

Candidate genes and microRNAs for glioma pathogenesis and prognosis based on gene expression profiles

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Abstract. Glioma is the most common malignant brain tumor, and the incidence of glioma demonstrates an upward trend. It is vital to elucidate the pathogenesis of glioma and seek effective therapies. The aim of the present study was to identify the potential gene markers associated with glioma based on GSE31262 gene expression profiles, and to explore the underlying mechanism of glioma progression by analyzing the gene markers. The microarray dataset GSE31262 was downloaded and neural stem cell samples (control group) and glioma samples (glioma group) were analyzed to identify the differentially expressed genes (DEGs) between the two groups. Gene Ontology functional and Kyoto Encyclopedia of Genes and Genomes pathway enrichment analyses were performed using DAVID software. Subsequently, a protein-protein interaction (PPI) network was constructed and important modules were extracted from this network. Additionally, the miRNA-target regulatory network was established. In total, 1377 DEGs with $P < 0.01$ and $|\log_2 \text{fold change}| \geq 2$ were identified between the control and glioma groups. The DEGs that were upregulated in glioma samples compared with controls were primarily associated with functions such as the M phase and cell cycle pathway, while the downregulated genes were associated with functions such as nerve impulse and the axon guidance pathway. The results also indicated that certain DEGs, including cyclin-dependent kinase 1 (CDK1) and cadherin 1 (CDH1), had important roles in the PPI network. The MCODE tool in Cytoscape software was used to identify upregulated and downregulated modules in the PPI network, and 5 upregulated

and 2 downregulated modules were extracted. Furthermore, the WebGestalt online tool was used to identify potential interactions of the upregulated and downregulated genes with microRNAs (miRNA/miR), and miR-135A/B and its two targets, discs large MAGUK scaffold protein 2 and forkhead box O1 (FOXO1), had the highest number of connections in the miRNA-target regulatory network. In addition, cell division cycle 20 and FOXO1 were confirmed to be upregulated in U87 glioma cells compared with normal human astrocytes (HA1800) by reverse transcription-quantitative polymerase chain reaction. In conclusion, M phase function and the axon guidance pathway may be vital for glioma progression. In addition, CDK1 and CDH1 may be associated with the process of glioma. Furthermore, miR-135A/B, and the target FOXO1, may be potential therapy targets for glioma treatment.

Introduction

Glioma is the most common malignant brain tumor, and inflicts personal distress and social and financial burdens (1). The incidence of glioma is increasing and the median survival rate for patients with glioblastomas is <2 years (2,3). Although certain techniques based on oncolytic viral therapy and neural precursor cells are promising in the treatment of glioma, their therapeutic effects are limited (4-6). Despite improvements in current therapeutic strategies, the overall prognosis for patients with glioma remains poor (7,8). Therefore, novel therapies are required to specifically target tumor cells, particularly cells that have important roles in the potential pathogenesis of glioma.

Genes and genetic factors are strongly associated with the development of glioma (9). Gene expression analyses may be used to investigate the biomarkers and potential therapeutic targets for glioma (10). Gene expression-based classification of malignant glioma is thought to be a better indicator of survival than histological classification (11). A previous study demonstrated that downregulation of cyclin-dependent kinase 1 (CDK1) expression may inhibit the proliferation of human malignant glioma (12). In addition, lentivirus-mediated knockdown of cyclin Y inhibits the proliferation of glioma cells (13). Furthermore, large-scale gene expression analysis

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and subset analysis of glioma revealed unrecognized heterogeneity of tumors and were efficient methods of predicting prognosis-associated genes (14).

A microRNA (miRNA/miR) is a small non-coding RNA molecule that functions in RNA silencing and post-transcriptional regulation of gene expression (15). In total, >50% of mRNAs are regulated by miRNAs, and one miRNA may target hundreds of different genes (16). Of all miRNA species, ~60% are present in the brain (17). As a result, miRNAs are reported to be involved in various functions associated with the brain, including learning, memory, neurological diseases (18) and neuroprotection (19). It was previously reported that the dysregulation of miRNA-21 and miRNA-10b may disrupt the migration of glioma cells and inhibit glioma cell migration and invasion (20). In addition, miRNA-16 was reported to suppress epithelial-mesenchymal transition-associated gene expression in human glioma (21). Therefore, alterations in miRNA expression may have an important role in glioma progression (22). However, potential gene markers associated with glioma based on gene or miRNA expression remain unclear.

