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PRIM2: A Marker of MYC-driven Hyper-proliferation, Disease Progression, Tumor Aggressiveness and Poor Survival in Glioma Patients

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Abstract. *Background/Aim: Gliomas are the most prevalent brain tumors with metabolic alterations playing a pivotal role in disease progression. However, the precise coordination of metabolic alterations with tumor-promoting cellular mechanisms, leading to tumor initiation, progression, and aggressiveness, resulting in poor outcomes, remains poorly understood in gliomas. Materials and Methods: We conducted a metabolism-targeted differential gene expression analysis using glioma patients' expression profiling data from The Cancer Genome Atlas (TCGA) database. In addition, pathway enrichment analysis, gene set enrichment analysis (GSEA), transcription factor prediction, network construction, and correlation analyses were performed. Survival analyses were performed in R. All results were validated using independent GEO expression datasets. Results: Metabolism-targeted analysis identified 5 hits involved in diverse metabolic processes linking them to disease aggressiveness in gliomas. Subsequently, we established that cell cycle progression and hyper-proliferation are key drivers of tumor progression and aggressiveness in gliomas. One of the identified metabolic hits, DNA primase 2 (PRIM2), a gene involved in DNA replication was found directly associated with cell cycle progression in gliomas. Furthermore, our analysis indicated that PRIM2, along with other cell cycle-related genes, is under the control of and regulated by the oncogenic MYC transcription factor in gliomas. In addition, PRIM2 expression alone is enough to predict MYC-driven cell cycle progression and is associated with tumor progression, aggressive disease state, and poor survival in glioma patients. Conclusion: Our findings highlight PRIM2 as a marker of MYC-driven cell cycle progression and hyper-proliferation, disease onset and progression, tumor aggressiveness, and poor survival in glioma patients.*

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Cancer, a multifaceted and formidable disease, spans a wide spectrum of malignancies and continually propels the boundaries of medical research (1). Among the intricate cancer types, gliomas emerge as a particularly complex and demanding subset of tumors. These primary brain tumors originate from neuroglial stem or progenitor cells, constituting 30% of all brain tumors and over 80% of malignant brain tumors (2). With an annual incidence rate of 6.6 cases per 100,000 individuals, gliomas exhibit a notable clinical heterogeneity, encompassing both slow-growing and highly aggressive forms, resulting in diverse disease progressions and clinical outcomes (3). Gliomas have been categorized into astrocytic, oligodendroglial, or ependymal tumors based on their histological characteristics and are assigned WHO grades 1-4 accordingly which indicate different degrees of malignancy (4). Tragically, glioblastoma (GBM), the exceptionally malignant form, accounts for half of newly diagnosed gliomas, with a median patient survival duration typically ranging from 14 to 17 months. Low-grade gliomas also carry the potential to progress into more aggressive

forms (5). Recent scientific interests in gliomas have primarily centered on unraveling the intricate molecular underpinnings that drive disease aggressiveness and contribute to adverse patient outcomes (6, 7). As a result, significant strides have been made in comprehending the molecular intricacies of gliomas over the past decade. Critical biomarkers, including isocitrate dehydrogenase (IDH) mutations, chromosome 1p/19q deletions, O6-methylguanine-DNA methyltransferase (MGMT) promoter methylation, telomerase reverse transcriptase (TERT) promoter mutations, and histone mutations, have emerged as pivotal factors in the classification of gliomas and the guidance of treatment decisions (8-10). However, our current understanding of gliomas at the molecular level remains somewhat constrained. This limitation poses considerable challenges in the treatment of these tumors, ultimately resulting in a less than optimistic prognosis for glioma patients (11). Consequently, identifying molecular biomarkers associated with disease onset, progression, tumor aggressiveness, and patient outcomes is of paramount importance (12, 13). Such insight forms the cornerstone for the development of more effective diagnostic tools and therapeutic strategies that can significantly benefit glioma patients.

Metabolic alterations have earned recognition as a fundamental molecular hallmark of cancer (14). These metabolic changes are not mere bystanders; they play an active role in fueling uncontrolled tumor cell growth and exert a profound influence on the course of disease progression, therapeutic response and patients' survival (15). Recent research endeavors have embarked on an in-depth exploration of intricate metabolic changes that underpin cancer. Despite significant progress, such as the recognition of aberrantly regulated glucose and lipid metabolisms as pivotal metabolic biomarkers associated with disease aggressiveness (16, 17), there remains a noticeable gap in 1) the identification of key metabolic hits that are associated with high proliferative characteristics of these tumors, and 2) the development of comprehensive metabolic biomarkers and associated signatures that could offer more precise prognostic insights for glioma patients (18). Furthermore, it is crucial for unraveling the intricate interplay between metabolic alterations and tumor promoting molecular mechanism that collectively drive the aggressive behavior of these tumors.

The advent of advanced expression profiling techniques and the accessibility of vast datasets have significantly empowered scientists to swiftly delve into the molecular mechanisms underpinning diseases. Furthermore, this progress has played a pivotal role in advancing biomarker research (19, 20). Here, we performed a targeted differential expression analysis to identify the metabolic markers of disease onset and progression, tumor aggressiveness and survival in glioma patients. Our rigorous analysis pipeline identified primase 2 (PRIM2) as a key molecular hit and biomarker of cell cycle progression and MYC driven proliferation in gliomas. This study contributes to the growing body of knowledge aimed at identifying novel biomarkers and therapeutic targets for glioma patients, with the ultimate goal of improving clinical outcomes and patient survival.

Materials and Methods

Data retrieved. Glioma patients' gene expression and DNA methylation data at The Cancer Genome Atlas (TCGA) database were retrieved online from Broad GDAC Firehose: https://gdac.broadinstitute.org/. Gene mutation data was retrieved from cBioportal for Cancer Genomics: https://www.cbioportal.org/. Genes associated with metabolic pathways were retrieved from the Mammalian Metabolic Enzyme Database: https://hpcwebapps.cit.nih.gov/ESBL/Database/MetabolicEnzymes/MetabolicEnzymeDatabase.html. In addition, expression data of glioma patients was also retrieved from GEO database for the following datasets: GSE4290 (21), GSE7696 (22), GSE10878 (23), GSE16011 (24), GSE21354 (25), GSE43289 (26), GSE45921 (27), GSE52009, and GSE107850 (28). Protein expression data of metabolic hits in Clinical Proteomic Tumor Analysis Consortium (CPTAC) database was retrieved from cProcite (https://cprosite.ccr.cancer.gov/).

Patients' tumor aggressiveness classification. Clinical data of pathological tumor stage/grade and pathological metastatic stage was not available for all the patients in the TCGA Glioma data. For this reason, tumor tissues were planned to be classified into low, intermediate and high aggressiveness groups using gene signature-based classification. Epithelial-to-mesenchymal transition (EMT) has been established as a prime marker for tumor aggressiveness and metastasis (29-32). We downloaded the Hallmark_EMT gene signature from the Gene Set Enrichment Analysis (GSEA) portal at: https://www.gsea-msigdb.org/ and calculated Z-scores of all the 200 genes in this signature for each patient. Thereafter, Z-scores were summed to calculate the tumor aggressiveness score for each patient. Lastly, patients were sorted according to their tumor aggressiveness score and were divided into three equal groups: low, intermediate and high.

Identification of differentially expressed metabolic genes associated with disease progression, tumor aggressiveness and survival. mRNA expression of genes associated with metabolic pathways were compared 1) between normal and primary tumor tissue, 2) among tumor aggressiveness groups and 3) between primary and recurrent tumor tissues. A significance cutoff of false discovery rate (FDR) <0.05 was used in these analyses. In addition, survival analysis was performed for genes associated with metabolic pathways. Later, the common genes among those 1) down-regulated in primary tumors compared to normal tissues, 2) successively down-regulated from low to high aggressiveness group, 3) down-regulated in recurrent tumors compared to primary tumors and 4) associated with better survival, were considered as tumor suppressive metabolic hits in gliomas. On the other hand, the common genes among those 1) up-regulated in primary tumors compared to normal tissues, 2) successively up-regulated from low to high aggressiveness group, 3) up-regulated in recurrent tumors compared to primary tumors and 4) associated with poor survival, were considered as oncogenic metabolic hits in gliomas.

