Frontiers in Immunology, 16, 1583039.
28. Zhang, L., Li, Y., Li, L., Yao, F., Cai, M., Ye, D., & Qu, Y. (2025). Detection, molecular function and mechanisms of m5C in cancer. Clinical and Translational Medicine, 15(3), e70239.
29. Zaccara, S., Ries, R. J., & Jaffrey, S. R. (2019). Reading, writing and erasing mRNA methylation. Nature reviews Molecular cell biology, 20(10), 608-624.
30. He, Y., Yu, X., Zhang, M., & Guo, W. (2021). Pan-cancer analysis of m5C regulator genes reveals consistent epigenetic landscape changes in multiple cancers. World Journal of Surgical Oncology, 19(1), 224.
31. Bertorello, J., Sesen, J., Gilhodes, J., et al. (2020). Translation reprogramming by eIF3 linked to glioblastoma resistance. NAR cancer, 2(3), zcaa020.
32. Cimmino, L., Dawlaty, M. M., Ndiaye-Lobry, D., et al. (2015). TET1 is a tumor suppressor of hematopoietic malignancy. Nature immunology, 16(6), 653-662.
33. Janin, M., Ortiz-Barahona, V., de Moura, M.C., et al. (2019). Epigenetic loss of RNA-methyltransferase NSUN5 in glioma targets ribosomes to drive a stress adaptive translational program. Acta neuropathologica, 138(6), 1053-1074.
34. Zhang, W., & Xu, J. (2017). DNA methyltransferases and their roles in tumorigenesis. *Biomarker research*, *5*(1), 1.
35. Zhang, C., Zheng, J., Liu, J., Li, Y., Xing, G., Zhang, S., ... & Wu, W. (2024). Pan-cancer analyses reveal the molecular and clinical characteristics of TET family members and suggests that TET3 maybe a potential therapeutic target. *Frontiers in Pharmacology*, *15*, 1418456.
36. Wang, B., Niu, L., Wang, Z., & Zhao, Z. (2021). RNA m1A methyltransferase TRMT6 predicts poorer prognosis and promotes malignant behavior in glioma. Frontiers in molecular biosciences, 8, 692130.
37. Garcia-Fabiani, M. B., Haase, S., Comba, A., et al. (2021). Genetic alterations in gliomas remodel the tumor immune microenvironment and impact immune-mediated therapies. Frontiers in Oncology, 11, 631037.
38. Chen, R., Smith-Cohn, M., Cohen, A. L., & Colman, H. (2017). Glioma subclassifications and their clinical significance. Neurotherapeutics, 14(2), 284-297.
39. Neri, F., Krepelova, A., Incarnato, D., et al. (2013). Dnmt3L antagonizes DNA methylation at bivalent promoters and favors DNA methylation at gene bodies in ESCs. Cell, 155(1), 121-134.
40. Qin, L., Qiao, C., Sheen, V., Wang, Y., & Lu, J. (2021). DNMT3L promotes neural differentiation by enhancing STAT1 and STAT3 phosphorylation independent of DNA methylation. Progress in neurobiology, 201, 102028.
41. Sojka, C., & Sloan, S. A. (2024). Gliomas: a reflection of temporal gliogenic principles. Communications Biology, 7(1), 156.
42. Xiong, W., Zhao, Y., Wei, Z., Li, C., Zhao, R., Ge, J., & Shi, B. (2023). N1-methyladenosine formation, gene regulation, biological functions, and clinical relevance. Molecular Therapy, 31(2), 308-330.
43. Tumia, R., Wang, C. J., Dong, T., et al.. (2020). Eif3a regulation of nhej repair protein synthesis and cellular response to ionizing radiation. Frontiers in cell and developmental biology, 8, 753.
44. Gomes-Duarte, A., Lacerda, R., Menezes, J., & Romão, L. (2018). eIF3: a factor for human health and disease. RNA biology, 15(1), 26-34.
45. Meleiro, M., & Henrique, R. (2025). Epigenetic Alterations in Glioblastoma Multiforme as Novel Therapeutic Targets: A Scoping Review. International Journal of Molecular Sciences, 26(12), 5634.
46. Johnson, K. C., Houseman, E. A., King, J. E., Von Herrmann, K. M., Fadul, C. E., & Christensen, B. C. (2016). 5-Hydroxymethylcytosine localizes to enhancer elements and is associated with survival in glioblastoma patients. Nature communications, 7(1), 13177.

