Identification of potential core genes as potential biomarkers for predicting progress and prognosis in glioblastoma

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Research Article

Keywords: Glioblastoma, Expression profiling data, Hub genes, Prognosis, NDC80

Posted Date: April 12th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1522661/v1

Abstract

Background

Glioblastoma is a common malignant neuroepithelial neoplasm with poor clinical outcome and limited treatment options. It is extremely important to search and confirm diverse hub genes that are effective in the advance and prediction of glioblastoma.

Methods

We analyzed three microarray datasets (GSE50161, GSE4290, GSE68848), which was derived from the GEO database. GO function and KEGG pathway enrichment analysis for Differentially Expressed Genes (DEGs) were performed using the DAVID. PPI network of these DEGs was analyzed based on the Search Tool for the Retrieval of Interacting Genes database and visualized by Cytoscape software. Hub genes were identified through PPI network and Robust Rank Aggregation method. The Cancer Genome Atlas (TCGA) and Oncomine database were used to perform the validation of hub genes. In addition, the survival curve analysis was also conducted to verify the correlation between the expression of hub genes and prognosis. The human glioblastoma cells and normal cells were collected and then RT-PCR, Western-blot and immunofluorescence were conducted to validate the expression of NDC80. A Cell Counting Kit-8 (CCK-8) assay were used to detect the proliferative of glioma cells. The effects of NDC80 on migration and invasion of GBM cell lines were evaluated by conducting scratch assay and transwell assay.

Results

A total of 716 overlapping genes were in the common region, containing 188 upregulated DEGs and 528 downregulated DEGs. Furthermore, we also found among the overlapping DEGs, ten hub genes with a high degree of connectivity. The expression of ten hub genes in TCGA and Oncomine database were significantly overexpressed in glioblastoma compared with normal ones. Besides, the survival analysis showed that the patients with low expression of six genes (BIR5C, CDC20, NDC80, CDK1, TOP2A and MELK) had a significant favorable prognosis ($P < 0.01$). We firstly discovered that NDC80, an important molecule in other cancers, also plays an important role in malignant gliomas. The RT-PCR, western blot and immunofluorescence results showed that the expression level of NDC80 was significantly higher in human glioblastoma cells than in normal cells. Moreover, we identified that NDC80 up-regulates the proliferation and invasion abilities of human glioblastoma cells.

Conclusions

The distinguished six genes may be utilized to form a board of progressive and predictive biomarkers of glioblastoma for clinical purpose. Especially, NDC80 was discovered that may play an important role in

glioblastoma.

Background

Glioblastoma multiforme (GBM) is considered as the most malignant brain tumor, which has strong proliferative capacity and high invasion characteristics, resulting in rapid progression and high degree of malignancy. GBM is classified as Grade IV by the World Health Organization (WHO), and the mortality rate of patients in the first year after diagnosis is close to 80% [1]. GBM is also the most common and fatal malignant primary brain tumor in adults [2], and the 5-year survival rate of patients diagnosed with GBM is less than 6% [3]. Currently, the standard treatment for GBM is surgical resection followed by radiotherapy combined with concurrent and/or adjuvant temozolomide (TMZ) chemotherapy[4, 5]. Tumor-treating fields, delivering low-intensity alternating electric fields, can also be given concurrently with adjuvant temozolomide. While there have been many reports on immunotherapy and gene therapy for GBM, the effects are not completely affirmed due to the inconsistency in treatment methods and evaluation criteria. Gene expression profiling that provides rich data on genetics, gene expression and promoter methylation, will aid in the early diagnosis and validation of specific biomarkers [6]. However, several studies using a single or small sample dataset for gene expression analysis require further reproducibility and independent validation or experimental studies [7–9]. In order to show our results' robustness, we performed Gene expression profiling analysis across different expression datasets to explore sensitive and specific biological markers that would help for early diagnosis and validation of interventions among the GBM patients. Furthermore, we conducted the experiments of Quantitative real-time PCR, Western-Blotting analysis and Immunofluorescence between GBM cells and normal control cells, with the purpose of verifying the newly valuable mark of our previous analysis.

Materials And Methods

Microarray data source

In our study, the gene expression datasets were obtained from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih. gov/geo/). There are 50044 series about human GBM cancer retrieved from the GEO database. After a careful review, three gene expression profiles (GSE50161, GSE4290, and GSE68848) were selected. All of them are based on platform GPL570 ([HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array). All of the data were freely available online.

Data processing of DEGs

The DEGs between GBM and normal samples were detected by using online analysis tool GEO2R (https://www.ncbi.nlm. nih.gov/geo/geo2r/), for the GEO database, based on R language. We considered DEGs as differentially expressed if genes that met the cutoff criteria, adjusted $P < 0.05$ and $|logFC| \geq 2.0$ [10, 11]. The Venn diagram webtool (bioinformatics.psb.ugent.be/webtools/Venn/) was used to identify the overlapping DEGs.

GO and KEGG pathway analysis of DEGs

GO function and KEGG pathway enrichment analysis were performed using the Database (DAVID 6.8, http://david-d.ncifcrf.gov/) for Annotation, Visualization and Integrated discovery to identify the pathways of DEGs [12]. P-value < 0.05 was considered as statistically significant.

Protein-protein interaction network construction and hub gene identification

STRING database (http://string-db.org/) aims to collect and integrate the knowledge of all functional interactions between the expressed proteins, by consolidating known and predicted protein-protein association data for a large number of organisms [13]. We uploaded the overlapping DEGs to the online tool STRING, then we got the TSV data of PPI network. Next, we further used Cytoscape software to construct a PPI network. We screened the modules of PPI network by a plug-in MCODE in Cytoscape. Modules inferred using the default settings that the degree cutoff was set at 2, nodes core cutoff was set at 0.2, K-core was set at 2, and max. depth was 100. Generally, we always decide the most highly connected genes in the PPI network as hub genes, which are expected to play an important role in understanding the biological mechanism of response under conditions [14]. In order to identify the hub genes, CytoHubba, a Plugins in Cytoscape was used to analyze the above TSV data. Finally, we got ten top hub genes.

Validation of hub genes expression levels

Gene Expression Profiling Interactive Analysis (GEPIA) (http://gepia.cancer-pku.cn) and Oncomine (https://www.oncomine.org) were used to carry out validation of expression of the candidate hub genes [15, 16].

Survival analysis of hub genes

The gene expression data of 325 patients (203 males and 122 females), with an average age of 43.38 years, were downloaded from the Chinese Glioma Genome Atlas (CGGA) (http://www.cgga.org.cn). Those patients were categorized into a high-expressed group and low-expressed group according to the expression level of the 10 Hub genes. We regarded OS as the prognostic outcome of patients with glioblastoma.

Cell Culture

The human glioblastoma U251 and U-87MG cells, and normal control HA1800 cells were offered by G.F.Vande Woude, Van Andel Research Institute, Grand Rapids, MI. The cells were cultured in DMEM containing 10% fetal bovine serum and placed in 5% carbon dioxide and 37-degree cell incubator.

