# m1A RNA Methylation Regulators Contribute To Malignant Progression And Have Clinical Prognostic Impact In Gliomas

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# Abstract

**Background** Glioma is the majority of primary malignant brain tumors in adults, accounting for over 70% of malignant brain tumors. RNA modification has been proved to be closely related to the development of various carcinomas including glioma. N1-methyladenosine (m1A) is a crucial and newly validated posttranscriptional modification in RNA. Its influence on glioma is still under elucidation.

**Results** Gene expression and clinicopathological data of glioma were downloaded from TCGA and CGGA databases. The m1A regulators' gene expression and its relationship with tumor malignancy grade or IDH mutation or 1p19q co-deletion status in glioma, as well as its related signaling pathway were investigated. We found that m1A regulators were dysregulated and positively associated with the WHO grade of glioma. The YTHDF2 expression was associated with the overall survival rate of glioma patients and was significantly lower in IDH mutation and the 1p/19q codeletion group. YTHDF2 mainly involved in RNA splicing, mRNA processing functions, or cell cycle relate pathways in glioma.

**Conclusion** This study elucidated the dysregulation of m1A regulators and its potential signaling pathways in glioma, which will contribute to the further understanding of m1A RNA modification in glioma.


# Background

Glioma is the majority of primary malignant brain tumor in adults, accounting for over 70% of malignant brain tumors[1, 2]. And it's mainly diagnosed based on histopathology and molecular features according to the 2016 WHO classification. Despite the development of surgical resection, radiotherapy and chemotherapy, the survival rate of glioma patients remains far from satisfactory, especially for glioblastoma (the WHO grade Ⅳ)[2, 3]. Although, over the past years, researchers have done a lot of work on glioma, the detailed molecular mechanism involved in it remains to be elucidated.

In recent years, reversible RNA modifications was found to play important roles in gene expression regulation and affects the biogenesis, structural integrity, function, and metabolism of RNA molecules[4, 5]. More than 100 types of RNA modifications were found with the development of high-throughput sequencing[6, 7]. Post-transcriptional modification of RNA forms an emerging layer of genetic expression regulation and may even plays a crucial role in posttranslational modification of proteins level[8, 9]. RNA modification has been proved to be closely related to the development of various carcinomas including glioma. The N6-methyladenosine (m6A) methyltransferase METTL3 sustained its oncogenic role by modulating nonsense-mediated mRNA decay (NMD) of splicing factors and alternative splicing isoform switches in the Glioblastoma (GBM)[10].

N1-methyladenosine (m1A) is a crucial posttranscriptional modification in RNA which were found more than five decades ago[11]. Similar to the m6A, the m1A was regulated by"writer", "eraser", and "reader"[12]. The writers (TRMT10C, Trmt61B, TRMT6/61A) catalyze formation of m1A of tRNA, rRNA, or mRNA at different position, while erasers (ALKBH1, ALKBH3) catalyze demethylate m1A to adenosine.

The readers (YTHDF1, YTHDF2, YTHDF3, YTHDC1) can bind to m1A modified RNA and modulate various biological processes[6]. Recent studies suggested that m1A was closely related to carcinoma development[9]. However, to our knowledge, the role of m1A in glioma development was still under investigated.

In this study, we capitalized on the nine m1A regulators in glioma through The Cancer Genome Atlas (TCGA) and The Chinese Glioma Genome Atlas (CGGA) database. We found that m1A regulators were upregulated in glioma, especially in high grade (WHO IV grade) glioma. Additionally, the upregulation of YTHDF2 was closely related to higher overall survival rate and positively corelated with the IDH mutation or 1p19q codeletion status. Moreover, YTHDF2 co-expressed genes were enriched in RNA splicing, RNA processing, et al. These results suggest the potential role of m1A in regulating glioma.

## Results

## 2.1 Expression of m1A related proteins and its correlation with pathology stage in glioma

Based on GEPIA, a total of 163 glioma and 207 normal samples were included in TCGA data and the GTEx projects. The expression patterns of m1A related proteins in glioma were explored. As shown in Fig.1A, among the nine m1A regulators composed of writer (TRMT6, TRMT61A, TRMT10C), eraser (ALKBH1, ALKBH3), and reader (YTHDF1-3, YTHDC1), TRMT10C, ALKBH1, YTHDF1, YTHDF2, and YTHDF3 were significantly upregulated ($P<0.05$) in glioma compared with normal samples (Fig. 1A).

Next, the correlation between the expression levels of m1A regulators and clinical-pathological characteristic was also investigated with data from CGGA (the Chinese Glioma Genome Atlas) and TCGA. We found that, in TCGA database, the m1A regulators were significantly upregulated in WHO IV glioma compared to WHO grade II/III glioma except TRMT61A (Fig 1B, $p < 0.05$). Similarly, the TRMT6, TRMT10C, ALKBH1, YTHDF1, YTHDC1 in CGGA dataset were also significantly upregulated in WHO grade IV glioma ($p<0.05$), though no significant difference was observed in the expression levels of TRMT61A, ALKBH3, YTHDF3 in each stage group. Moreover, in both CGGA and TCGA database, YTHDF2, was significantly upregulated in WHO grade IV glioma compared to WHO grade III/ II glioma (Fig. 1B, $p < 0.001$). These results suggested that m1A regulators, especially YTHDF2, were remarkably upregulated in glioma and were positively correlated with pathology stage.

