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Original Article

Integrative analysis defines DDIT3 amplification as a correlative and essential factor for glioma malignancy

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Abstract: Glioma, particularly glioblastoma multiforme (GBM), is a highly aggressive and lethal primary brain tumor with poor prognosis. Metabolic reprogramming and endoplasmic reticulum (ER) stress are two crucial factors contributing to glioma pathogenesis. However, the intricate coordination between these processes remains incompletely understood. Here, we conducted an integrative analysis to elucidate the nodal role of DNA Damage Inducible Transcript 3 (DDIT3) to couple metabolisms and stress responses in glioma. We demonstrated a positive association between DDIT3 amplification/enhanced expression with glioma malignancy, indicating its potential as a novel biomarker for prognosis and treatment stratification. Genomic and transcriptomic analyses further revealed the involvement of DDIT3 enhancement in glioma progression. Moreover, immune infiltration analysis showed that distinct DDIT3 expression groups had different immune microenvironment. Finally, *in vitro* validations confirmed the impact of DDIT3 on proliferation and migration of glioma cells. Our findings provide novel insights into the complex interplay between metabolic reprogramming and ER stress, and defines DDIT3 as a promising therapeutic target in glioma.

Keywords: Glioma, metabolic reprogramming, ER stress, DDIT3, coordination

Introduction

Glioma, especially glioblastoma multiforme (GBM), represents a devastating and lethal form of primary tumors in the brain [1, 2]. Characterized by its high aggressiveness and unfavorable prognosis [3], GBM accounts for a considerable number of deaths worldwide [4]. Despite substantial advancements in clinical interventions, including surgical resection, radiotherapy, and chemotherapy, the overall survival (OS) of GBM patients remains poor, with an average survival rate of a mere 15 months [5]. The poor clinical outcomes in glioma patients can be attributed to two critical factors, tumor heterogeneity (e.g., metabolic reprogramming) [6] and tumor resistance (e.g., to environment stress and chemotherapy) [7], which drives active stress response and protective mechanisms. Thus, exploring molecular mechanisms underlying the heterogeneity and resistance in glioma is of great significance, as it enables a deeper understanding of glioma pathogenesis and facilitates the identification of therapeutic targets.

The distinct feature of glioma lies in its remarkable capacity to adapt to stimulations imposed by internal and external stresses on tumor cells, particularly through metabolic reprogramming and endoplasmic reticulum (ER) stress. The metabolic reprogramming is exemplified by the shift towards aerobic glycolysis from mitochondrial oxidative phosphorylation [8]. Furthermore, glioma cells also exhibit a distinct utilization of the tricarboxylic acid cycle/oxidative phosphorylation [9]. The enhanced aerobic glycolysis in glioma not only meets the energetic demand to support tumor growth, but also furnishes essential building blocks for nucleotide, lipid, and protein synthesis [10]. Concurrently, these synthetic processes predominantly occur within the endoplasmic reticulum (ER), and thus unavoidably triggers cellular stress. For exam-

ple, glioma cells bear a considerable vulnerability to protein misfolding, prompting their activation of the unfolded protein response (UPR), a pro-survival mechanism triggered by ER stress [11]. Therefore, it's reasonable that metabolic reprogramming and ER stress response should be coupled in glioma progression [12].

The coordination between metabolic reprogramming and endoplasmic reticulum (ER) stress in glioma cells is evident. For instance, the transcription factor X box-binding protein 1 (XBP1), a central regulator of UPR, exhibits significant activation in glioma tissues. Silencing XBP1 leads to inhibited glioma cell viability and tumor growth by suppressing the expression of HK2 and glycolysis under hypoxic conditions [13]. Additionally, PERK, a crucial downstream component of the UPR, is activated in glioma cells. Silencing PERK results in reduced glioma cell viability and diminished ATP/lactate production under low glucose stress, partly mediated by inhibiting the mitochondrial translocation of HK2 [14]. Despite these findings, the precise mechanisms underlying the coordination between ER stress and metabolic reprogramming in glioma remain incompletely understood.

DNA Damage Inducible Transcript 3 (DDIT3), also known as GADD153 or CHOP, serves as a transcription factor that is prominently induced under diverse stress conditions, encompassing DNA damage, ER stress, hypoxia, and nutrient starvation [15, 16]. DDIT3 has long been recognized as a pivotal instigator of apoptosis linked to ER stress, and various anti-tumor reagents induce cell death through the activation of DDIT3 [17-19]. Intriguingly, a recent work demonstrates the role of DDIT3 in energetic production by promoting glycolysis [20]. Therefore, DDIT3 is assumed as the central nexus that coordinates metabolic reprogramming and stress response pathways within glioma cells. However, precise mechanisms underlying the specific contribution of DDIT3 to glioma remain incompletely understood.

In this study, we conducted a comprehensive integration of bioinformatic analyses and experimental investigations, unveiling the indispensable role of DDIT3 in glioma pathogenesis. Through the elucidation of the genomic and transcriptomic signatures associated with DDIT3 in glioma progression, our study demonstrates a novel mechanism by which couples metabolic reprogramming and stress response pathways within glioma cells. Our work defines amplified DDIT3 as a promising therapeutic target, presenting a new avenue for glioma interventions.

Material and methods

Genomic mutation analysis

To assess the genomic alterations of DDIT3 in glioma, the expression profiles of DDIT3 were examined utilizing the cBioPortal database (http://www.cbioportal.org/) [21], which facilitates the detection of genomic events across multiple cancer types, including glioma. The frequency and types of distinct DDIT3 mutations were revealed. Furthermore, the Kaplan-Meier survival analysis was performed to assess the impact of DDIT3 mutations on patient survival. Thus, glioma patients were categorized into the group with DDIT3 mutations (especially amplifications) and the non-mutation group, to examine the difference in OS between patients with and without DDIT3 mutations using the survminer R packages [22].

Transcriptional expression analysis

To investigate the correlation between DDIT3 mRNA expression levels and the malignant progression of glioma, the TCGA database was analyzed for DDIT3 expression. Biomarker data and clinical information were obtained from the TCGA repository at https://portal.gdc.cancer.gov. The R programming language was utilized, along with the TCGAbiolinks package [23], to download normalized RNA-seq data for a total of 681 samples in the TCGA glioma cohorts. IDH mutations were identified by considering mutations in IDH1 codon 132 and IDH2 codon 172. Co-deletion of chromosome 1p19q was determined by focal copy number variation (CNV) scores below -0.3. Methylation levels of the MGMT promoter were categorized based on the 10% and 40% percentiles of the mean beta value of probes within the chromosomal region 131264896 to 131265737. Additionally, DDIT3 expression was quantified in RNA-seq data for specific tumor anatomic structures, identified through H&E staining, obtained from the Ivy Glioblastoma Atlas Project (http://glioblastoma.alleninstitute.org/).