Supplementary Figures

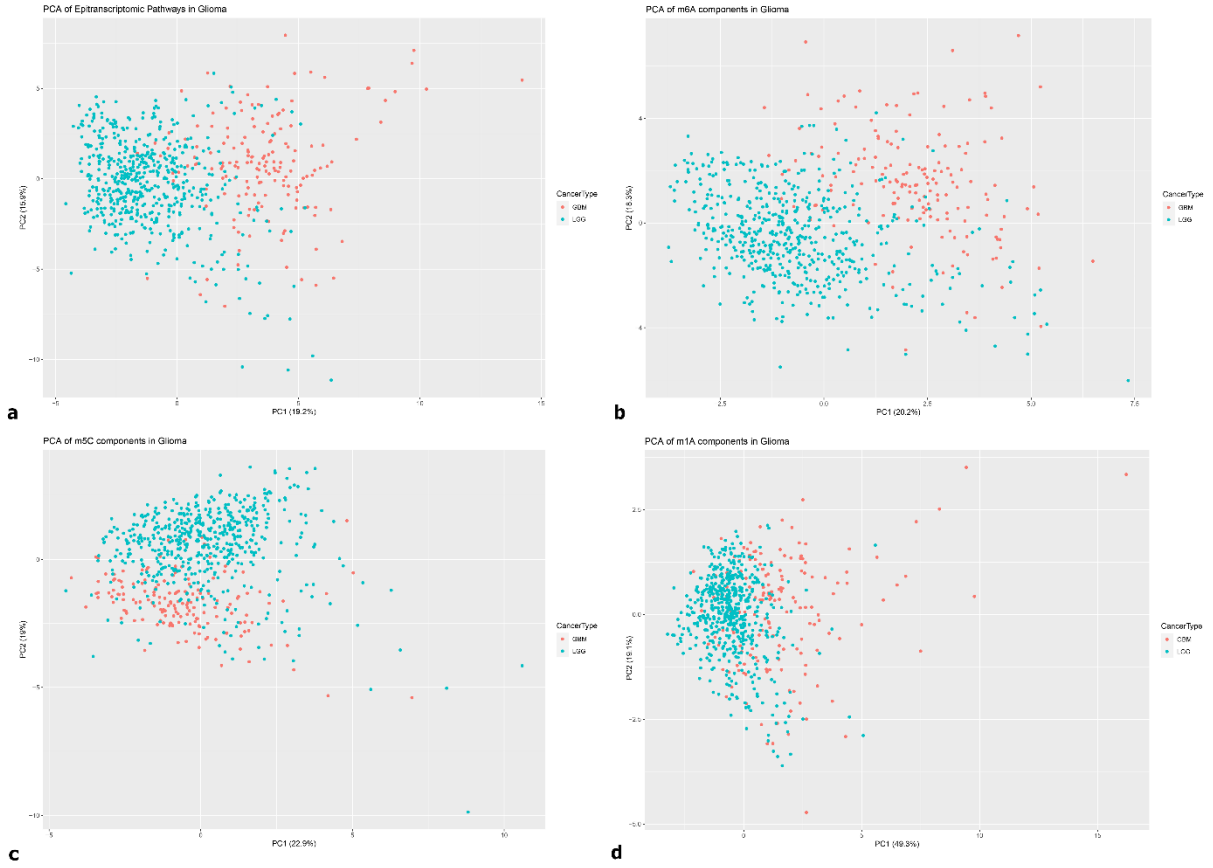

Figure S1. Principal compopnent analysis(PCA) was conducted using RNA-seq data, to elucidate the spatial distrubution of m6A,m5C and m1A regulators in GBM and LGG cases. (a) PCA of regulators in all m6A, m5C and m1A pathways, (b) PCA of m6A pathway regulators, (c) PCA of m5C pathway regulators, (d) PCA of m1A pathway regulators. GBM samples were marked with pink dots, LGG sapmles were marked with blue dots.