## 2.2. Survival analysis of m1A regulators in glioma

Kaplan-Meier analysis and a log-rank test were performed. Data samples were classified into high-expression group and low-expression group according to the optimal cut-off value of m1A regulators gene expression levels in CGGA and TCGA, respectively. As shown in Fig. 2, in data from CGGA database, high expression of YTHDF1 (Fig. 2A, $p$=0.047) and YTHDF2 (Fig. 2A, $p$=0.013) was associated with

shorter overall survival rate (OS) of glioma. Consistently, in TCGA database, high-expression of ALKBH1 (Fig. 2B, $p$=0.031), ALKBH3 (Fig. 2B, $p$=0.027, YTHDF1 (Fig. 2B, $p$=0.039) and YTHDF2 (Fig. 2B, $p$=0.017) was also significantly correlated with shorter overall survival rate. However, no significant correlation was observed between the OS and the expression level of TRMT6, TRMT61A, TRMT10C, YTHDF3 and YTHDC1 in both CGGA and TCGA database (Fig. 2).

## 2.3. YTHDF1 and YTHDF2 were dysregulated in IDH mutated or 1p19q co-deleted glioma

IDH mutation was found to be a common genetic mutation promotor in glioma, while chromosome 1p/19q codeletion is increasingly being recognized as the crucial genetic marker for glioma patients and have been included in WHO classification of glioma in 2016[13, 14]. Hence, the expression level of YTHDF1 and YTHDF2 in IDH mutated or 1p19q co-deleted glioma were also explored. As shown in Fig. 3A, compared with wild type glioma patients, the YTHDF1 and YTHDF2 expression level were downregulated in IDH mutated patients both in CGTA and TCGA dataset. Consistently, the YTHDF2 expression level in 1p19q codeletion group was also significantly downregulated compared with 1p19q intact group (Fig. 3A). There was a general m1A regulator gene alteration in glioma, though no regular routine was observed (Fig. 3B). YTHDF1 and YTHDF2 had the highest alteration frequency of 4% and 3%, respectively. Followed by TRMT6 and TRMT61A, which presented an alteration frequency of 2.4%. ALKBH1 and YTHDF3 had an alteration frequency of 2%, while TRMT10C and ALKBH3 altered in 1.7% of all samples. Meanwhile, there was a rare alteration of YTHDC1 in glioma with a lowest alteration frequency at only 1.2% (Fig. 3B).

## 2.4. YTHDF2 co-expressed gene analysis in glioma

As shown in Fig. 4A, the YTHDF2 co-expressed gene in glioma were analyzed via LinkedOmics. In the TCGA and CGGA dataset, genes whose correlation coefficient against YTHDF2 expression bigger than 0.5 were selected. Next, the intersection of YTHDF2-related genes in TCGA and CGGA dataset were turned to GO enrichment and KEGG pathway analysis. The co-expressed genes were divided into three function groups: cellular component group, molecular function group, and biological process group. As depicted in Fig. 4B, YTHDF2 co-expressed genes were mainly involved in function related to ‘chromatin binding’, ‘catalytic activity, acting on RNA or DNA’, ‘histone binding’, ‘helicase activity’, ‘ATPase activity’ and ‘structural constituent of nuclear pore’.  Furthermore, the co-expressed genes were mainly enriched in several bioprocesses, including mRNA processing or mRNA splicing, RNA splicing, chromosome segregation and mitotic nuclear division (Fig. 4B). Additionally, in the molecular function group, the genes were most likely the component of chromosome region, chromatin, nuclear speck, spindle, spliceosomal complex or kinetochore of cells. The KEGG analysis showed that these genes mainly enriched in pathways related to cell cycle, spliceosome, RNA transport, or mRNA surveillance pathway.

## 2.5. gene set enrichment analysis (GSEA) and Protein interaction analysis

Gene enrichment analysis indicated that the following gene sets including: GCNP_SHH_UP_LATE.V1_UP, E2F1_UP.V1_UP, VEGF_A_UP. V1. DN, BIOCARTA, IGF1MTOR_pathway, ERBB2_up.V1_DN, MYC_UP.V1_UP, BIOCARTA_G2_pathway and BIOCARTA_G1_pathway were enriched in YTHDF2 high expression group (Fig5A). The downstream candidate genes of YTHDF2 in glioma were analyzed by PPI network based on STRING database and Cytoscape. As shown in Fig. 5B, a total of 15 genes including HMGB2, ZNF644, DUSP12, UBAP2L, SRSF10, SFPQ, ELAVL1, NCBP1, SKIV2L2, CNOT6, CNOT1, PTBP1, HNRNPA2B1 and YTHDC1 were found to interact with YTHDF2. Additionally, the protein expression level of YHTDF2 was also found to be upregulated in glioma according to the immunohistochemical data from the Oncomine and HPA databases（Fig 5C）.

## Discussion

More than a hundred types of posttranscriptional modifications, including N(6)-methyl-adenosine (m(6)A), 5-methylcytosine (m(5)C), and N(1)-methyl-adenosine (m(1)A) have been reported up to date[15]. Dynamic modification of RNA, represents a novel layer of genetic information, has been proved to participate in modulating gene expression and controlling cell fate. More importantly, RNA modification was found to play a vital role in human disease and especially in tumor initiation and progression[16]. Studies have shown that m6A RNA methylation is associated with self-renewal of Glioblastoma stem cells (GSCs) and abnormal m6A modification in GSCs plays an important role in the development of Glioblastoma (GBM) and is a potential target for GBM treatment [17]. Apart from N6-methyladenosine (m6A), N1-methyladenosine (m1A) has been found as a reversible modification in tRNA, mRNA and lncRNA[11, 18]. Up to now, about 2570 m1A modification sites have been validated in humans[19]. However, the relationship between m1A modification and glioma remains under elucidation.