Quantitative real-time PCR

Total RNA was extracted from cells by using TRIzol reagent (Invitrogen, U.S.A.) following the manufacturer's protocol. The cDNA Synthesis Kit (Takara, China) was used for the synthesis of cDNA

according to the manufacturer's instructions. Reverse transcription polymerase chain reaction (RT-PCR) was performed using The PrimeScript TM II Reverse Transcriptase kit of Takara. The sequence of primers was as follows: NDC80 sense chain: 5'-ATCAAGGACCCGAGACCACT-3', NDC80 antisense chain: 5'-ATGTATGAGGAGCCCCCACT-3'; β-actin sense chain: 5'-CTGGAACGGTGAAGGTGACA-3', β-actin antisense chain: 5'-CGGCCACATTGTGAACTTTG – 3'.

Western blot assay

Cells were lysed and protein in supernatant extracts was quantified using a BCA Protein Assay Kit (Beyotime Institute of Biotechnology). Fifty micrograms per lane of total cell lysates were resolved on SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) gels and transferred onto polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, MA, USA). Blots were incubated with the primary antibody overnight at 4°C. Furthermore, the blots were incubated with horseradish peroxidase-linked secondary anti-rabbit or anti-mouse antibody (Bio-Rad). Immunoreactivity was detected using the enhanced chemiluminescence (Amersham Biosciences, Piscat-away, NJ, USA), the Chemidoc and Quantity One software (Bio-Rad). Densitometric analysis was performed by using Quantity One software. β-actin was used as a loading control. NDC80 antibodies were purchased from the US Abcam company.

Immunofluorescence

The cells were seeded on coverslips and incubated for 24 h under normoxic conditions. Subsequently, the cells were fixed with 4% paraformaldehyde at room temperature and permeabilized with 0.2% Triton X-100 for 10 min. Consequently, cells were washed with PBS and blocked in PBS containing 5% bovine serum albumin (Sigma-Aldrich, St. Louis, MO, USA) for 90 min. The cells were washed with PBS and the primary antibodies were diluted with PBS containing 2% BSA as follows: rabbit anti-NDC80 (1: 200; Abcam, USA) and incubated for overnight at 4°C. Then washed with PBS and incubated for 2 hours with an anti-rabbit fluorescent secondary antibody (Amersham) at room temperature. Finally, DAPI (Beijing ComWin Biotech Co., LTD., China) was added to each sample for nuclear counterstaining. The coverglass was observed and photographed to show representative cells using an Olympus BX61WI-FV1200MPE confocal microscope.

Cell proliferation assay

Cell viability was measured using a Cell Counting Kit-8 (CCK-8) assay (Dojindo Molecular Laboratories, Inc.) according to the manufacturer's instructions. Transfection of NDC80 or the non-specific control was performed in 96-well plates in quadruplicate. Cell culture medium was replaced at 24 h following transfection. CCK-8 (10 μl) was added to each well, and the absorbance at 450 nm was measured following incubation for 2 h at 37˚C. Each experiment was repeated in triplicate.

Scratch assay

Briefly, cells were grown to full confluence in 6-well culture plates. After reaching confluency, monolayers were scratched with a sterile pipette tip to make a scratch of approximately 0.4–0.5 mm in width and

cells were cultured in serum deprived medium. After 24 h, the wound gap was observed and images were captured. All scratch assays were performed in triplicate.

Transwell invasion assay

Transwell invasion was performed with 24-well Matrigel-coated chambers (8-μM pore size) from BD Biosciences. According to the manufacturer's protocol, cells were permitted to grow to ~ 75–80% confluence and were starved for 24 h by serum. Then the nonmotile cells were removed with a cotton swab. The remaining cells at the lower surface of the filter were fixed with cold methanol and stained with 0.1% (w/v) crystal violet (Sigma). The invading cells were quantified by counting ten random fields at ×200 magnification. All **tanswell invasion assay**s were performed in three independent experiments.

Statistical analysis

In this study, at least 3 separate experiments were carried out. Data are presented as mean ± Standard Error of Mean (SEM). Statistical tests were performed using SPSS version 19.0 software for Windows (SPSS Inc, Chicago, USA). Two-tailed Student's *t*-test was used for comparisons between groups. $P < 0.05$ was considered to be a significant difference.

Results

Identification of DEGs

Our study consisted of three datasets, they were GSE50161, GSE4290 and GSE68848. GSE50161 contained 34 GBM samples and 13 normal samples, GSE4290 composed of 77 GBM samples and 23 normal samples and GSE68848 included 228 GBM samples and 28 normal samples (Table 1). Based on the criteria of $P < 0.05$ and $|logFC| \geq 2$, a total of 2116 DEGs were identified from GSE50161, including 876 upregulated genes and 1240 downregulated genes. There are 1175 DEGs identified in gene chip GSE4290; 400 genes were upregulated, and 775 genes were downregulated. What's more, GSE68848 has 1087 DEGs composing of 360 upregulated genes and 727 downregulated genes. Subsequently, Venn analysis was performed to get the overlapping part of the DEG profiles (Fig. 1). Finally, 716 DEGs were significantly differentially expressed among all three groups, of which 188 were significantly upregulated genes and 528 were downregulated.

Gene Ontology (GO) Enrichment Analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of DEGs

After GO analysis of abnormal expression genes, we detected that those genes were mainly associated with biological information transfer or cellular function alternation, including chemical synaptic transmission, neurotransmitter secretion, nervous system development, regulation of exocytosis, glutamate secretion, cell junction, synaptic vesicle membrane, axon, synapse, synaptic vesicle, calcium ion binding, calcium-dependent phospholipid binding.

Furthermore, the results of KEGG analysis showed that the mutual DEGs were mainly enriched in Retrograde endocannabinoid signaling, GABAergic synapse, Morphine addiction, Calcium signaling pathway, Synaptic vesicle cycle (Table 2).

PPI network construction and hub gene identification

To construct the network of PPI relationships of the DEGs, we used the online tool STRING. We obtained the PPI relationships with a combined score > 0.4. Based on the information obtained from the STRING database, we produced a network diagram. There were 501 nodes and 3030 edges in the network. Cytoscape was used to evaluate primary modules of the PPI sub-network using the plug-in molecular complex detection (MCODE) (Fig. 2). There are 42 genes involved in sub-network a, 39 genes involved in sub-network b, 11 genes involved in sub-network c, 13 genes involved in sub-network d, 6 genes involved in sub-network e and 14 genes involved in sub-network f. Base on the degree, we use CytoHubba in Cytoscape to calculate top ten hub genes among 716 DGEs (Table 3). The top 10 genes also constructed a network (Fig. 3). The most understanding gene is DNA topoisomerase II alpha (TOP2A; degree = 78), followed by cyclin-dependent kinases 1 (CDK1; degree = 64), budding uninhibited by benzimidazoles 1 (BUB1; degree = 53), cell-division-cycle protein 20 (CDC20; degree = 53), baculoviral IAP repeat containing 5 (BIRC5; degree = 53), maternal embryonic leucine zipper kinase (MELK; degree = 53), kinesin family member 4A (KIF4A; degree = 52), PDZ binding kinase (PBK; degree = 52), NDC80 kinetochore complex component (NDC80; degree = 51), TTK protein kinase (TTK; degree = 51). All of them are upregulated genes.