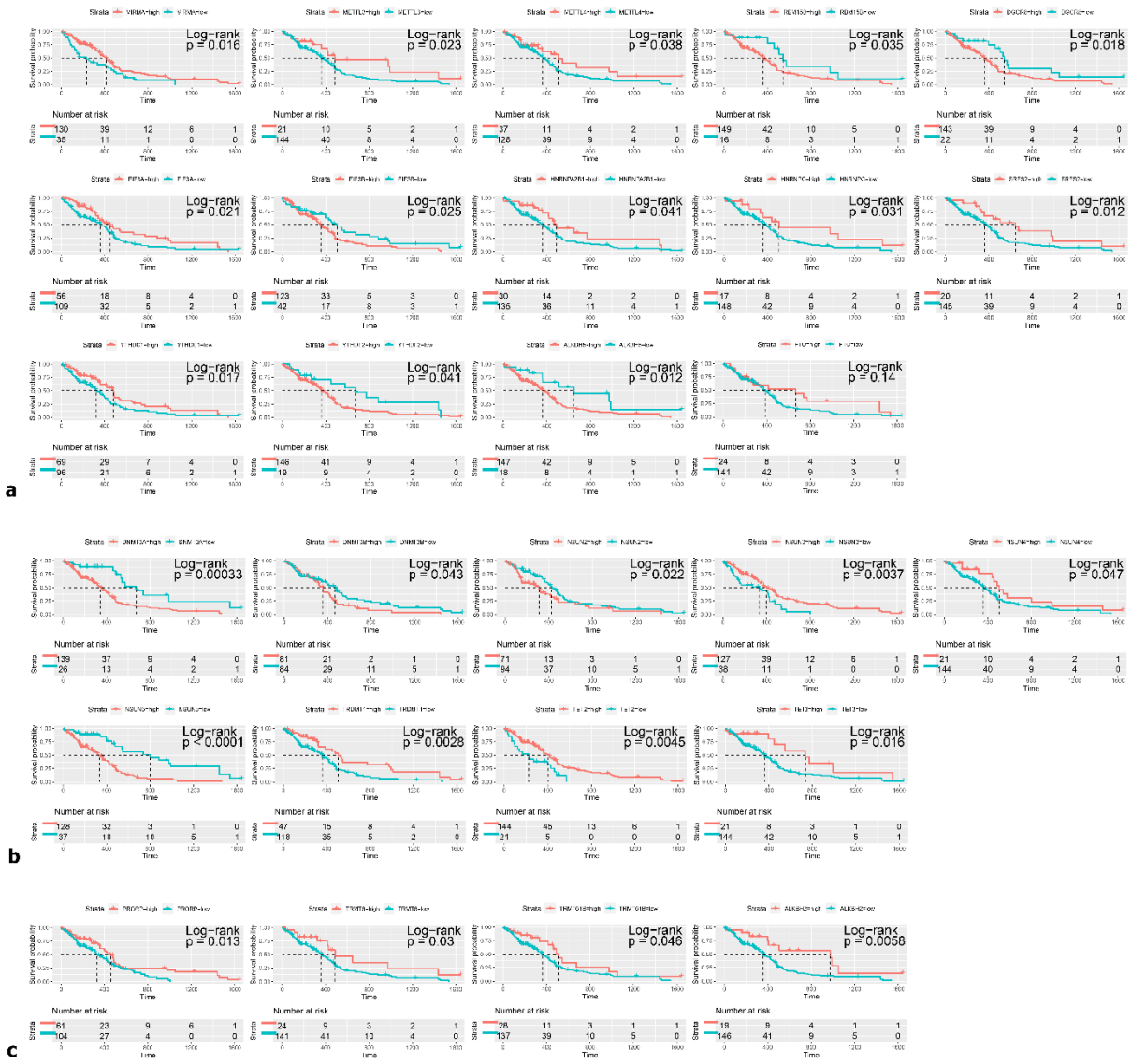

Figure S2. Kaplan-Meier overall survival (OS) curves were drawn, utilize together RNA-seq data with clinical data of GBM patients. Datas collected by RTCGA package. OSs were drawn for all m6a (a), m5C (b) and m1A(c) pathway regulators, but only signiticant ones were visualized. Pink curves represent high expression levels and blue curves represent low expression levels.