The m1A modification of mRNAs and ncRNAs was regulated by the “writer” (TRMT10C, Trmt61B, TRMT6/61A), “reader” (YTHDF1, YTHDF2, YTHDF3, andYTHDC1), and “eraser” (ALKBH1, ALKBH3) proteins[9, 20]. TRMT10C catalyzes m1A at position 9 of human cell mitochondrial and cytoplasmic tRNA, while Trmt61B and TRMT6/61A catalyze it at position 58[21]. YTHDF1, YTHDF2, YTHDF3, and YTHDC1 can directly bind to m1A-bearing RNA as readers[22, 23]. But ALKBH1 or ALKBH3 function as demethylase of m1A in single-stranded (ss) DNA and RNA[24]. It has been proved that the dysregulation of m1A hTrm6p/hTrm61p, trans-methylase of m1A, promotes urinary bladder cancer development[25]. Additionally, previous studies shown that high expression of ALKBH3 is positively associated with advanced tumor stage in pancreatic cancer, while overexpression of ALKBH1 is significantly associated with poor prognosis and metastasis in gastric cancer[26, 27]. Moreover, m1A regulator gene expression levels were significantly higher in liver hepatocellular carcinoma (LIHC) than normal, and the higher m1A regulator gene expression level is associated with poor prognosis in LIHC[9]. Thus, dysregulation of m1A-related genes may also closely be related with initiation and progression of glioma.

In this study, with the data in TCGA, the expression levels of some m1A regulators in glioma were found to be upregulated (Fig. 1A). Notably, using the publicly available clinical data from TCGA and CGGA, we also found that the expression levels of m1A regulator genes (except TRMT61A) were significantly higher in grade IV glioma compared to grade III/ II glioma (Fig. 1B). Thus, it is speculated that m1A regulators are positively linked to the tumor malignance of glioma and involved in the pathogenesis of it, especially in grade IV glioma (Glioblastoma). Additionally, upregulation of YTHDF1 and YTHDF2 was negatively associated with the overall survival rate in glioma in both TCGA and CGGA dataset (Fig. 2B), suggesting that YTHDF1 or YTHDF2 may affect the prognosis of glioma. Previous study showed that YTHDF2 facilitates the degradation of m1A-modified RNA, and besides, depletion of cellular YTHDF2 increased the abundance of m1A-modified transcripts[23]. Moreover, YTHDF1 and YTHDF2 were also function as “readers” of m6A-modified RNA[28] in glioma. Therefore, YTHDF2 probably affect the progression of glioma by not only “m6A pathway”, but also by “m1A pathway”. But the specific mechanism about how YTHDF2 function in glioma deserves further investigation.

Accumulating evidence show that isocitrate dehydrogenase (IDH) mutations is tightly corelated with the genesis of glioma, and have a major impact on tumor biology, and also have clinical and prognostic importance[14, 29]. Meanwhile, co-deletion of 1p and 19q (1p/19q codeletion) represents a codeletion of the short arm of chromosome 1 and the long arm of chromosome 19, which have also been suggested to indicate a favorable prognosis in gliomas[30, 31]. In our study, we separated patient data into IDH mutation group and IDH wild-type group and investigated the association between YTHDF1/2 expression levels and IDH status. Besides, the relationship between 1p/19q codeletion status in glioma and YTHDF1/2 expression levels with were also analyzed. In both TCGA and CGGA cohort, the YTHDF2 expression level was significantly lower in IDH mutation and 1p/19q codeletion group. This result was consistent with that reported in a former study[32]. Since IDH mutation is an early event in glioma formation and occurs prominently in low grade tumors, and glioma with IDH mutation shows better prognosis[29], it‘s reasonable to speculate that YHDF2 might promote the development of glioma.

It has been reported that missense mutation of TRMT10C decreases mitochondrial ribonuclease P protein 1 (MRPP1) in mitochondria and caused the mitochondrial disease by affecting the protein stability and mt-tRNA processing[33]. Hence, the mutation of m1A regulator genes can not only affect the m1A function, but also contribute to other phenomenon like carcinogenesis and aging[9, 34]. Thus, it’s important to analyze the m1A regulator alteration to better understanding of the role of m1A in glioma tumorigenesis. In our study, the genetic alteration frequencies of the m1A RNA methylation regulators were very Low (1.2%~4%) in gliomas (Fig. 3B), which indicate that dysregulation of the m1A RNA methylation regulators were not caused by the genetic changes of the corresponding genes.

In this study, among the nine m1A regulators, overexpressed YTHDF2 in glioma was positively related with WHO grade, decreased overall survival rate, and was negatively related with IDH mutation status and 1p/19q codeletion status in both TCGA and CGGA cohort (Fig. 1–3). So YTHDF2 was further studied in the following section of this study. The YTHDF2 co-expressed genes were identified and annotated their function using gene ontology (GO) and KEGG pathway analysis. They were categorized into molecular

function, biological processes, and cellular component. The co-expressed genes were mainly enriched in biological processes related to RNA binding and splicing and chromosome segregation, whereas the most significant cellular components were ‘chromatin binding’, ‘catalytic activity, acting on RNA or DNA’, ‘histone binding’, ‘helicase activity’, ‘ATPase activity’. GSEA analysis enriched the genes in cell cycle pathways and oncogene signatures like MYC, ERBB2, VEGF, E2F1 and et al. ERBB2 was reported as a hub gene of hypomethylated genes in glioma and was associated with survival[35]. Research also reveal that ErbB pathway is associated with m1A methylation in gastrointestinal cancer [9]. These results suggested that YTHDF2 is closely related with oncogenes and it might be associated with glioma development, through regulation of the oncogene activation.