Assessment of ten hub genes in TCGA and Oncomine database

To assess the roles of these ten hub genes in GBM, gene expression validations were performed, and all of ten hub genes were also upregulated in the TCGA and Oncomine data (Fig. 4, 5). **Survival analysis of ten hub genes**

Each case enrolled was treated by members of the CGGA group. Tumor tissue samples were collected at the time of surgery after informed consent. Neuropathologists established the diagnosis and ensured the quality of the tissue for molecular testing. Overall survival (OS) was calculated from the date of diagnosis until death or the end of follow-up. The point of death is defined by death certification, which could be obtained from local hospitals and police stations. We assessed the prognostic effect of the 10 hub genes. According to the analysis, we found that only the downregulation of TOP2A, CDK1, CDC20, BIRC5, MELK, and NDC80 were closely associated with a decreased overall survival among patients with GBM (Fig. 7). The remaining 4 hub genes (BUB1, KIF4A, PBK, TTK) had no statistical significance between gene expression and clinical outcome of GBM (Fig. 6).

NDC80 expression in human glioblastoma cells is increased compared with normal cells

NDC80 plays an important role in cancers such as liver cancer and breast cancer, but it is rarely reported in GBM. Our study found that NDC80 was highly expressed in GBM. Then we further examined the expression of NDC80 in different malignant glioma cells. NDC80 mRNA level was determined by quantitative real time RT-PCR between human glioblastoma cells and normal control cells (Fig. 7A). RT-PCR showed NDC80 ($p < 0.001$) was augmented significantly in human glioblastoma cells comparing with the normal astrocyte (HA1800). In addition, we also found that the translation level of NDC80 ($p < 0.001$) was also significantly increased in malignant glioma cells by western-blotting analysis (Fig. 7B). In order to make our results more reliable, we also observed the expression of NDC80 in cells by immunofluorescence. The results of immunofluorescence were consistent with the results of RT-PCR and Western-blotting. NDC80 can be visualized by immunofluorescence in cells. U251 and U-87MG cells displayed a higher red fluorescence compared with HA1800 cells (Fig. 7C), suggesting NDC80 expression in malignant glioma cells is higher than normal cells.

NDC80 up-regulates the proliferation and invasion abilities of human glioblastoma cells.

First of all, Western blotting experiments confirmed that si-NDC80 designed by us could knock down the expression of NDC80 in cells. See Fig. 8 for details. After that, the CCK-8 test results showed that the proliferation ability of U251, U118 and A172 cells in the Si-NDC80 group was lower than that in the control group ($P < 0.05$). However, there was no significant difference between the Si-NDC80 group of normal astrocytes (HA1800) and its control group ($P > 0.05$). See Fig. 9A for details. Collectively, the results demonstrated that the proliferation ability of GBM cells was significantly affected by the expression of NDC80.In order to determine the role of NDC80 in GBM migration, we first conducted a scratch test and a Transwell invasion test. As shown in the scratch test of Fig. 9B, knockdown of NDC80 can reduce the migration ability of human glioblastoma cells (U251, U118, A172) compared with negative control cells. As shown in the results of the Transwell invasion experiment in Fig. 9C, the number of invasive cells in the Si-NDC80 group of human glioblastoma cells (U251, U118, A172) was significantly lower than that of the control group, indicating that Down-regulation can significantly inhibit the invasive ability of glioblastoma cells.

Discussion

Malignant brain tumors are among the most feared types of cancer, because of their poor prognosis, as well as because of their direct repercussions on the quality of life and cognitive function [17]. Malignant glioma is the most common type of primary malignant brain tumor accounting for 80% of patients and an annual incidence of 5.26 per 100 000 population, or 17 000 new cases diagnosed per year [18]. Glioblastoma, as the Ⅳ-grade glioma, is the most common and most malignant tumor in glioma. Because of the limited efficacy of conventional treatments such as surgery, chemotherapy, and radiotherapy, it is urgent to study a new and effective treatment. Over the past decade, many studies have made great progress in genetics and epigenetics of GBM [19]. Identification of relevant biomarkers for appropriate patient selection is essential for the successful development of novel therapies. In this study, we aimed to find potential biomarkers for predicting progress and prognosis in GBM.

In the present study, we obtained 716 DEGs among all three groups including 188 upregulated genes and 528 downregulated genes. These DEGs were associated with the GO gene functions involving cellular protein modification, regulation of cell communication, and regulation of signaling. Many studies reported that glioma cells can regulate cell communication and influence signaling pathway [20]. They even can form the network, which allows multicellular communication through microtube-associated gap junctions [21]. In addition, through KEGG pathway analysis, we found that these DEGs involved in Retrograde endocannabinoid signaling and Calcium signaling pathway, among which endocannabinoid was reported that it can act retrogradely and mediate synaptic modulation through release 2-arachidonoylglycerol (2-AG) as well as mediate long-term depression (LTD) [22]. Furthermore, many altered calcium-binding proteins have been observed in glioblastoma multiforme, which implicated the deregulation of calcium signaling and homeostasis in GBM [23]. In addition, glioblastoma can make patients present with epilepsy. It is known that glioma cells extrude pathological concentrations of glutamate which are thought to play a role in tumor progression and the development of epilepsy [24]. Furthermore, the synaptic vesicle cycle involves important extracellular/endocytic processes, as well as protein complex formation/decomposition processes, which have been reported to be associated with GBM [25, 26].

We constructed PPI network to investigate the interrelationship of the DEGs, and ten hub genes were identified, including TOP2A, CDK1, BUB1, CDC20, BIRC5, MELK, KIF4A, PBK, NDC80 and TTK. All of them were upregulated genes. Their gene expression was also validated via the TCGA and Oncomine database. We conducted survival analysis for 10 hub genes. In our study, only 6 genes showed significant results, they were TOP2A, CDK1, CDC20, BIRC5, MELK and NDC80. This finding implied that the prognosis of patients with glioblastoma could be predicted by detecting the expression level of those 6 genes. Furthermore, the results of the present study provided biomarkers and targets, which may be applied in the diagnosis and treatment of patients with glioblastoma for accurate therapy.