To investigate the association between DDIT3 expression and OS, patients were stratified into groups with low and high levels of DDIT3 gene expression, based on the median DDIT3 expression value (FPKM). The log-rank test was employed to examine the association of expressed hazard ratios. Gene expression was normalized as FPKM, and correlation and survival analyses were conducted in R using the grid and survminer packages [22].

Biological function analysis

To elucidate the potential biological functions of DDIT3 in glioma, the glioma cohort of TCGA was stratified into distinct groups based on their DDIT3 expression levels. Specifically, the patients were segregated into DDIT3 low and DDIT3 high groups by employing the median DDIT3 expression level as the threshold. Subsequently, a comparative analysis of the differentially expressed genes between the DDIT3 low and DDIT3 high groups was conducted to unravel potential pathways enriched in this context. For this purpose, gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were performed using the "clusterprofiler" package within the R software [24].

Immune infiltration analysis

To investigate the relationship between DDIT3 and immune infiltration in glioma, we examined the differences in immune infiltration among different DDIT3 expression groups. Using RNA-seq data, the infiltration abundance of 22 immune cell types in glioma samples was estimated using the "CIBERSORT" R package, which utilizes gene expression profiles provided by CIBERSORT for sample deconvolution [25]. The global *p*-value for each sample deconvolution was determined, and samples with $P < 0.05$ were selected for further analysis. To predict the tumor microenvironment (TME), immune scores, stromal scores, tumor purity, and estimated scores were calculated using the "ESTIMATE" R package [26].

Cell culture and DDIT3 knockdown

The human glioblastoma cell lines, namely U-87MG and U-251, were obtained from the Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences. These cell lines were authenticated through short tandem repeat (STR) analysis to ensure their validity. The cell lines were cultured in DMEM medium (Hyclone, USA) supplemented with 10% FBS (PAN, USA) and maintained in a controlled environment at 37°C with a 5% CO_2 atmosphere. To achieve DDIT3 knockdown, the cultured glioma cells were transfected with specific target siRNA using Lipofectamine RNAiMAX reagent (Invitrogen) in Opti-MEM (Invitrogen). The transfected cells were then incubated for 72 hours. The siRNA sequences employed were as follows: siRNA DDIT3#1: Sense 5'-UUCACCAUUCGGUCAACAGAGCUC-3'; Antisense 5'-GAGCUCUGUUGACCGAAUGGUGAA-3'; siRNA DDIT3#2: Sense 5'-AAGAACCAGCAGGUCACAA-3'; Antisense 5'-UUGUGACCUCUGCUGGUUCUU-3'.

mRNA extraction and real-time PCR

Total RNA from the cell samples was extracted using an RNA extraction kit from Foregene (Chengdu), following the manufacturer's protocol. Subsequently, cDNA conversion was performed using a reverse transcription kit. Quantitative PCR reactions and analysis were conducted using the Takara kit in the Bio-Rad iQ5 system. The expression levels of the target mRNA were normalized using the housekeeping gene GAPDH.

Wound healing assay

To assess the impact of DDIT3 on glioma cell migration, a wound healing assay was conducted following a previously described protocol. Cells were seeded overnight in a 6-well plate at a density of 2.5×10^4 cells/well in 2 mL medium supplemented with 10% FBS. Once the cells reached a confluency of at least 90%, siRNA targeting DDIT3 was transfected into the cells. After 2 days, a monolayer of cells was mechanically scratched using a sterile 200 µL pipette tip to create a wound. The detached cells were eliminated by gently washing with PBS, and the cells were subsequently cultured in a serum-free medium. Images of the wound area were captured at 0-72 hours after the treatment. The acquired images were then analyzed using ImageJ software to quantify the extent of wound closure and assess cell migration dynamics.

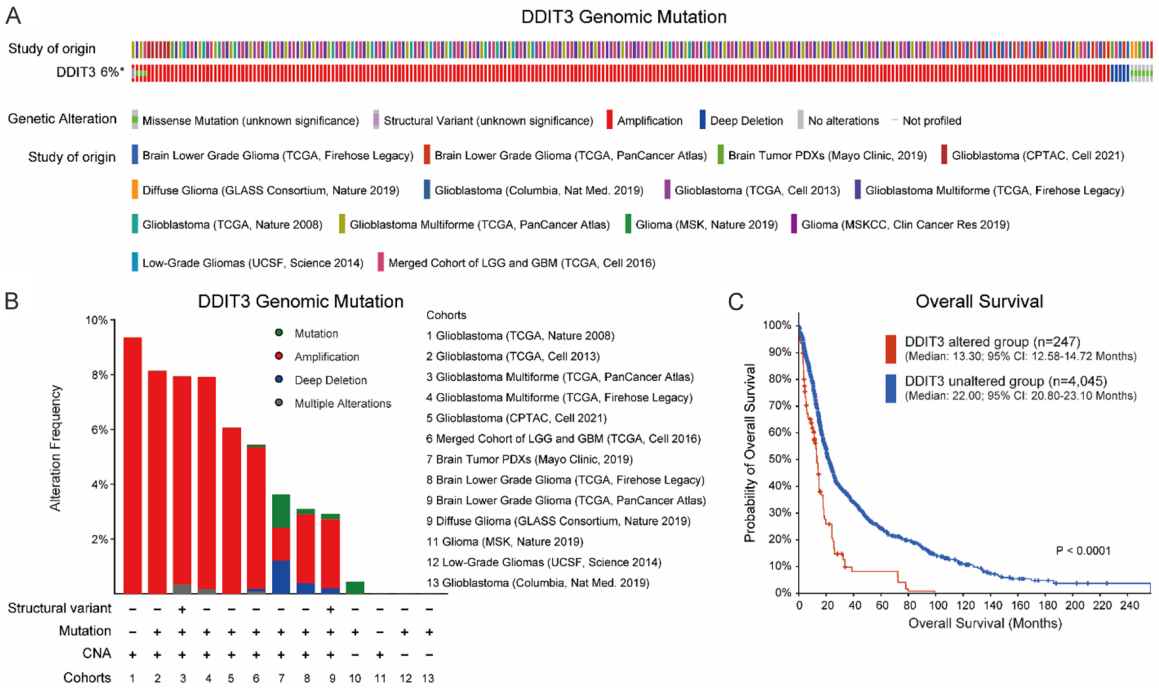

Figure 1. Amplification of DDIT3 in glioma. A, B. Diagrams showing the genomic amplification of DDIT3 in glioma. C. Survival analysis of DDIT3 mutation with glioma. Noted that patients were stratified into DDIT3 mutant and non-mutant groups.