Through protein-protein interaction (PPI) network analysis, fifteen hub proteins including HMGB2, RBMB15, SRSF10, DUSP12, UBAP2L had a high degree of connectivity with YTHDF2(Fig. 5B). HMGB2 was reported to be able to promote cell viability, invasion, and chemotherapeutic resistance in glioblastoma[36], thus YTHDF2 might promote glioma development through regulation expression of HMGB2. While RBMB15, SRSF10, DUSP12, UBAP2L were either RNA metabolism related or cell cycle associated proteins, most of these proteins were implicated in tumor initiation or progression[36–38]. These findings indicated that YTHDF2 might influence glioma development through regulation of RNA metabolism and cell proliferation and invasion.

## Conclusion

Our study depicted the dysregulation of m1A regulators and its association with clinicopathological parameters in glioma for the first time, and highlighted the importance of this newly validated RNA modification in glioma development. Results suggested that YTHDF2 might function most in glioma and probably modulate oncogene expression or cell cycle pathways in glioma. However, the specific mechanism of m1A regulators in glioma genesis and prognosis still needs further validation. Besides, the expression of m1A regulators and YTHDF2 related pathways still need to be validated in a large quantity of clinical glioma specimens.

## Methods

# 5.1. Patients and clinicopathological data

The GPEIA (Gene Expression Profiling Interactive Analysis) dataset ( http://gepia.cancer-pku.cn/ ) was employed to analyze the expression level of m1A regulators in glioblastoma (GBM). The gene expression data (RNA-sequence) data, together with its clinicopathological data of a total of 1082 glioma samples (named TCGA_GBM, TCGA_LGG) were downloaded from The Cancer Genome Atlas (TCGA, http://cancergenome.nih.gov) database and transcripts per million (TPM) normalized. Another 693 glioma samples were downloaded from the Chinese Glioma Genome Atlas (CGGA, http://www.cgga.org.cn/) database. The IDH mutation data (amplification, deep deletion, and missense mutations) and 1p19q codeletion data of glioma were taken from TCGA through cBioPortal.

## 5.2. Analysis of the correlation between the m1A regulators expression level and the overall survival rate

In order to clarify the relationship between the m1A regulators expression level and the clinical prognosis. The Kaplan Meier analysis and the log-rank test were used to analysis relationship between the overall survival rate and the m1A regulators expression level in both TCGA and CGGA datasets via R package (survival, survminer). $P < 0.05$ represented a statistically significant difference.

## 5.3. Association investigation between expression level of m1A regulators and IDH mutation or 1p19q codeletion status in glioma.

As IDH mutation and 1p/19q codeletion are two most important gene alteration in glioma[29, 31], we further investigated the expression level of YTHDF1 and YTHDF2 in glioma with or without IDH mutation. Additionally, their expression status was also analyzed in 1p/19q codeletion glioma. Furthermore, the genetic alteration of m1A regulators in glioma was also studied. The cBio Cancer Genomics Portal (cBioPortal) database was accessed to analyze the genetic alteration of nine m1A regulator genes.

## 5.4. Co-expressed gene GO and KEGG pathway analysis

In this study, as YTHDF2 is the most significantly up regulated factor in glioma, its co-expression analysis was performed on the TCGA and CGGA datasets by calculating the Pearson correlation coefficient of paired genes. The intersection of YTHDF2 related genes with a Pearson correlation coefficient $\geq 0.5$ were selected for GO and KEGG analysis. To further investigate the function of YTHD2 co-expressed genes, GO enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis was performed by R package (cluster Profiler). $P < 0.05$ was considered to indicate a statistically significant difference.

## 5.5. GSEA and PPI network construction

To investigate the potential function of YTHDF2 in glioma, the samples were divided into two groups according to the YHTDF2 expression level. And the DEGs were identified and ranked according to their expression level. Then GSEA was conducted to detect whether a series of priori defined biological processes were enriched in the gene rank derived from DEGs between the two groups[39].

Protein-protein interaction (PPI) refers to the formation of protein complex by two or more protein molecules through non-covalent bonds. The PPI of YTHDF2 co-expressed genes were analyzed by utilizing the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING; string-db.org/) database. Then the PPI network were constructed by applying Cytoscape software.

## 6.6. Statistical analysis

Numeric data was presented as mean ± standard deviation. Students' *t*-test was used to compare two-group difference, one-way ANOVA was applied to compare multiple group and Pearson analysis was employed to calculate the correlation between two classes. Overall survival analysis was performed by Kaplan-Meier with P value calculated using the log-rank test. *P* values ≤ 0.05 were considered statistically significant.

## Abbreviations

m1A, N1-methyl adenosine; m6A, N6-methyl adenosine; TCGA, The Cancer Genome Atlas; CGGA, the Chinese Glioma Genome Atlas; IDH, isocitrate dehydrogenase, GSEA, gene set enrichment analysis.

## Declarations

# Availability of data and materials

The mRNA-seq data of patients with LGG/GBM can be directly downloaded from TCGA by the tool named SangerBox (https://shengxin.ren/softs/Sanger_V1.0.8.zip) without logging in the accession numbers or visiting website of TCGA. Another dataset can be downloaded in the Chinese Glioma Genome Atlas repository (CGGA, http://www.cgga.org.cn/download.jsp, Accession number/Dataset ID: mRNAseq_693).