Interesting, TOP2A, CDK1, CDC20, MELK, and NDC80 genes were all associated with the cell cycle. Cell cycle involves multiple molecular pathways that appear to be the essential mechanism of the indefinite proliferation of malignant glioma. If the gene that regulates the cell cycle progression of malignant glioma is deregulated, the development of glioma will be promoted [27]. Most of them were reported as an essential factor involved in cell division and proliferation. In mammals, TOP2A has an important role in altering DNA topology and it is expressed in proliferating cells in late S phase, with a high in the G2 to M phases, which suggests that it has potential as a proliferation marker [28]. Compared with lower grade astrocytomas and normal brain tissues, TOP2A transcription level in GBM patients increased significantly, which is a good prognostic indicator and can guide temozolomide chemotherapy [29]. Cyclin-dependent kinases 1 (CDK1), located on 10q21.2, is one of the gene Cyclin-dependent kinases (CDKs) that are important regulators of cell cycle progression and cell cycle regulation [30]. When it comes to cell proliferation, we think of the important role of CDK1 in G1/S and G2/M phase transitions, which promote the M-phase process. In addition to glioma-related, there are many cancers-related, such as lung adenocarcinoma, oral squamous cell carcinoma, etc [31–34]. CDC20, a central regulator of the cell cycle in numerous cancers, plays an essential role in the regulation of glioblastoma tumor initiating

cells (TIC) proliferation, self-renewal and survival [35]. CDC20 knockdown by transducted with shCDC20 caused loss of tumor initiating cells (TICs) in the S, M and G_2cell cycle phases and accumulation in the G_1 phase [36]. In addition, silencing CDC20 expression in TIC accelerated a significant increase in apoptotic cell death [36]. BIRC5 (Survivin) is associated with proliferation markers, histological malignancy grade, and are inversely associated with prognosis [37, 38]. Recent comprehensive studies have elaborated that knockdown of Survivin in permanent as well as primary glioma cell lines lead to immense cellular polyploidy with cells having DNA contents up to 32n, poly-merotelic kinetochore-microtubuli connections, DNA damage, DNA damage response [39–41]. Knocking down the BIRC5 in GBM cells can lead to transient G1 cell cycle arrest which was not able to halt endoreplication of DNA [38]. MELK, paralleled with the increasing degree of malignancy in astrocytomas, is a member of the subfamily that activates the serine/threonine protein kinase [42]. Kig C showed that siRNA-mediated loss of MELK in glioblastoma cells causes a G1/S phase cell cycle arrest accompanied by cell death or a senescence-like phenotype implied that MELK inhibitors hold great potential for the treatment of glioblastomas as such or in combination with DNA-damaging therapies [43].

As one of the key elements of outer kinetochore, NDC80, which has a molecular weight of 74 kDa, is a heterotetrameric protein complex that plays an important role in cell mitosis [44]. Abnormal expression of the NDC80 causes chromosomal abnormalities, leading to instability of the genome, and genomic instability is a major factor in all tumorigenesis [45]. Numerous studies have found that the components of the NDC80 complex are highly expressed in tumors, which can be used as a diagnostic marker for certain tumors and may even be an indicator for evaluating prognosis [46]a. Therefore, the role of the NDC80 complex in the development of tumorigenesis has received increasing attention. However, NDC80 has rarely been reported in GBM. Our results showed the gene expression of NDC80 was upregulated in the GBM cell lines comparing the normal cells. Interestingly, the expression level of NDC80 in glioma cells (U251, U-87MG and A172) was significantly higher than that in normal astrocytes (HA1800). Moreover, its expression level is positively correlated with the degree of malignancy. These results also suggest that NDC80 plays a role in the pathogenesis and progression of GBM.

Conclusions

In summary, the distinguished six genes may be utilized to form a board of progressive and predictive biomarkers for GBM for clinical purpose. Especially, NDC80, was discovered that may play an important role in GBM, need to be further studied.

Abbreviations

Glioblastoma (GBM), Gene Expression Omnibus (GEO), Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), Protein-Protein Interaction (PPI), Differentially Expressed Genes (DEGs), The Cancer Genome Atlas (TCGA), the World Health Organization (WHO), Central Nervous System (CNS), temozolomide (TMZ), Gene Expression Profiling Interactive Analysis (GEPIA), The Chinese Glioma

Genome Atlas (CGGA), Reverse transcription polymerase chain reaction (RT-PCR), polyvinylidene fluoride (PVDF)，Overall survival (OS)，2-arachidonoylglycerol (2-AG)，long-term depression (LTD).

Declarations

Acknowledgment

We apologize to the authors whose publications could not be included in this review due to limited space. The authors acknowledge Yu Zhu for reviewing the article. We are grateful to the contributors of data to Oncomine, GEPIA, CGGA.

Author's contributions

Conceptualization: Jianping Zeng, Jiugeng Feng. Data curation: Jin Liu. Formal analysis: Jianping Zeng. Funding acquisition: Jiugeng Feng. Methodology: Jing Liu. Project administration: Yongsheng He. Supervision: Jiugeng Feng. Writing-original draft: Shushan Hua. Writing-review & editing: Rajneesh Mungur, Jiugeng Feng.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that there is no conflict of interest.

Funding

This work was supported by the Traditional Chinese Medicine Science and Technology Plan of Zhejiang Province (2019ZB030); the Scientific Research Fund of Jiangxi Provincial Education Department (GJJ200110) and the Youth Scientific Research Cultivation Fund of the First Affiliated Hospital of Nanchang University (YFYPY2020).

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Tables

Tables 1-3 are not available with this version.

Figures

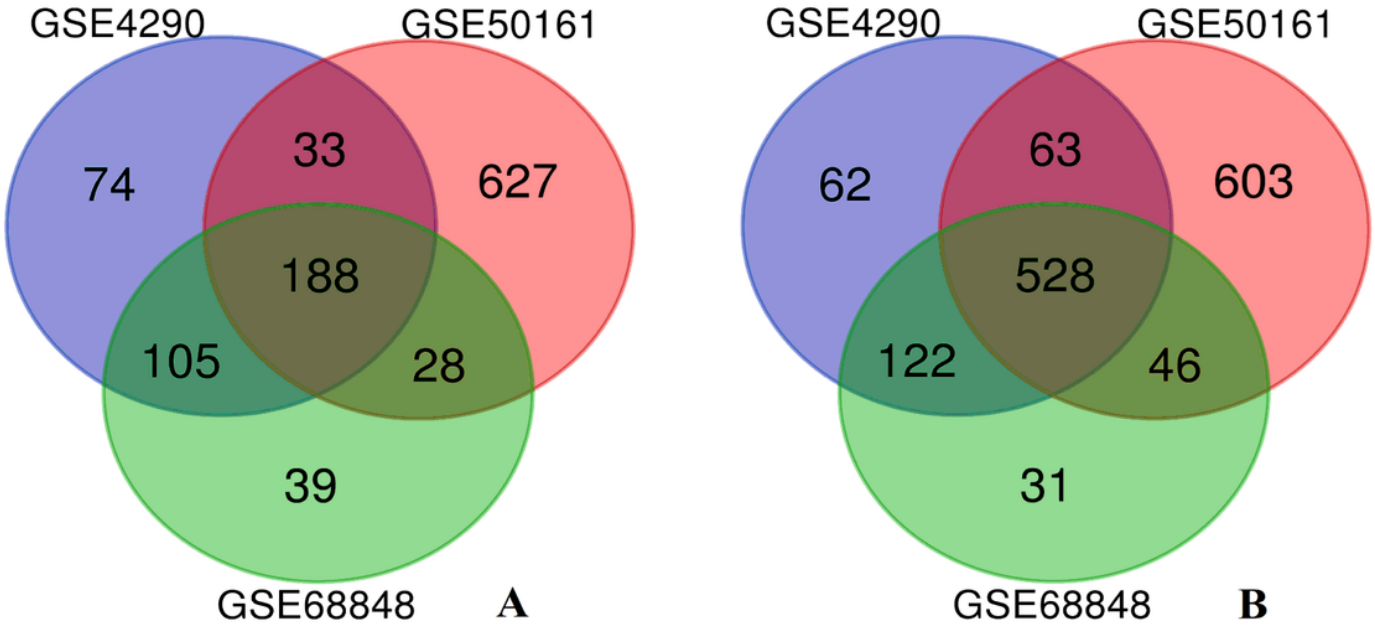

Figure 1

Venn plot of differentially expressed genes overlapping of all three GEO datasets.