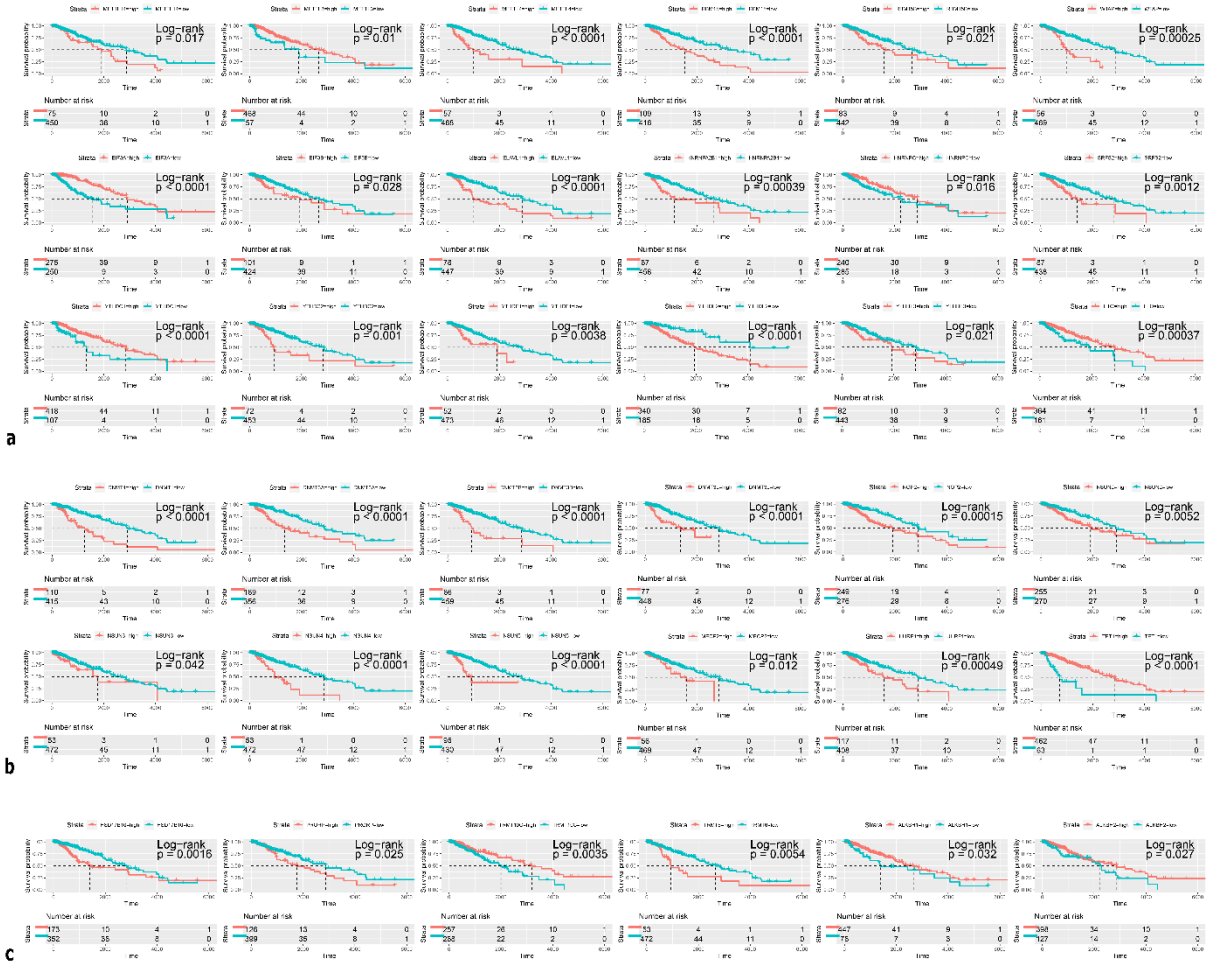

Figure S3. Kaplan-Meier overall survival (OS) curves were created based on expression levels of m6A, m5C and m1A pathway regulators and their clinical data in R environment for LGG patients., Only significant genes were viewed, in m6A (a), m5C (b) and m1A (c) pathway regulators. Pink curves represent high expression levels and blue curves represent low expression levels.