Transwell migration assay

Transwell migration assays were conducted using 8.0 μm pore size chambers in 24-well plates (Corning, USA). U87 cells were seeded in the upper chamber at a density of 1×10^4 cells/well, in FBS-free DMEM, while the lower chamber was filled with DMEM containing 10% FBS. After an 8-hour incubation period, the upper surface of the membrane was gently scrubbed to remove any non-invaded cells. The cells that had migrated to the lower surface of the membrane were fixed and stained using a 0.5% crystal violet solution. The migrated cells were quantified using ImageJ, and data from three independent experiments were statistically analyzed.

Statistical analysis

Statistical analysis was conducted using GraphPad Prism software (version 9.0, GraphPad Software, Inc.). The data were presented as mean ± SEM. To assess the significance of differences between groups, Student's t-test was employed for two-group comparisons, while one-way analysis of variance (ANOVA) followed by Tukey post hoc tests was utilized for multi-group comparisons. A p-value < 0.05 (*) was considered statistically significant, while $P < 0.01$ (**) and $P < 0.001$ (***) were denoted as highly significant.

Results

Positive association between DDIT3 expression and glioma malignancy

To examine the potential relevance of DDIT3 in glioma, we first conducted an examination of DDIT3's genomic expression profiles utilizing the cBioPortal database, a comprehensive platform that enables the detection of genomic events across various cancer types, including glioma. Results showed a mutation frequency of 6% in DDIT3, with a substantial amplification in glioma patients (**Figure 1A**, **1B** and Supplementary Figure 1A, 1B). To further explore the association between DDIT3 mutations and glioma prognosis, we performed Kaplan-Meier analysis and demonstrated a significantly shorter OS in the group with DDIT3 mutations (mOS=13.30 months) compared with the group without DDIT3 mutations (mOS=22.00 months) (**Figure 1C** and Supplementary Figure 1C). These findings strongly

indicate that amplification of DDIT3 may serve as a critical mechanism in glioma malignancy.

To further explore the relationship between DDIT3 expression and glioma clinical features, we analyzed the DDIT3 gene expression data and associated clinical information sourced from TCGA. Results indicated a positive correlation between DDIT3 expression and glioma grade, with elevated DDIT3 expression being more prevalent in the glioblastoma (GBM) subtype (**Figure 2A**, **2B**). In glioma, IDH mutation, chromosome 1p/19q co-deletion, and MGMT promoter methylation are considered pivotal biomarkers for prognosis and treatment guidance. We found a strong association between DDIT3 expression and the IDH wild-type, 1p/19q non-codeletion and MGMT promoter unmethylated groups, indicating a poorer prognosis for glioma patients (**Figure 2C-E**). Next, we found that elevated expression of DDIT3 in pseudopalisading cells and the perinecrotic zone of GBM samples through IVY database, implying distinct DDIT3 expression within anatomical features of glioma (**Figure 2F**). Finally, consistent with the association between DDIT3 and highly malignant gliomas, Kaplan-Meier survival analysis showed a significant correlation between DDIT3 expression and patient prognosis. Specifically, patients with lower DDIT3 expression exhibited notably better overall survival compared to those with higher DDIT3 expression (**Figure 2G**).

To confirm the correlation between DDIT3 and glioma malignancy, we conducted additional analyses using the CGGA (the Chinese Glioma Genome Atlas) dataset. Consistently, we observed an upregulation of DDIT3 mRNA expression in WHO IV compared with WHO grade II/III gliomas (**Figure 2H**). Furthermore, Kaplan-Meier survival curves demonstrated that high DDIT3 expression was associated with poor survival in the CGGA dataset (**Figure 2I**). Altogether, these results demonstrate the pronounced expression of DDIT3 in human gliomas and defines DDIT3 as an indicator of glioma malignancy and poor prognosis.

Identification of DDIT3-related co-expressed gene networks in glioma

To further elucidate the potential mechanisms by which DDIT3 regulates glioma malignancy, we conducted an in-depth investigation of the genomic and transcriptomic disparities between subsets with high and low DDIT3 expressions. First, we examined somatic mutations derived from the TCGA datasets. Overall, there were subtle differences in the genomic mutation landscape among the glioma subgroups with different DDIT3 expression levels. In the low DDIT3 expression group, the predominant mutated genes were IDH1 (76%), followed by TP53 (48%), ATRX (39%), and CIC (20%), with mutation frequencies below 10% for other genes (**Figure 3A**). In contrast, the high DDIT3 expression group exhibited a different mutation pattern, with IDH1 remaining a prevalent mutation but with a reduced frequency of 46%. This was followed by TP53 (36%), ATRX (21%), TTN (16%), PTEN (15%), EGFR (13%), and CIC (12%), with mutation frequencies below 10% for other genes (**Figure 3B**). These findings suggest that although the genomic mutation repertoire is relatively consistent between glioma subgroups with different DDIT3 expression levels, the mutation frequencies differ, with the high DDIT3 expression group exhibiting a greater diversity of mutations. Altogether, all these results demonstrate the higher heterogeneity of gliomas in the subgroup with elevated DDIT3 expression.

Next, we analyzed the differential gene expression between the subsets with distinct DDIT3 expressions. The volcano plot illustrated the presence of differentially expressed genes (DEGs) between the two groups (**Figure 3C**). Further examination through KEGG and GO biological process analyses revealed enrichment of these DEGs in specific pathways, including inflammation (IL-17 and TNF signaling pathways), transcriptional mis-regulation in cancer, and extracellular matrix (ECM) remodeling (**Figure 3D**, **3E**). This observation suggests that DDIT3 may exert regulatory control over gene transcription, potentially influencing tumor inflammation and ECM-related tumor behaviors.