Notes: Different color areas represent different datasets. DEGs were identified using a classical t-test and statistical significance defined by P<0.05 and [logFC] > 2 as the cut-off criterion. (A) Upregulated genes. (B) Downregulated genes.

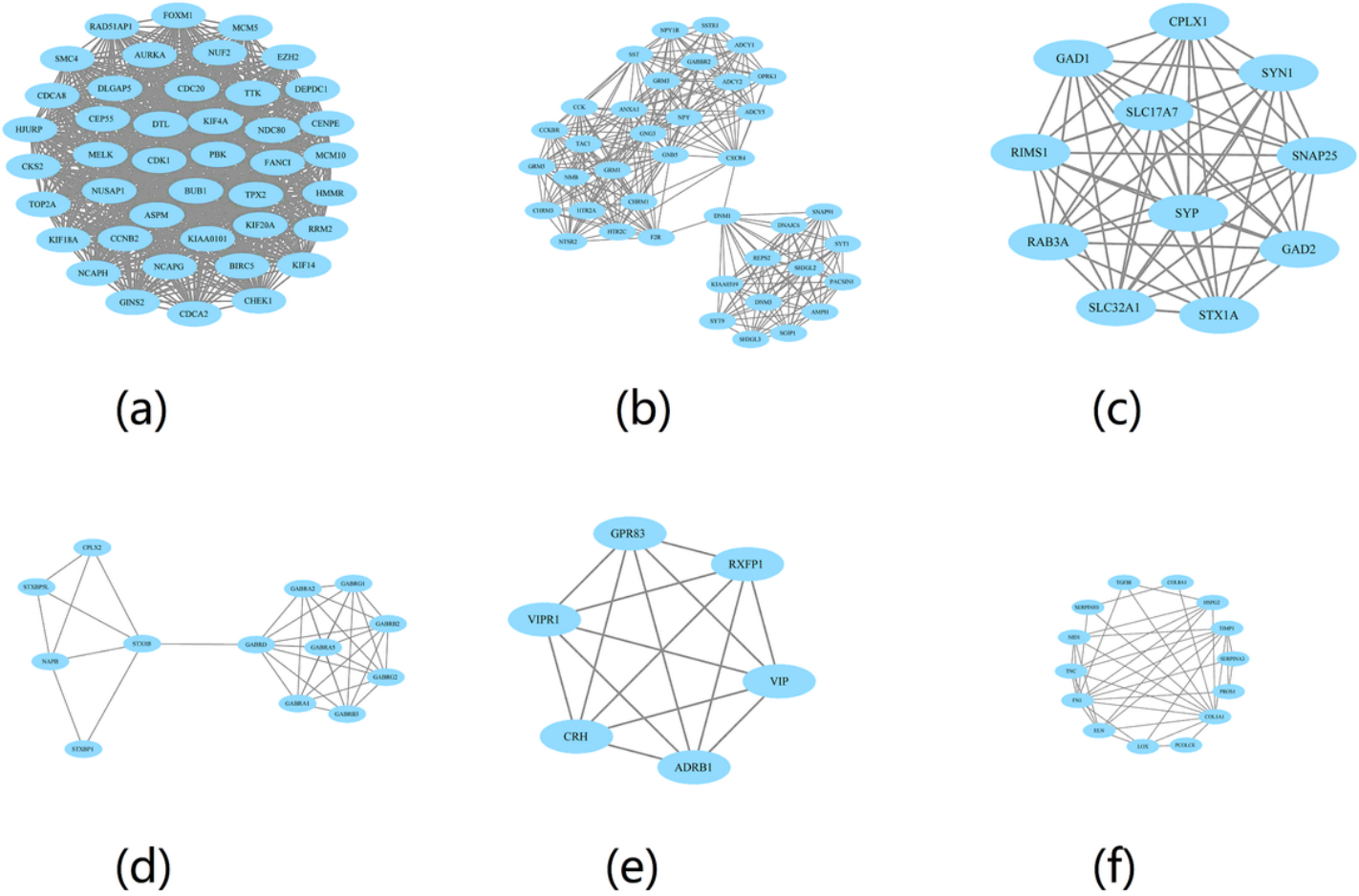

Figure 2

Top 6 primary modules of PPI sub-network by plug-in MCODE in Cytoscape software. (a) module 1; (b) module 2; (c) module 3; (d) module 4; (e) module 5; (f) module6; PPI, protein-protein interaction; MCODE, Molecular Complex Detection.PPI network of top 10 hub genes