Pathway enrichment analysis and network construction. Pathway enrichment analysis was performed using the online freely available

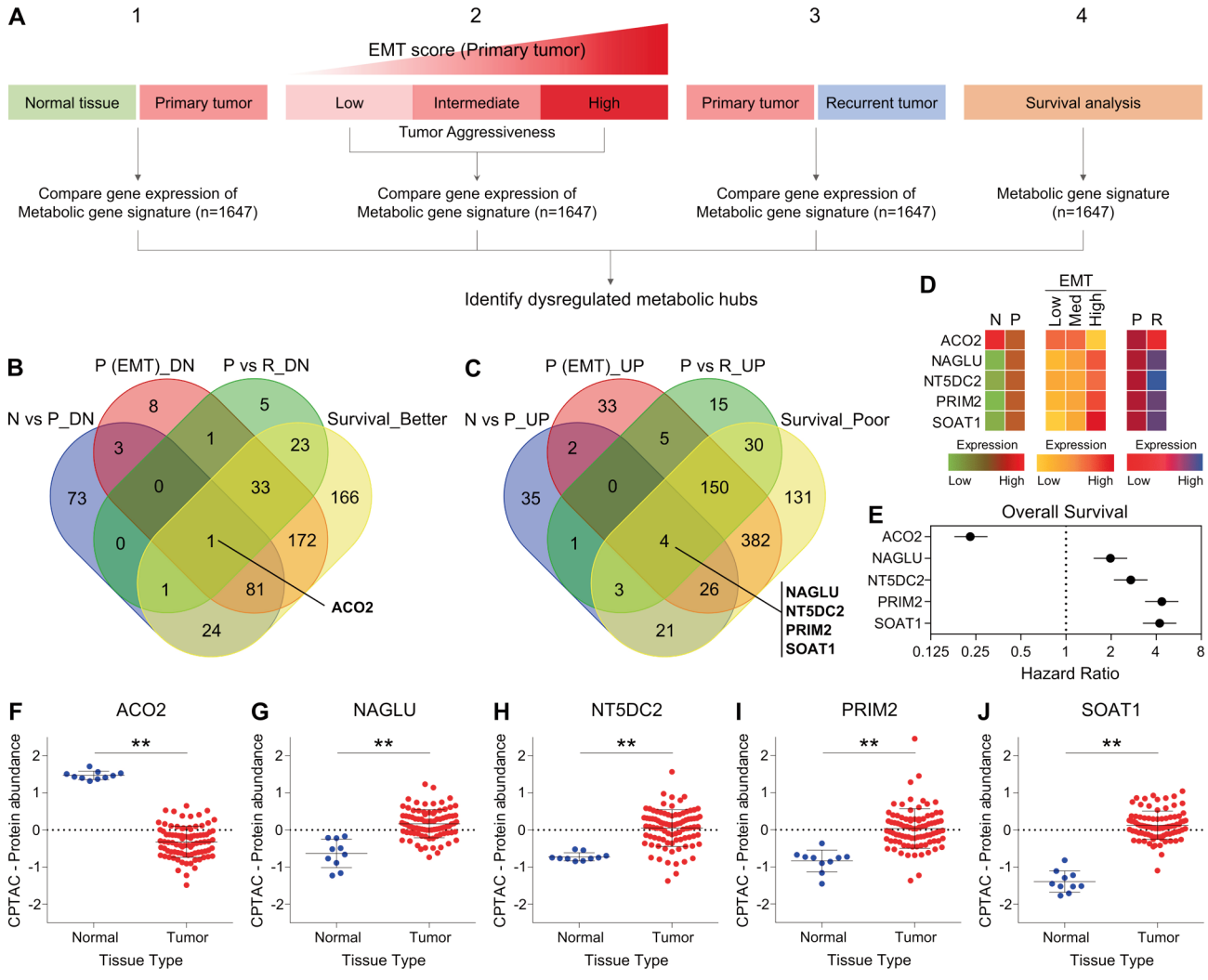

Figure 1. *Metabolism-targeted expression analysis identifies key metabolic hits associated with disease progression, tumor aggressiveness and survival in glioma patients. (A) Illustration showing the analysis pipeline used to identify dysregulated metabolic hubs in the TCGA database. Briefly, expression of metabolic genes was compared in glioma tissue 1) between normal and primary tumor tissues, 2) among EMT score-based tumor aggressiveness groups (low, intermediate, high) of primary tumors, and 3) between primary and recurrent tumors. In addition, 4) association with survival analysis was also checked. (B and C) Venn diagrams showing the number of deregulated metabolic hits identified through the pipeline in (A). (B) shows the down-regulated genes whose expression is also associated with better survival in glioma patients from the TCGA database, whereas (C) shows the up-regulated genes whose expression is also associated with poor survival in glioma patients from the TCGA database. (D) Heatmaps showing expression Z-score changes in the identified metabolic hubs between normal tissue and primary tumors (left), among EMT score-based tumor aggressiveness groups (low, intermediate, high) of primary tumors (middle), and between primary and recurrent tumors (right). (E) Forest plot showing the survival association of identified metabolic hits in glioma patients in the TCGA database. (F-J) Dot-plots showing protein expression comparison of ACO2 (F), NAGLU (G) NT5DC2 (H), PRIM2 (I) and SOAT1 (J) between normal and tumor (glioma) tissues using the CPTAC database. EMT: epithelial-to-mesenchymal transition, N: Normal tissue; P: primary tumor; R: recurrent tumor; UP: up-regulated; DN: down-regulated. **p<0.01.*

DAVID functional annotation tool: https://david.ncifcrf.gov/summary.jsp. Briefly, the intersecting genes 1) successively up-regulated from normal tissue to primary tumors to recurrent tumors and 2) successively up-regulated from low to high aggressiveness groups were identified. Later, these genes were filtered through survival analysis threshold, and the list of genes which was associated with poor survival was uploaded to the DAVID platform to identify the associated KEGG pathways. A significance cutoff of FDR <0.01 was used. The cell cycle associated network was developed using the String database (https://cn.string-db.org/).

Gene set score calculation and gene set enrichment analysis (GSEA). Gene set scores for patients were calculated by summing the Z-scores of genes in a given gene set for each patient. GSEA

was performed using gene sets related to 1) cell cycle progression, 2) MYC targets, and 3) CREB1 targets downloaded from the GSEA website: http://software.broadinstitute.org/gsea/index.jsp.

Transcription factor analysis. In order to predict the transcription factor (TF), cell cycle related gene signature from our results was uploaded to freely available TF prediction tools: hTFtarget (33) and Chea3 (34). In particular, while performing these analyses, ENCODE-ChIP-seq and Literature-ChIP-seq data options were selected in the Chea3 tool. Later, the data from 3 outputs were combined to find common regulators of the input genes.

Survival analysis. Kaplan-Meier survival curves were generated using R. Patients without any available survival time or event were excluded from the corresponding patient groups. All separations were done using the best cut-off threshold. Significance of the differences in survival between two groups was calculated by the Log-rank (Mantel-Cox) test. Comparisons between two groups were made by two tailed student *t*-test. The significance cut-off was set at $p<0.05$.

Statistical analysis. In order to compare data between two groups, the Student's *t*-test was performed. One-way ANOVA was performed to compare data among more than two groups followed by Tukey's multiple comparison test. For correlation analysis, Pearson correlation coefficient was calculated. Bar-graphs, dot-plots, forest-plots and survival plots were made using GraphPad Prism v6 (Dotmatics, Boston, MA, USA). Venn diagrams were made online at: http://bioinformatics.psb.ugent.be/webtools/Venn/.

Results

Metabolism-targeted expression analysis identifies key metabolic hits associated with disease progression, tumor aggressiveness and survival in glioma patients. In order to identify the metabolic pathways associated with disease progression and tumor aggressiveness, we first downloaded the gene expression data of gliomas from the TCGA database, and compared the expression of genes (enzymes) involved in different metabolic pathways between (1) normal and primary tumor tissue, (2) among low, intermediate and highly aggressive primary tumor groups (based on EMT scores) (see Materials and Methodsfor details), and (3) between primary and recurrent tumors. In addition, survival association was also assessed for all these genes (Figure 1A). As a result of these analyses, one hit, Aconitase 2 (ACO2), was identified as key metabolic enzyme whose expression is down-regulated in primary tumors compared to normal brain tissues. It is also successively down-regulated from low to high tumor aggressiveness groups and is further down-regulated in recurrent tumors compared to primary ones (Figure 1B and D). In addition, high ACO2 expression is associated with better survival in glioma patients (Figure 1E). On the other hand, four hits, namely N-acetyl-alpha-glucosaminidase (NAGLU), 5'-nucleotidase domain-containing 2 (NT5DC2), DNA primase subunit 2 (PRIM2), and sterol O-acyltransferase 1 (SOAT1) were identified as key metabolic enzymes whose expression is up-regulated in primary tumors compared to normal brain tissues. These are also successively up-regulated from low to high tumor aggressiveness groups and are further up-regulated in recurrent tumors compared to primary ones (Figure 1C and D). In addition, high expression of these metabolic hits is associated with poor survival in glioma patients (Figure 1E). In order to validate our findings at the protein level, we also checked the protein expression of these metabolic hits in the CPTAC protein abundance database; we found that protein expression of these metabolic hits aligns well with RNA expression as ACO2 is down-regulated in gliomas compared to normal tissue whereas NAGLU, NT5DC2, PRIM2 and SOAT1 are up-regulated in gliomas compared to normal tissues (Figure 1F-J). Interestingly, two of these metabolic hits have already been established as biomarker of disease progression in gliomas through their involvement in lipid metabolism (35, 36), thereby validating our analysis pipeline and findings. ACO2 is a key enzyme in tricarboxylic cycle (TCA) and tightly regulates the production of mitochondrial citrate that is precursor metabolite for *de novo* fatty acid biosynthesis and lipogenesis. Low ACO2 expression has been established as prognostic and immunotherapeutic biomarker for multiple cancers including gliomas (35). In addition, SOAT1 is also involved in lipid metabolism as it esterifies cholesterol with fatty acids to make cholesterol esters and store fat in the form of lipid droplets. Inhibition of SOAT1