Association between DDIT3 expression immune cell infiltration in glioma

Based on above analysis, we then investigated the potential involvement of DDIT3 in immune regulation within the context of GBM. We evaluated immune infiltration within the tumor microenvironment (TME) using the CIBERSORT algo-

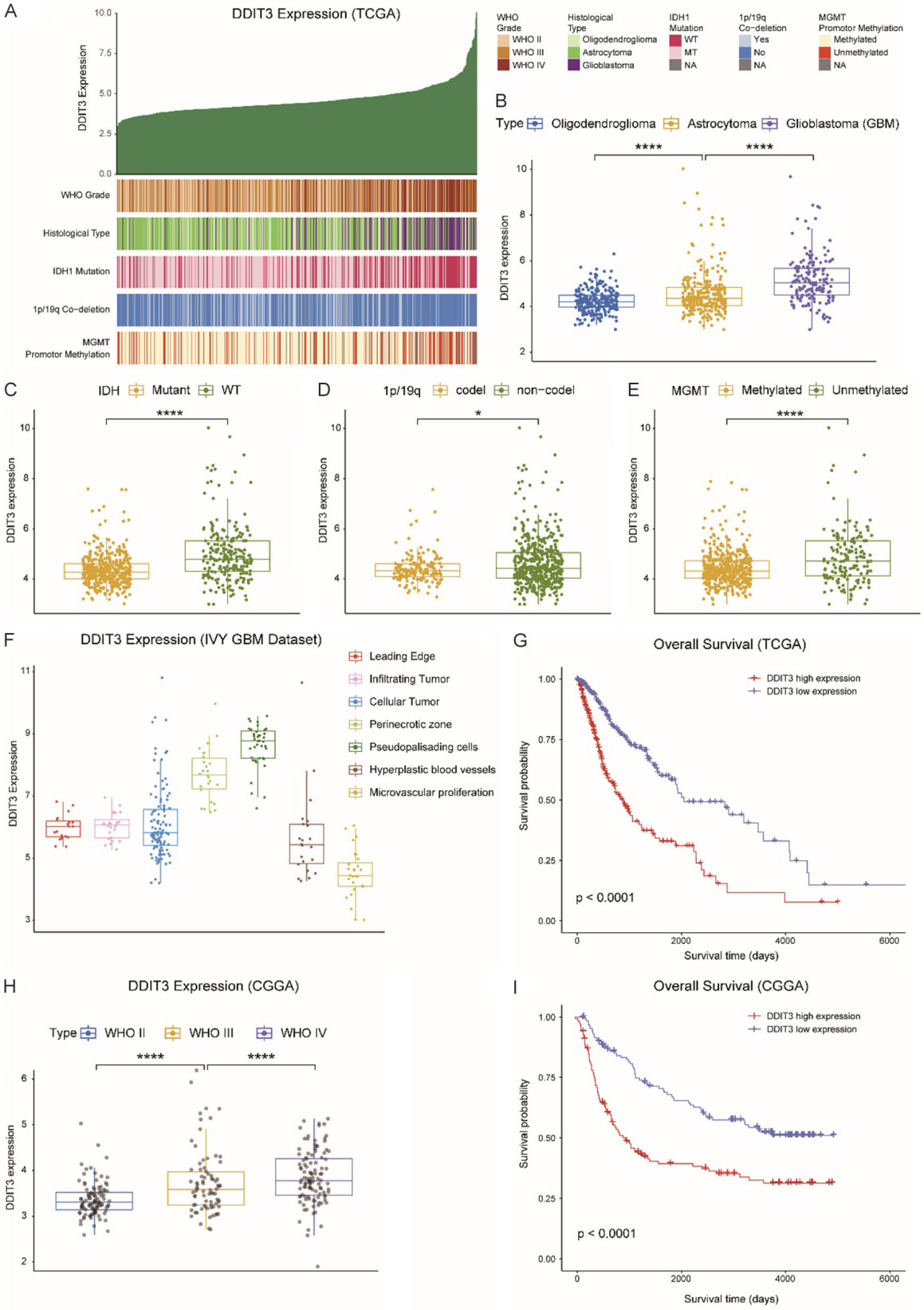

Figure 2. Positive association between DDIT3 expression and glioma malignancy. A. Correlation between DDIT3 expression (FPKM) and glioma prognostic biomarkers in TCGA. B. Diagrams showing DDIT3 expressions in different histological gliomas in TCGA. C-E. Diagrams showing the correlations of DDIT3 expressions with glioma markers. F.

Diagrams showing DDIT3 expressions in GBM anatomical zones. G. Survival analysis of DDIT3 with glioma in TCGA, cut by median expression level. H. Diagrams showing DDIT3 expressions in different grade gliomas in CGGA. I. Survival analysis of DDIT3 with glioma in CGGA, cut by median expression level.