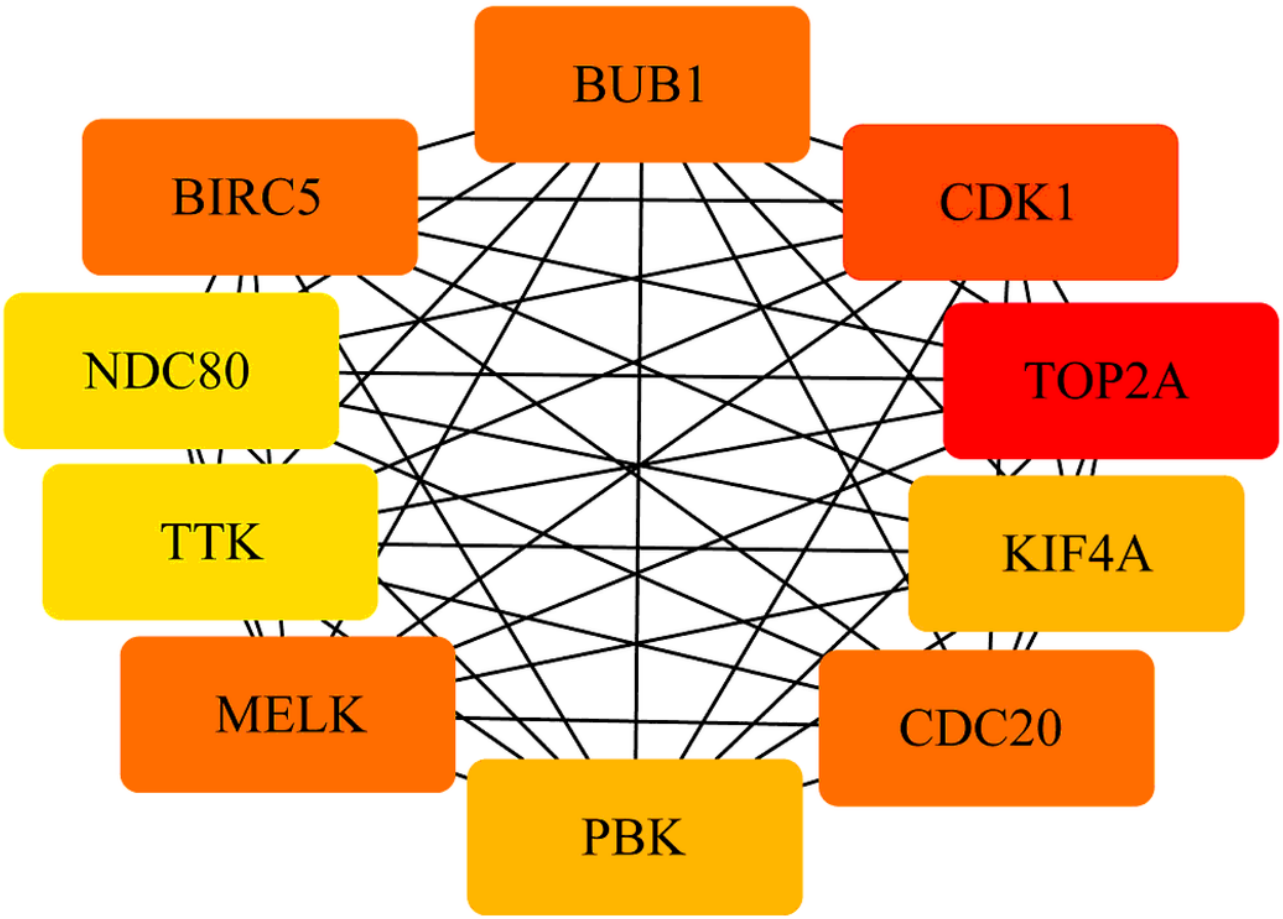

Figure 3

PPI network of top 10 hub genes

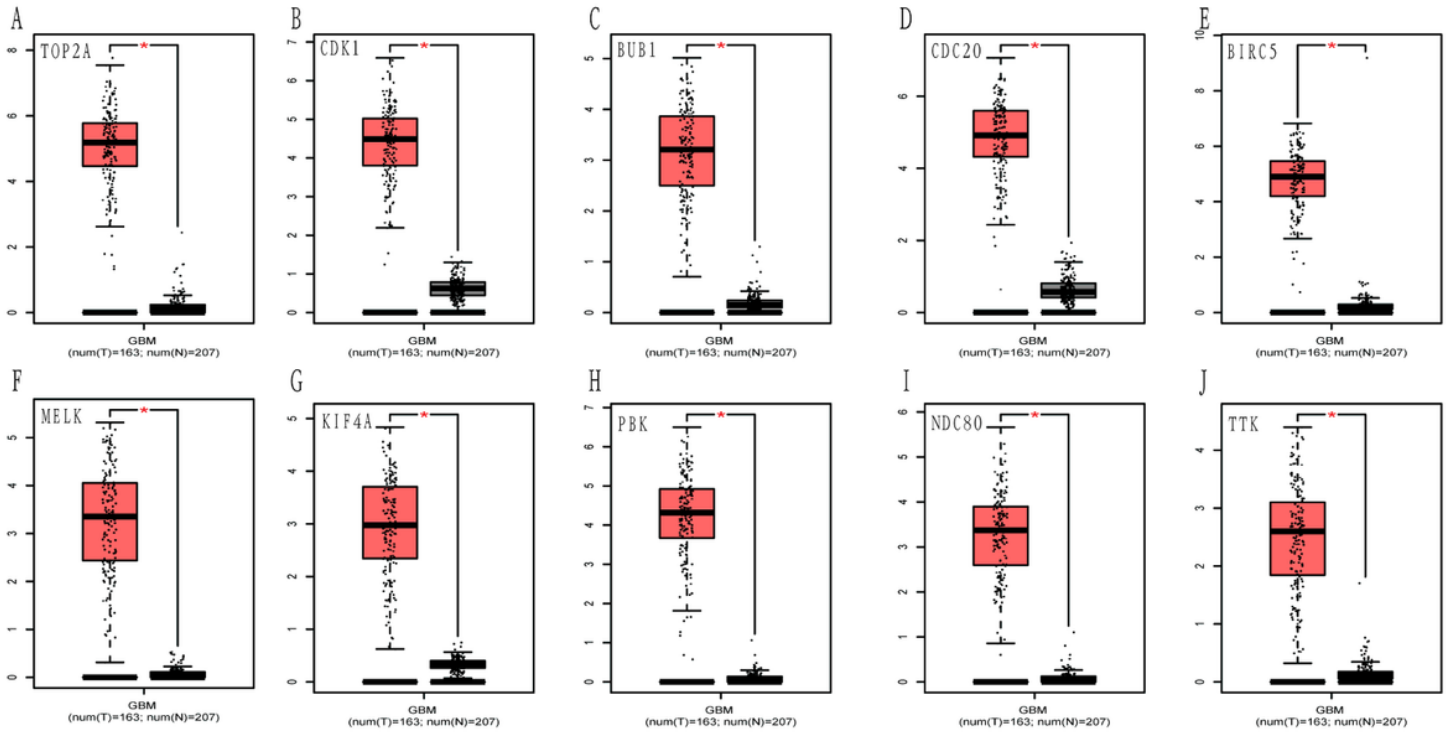

Figure 4

Validation of the ten hub genes' expression levels between normal brain samples and GBM samples based on TCGA and GTEx data in GEPIA. (A) TOP2A, (B) CDK1, (C) BUB1, (D) CDC20, (E) BIRC5, (F) MELK, (G) KIF4A, (H) PBK, (I) NDC80 and (J) TTK.