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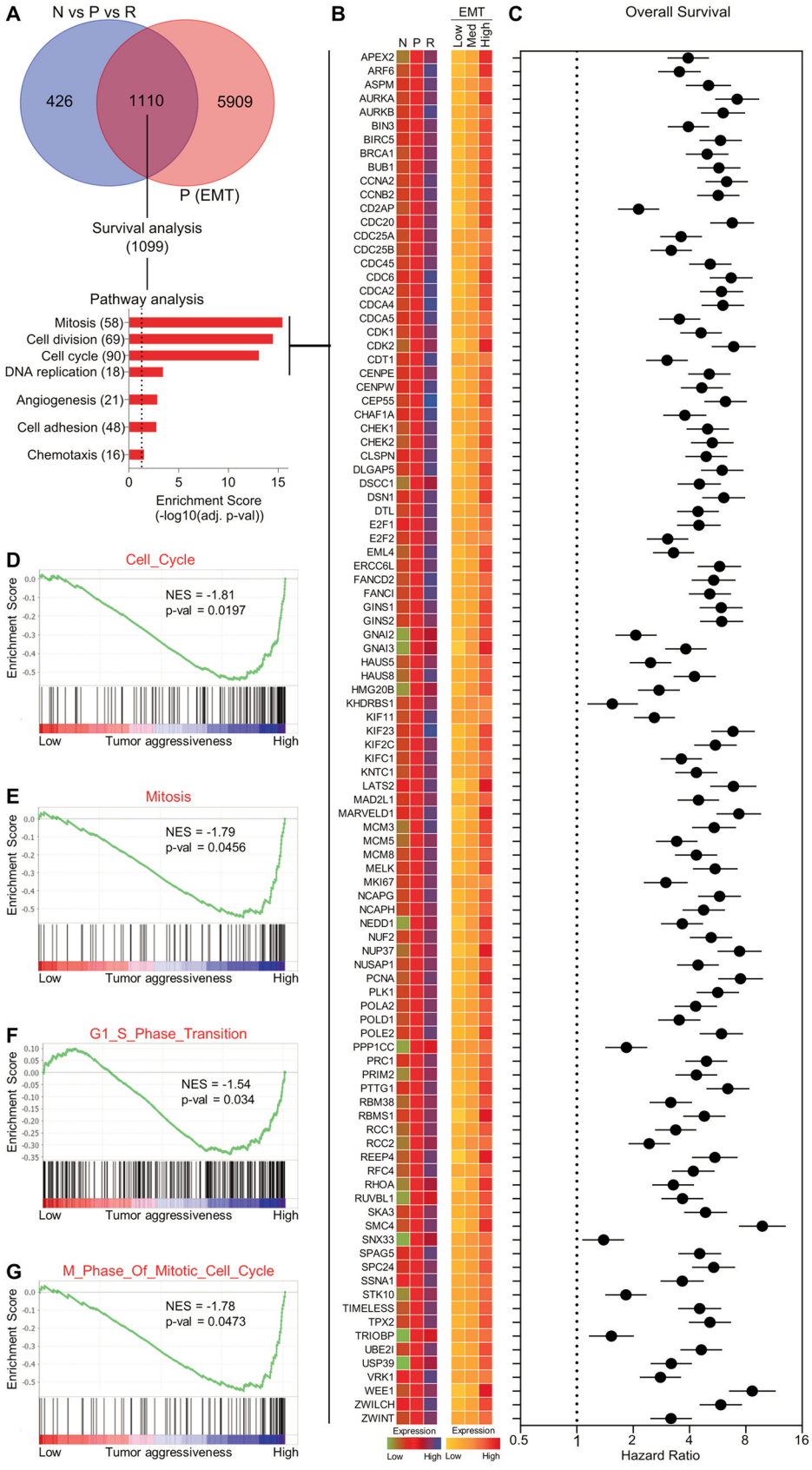
Figure 2. *Transcriptomic analysis identified hyperactive cell cycle progression as key mechanism associated with tumor progression and aggressiveness in gliomas. (A) Illustration showing the analysis pipeline and pathway enrichment analysis used to identify the cellular mechanisms involved in tumor progression and aggressiveness in gliomas. Briefly, intersecting genes (1,110) between those successively up-regulated 1) among normal tissue to primary tumors to recurrent tumors in the TCGA database, and 2) among EMT score-based tumor aggressiveness groups (low, intermediate, high) of primary tumors were identified. Their survival association was assessed, and 1,099 hits were shortlisted. Pathway analysis was then performed with the shortlisted hits using the DAVID tool. Bar graph shows the top up-regulated cellular mechanisms associated with tumor progression and aggressiveness in gliomas. (B) Heatmaps showing expression Z-score changes in deregulated cell cycle associated genes (hits) among normal tissue to primary tumors to recurrent tumors (left), and among EMT score-based tumor aggressiveness groups (low, intermediate, high) of primary tumors (right) from glioma patients from TCGA database. (C) Forest plot showing the survival association of deregulated cell cycle associated genes (hits) in glioma patients from the TCGA database. (D-G) GSEA analysis showing the enrichment of cell cycle related gene signatures, Cell cycle (D), mitosis (E), G1_S_Phase_Transition (F) and M_Phase_Of_Mitotic_Cell_Cycle (G) between low and high tumor aggressiveness groups of gliomas from the TCGA database. EMT: Epithelial-to-mesenchymal transition; N: normal tissue; P: primary tumor; R: recurrent tumor; NES: normalized enrichment score.*


A
N vs P vs R
426
1110
5909
P (EMT)
Survival analysis
(1099)
Pathway analysis
Mitosis (58)
Cell division (69)
Cell cycle (90)
DNA replication (18)
Angiogenesis (21)
Cell adhesion (48)
Chemotaxis (16)
0
5
10
15
Enrichment Score
(-log10(adj. p-val))
B
N P R
EMT
Low
Med
High
APEX2
ARF6
ASPM
AURKA
AURKB
BIN3
BIRC5
BRCA1
BUB1
CCNA2
CCNB2
CD2AP
CDC20
CDC25A
CDC25B
CDC45
CDC6
CDCA2
CDCA4
CDCA5
CDK1
CDK2
CDT1
CENPE
CENPW
CEP55
CHAF1A
CHEK1
CHEK2
CLSPN
DLGAP5
DSCC1
DSN1
DTL
E2F1
E2F2
EML4
ERCC6L
FANCD2
FANCI
GINS1
GINS2
GNAI2
GNAI3
HAUS5
HAUS8
HMG20B
KHDRBS1
KIF11
KIF23
KIF2C
KIFC1
KNTC1
LATS2
MAD2L1
MARVELD1
MCM3
MCM5
MCM8
MELK
MKI67
NCAPG
NCAPH
NEDD1
NUF2
NUP37
NUSAP1
PCNA
PLK1
POLA2
POLD1
POLE2
PPP1CC
PRC1
PRIM2
PTTG1
RBM38
RBMS1
RCC1
RCC2
REEP4
RFC4
RHOA
RUVBL1
SKA3
SMC4
SNX33
SPAG5
SPC24
SSNA1
STK10
TIMELESS
TPX2
TRIOBP
UBE2I
USP39
VRK1
WEE1
ZWILCH
ZWINT
Expression
Low
High
Expression
Low
High
C
Overall Survival
0.5
1
2
4
8
16
Hazard Ratio
D
Cell_Cycle
Enrichment Score
NES = -1.81
p-val = 0.0197
Low
Tumor aggressiveness
High
E
Mitosis
Enrichment Score
NES = -1.79
p-val = 0.0456
Low
Tumor aggressiveness
High
F
G1_S_Phase_Transition
Enrichment Score
NES = -1.54
p-val = 0.034
Low
Tumor aggressiveness
High
G
M_Phase_Of_Mitotic_Cell_Cycle
Enrichment Score
NES = -1.78
p-val = 0.0473
Low
Tumor aggressiveness
High

suppresses GBM growth by limiting lipogenesis (36). NT5DC2 is suggested to harbor nucleotidase activity but the actual molecular function of this gene is still not clear. It has also been shown to promote stemness in glioma cells by up-regulating Fyn (37). Out of the remaining two metabolic hits, NAGLU is primarily located in lysosomes where it plays a critical role in the breakdown of complex sugars called glycosaminoglycans (GAGs), specifically heparan sulfate. Mutations in these genes are associated with Sanfilippo syndrome, a rare autosomal recessive lysosomal storage disorder that affects brain and spinal cord (38), though its role in cancer is not well-established. PRIM2, as a part of DNA primase enzyme complex, is responsible for synthesizing RNA primers that serve as starting points for DNA synthesis during replication. Although it is not directly involved in cellular metabolism, it can direct nucleotide metabolism due to the energy and nucleotide requirements for DNA synthesis in rapidly dividing cancer cells. PRIM2 has been shown to promote cell cycle progression and proliferation in lung cancer cells (39). Overall, our analysis pipeline identified key metabolic hits related to gliomas, with NAGLU and PRIM2 as novel biomarkers of disease progression, tumor aggressiveness and survival in glioma patients.

Transcriptomic analysis identified the hyperactive cell cycle progression as a key mechanism associated with tumor progression and aggressiveness in gliomas. Next, we sought to characterize the molecular mechanisms through which our identified metabolic hits may take part in disease onset, progression, and tumor aggressiveness in gliomas. In this context, we aimed to identify the molecular mechanism hyperactive in gliomas as disease progresses. First, we identified genes successively up-regulated from normal tissue to primary tumor to recurrent tumors and also with tumor aggressiveness. This resulted in 1,110 hits. Later, we checked their association with glioma patients' survival and found that most of these genes (1099) were associated with poor survival in glioma patients. Later, we performed pathway analysis and found that cell cycle associated terms, such as mitosis, cell division, cell cycle and DNA replication are enriched as hyperactive mechanisms in gliomas (Figure 2A-C). In order to further confirm, we performed gene set enrichment analysis (GSEA) and found that cell cycle associated gene signatures (Cell_Cycle, Mitosis, G1_S_Phase transition and M_Phase_Of_Mitotic_Cell_Cycle) are highly enriched in patients with highly aggressive gliomas compared to those with less aggressive tumors (Figure 2D-G). These results suggest that hyperactive cell cycle and associated proliferation are key determinants of glioma progression and disease aggressiveness.