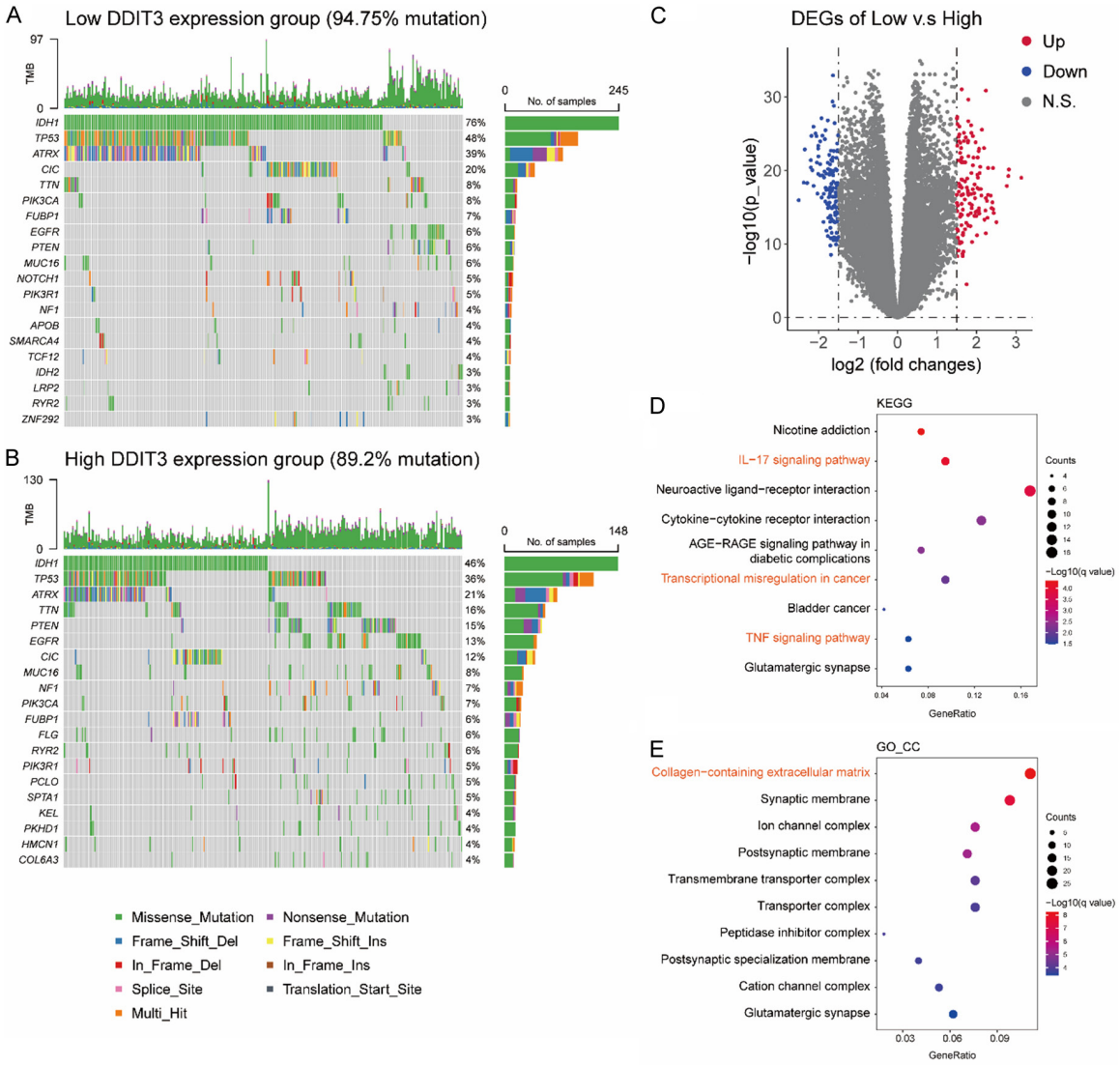

Figure 3. DDIT3-related co-expressed gene networks in glioma. A, B. Waterfall plots showing genomic alterations in DDIT3 high and low groups in TCGA. C. Volcano blot showing different expression genes in DDIT3 high vs. low groups in TCGA. D, E. KEGG and GO analysis showing the functional enrichment of the DEGs in DDIT3 high vs. low groups in TCGA.

rithm. The analysis revealed substantial variations in the proportions of 22 immune cell types among different samples and cell types (**Figure 4A**). Notably, statistical analysis identified 10 immune cell types that exhibited significantly different infiltration degrees between the high and low DDIT3 expression groups ($P < 0.05$) (**Figure 4A**). The high DDIT3 expression group demonstrated higher abundance of M2 macrophages and lower abundance of monocytes compared with the low DDIT3 group, suggesting an immunosuppressive tumor microenvironment characterized by the infiltration of inactivated immune cells. Moreover, it's noted that in LGG, the high DDIT3 expression group exhibited more M2 macrophages and fewer monocytes (Supplementary Figure 2A), consistent with the overall glioma results (**Figure 4A**).

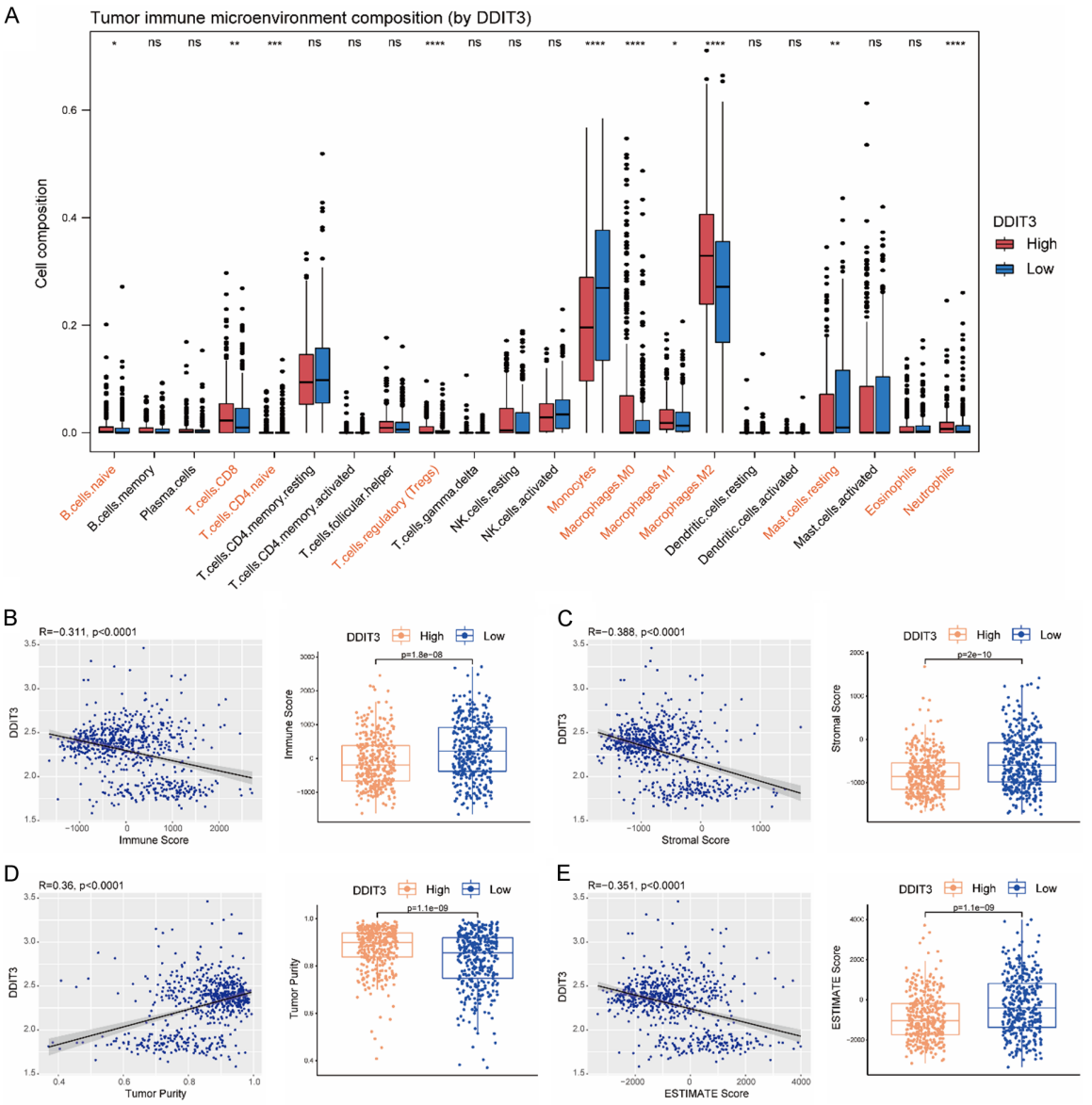

Figure 4. Association between DDIT3 expression immune cell infiltration in glioma. A. Diagrams showing distinct immune cell abundance in DDIT3 high vs. low groups in TCGA. B-E. Diagrams showing the comparison of immune score, stromal score, tumor purity and ESTIMATE score in DDIT3 high vs. low groups in TCGA.