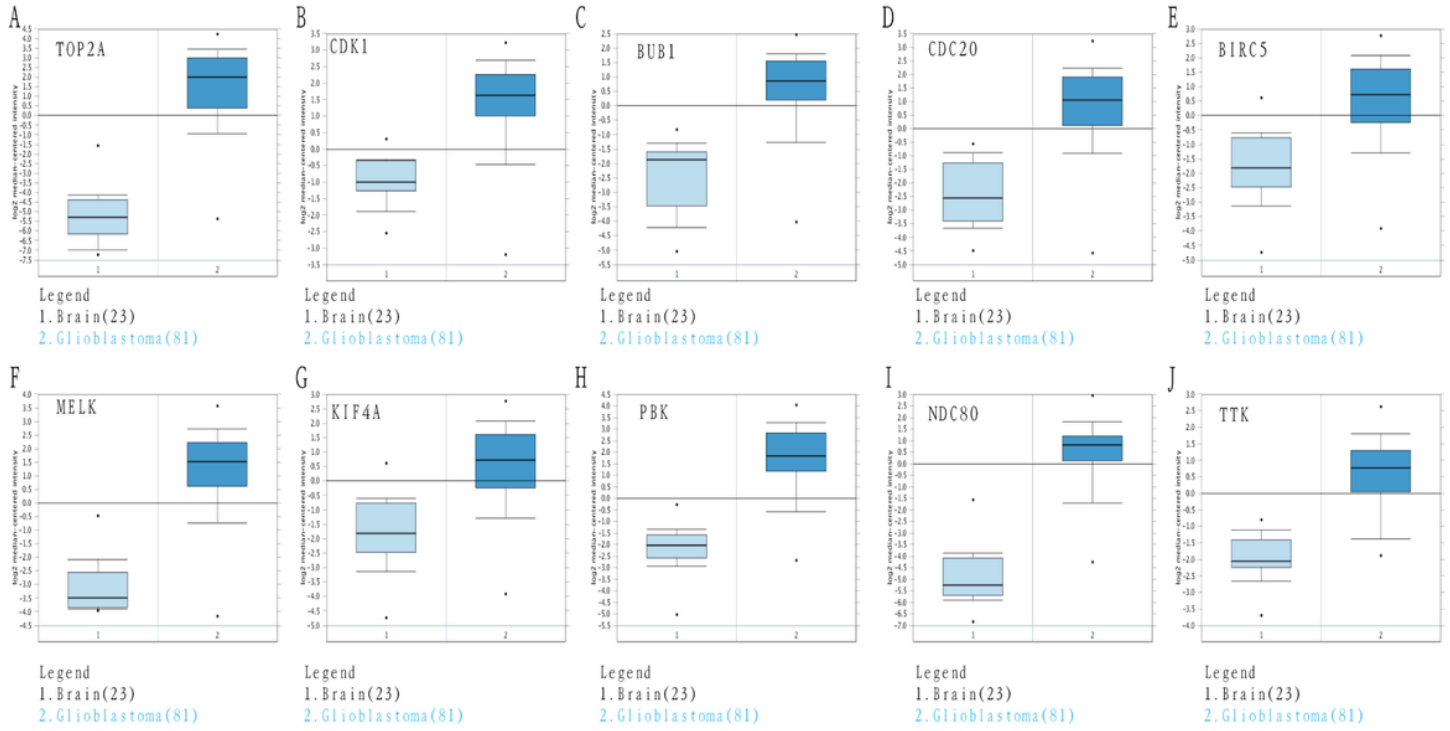

Figure 5

Validation of the ten hub genes' expression levels between normal brain samples and GBM samples based on Oncomine data. (A) TOP2A, (B) CDK1, (C) BUB1, (D) CDC20, (E) BIRC5, (F) MELK, (G) KIF4A, (H) PBK, (I) NDC80 and (J) TTK.

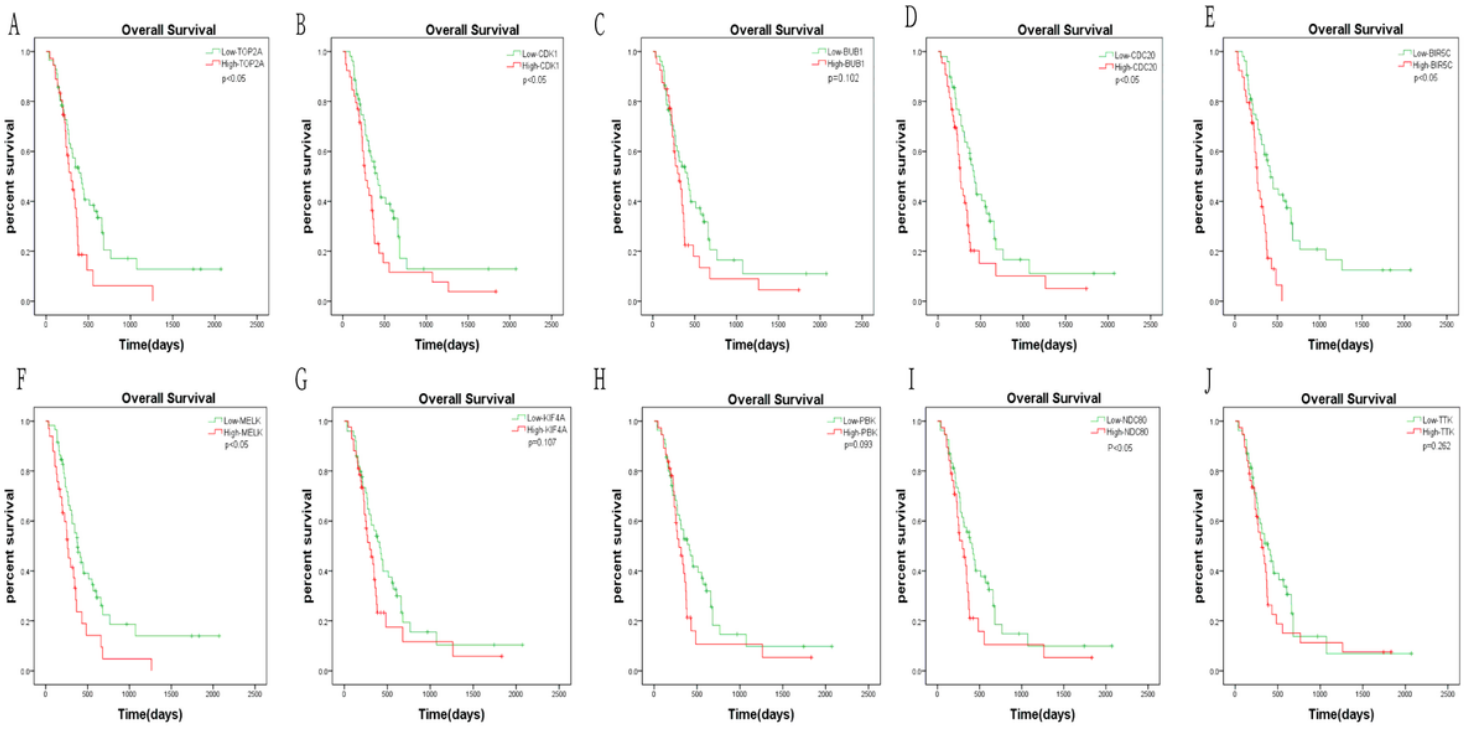

Figure 6

The prognostic values of hub genes in GBM. High mRNA level of any one gene of BIRC5, CDC20, NDC80, CDK1, TOP2A and MELK genes was associated with overall survival (OS) in all GBM patients.