PRIM2 is a marker of cell cycle progression and tumor aggressiveness in gliomas. One hundred genes were found to be associated with the cell cycle from our transcriptomic analysis and interestingly, one of our metabolic hits, PRIM2, was also among them (Figure 2A-C). Cell division and proliferation starts from DNA replication during S phase that subsequently leads to cell cycle progression into M phase when the cell divides into two (40). As PRIM2 is involved in and regulates a critical step during DNA replication (the earliest step of cell cycle progression and cell division) (Figure 3A, B), we hypothesized that PRIM2 expression alone could predict cell cycle progression in gliomas. In line with this, we found that PRIM2 expression is positively correlated with almost all the cell cycle associated genes identified in the transcriptomic analysis (Figure 3C). Next, in order to further confirm, we performed GSEA using cell cycle related gene signatures. Notably, PRIM2 expression alone is enough to predict cell cycle progression and tumor aggressiveness in gliomas as multiple cell cycle progression associated gene signatures (including both related to early and late stages of cell cycle and division) are enriched in patients having high PRIM2 expression (Figure 3D-I). These finding suggest that PRIM2 alone can work as a marker of cell cycle progression and tumor aggressiveness in gliomas.

MYC-driven PRIM2 expression and cell cycle progression promotes tumor aggressiveness. Next, we aimed to find that how this cell cycle progression is regulated in gliomas. We checked the mutation status of cell cycle related genes identified in our transcriptomic analysis but found that these genes are not significantly mutated in gliomas (Figure 4A). Next, we checked whether changes in DNA methylation levels are related to the up-regulation of PRIM2 and other cell cycle related genes in gliomas. Although gene expression of most of tumor aggressiveness associated cell cycle related genes was

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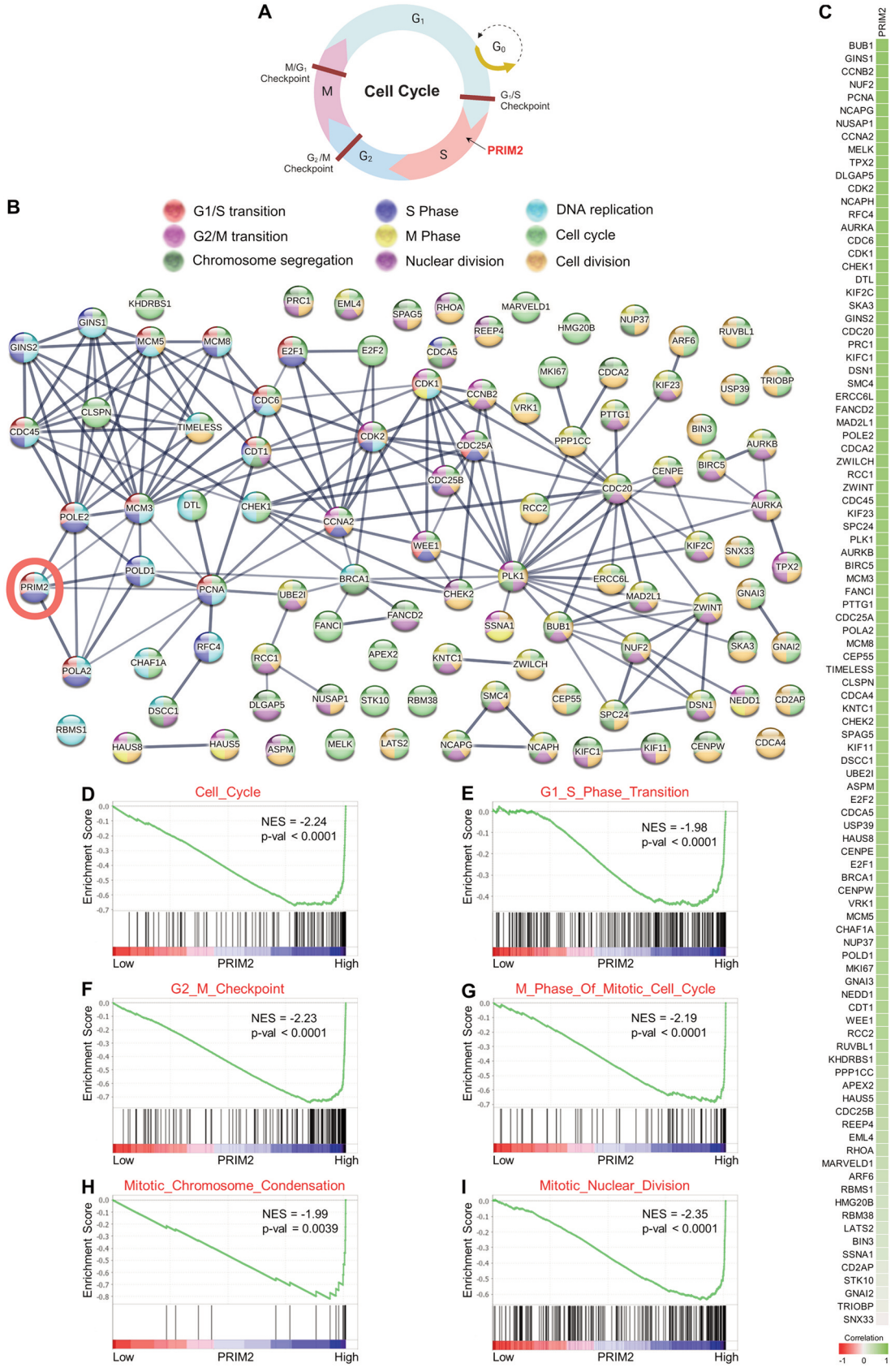
Figure 3. *PRIM2 is a marker of cell cycle progression and tumor aggressiveness in gliomas. (A) Illustration depicting the cell cycle with its phases and checkpoints highlighted. PRIM2 is shown to take part in early steps of the S phase during cell cycle progression. (B) Network showing interactions among the hits identified from transcriptomic analysis that are associated with hyperactive cell cycle progression in gliomas. Network was developed using the STRING database. The color code is used to highlight genes involved in different phases of cell cycle. The PRIM2 node is highlighted with a red circle. (C) Heatmap showing Pearson correlation between PRIM2 expression and cell cycle related hits identified from transcriptomic analysis of gliomas in the TCGA database. (D-I) GSEA analysis showing the enrichment of cell cycle related gene signatures, Cell cycle (D), G1_S_Phase_Transition (E), G2_M_Checkpoint (F), M_Phase_Of_Mitotic_Cell_Cycle (G), Mitotic_Chromosome_Condensation (H) and Mitotic_Nuclear_Division (I) between low and high PRIM2 expressing gliomas from the TCGA database. NES: Normalized enrichment score.*


A
Cell Cycle
G1
G0
G1/S Checkpoint
S
PRIM2
G2
G2/M Checkpoint
M
M/G1 Checkpoint
B
G1/S transition
G2/M transition
Chromosome segregation
S Phase
M Phase
Nuclear division
DNA replication
Cell cycle
Cell division
C
PRIM2
Correlation
D
Cell_Cycle
NES = -2.24
p-val < 0.0001
E
G1_S_Phase_Transition
NES = -1.98
p-val < 0.0001
F
G2_M_Checkpoint
NES = -2.23
p-val < 0.0001
G
M_Phase_Of_Mitotic_Cell_Cycle
NES = -2.19
p-val < 0.0001
H
Mitotic_Chromosome_Condensation
NES = -1.99
p-val = 0.0039
I
Mitotic_Nuclear_Division
NES = -2.35
p-val < 0.0001
Enrichment Score
Low
PRIM2
High

inversely correlated with their methylation (Figure 4B), the overall DNA methylation level respective to these genes was not significantly changed among normal, primary, and recurrent tumors, as well as among different tumor aggressiveness groups (Figure 4C). Next, we aimed to check whether PRIM2 and other cell cycle related genes are regulated through some common transcription factors (TF) in gliomas. To this end, we performed TF analysis using literature based and ENCODE project ChIP-seq data along with transcription factor prediction by the hTFtarget tool. Four TF hits were found to regulate the cell cycle associated genes in gliomas (Figure 5A). We found that MYC targets (Figure 5B-D), but not E2F1 (Figure 5E-G), CREB1 (Figure 5H and I) or SPI1 (Figure 5J and K) targets, were enriched in highly aggressive glioma compared to low aggressiveness groups. Surprisingly, MYC target genes were also enriched in tumors with high PRIM2 expression compared to low PRIM2 expression (Figure 5L-N) suggesting that PRIM2 can be marker of MYC-driven cell cycle progression and tumor aggressiveness in gliomas. Notably, PRIM2 is predicted to have two MYC binding sites within 5kb of downstream regulatory range (Data retrieved from ChIPBase v.2) (Figure 5O), ensuring that PRIM2 is regulated by MYC to promote cell cycle progression, proliferation, and tumor aggressiveness in gliomas.