However, there's no significant difference in immune cell composition between high and low DDIT3 expression groups in GBM (Supplementary Figure 2B).

To further elucidate the immune landscape associated with different DDIT3 expression groups in glioma, we calculated the immune score, stromal score, tumor purity, and ESTIMATE score using the ESTIMATE algorithm. The low DDIT3 group exhibited significantly higher immune, stromal, and ESTIMATE scores, and lower tumor purity compared with the high DDIT3 group (**Figure 4B-E**), indicating that the poor prognosis observed in the high DDIT3 group may be partly attributed to higher tumor purity and immunosuppressive microenvironment.

DDIT3 regulates glioma cell proliferation and migration in vitro

In order to delineate the functional role of DDIT3 within glioma, we employed targeted

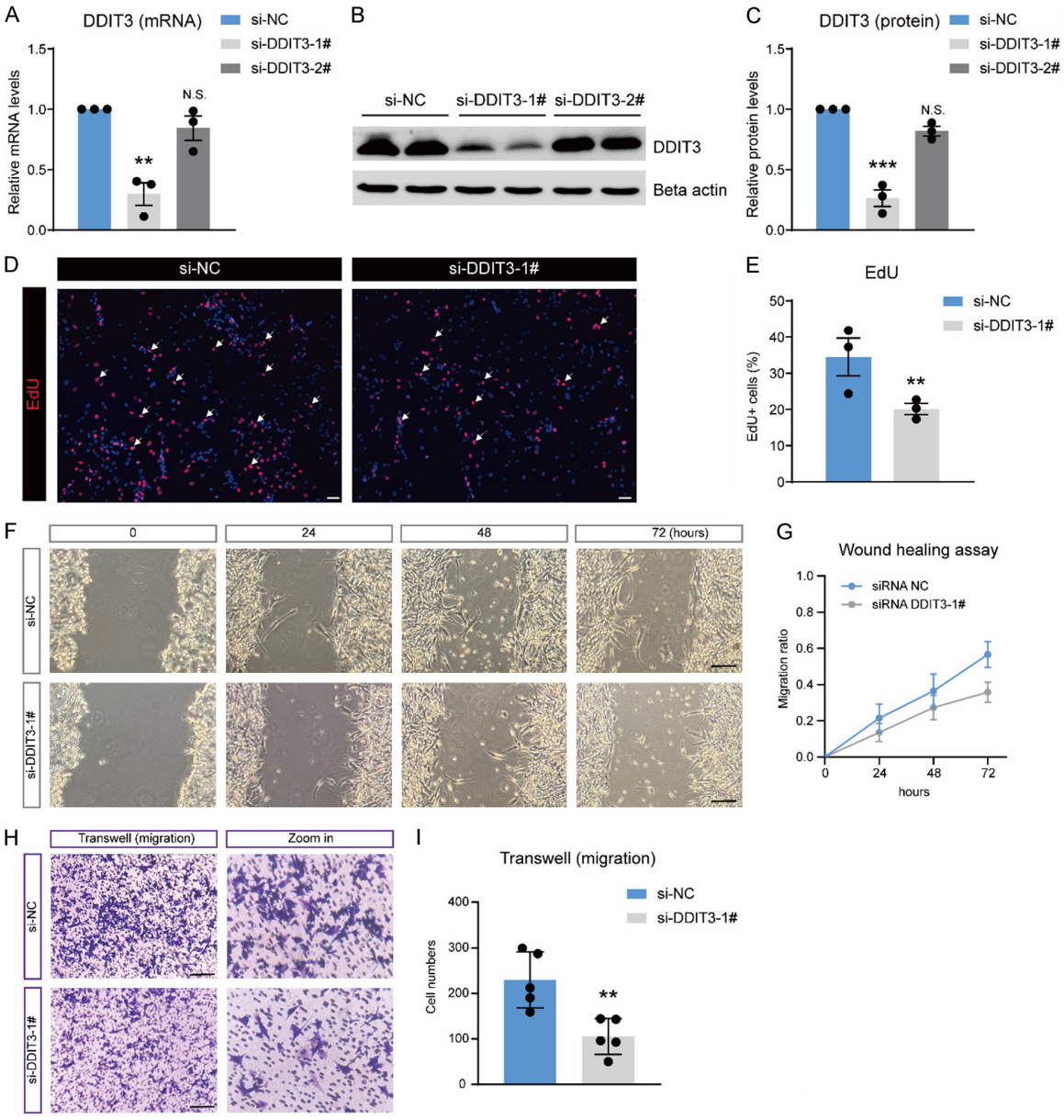

Figure 5. DDIT3 regulates glioma cell proliferation and migration *in vitro*. (A-C) qPCR (A), Western blots and quantifications (B, C) showing the gene knockdown of DDIT3 in U87 glioma cells. (D, E) Images and quantifications of EdU assay showing the decreased cell proliferation by DDIT3 knockdown in U87 glioma cells. Scale bar, 100 μm. (F, G) Images and quantifications of wound healing assay showing the decreased cell migration by DDIT3 knockdown in U251 glioma cells. Scale bar, 100 μm. (H, I) Images and quantifications of transwell assay showing the decreased cell migration by DDIT3 knockdown in U87 glioma cells. Scale bar, 100 μm.

manipulation of DDIT3 expression in U87 and U251 GBM cells through siRNA-mediated knockdown. The effectiveness of DDIT3 knockdown was validated using qPCR and Western blot (**Figure 5A-C**). It's noted that, among the siRNA candidates, siRNA 1# exhibited the most pronounced reduction in DDIT3 expression and therefore was selected for subsequent *in vitro* experiments. First, we examined the influence of DDIT3 on glioma cell proliferation. Through an EdU labeling assay, results revealed that knockdown of DDIT3 yielded a slight but significant reduction in the fraction of EdU+ U87 cells, suggesting that DDIT3 is required for glioma cell proliferation (**Figure 5D**, **5E**). Next, we elucidated the role of DDIT3 in the regulation of

glioma cell migration. Using the wound healing assay, we demonstrated that DDIT3 knockdown dramatically attenuated the migratory rate of U251 glioma cells (**Figure 5F**, **5G**). To further confirm these findings, we used the transwell assay, and found that DDIT3 knockdown led to an impaired infiltration of U87 cells (**Figure 5H**, **5I**). Thus, all these results clearly demonstrate that DDIT3 is required for cell proliferation and migration of glioma cells.