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Availability of data and materials

The datasets generated and/or analysed during the current study are available in the Cancer Genome Atlas (TCGA, http://cancergenome.nih.gov) and the Chinese Glioma Genome Atlas (CGGA, http://www.cgga.org.cn/) database.

### Competing interests

The authors declare that there is no conflict of interest regarding the publication of this paper

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data or writing the manuscript.

### Authors' contributions

BH and ZL designed the study and had full access to all data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. JG, HS contributed to data collection and analysis. JG, HS, and MS have drafted the manuscript. JG, HS, MS, YC, JC, and HC have substantively revised the manuscript. All authors have read and approved the final vision of this manuscript.

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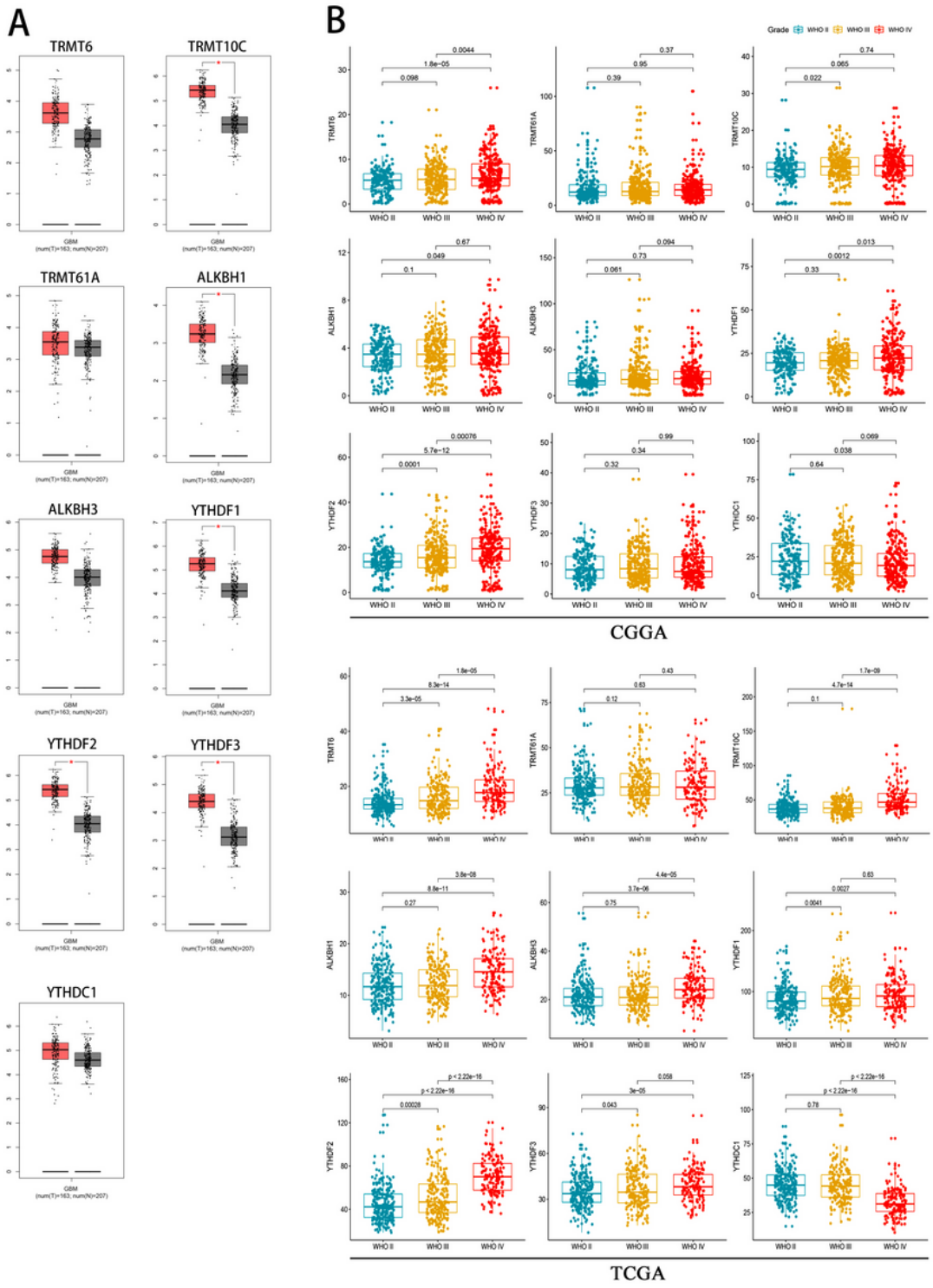
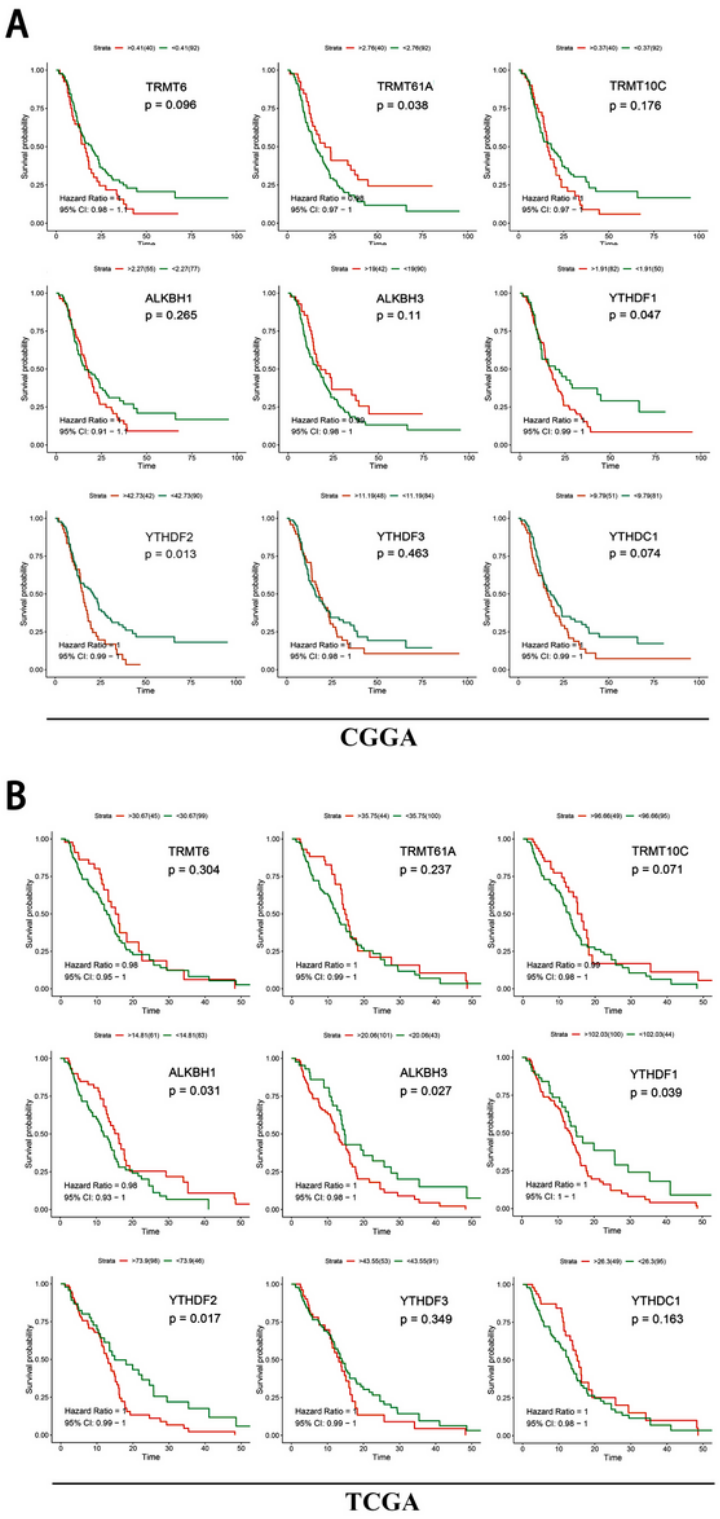
# Figures