PRIM2 is associated with MYC-driven hyperactive cell cycle, disease onset and progression in gliomas. Next, we aimed to validate our findings in independent datasets. We found that PRIM2 expression is up-regulated in gliomas (primary and recurrent tumors) compared to normal tissues in multiple GEO datasets entailing profiling data from gliomas (Figure 6A-E). PRIM2 expression is highly positively correlated with MYC-GS and cell cycle gene signature in multiple GEO datasets (Figure 6F). All this aligns with hyperactive MYC signaling and cell cycle progression as a MYC gene signature (Figure 7A-D) and our cell cycle signature (Figure 7E-I) is also up-regulated in gliomas (primary and recurrent tumors) compared to normal tissues in multiple GEO datasets. These findings suggest that PRIM2 is marker of MYC-driven hyper-proliferation and disease progression in gliomas.

PRIM2 is associated with aggressive disease state and poor survival in glioma patients. Lastly, we aimed to confirm whether RPIM2 expression is associated with clinical aspects of disease in glioma patients. Along these lines, we found that high PRIM2 expression is associated with higher tumor grade in multiple GEO datasets entailing profiling data from gliomas (Figure 8A-C). This also aligns with hyperactive MYC signaling and cell cycle progression as high MYC gene signature (Figure 8D-F) and cell cycle signature (Figure 8G-I) scores are also associated with higher tumor grade in multiple GEO datasets. Karnofsky score is a scoring plan for the performance of cancer patients in daily life: the better the performance, the higher the score. We found that PRIM2 expression is high in patients with low Karnofsky score (Figure 9A and B) suggesting that PRIM2 expression is associated with poor performance in glioma patients. This also aligns with hyperactive MYC signaling and cell cycle progression as high MYC gene signature (Figure 9C) and cell cycle signature (Figure 9D and E) scores are also high in patients with low Karnofsky scores. Lastly, in addition to being associated with poor overall survival in glioma patients (Figure 1E), PRIM2 expression is associated with poor progression free survival in glioma patients (Figure 9F). These findings confirm that PRIM2 is associated with an aggressive disease state and a poor clinical outcome in glioma patients.

Discussion

This study emphasizes the importance of metabolic alterations in gliomas, aligning with the growing recognition of metabolic reprogramming as a hallmark of cancer (14). These metabolic changes often fuel tumor growth and influence clinical outcomes, a phenomenon that has garnered substantial attention in cancer research (15). Here, we identified 5 metabolic hits associated with disease onset, progression, tumor aggressiveness, and survival in glioma patients (Figure 1). As mentioned earlier, two of the metabolic hits we identified, namely ACO2 and SOAT1, have already been established as biomarkers of disease progression in gliomas through their involvement in lipid metabolism (35, 36). This also aligns with previous research showing that metabolic changes, such as aberrantly regulated glucose and lipid metabolism, play a pivotal role in disease aggressiveness in gliomas (18). Another hit, NT5DC2, has also been shown to promote stemness in glioma cells (37)

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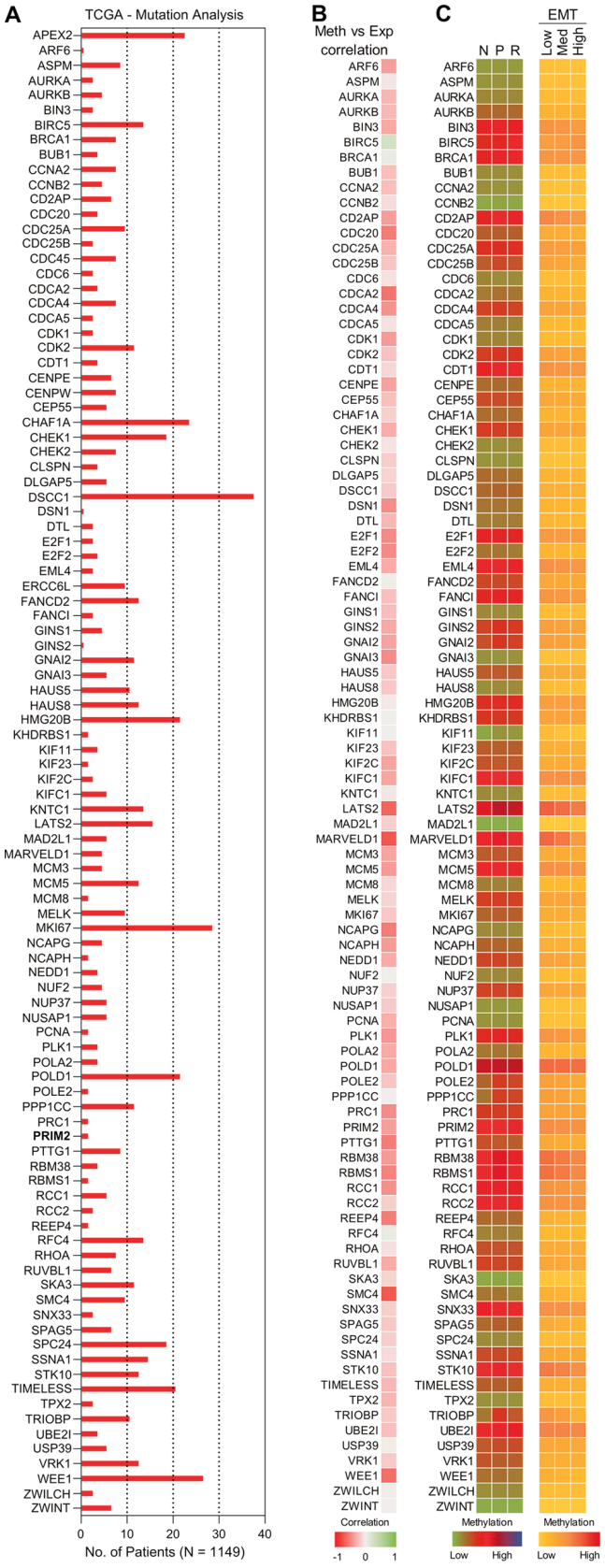
Figure 4. *Tumor aggressiveness associated changes in gene expression of PRIM2 and other cell cycle related hits are not related to their mutation and DNA methylation status in gliomas. (A) Bar graph showing number of glioma patients having mutations in PRIM2 and other cell cycle related hits identified from transcriptomic analysis of glioma patients from the TCGA database. (B) Heatmap showing Pearson correlation between DNA methylation and gene expression of cell cycle related hits identified from transcriptomic analysis of glioma patients from the TCGA database. (C) Heatmaps showing change in methylation score of cell cycle associated hits among normal tissue to primary tumors to recurrent tumors (left), and among EMT score-based tumor aggressiveness groups (low, intermediate, high) of primary tumors (right) of glioma patients from the TCGA database. Meth: Methylation; Exp: expression; EMT: epithelial-to-mesenchymal transition; N: normal tissue; P: primary tumor; R: recurrent tumor.*


A
TCGA - Mutation Analysis
APEX2
ARF6
ASPM
AURKA
AURKB
BIN3
BIRC5
BRCA1
BUB1
CCNA2
CCNB2
CD2AP
CDC20
CDC25A
CDC25B
CDC45
CDC6
CDCA2
CDCA4
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CDK2
CDT1
CENPE
CENPW
CEP55
CHAF1A
CHEK1
CHEK2
CLSPN
DLGAP5
DSCC1
DSN1
DTL
E2F1
E2F2
EML4
ERCC6L
FANCD2
FANCI
GINS1
GINS2
GNAI2
GNAI3
HAUS5
HAUS8
HMG20B
KHDRBS1
KIF11
KIF23
KIF2C
KIFC1
KNTC1
LATS2
MAD2L1
MARVELD1
MCM3
MCM5
MCM8
MELK
MKI67
NCAPG
NCAPH
NEDD1
NUF2
NUP37
NUSAP1
PCNA
PLK1
POLA2
POLD1
POLE2
PPP1CC
PRC1
PRIM2
PTTG1
RBM38
RBMS1
RCC1
RCC2
REEP4
RFC4
RHOA
RUVBL1
SKA3
SMC4
SNX33
SPAG5
SPC24
SSNA1
STK10
TIMELESS
TPX2
TRIOBP
UBE2I
USP39
VRK1
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No. of Patients (N = 1149)
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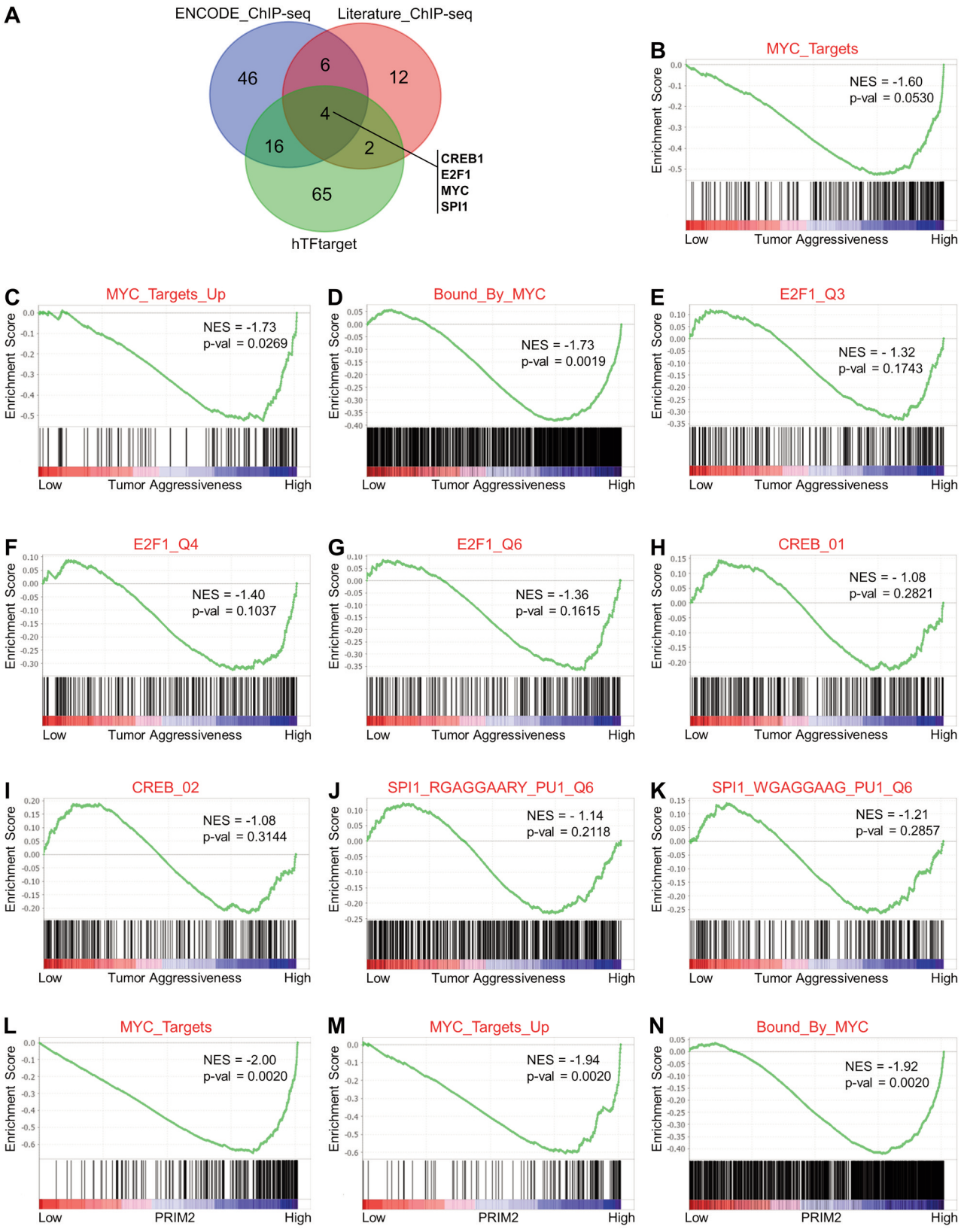