Discussion

In this study, we investigated the clinical and biological significance of DDIT3 in glioma. Our findings revealed that DDIT3 expression is positively associated with glioma malignancy, making it a potential novel biomarker for glioma prognosis and treatment stratification. High DDIT3 expression predicted poor overall survival in glioma patients. At the biological level, we demonstrated the involvement of DDIT3 in glioma progression through genomic, transcriptomic, and proteomic analyses. Experimental validations further confirmed the impact of DDIT3 on glioma malignancies, including proliferation, migration, and invasion. Our study positioned DDIT3 as a nodal point in the interplay between ER stress and metabolic reprogramming in glioma, highlights its clinical and biological significance.

Glioma, particularly GBM, is characterized by high aggressiveness and poor prognosis [27]. The pathogenesis of glioma involves a complex interplay of various tumor-promoting and tumor-suppressive signaling pathways [28-30]. Dysregulation of these pathways leads to uncontrolled cell proliferation, enhanced cell survival, and the acquisition of invasive properties. Previous studies have highlighted the importance of two key factors in glioma pathogenesis: ER stress and metabolic reprogramming [31]. Metabolic reprogramming, particularly increased glycolysis, can induce ER stress due to the high demand for protein synthesis and folding [32]. In turn, ER stress can influence glycolysis by modulating the expression and activity of key enzymes involved in glucose metabolism [33]. This reciprocal regulation creates a feedback loop that contributes to the adaptation and survival of glioma cells under stressful conditions. Thus, the coordination between these pathways allows cancer cells to adapt to stressful conditions and meet the increased demands of proliferation and survival. Recent studies have identified several nodal points that connect ER stress and glycolysis in glioma [13, 14]. In this study, we provide additional insights into current understandings to the coupling of ER stress and metabolic reprogramming in glioma, with a specific focus on the role of DDIT3. Our results position DDIT3 as a nodal point in the interplay between ER stress and glycolysis in glioma.

We argue that excessive elevation in DDIT3 levels may trigger cell death; however, moderate upregulation of DDIT3 expression can play a role in promoting cell survival. This survival-promoting effect is notably achieved through mechanisms correlated with an augmentation of glycolysis, which, in turn, increases the availability of energy resources essential for sustaining cells under adverse conditions. This suggests that appropriate DDIT3 expression not only contributes to cell survival mechanisms but also orchestrates metabolic adaptations crucial for cellular resilience during stress. Consequently, the absence of DDIT3 appears to hinder the optimal growth and viability of GBM cells. Previous studies have demonstrated that the induced expression of DDIT3 can facilitate apoptosis in GBM cells through an ER stress-independent pathway [19]. In contrast, our study primarily revealed the elevated expression of DDIT3 in GBM, emphasizing its role in GBM growth. These collective studies underscore the dual role of DDIT3 in GBM: on the one hand, its high expression in GBM directly correlates with poor prognosis and promotes GBM growth; on the other hand, excessive overexpression of DDIT3 induces cell death. Thus, our findings highlight the intricate role of DDIT3 in influencing cellular outcomes, revealing its impact on both survival pathways and metabolic adjustments.

Our study revealed a positive association between DDIT3 expression and glioma malignancy. Elevated DDIT3 expression was found to be prevalent in GBM, the most aggressive form of glioma. This observation suggests that DDIT3 could serve as a novel biomarker for glioma malignancy, aiding in the stratification of patients based on prognosis and treatment response. The correlation between DDIT3 expression and clinical features, such as IDH

wild-type, 1p/19q non-codeletion, and MGMT promoter unmethylation, highlights the potential of DDIT3 as a predictive biomarker for patient responses. These facts may guide treatment strategies, as patients with higher DDIT3 expression levels might require more aggressive therapeutic interventions to improve outcomes.

Outside the context of glioma, DDIT3 has been studied in various human cancers, exhibiting both tumor-promoting and tumor-suppressive roles depending on the cancer type and context. DDIT3, also known as CHOP, has been implicated in various human cancers, including gastric cancer (GC), lung cancer, chronic myeloid leukemia (CML), castration-resistant prostate cancer (CRPC), and cholangiocarcinoma. In GC, DDIT3 is upregulated in tumor tissues and cancer cell lines, promoting cell proliferation, colony-forming ability, sphere formation, and cancer stemness through the upregulation of CEBPβ and CSC markers [34]. Additionally, DDIT3 interacts with phospho-JUN and KAT2A, leading to the upregulation of TNFRSF10A and TNFRSF10B, which play a role in apoptosis induction in lung cancer cells [35]. In CML, aberrant methylation of the DDIT3 promoter is observed, correlating with white blood cell counts [36]. In CRPC, DDIT3 is upregulated and associated with poor disease-free survival, suggesting its involvement in disease progression [37]. Furthermore, in colon carcinoma cells, resveratrol induces the expression of DDIT3, contributing to apoptosis [38]. These divergent roles suggest that the functions of DDIT3 are highly context-dependent. The differential roles of DDIT3 in various human cancers can be attributed to several factors, including the specific genetic alterations present in each cancer type, the cellular context, and the interplay with other signaling pathways. These variations highlight the complexity of DDIT3's functions and emphasize the importance of considering the specific cancer type when investigating its role in tumor biology. In glioma, our findings support the notion of DDIT3 as a tumor-promoting factor. The positive correlation between DDIT3 amplification/enhanced expression with glioma malignancy, as well as the experimental validations demonstrating its impact on glioma cell behavior, provide strong evidence for the promotive role of DDIT3 in glioma.

Conclusions

In conclusion, our study provides comprehensive insights into the clinical and biological significance of DDIT3 in glioma. DDIT3 emerges as a novel biomarker for glioma malignancy and predicts patient responses, potentially guiding treatment strategies. Mechanistically, DDIT3 acts as a nodal point connecting ER stress and metabolic reprogramming in glioma. The coupling of these processes contributes to glioma progression and highlights DDIT3 as a promising therapeutic target. Future studies should focus on unraveling the precise mechanisms by which DDIT3 influences ER stress and glycolysis coupling and explore its therapeutic implications for glioma and other cancers.