Sandberg *et al.* (23) performed genome-wide analysis that directly compared the gene expression profile of glioblastoma stem cells from patients with glioma to stem cells from the normal adult. The study revealed 30 signature genes that were associated with clinical outcome and demonstrated the clinical relevance of glioblastoma stem cells in glioma. However, based on the large amount of information in the gene expression profile, the data concerning the role of potential gene markers based on genes or miRNAs in glioma were limited. In the present study, bioinformatics analysis based on the microarray data deposited by Sandberg *et al.* (23) was performed. Function and pathway analysis based on differentially expressed genes (DEGs) between neural stem cell samples (control group) and glioma samples (glioma group) was performed, followed by protein-protein interaction (PPI) network analysis. Subsequently, analysis was performed to identify potential miRNA-target regulation in the process of glioma. The present study aimed to determine a detailed mechanism of transcriptional regulation in glioma, and provide a novel strategy for therapy based on a comprehensive understanding of glioma progression.

Materials and methods

Samples. The gene expression profile of GSE31262 (23) was downloaded from the Gene Expression Omnibus (<http://www.ncbi.nlm.nih.gov/geo/>), which was based on the platform of GPL2986 ABI Human Genome Survey Microarray Version 2. In total, 5 neural stem cell samples (control group) and 9 glioma samples (glioma group) were included in this profile. Annotation information of all probe sets was also downloaded from the platform.

Data preprocessing and differential expression analysis. Probe-level data in CEL files were converted into expression values. In the case that >1 probe corresponded to one single gene, the average value was considered as the final gene expression. The missing values were imputed based on the estimation method of Troyanskaya *et al.* (24) and the complete data were subsequently standardized (25).

The non-pairing t-test in the Linear Models for Microarray Data (Limma; version 3.10.3; <http://www.bioconductor.org/packages/release/bioc/html/limma.html>) package in R language (version 3.3.2) (26) was performed to calculate the statistical significance for each gene. $P < 0.01$ and $|\log_2 \text{fold change (FC)}| \geq 2$ were selected as the threshold for the identification of DEGs. Hierarchical clustering analysis of DEGs was conducted using pheatmap package (version 1.0.8; <https://cran.r-project.org/web/packages/pheatmap/index.html>) in R (version 3.3.2) software (27).

Functional and pathway enrichment analysis of DEGs.

Based on the deficiency of individual gene analysis, gene set enrichment analysis was performed to evaluate differential expression patterns of gene groups. The Database for Annotation, Visualization and Integrated Discovery (DAVID; version 6.8; <http://david.abcc.ncifcrf.gov/>) is a gene functional classification tool that provides a comprehensive set of functional annotation tools for investigators to understand the biological meaning behind large lists of genes (28,29). Gene Ontology (GO) includes three functional categories: Molecular function (MF); biological process (BP); and cellular component (CC) (30). GO functional enrichment (31) was performed based on DAVID. The Kyoto Encyclopedia of Genes and Genomes (KEGG; <http://www.genome.jp/kegg/>) pathway database (32) comprises a collection of manually drawn pathway maps concerning molecular interaction and reaction networks. In the present study, KEGG pathway enrichment analysis was performed based on DAVID. A count (the number of DEGs associated with a target function or pathway) ≥ 2 and $P < 0.05$ were selected as the cut-off criteria for significant target functions and pathways.