Figure 5. *Continued*

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MYC: binding site[1]	
Regulatory range	within downstream 5kb
Gene reference id	ENSG00000146143.17
Official gene symbol	*PRIM2*
TSS	chr6:57314804
Gene type	protein_coding
MYC binding locus	chr6:57316708-57317007, Summit: 57316858
Binding site distance	-2053

MYC: binding site[2]	
Regulatory range	within downstream 5kb
Gene reference id	ENSG00000146143.17
Official gene symbol	*PRIM2*
TSS	chr6:57314804
Gene type	protein_coding
MYC binding locus	chr6:57317699-57317998, Summit: 57317849
Binding site distance	-3044

Figure 5. *MYC-driven PRIM2 expression and cell cycle progression promotes tumor aggressiveness. (A) Venn diagram showing intersecting transcription factors (TFs) predicted to regulate PRIM2 and other cell cycle related hits in gliomas. Data from 3 databases, namely ENCODE_ChIP-seq, Literature_ChIP-seq and hTFtarget, was used for analysis. (B-D) GSEA analysis showing the enrichment of MYC TF related gene signatures, MYC_Targtes (B), MYC_Targtes_Up (C) and Bound_By_MYC (D), between low and high tumor aggressiveness groups of gliomas from the TCGA database. (E-G) GSEA analysis showing the enrichment of E2F1 TF related gene signatures, E2F1_Q3 (E), E2F1_Q4 (F) and E2F1_Q6 (G) between low and high tumor aggressiveness groups of gliomas from the TCGA database. (H and I) GSEA analysis showing the enrichment of CREB TF related gene signatures, CREB_01 (H) and CREB_02 (I) between low and high tumor aggressiveness groups of gliomas from the TCGA database. (J and K) GSEA analysis showing the enrichment of SPI1 TF related gene signatures, SPI1_RGAGGAARY_PU1_Q6 (J) and SPI1_WGAGGAAG_PU1_Q6 (K) between low and high tumor aggressiveness groups of gliomas from the TCGA database. (L-N) GSEA analysis showing the enrichment of MYC TF related gene signatures, MYC_Targtes (L), MYC_Targtes_Up (M) and Bound_By_MYC (N) between low and high PRIM2 expressing gliomas from the TCGA database. (O) Table showing MYC binding sites in the downstream regulatory range of the PRIM2 gene. Data was retrieved from ChIPBase v2. NES: Normalized enrichment score.*

while two other hits, NAGLU and PRIM2, are novel biomarkers of the disease. As all this directly validates our analysis pipeline and findings, our study fills a gap in knowledge about metabolic alterations and their impact on the clinical course of gliomas, providing more targeted insights into the disease's molecular underpinnings.

Uncontrolled cell division and proliferation are key hallmarks of cancer (14). Our transcriptomic analysis unveiled the significant activation of cell cycle-associated genes in gliomas, indicative of uncontrolled proliferation (Figure 2 and Figure 3). This finding is consistent with established knowledge that gliomas are characterized by rapid and unregulated cell division, contributing to their aggressiveness (41-43). Interestingly, one of the identified metabolic hits, PRIM2, was found to be directly associated with cell cycle progression in gliomas (Figure 3), which underscores its central role in driving the aggressive behavior of these tumors. As PRIM2 is intimately associated with DNA replication, the earliest step is cell cycle progression and cell division, its overexpression suggests that glioma cells are engaging in rapid and uncontrolled replication, contributing to the tumor's aggressiveness. In addition, our study is in line with the findings that high PRIM2 expression is associated with hyperactive cell cycle and aggressive disease state in other cancer types such as lung cancer (39, 44).

One of the significant findings in our study is the regulation of PRIM2 by the MYC oncogenic transcription factor (Figure 4 and Figure 5). MYC is a master regulator of numerous genes and pathways, promoting cell cycle progression and uncontrolled proliferation (45-47). The identification of PRIM2 as a downstream target of MYC in gliomas provides a key mechanistic insight into how MYC promotes tumor aggressiveness. The MYC-driven up-regulation of PRIM2 (Figure 5) suggests that MYC plays a central role in accelerating the cell cycle and promoting proliferation at the earliest stages of cell cycle (DNA replication). In addition, MYC may regulate multiple other cell cycle related genes to boost the cell cycle progression and proliferation (Figure 5) (45-47). Although E2F1, CREB and SPI1 TFs were also predicted to be regulators of genes involved in cell cycle progression (Figure 5), and have been reported to be proto-oncogenes regulating tumor initiation and progression (48-50), our GSEA did not link these TFs to cell cycle progression associated tumor aggressiveness in gliomas (Figure 5E-K). In the context of MYC-driven cell cycle progression, PRIM2 expression alone is enough to predict MYC driven hyper-proliferation in gliomas as it aligned well with MYC and cell cycle-based gene signatures (Figure 3 and Figure 5). This alignment highlights the intricate molecular underpinnings of gliomas and further emphasizes the role of PRIM2 as a key indicator of MYC-driven hyper-proliferation in gliomas.

The clinical implications of our findings are of paramount importance. PRIM2's association with disease onset and progression (Figure 1D and Figure 6A-E), aggressive disease