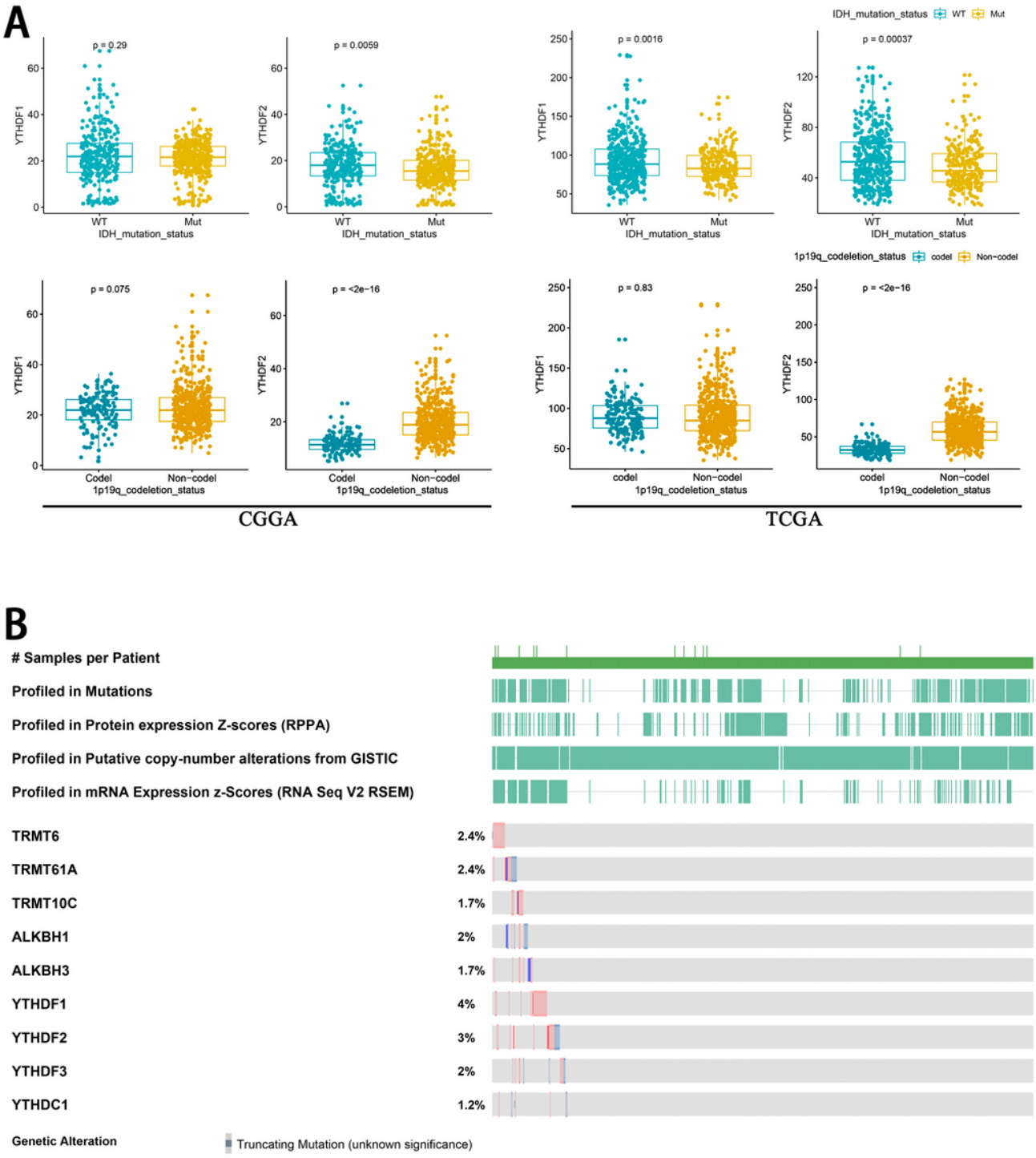

## Figure 1

Expression level of nine m1A regulators (TRMT6, TRMT10, TRMT61A, ALKBH1, ALKBH3, YTHDF1-3 and THHDC1) and its relationship with pathological stage in glioma in CGGA and TCGA dataset. (A) m1A regulators gene expression level in tumor (n=163) and normal tissue (n=207); (B) m1A regulators expression level in different glioma pathology stage in CGGA and TCGA database. YTHDF2 were up-regulated in WHO grade IV compared with WHO grade II or III in glioma in both dataset ($p<0.05$).