PPI network and module network analysis. The investigation of PPIs may aid the identification of protein functions at the molecular level and improve the understanding of various cellular activities, including growth, development, metabolism, differentiation and apoptosis (33). The Search Tool for the Retrieval of Interacting Genes (STRING; www.string-db.org/) is a database that provides experimental and predicted interaction information (34). The proteins associated with DEGs were selected according to the STRING database (version 10.0). When the required confidence (score) was > 0.4 , there was a protein-protein interaction, which was selected to establish the PPI network. The network was visualized by Cytoscape (version 3.2.0) software (35). Subsequently, the MCODE (version 1.4.2; <http://apps.cytoscape.org/apps/MCODE>) tool in Cytoscape (36) was used to analyze the clusters of the PPI network.

miRNA-target gene network construction. The potential targets of miRNAs were investigated by using WebGestalt (<http://www.webgestalt.org/option.php>) (37,38) software. The miRNA-target regulatory network was constructed with miRNAs that were associated with the top15 upregulated and the top15 downregulated DEGs, and was visualized by Cytoscape software.

Validation of gene expression by reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Based on the

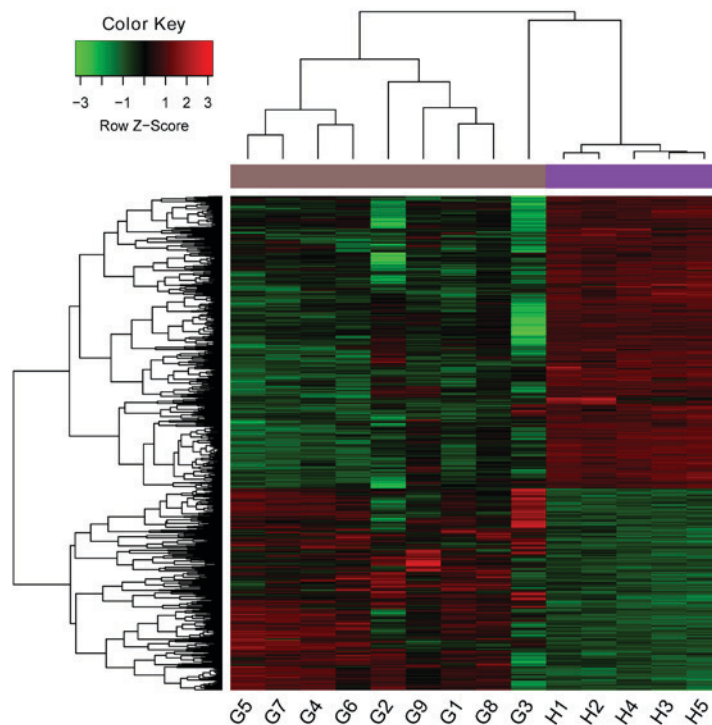


Figure 1. Heatmap of differentially expressed genes between neural stem cell samples and glioma stem cell samples. Red represents upregulated genes and green represents downregulated genes. G1-9, glioma stem cell samples; H1-5, neural stem cell samples.

results of bioinformatics analysis, the major DEGs were validated by RT-qPCR. Normal human astrocytes (HA1800) and U87 glioma cells were purchased from Cell Bank of Chinese academy of science (Shanghai, China), and cultured in the RPMI-1640 medium (Thermo Fisher Scientific, Inc., Waltham, MA, USA) and Dulbecco's modified Eagle's medium (Thermo Fisher Scientific, Inc.), respectively, supplemented with 10% fetal bovine serum (FBS; Gibco, Carlsbad, CA, USA), 1% streptomycin and 1% penicillin (Gibco; Thermo Fisher Scientific, Inc.) at 37°C in a humidified incubator containing 5% CO₂. Following the HA1800 and U87 glioma cells being serially passaged for 3 generations, total RNA was extracted from the cells using RNAiso Plus (Takara Biotechnology Co., Ltd., Dalian, China) reagent to detect the mRNA expression of DEGs. The total RNA was reversed transcribed using a PrimeScript RT Master Mix (Perfect Real-Time; Takara Bio, Inc., Otsu, Japan) according to the following procedure: 37°C for 1 h and 85°C for 5 sec. Following cDNA synthesis, mRNA expression levels were assessed using the Power SYBR Green PCR Master Mix (Applied Biosystems; Thermo Fisher Scientific, Inc.) and the GAPDH transcript was used as the reference gene. The qPCR protocol was: Denaturation (95°C for 3 min), cycling (95°C for 10 sec), annealing (60°C for 30 sec) for 40 cycles at a qPCR machine (model: ViiA7; Applied Biosystems; Thermo Fisher Scientific, Inc.). All experiments were carried out in triplicate. Relative gene expression was calculated using the 2^{-ΔΔC_q} method (39). The primer sequences for the genes were as follows: CDK1, 5'-CCCTTAGCGG GATCTACC-3' (forward) and 5'-ATGGCTACCACTTGA CCTGT-3' (reverse); cell division cycle 20 (CDC20), 5'-CAG CATCAAGGGGCTGTCAA-3' (forward) and 5'-GAGACC AGAGGATGGAGCAC-3' (reverse); polo-like kinase 1 (PLK1), 5'-CTGCCTGACCATTCCACCAA-3' (forward)