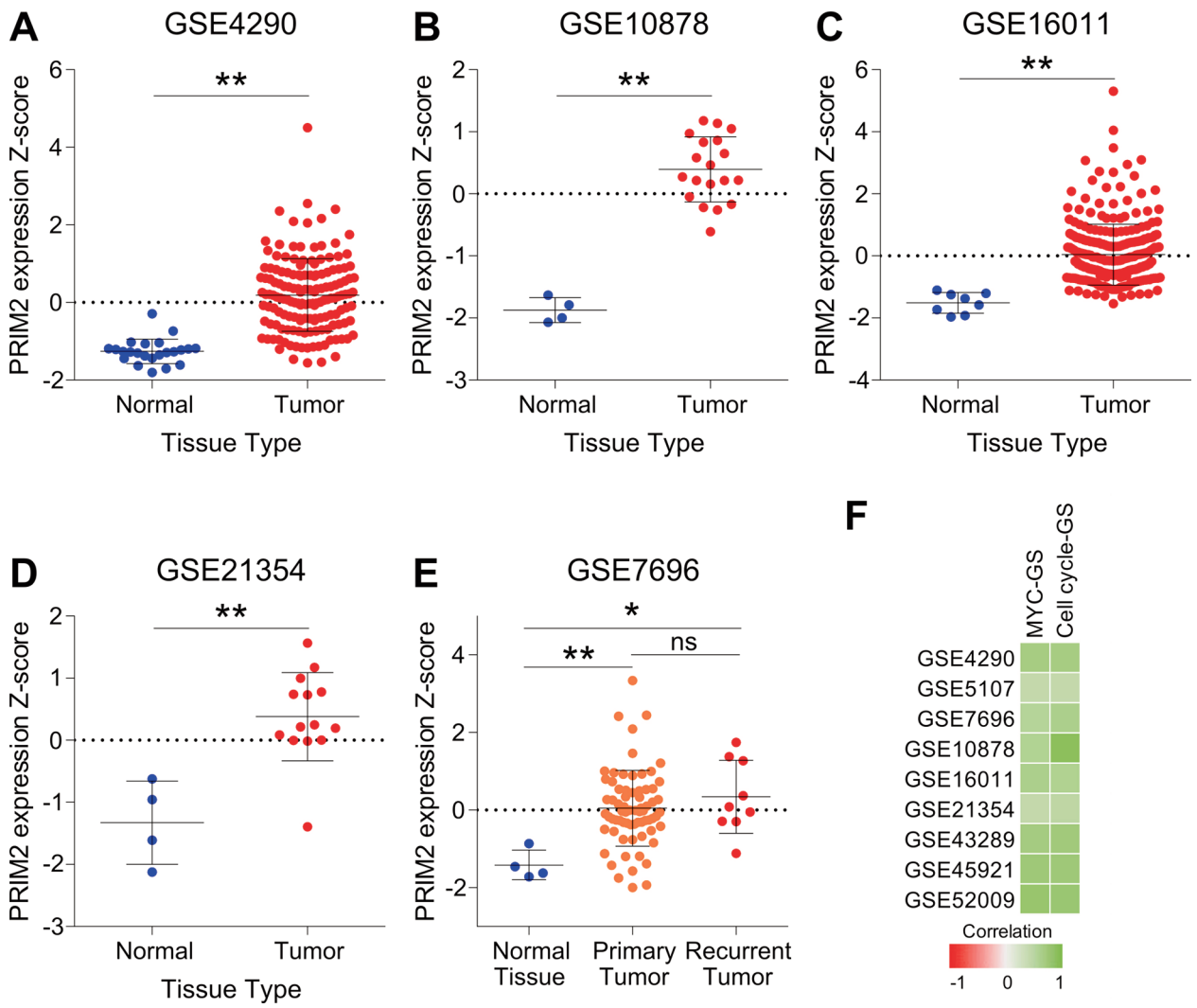

Figure 6. *PRIM2 is associated with MYC-driven hyperactive cell cycle, disease onset and progression in gliomas. (A-D) Dot-plots showing changes in PRIM2 expression between normal tissue and primary tumor tissue in glioma patients from GSE4290 (A), GSE10878 (B), GSE16011 (C) and GSE21354 (D). (E) Dot-plot showing changes in PRIM2 expression among normal tissues, and primary and recurrent tumor tissues in glioma patients from GSE7696. (F) Heatmap showing Pearson correlation of PRIM2 with MYC and cell cycle associated gene signatures in expression data from different GEO datasets. GS: Gene signature; ns: non-significant. *p<0.05 and **p<0.01.*

state, higher tumor grades, reduced Karnofsky performance scores, and poor survival (Figure 1E, Figure 8A-C, Figure 9A, Figure 9B and Figure 9F) underscores its potential as a prognostic marker. Our results suggest that PRIM2 expression levels could be used as an indicator of disease progression and aggressiveness, enabling clinicians to make informed decisions regarding patient management. Such insights can significantly impact the development of more effective diagnostic tools and therapeutic strategies for glioma patients, aligning with the broader goal of improving clinical outcomes in this challenging malignancy (12, 51). Furthermore, the connection between PRIM2 and MYC provides a promising avenue for the development of novel targeted therapeutic strategies aimed at targeting MYC-driven cell cycle progression (52, 53).

Conclusion

Our study advances our understanding of glioma biology by identifying PRIM2 as a marker for MYC-driven cell cycle progression, disease progression, tumor aggressiveness, and poor survival in glioma patients. By bridging the gap between metabolic alterations, MYC-cell cycle progression, and clinical outcomes in glioma patients, our research offers

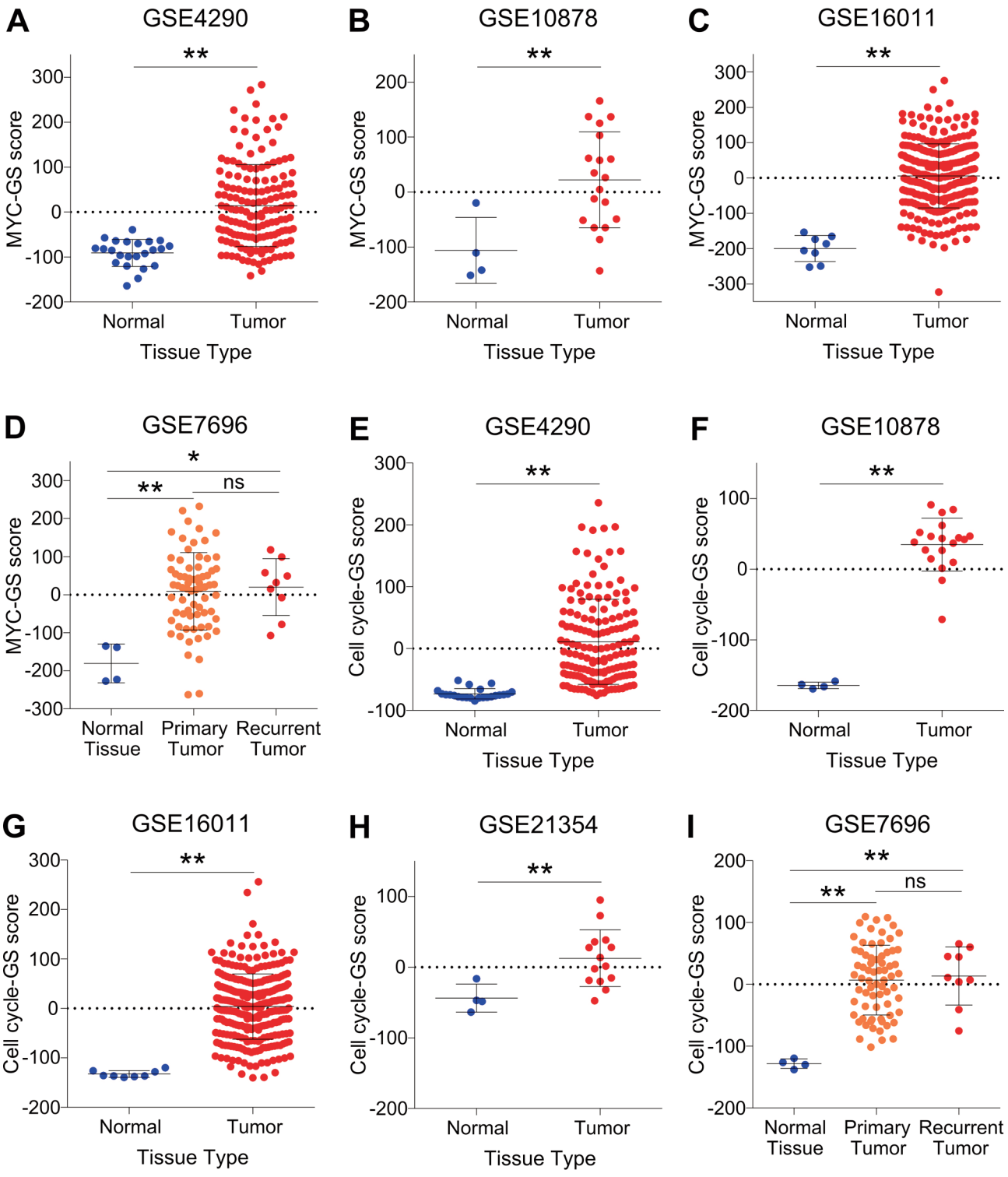

Figure 7. *MYC TF and identified cell cycle gene signature are associated with disease onset and progression in gliomas. (A-C) Dot-plots showing changes in MYC-GS expression between normal tissue and primary tumor tissue in glioma patients from GSE4290 (A), GSE10878 (B) and GSE16011 (C). (D) Dot-plot showing changes in MYC-GS expression among normal tissues, and primary and recurrent tumor tissues in glioma patients from GSE7696. (E-H) Dot-plots showing changes in cell cycle-GS expression between normal tissue and primary tumor tissue in glioma patients from GSE4290 (E), GSE10878 (F), GSE16011 (G) and GSE21354 (H). (I) Dot-plot showing changes in cell cycle-GS expression among normal tissues, and primary and recurrent tumor tissues in glioma patients from GSE7696. GS: Gene signature; ns: non-significant. *p<0.05 and **p<0.01.*