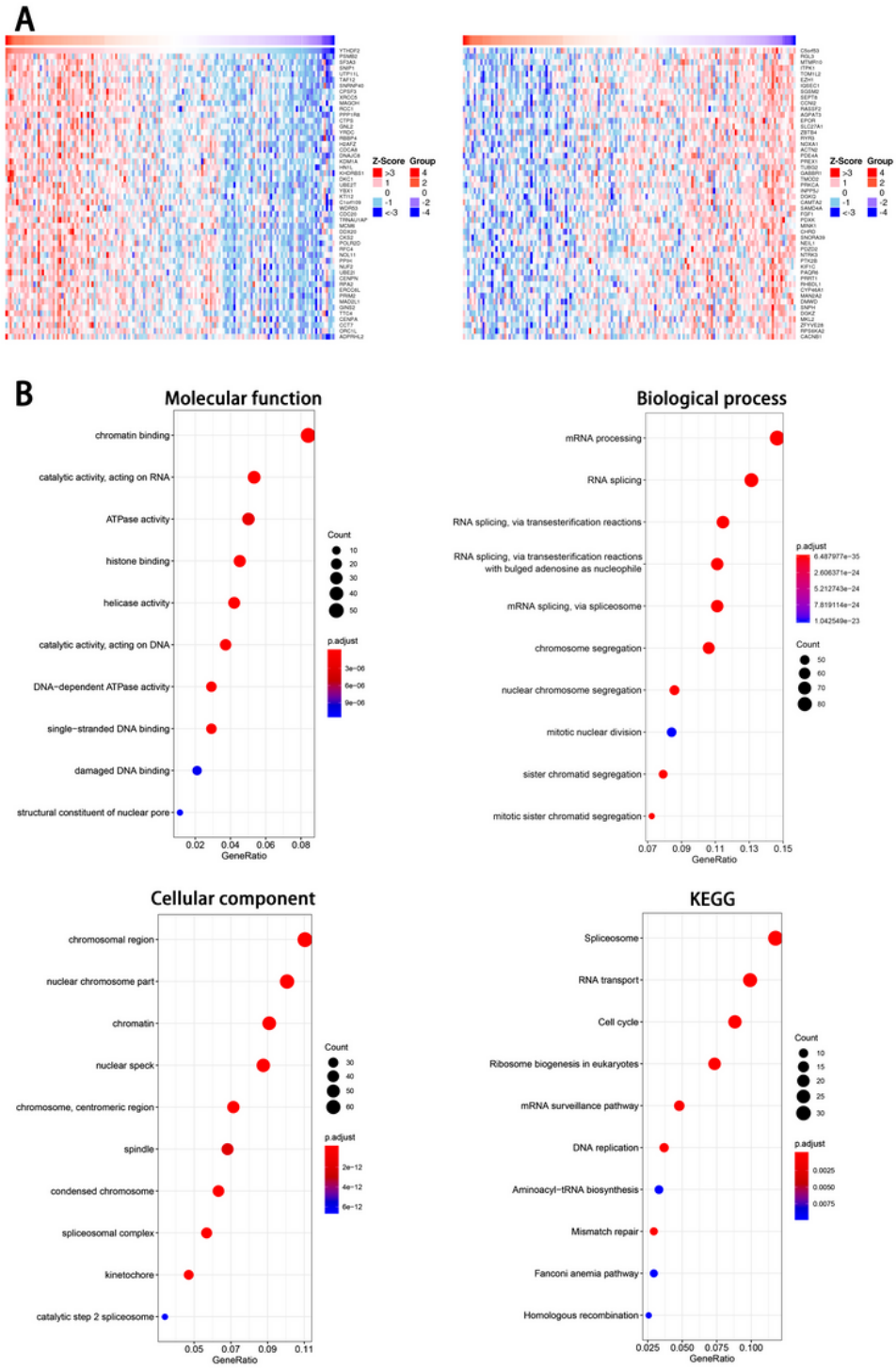

## Figure 2

The correlation between the expression level of m1A regulators and the overall survival rate (OS) in glioma. The high YTHDF1 and YTHDF2 expression in glioma was correlated with significantly shorter overall survival rate in both CGGA (A) and TCGA (B) dataset. No significant correlation was observed between the OS and the expression level of TRMT6, TRMT61A, TRMT10C, YTHDF3 and YTHDC1 in glioma in both datasets(p>0.05).