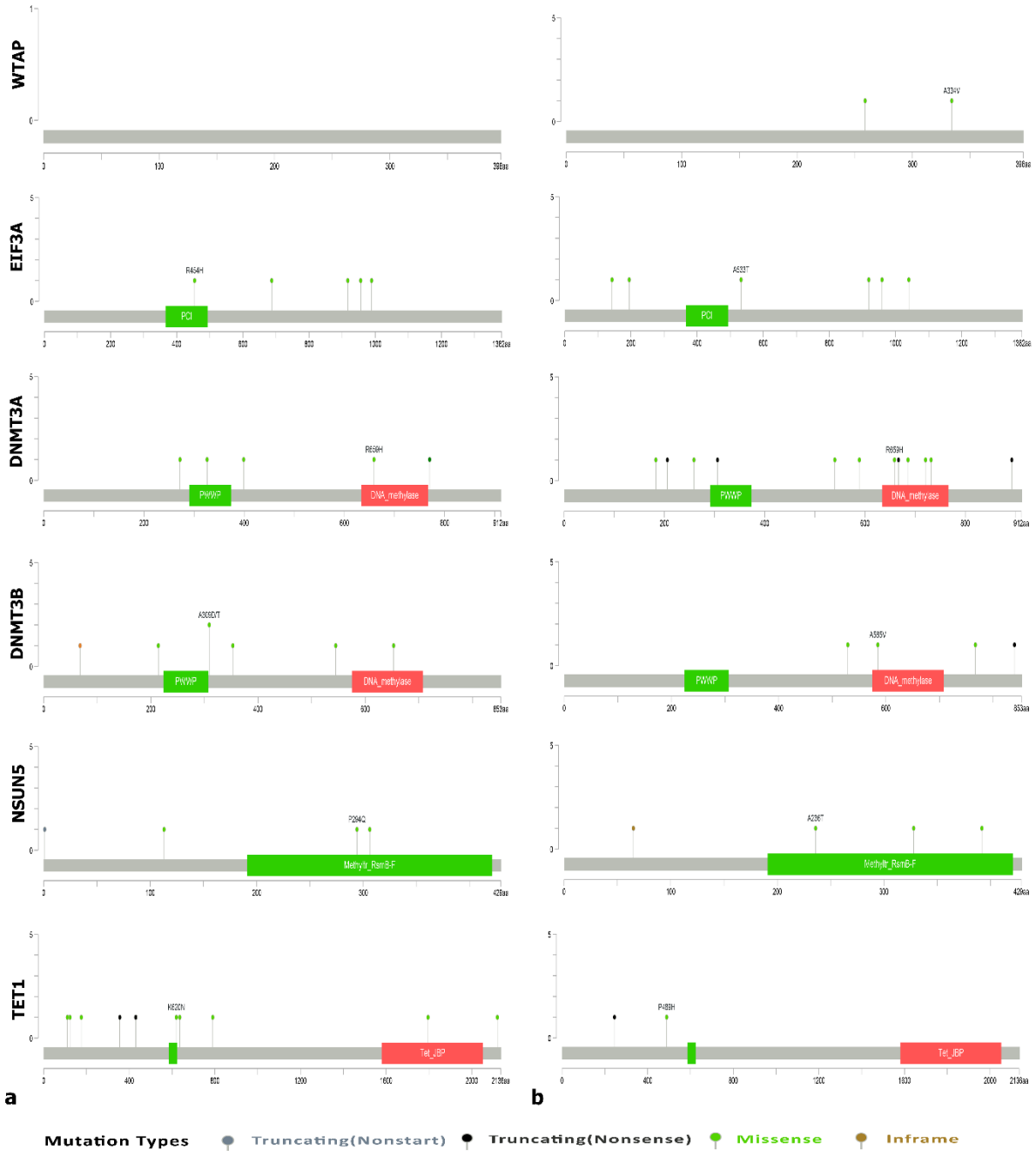

Figure S4. Lollipop visualization for indicated the protein structure alteration of EIF3A and TET1 genes in GBM (a) and LGG samples (b). Green and pink bold bars were represent protein domains of genes and lollipop like dots present the alteration such as truncating(nonsesnse) and missense according to colors that green and black respectively, on the grey bar.