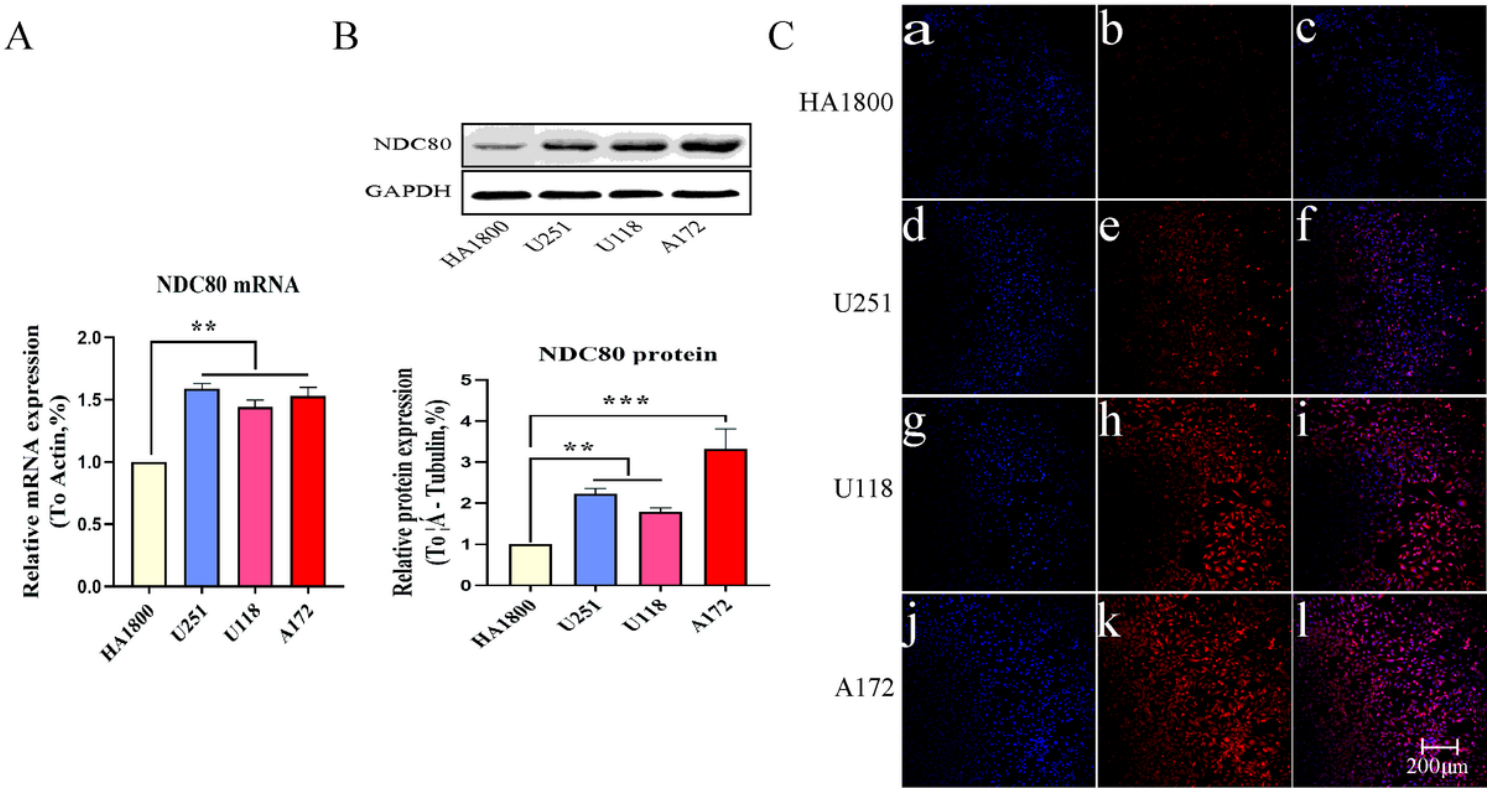

Figure 7

Expression of NDC80 in human glioblastoma cells and normal cells. (A) Transcriptional levels of NDC80 in human glioblastoma cells and normal cells. (B) Translational levels of NDC80 in human glioblastoma cells and normal cells. (C) Immunofluorescence of NDC80. (b), (e), (h) Cy3-immunofluorescence (red) indicates NDC80 was observed in cells. (a), (d), (g) DAPI (blue) indicates nuclear staining in cells. (c), (f), (i) Merged image (magnification × 100).

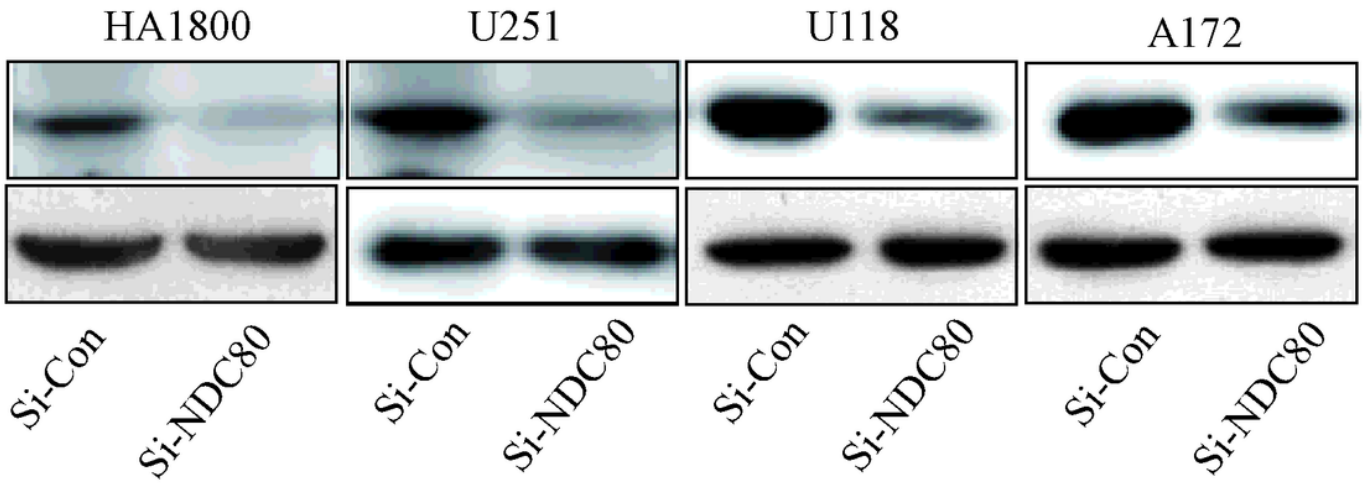

Figure 8

Si-NDC80 can inhibit the protein translation level of human glioblastoma cells and normal astrocytes NDC80. Western blotting detected NDC80 knockdown, NDC80 protein expression levels in human glioblastoma U251, U118, A172 cell lines and normal astrocyte cell line HA1800 were significantly down-regulated.

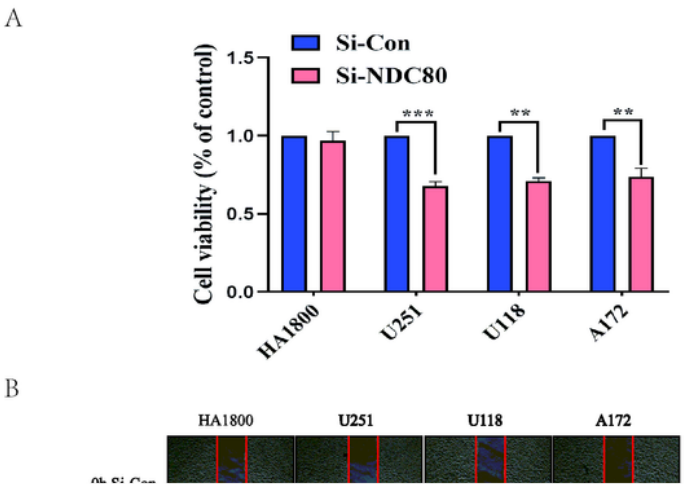

Figure 9

Compare the proliferative ability (OD value) and invasion ability of human glioblastoma cells with normal astrocytes in each group (x±S). A. CCK-8 test suggests that Si-NDC can down-regulate the proliferation of human glioblastoma cells. B. The scratch assay showed that the migration ability of human glioblastoma cells (U251, U118, A172) in the Si-NDC80 group was significantly lower than that of normal astrocytes. C. The number of invasive cells in the Si-NDC80 group of Transwell invading experimental human glioblastoma cells (U251, U118, A172) was significantly lower than that of the control.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supplementaryfile1.tif
- supplementaryfile2.tif