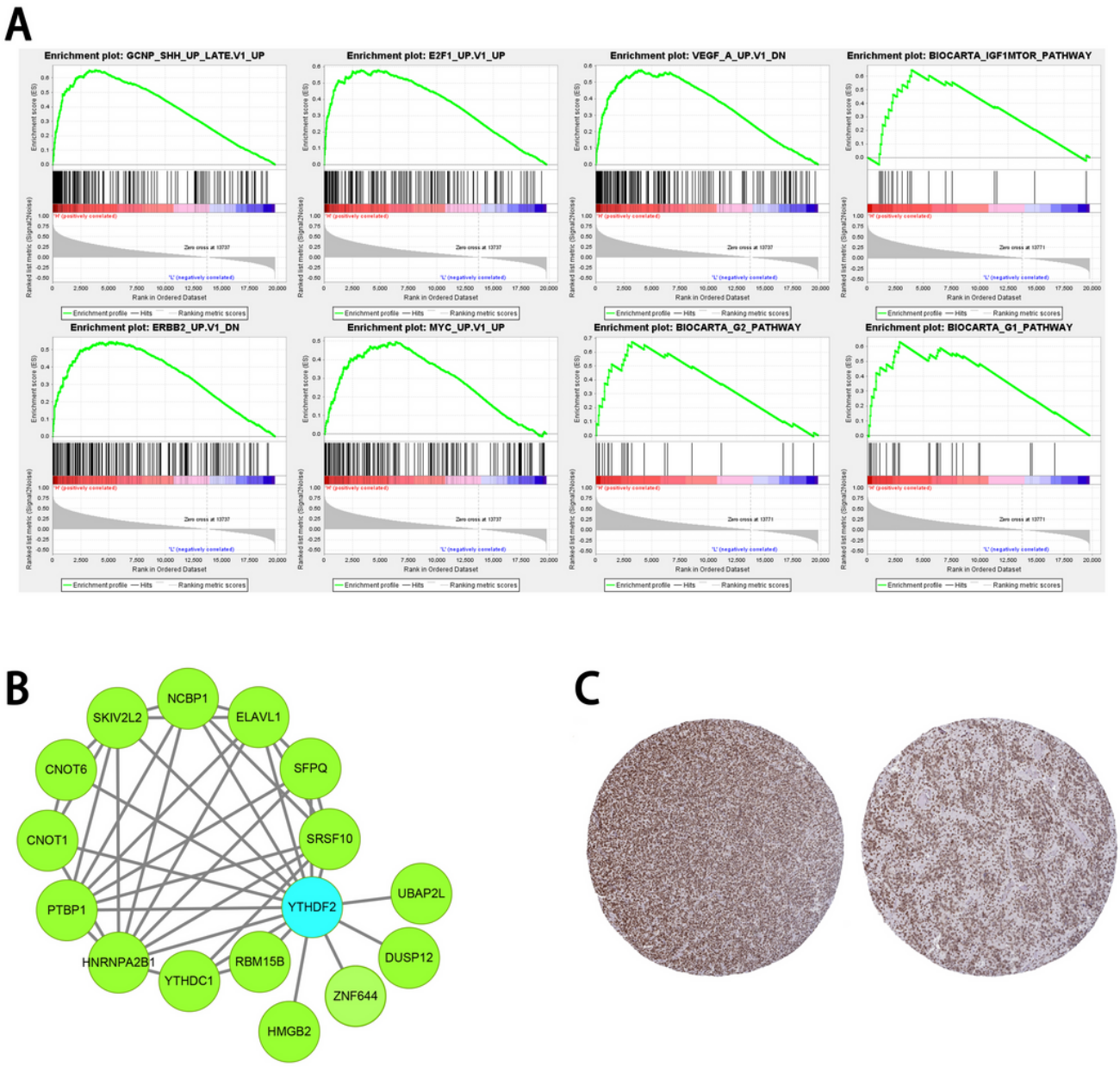

## Figure 3

m1A regulator expression level in IDH mutated and 1p19q codeletion glioma and its alteration frequency in glioma. (A) the expression level of YTHDF1 and YTHDF2 was downregulated in IDH mutated glioma in both TCGA and CGGA dataset. Similarly, compared with non-codeletion glioma, the YTHDF2 was significantly downregulated in 1p19q codeletion glioma. (B) the mutation profile and the m1A regulator alteration frequency in glioma.




## Figure 4

Function analysis of YTHDF2 co-expressed genes. (A) The heatmap of genes positively or negatively correlated with YTHDF2 in TCGA cohort through LinkedOmics. (B) GO and KEGG annotation of the co-expressed genes. The genes were categorized in to molecular function group, biological process group, cellular component group in GO annotation. The size of the cycle represents the number of genes enriched in each entry; the color of the size refers to the P value.




## Figure 5

Gene set enrichment analysis (GSEA) and the PPI network analysis. (A) Gene set enrichment analysis revealed that oncogene signature or cell cycle related pathways were enriched in YTHDF2 high expressed samples. (B)protein-protein interaction analysis showed that fifteen proteins were identified having connectivity with YTHDF2. (C) YTHDF2 protein expression in glioma using the Oncomine and HPA databases