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Disclosure of conflict of interest

None.

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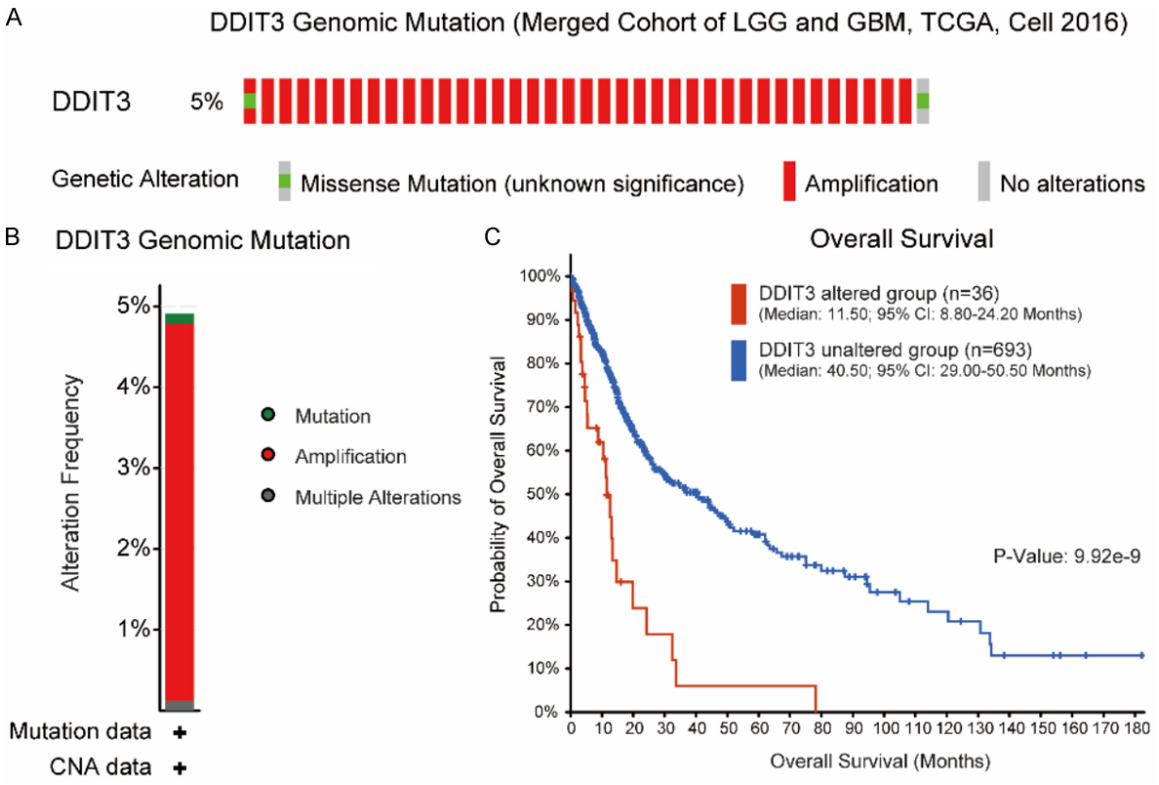
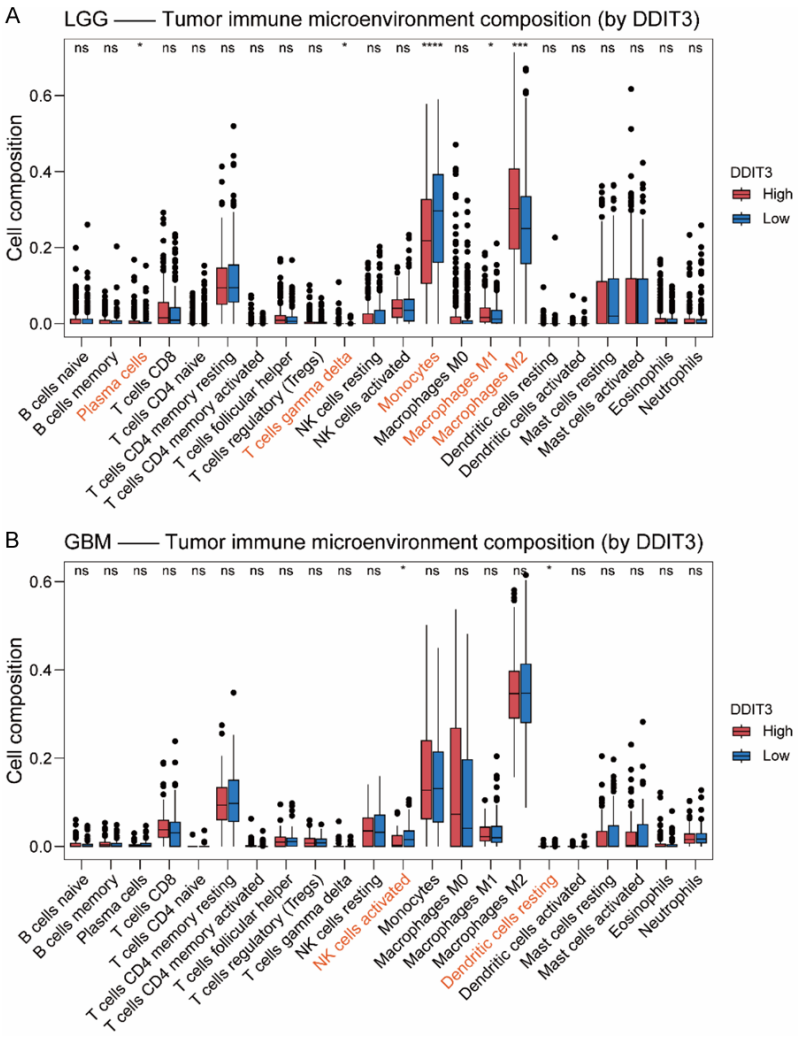


Supplementary Figure 1. Amplification of DDIT3 in glioma (TCGA dataset). A, B. Diagrams showing the genomic amplification of DDIT3 in glioma (TCGA dataset). C. Survival analysis of DDIT3 mutation with glioma. Noted that patients were stratified into DDIT3 mutant and non-mutant groups (TCGA dataset).



Supplementary Figure 2. Association between DDIT3 expression immune cell infiltration in glioma. A. Diagrams showing distinct immune cell abundance in DDIT3 high vs. low groups in LGG. B. Diagrams showing distinct immune cell abundance in DDIT3 high vs. low groups in GBM.