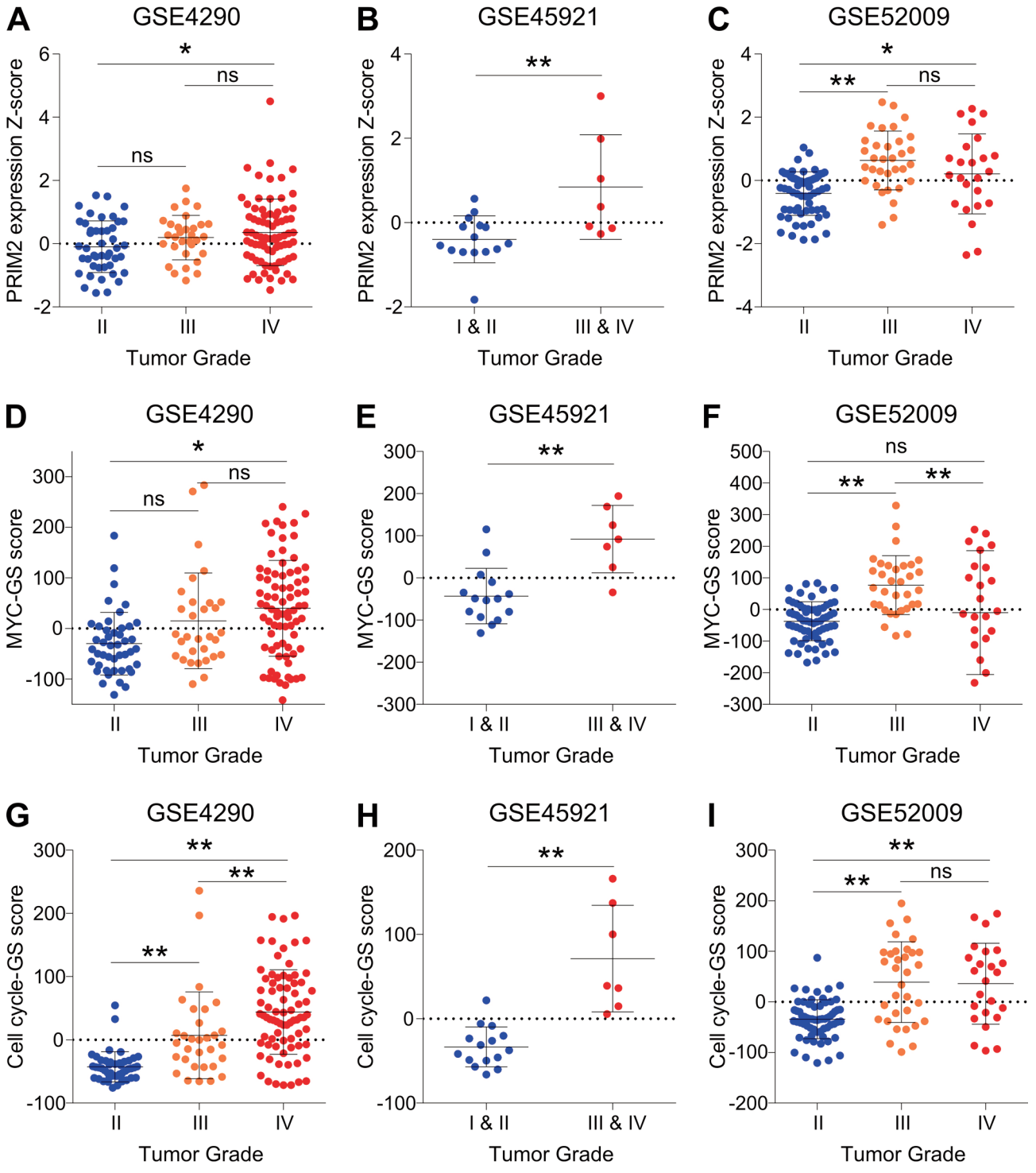

Figure 8. *PRIM2, MYC TF and identified cell cycle gene signature are associated with advanced tumor grade in glioma patients. (A-C) Dot-plots showing changes in PRIM2 expression among different tumor grades (II, III and IV) in glioma patients from GSE4290 (A), between different tumor grades (I and II to III and IV) in glioma patients from GSE45921 (B) and among different tumor grades (II, III and IV) in glioma patients from GSE52009 (C). (D-F) Dot-plots showing changes in MYC-GS expression among different tumor grades (II, III and IV) in glioma patients from GSE4290 (D), between different tumor grades (I and II to III and IV) in glioma patients from GSE45921 (E) and among different tumor grades (II, III and IV) in glioma patients from GSE52009 (F). (G-I) Dot-plots showing changes in cell cycle-GS expression among different tumor grades (II, III and IV) in glioma patients from GSE4290 (G), between different tumor grades (I and II to III and IV) in glioma patients from GSE45921 (H) and among different tumor grades (II, III and IV) in glioma patients from GSE52009. GS: Gene signature; ns: non-significant. *p<0.05 and **p<0.01.*

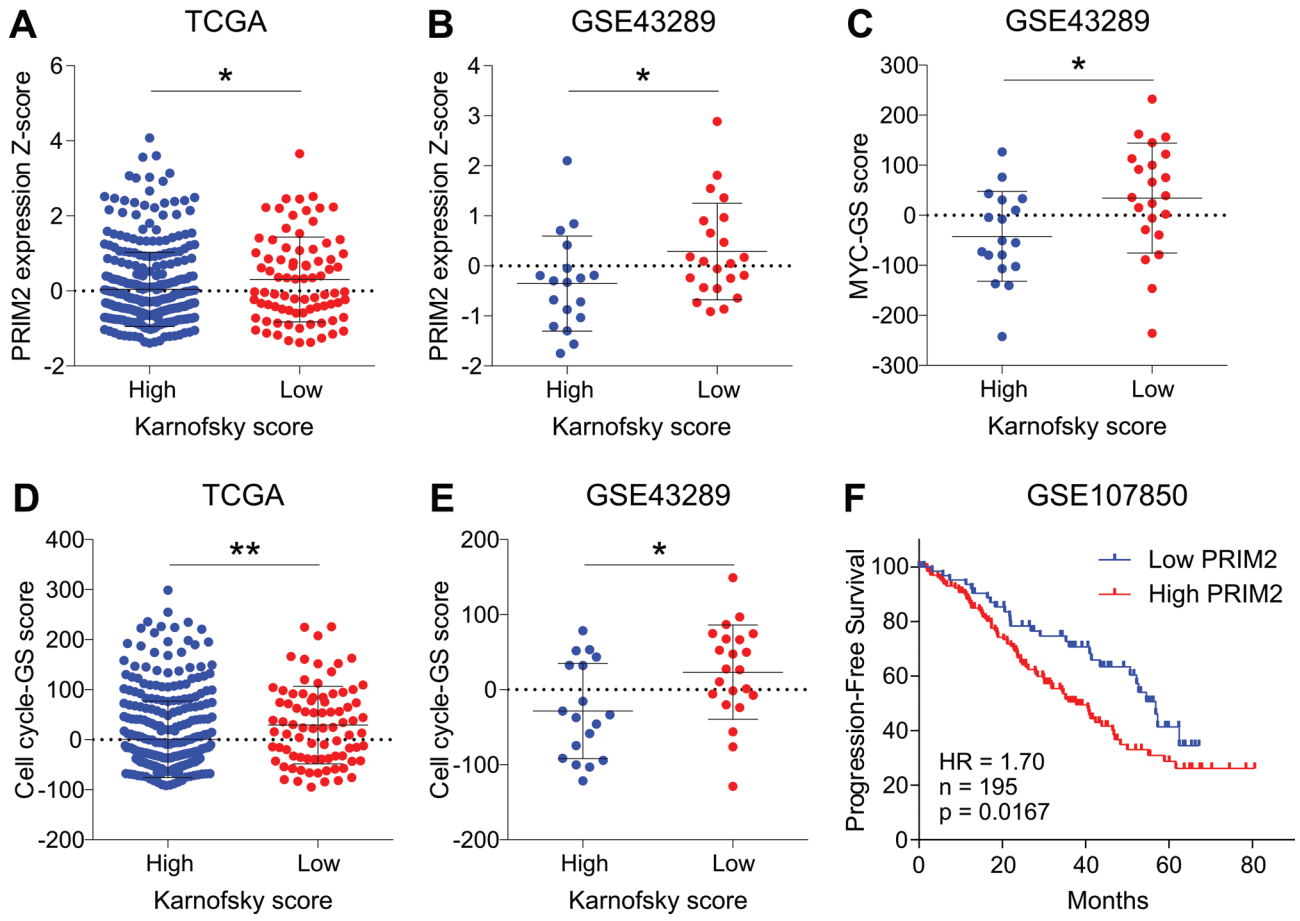

Figure 9. *PRIM2, MYC TF and identified cell cycle gene signature are associated with tumor aggressiveness and poor survival in glioma patients. (A and B) Dot-plot showing changes in PRIM2 expression between tumors associated with high and low Karnofsky score in glioma patients from the TCGA database (A) and from GSE43289 (B). (C) Dot-plot showing changes in MYC-GS expression between tumors associated with high and low Karnofsky score in glioma patients from GSE43289. (D and E) Dot-plot showing changes in cell cycle-GS expression between tumors associated with high and low Karnofsky score in glioma patients from the TCGA database (D) and from GSE43289 (E). (F) Kaplan-Meier survival plot showing progression-free survival analysis based on low and high PRIM2 expression in glioma patients from GSE107850. GS: Gene signature. *p<0.05 and **p<0.01.*

a novel and comprehensive perspective on this complex disease. Our findings regarding PRIM2's involvement in glioma aggressiveness, cell cycle progression, and MYC regulation provide a foundation for future research. Experimental validation of our findings and exploring potential therapeutic interventions targeting PRIM2 or its regulatory pathways, are promising areas for further study.

Conflicts of Interest

The Authors declare no conflicts of interest.

Authors' Contributions

RHS performed the data analysis and contributed to the writing of the manuscript. XDS, FA and KRZ performed data collection. RHS, XDS, FA, and KRZ performed data analysis. SY, LTM and GZX contributed to study design and project development. LTM and GZX contributed to funding acquisition. FA, SY, and GZX critically revised the manuscript. All Authors prepared and approved the final version of the manuscript.

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