and 5'-CCTCACCTGTCTCTCGAACC-3' (reverse); aurora kinase A (AURKA), 5'-CTCCGTCCCTGAGTGTCC TT-3' (forward) and 5'-AAATATCCCCGCACTCTGGC-3' (reverse); cadherin 1 (CDH1), 5'-CGAGAGCTACACGTT CACGG-3' (forward) and 5'-TTTGAATCGGGTGTCTGAG GG-3' (reverse); forkhead box O1 (FOXO1), 5'-TCAAGAGCG TGCCCTACTTC-3' (forward) and 5'-TCTTGCCACCCT CTGGATTG-3' (reverse); G-protein subunit α1 (GNAI1), 5'-ACAGGGTTCTGTCTCCGCTG-3' (forward) and 5'-TCC CCATAGCCCTAATGATAGC-3' (reverse); and GAPDH, 5'-TGACAACTTTGGTATCGTGGAAGG-3' (forward) and 5'-AGGCAGGGATGATGTTCTGGAGAG-3' (reverse).

Statistical analysis. Data are presented as the mean ± standard deviation. Differences between groups were analyzed statistically by Student's t-test using SPSS 19.0 software (IBM Corp., Armonk, NY, USA). $P < 0.05$ was considered to indicate a statistically significant difference.

Results

DEGs between glioma and control groups. Under the conditions of $P < 0.01$ and $|\log_2 \text{FC}| \geq 2$, a total of 1377 DEGs, including 562 upregulated and 815 downregulated genes, were identified. The heatmap constructed for DEGs in different samples is presented in Fig. 1.

GO function and KEGG pathway enrichment analyses. GO function and KEGG pathways enriched with DEGs were investigated with DAVID. The results of GO analysis demonstrated that upregulated DEGs were primarily associated with functions that included M phase ($P = 4.94 \times 10^{-30}$), spindle ($P = 1.73 \times 10^{-17}$) and chromatin binding ($P = 1.32 \times 10^{-5}$; Table I).

Table I. Top five significant BP, CC and MF items associated with genes that were differentially expressed between control and glioma samples in the GSE31262 microarray.

A, Top 5 BP, CC and MF items significantly associated with differentially expressed genes (DEGs) that were upregulated in glioma samples compared with controls

Item type	Function ID	Function name	Count	P-value	Gene
BP	GO:0000279	M phase	62	4.94×10^{-30}	KIF23, PRC1, TTK, PTTG2, AURKA
	GO:0022403	Cell cycle phase	68	2.81×10^{-29}	KIF23, PRC1, DBF4, TTK, AURKA
	GO:0000280	Nuclear division	49	4.88×10^{-27}	KIF23, NEK2, AURKA, PTTG2, AURKB
	GO:0007067	Mitosis	49	4.88×10^{-27}	KIF23, NEK2, AURKA, PTTG2, AURKB
	GO:0007049	Cell cycle	89	9.74×10^{-27}	GAS2L3, KIF23, STEAP3, PRC1, ZAK
CC	GO:0005819	Spindle	31	1.73×10^{-17}	KIF23, KIF4A, PRC1, NEK2, TTK
	GO:0000793	Condensed chromosome	29	3.84×10^{-17}	HMGB2, NEK2, CHEK1, AURKB, RCC1
	GO:0005694	Chromosome	46	6.87×10^{-13}	HMGB2, HIST1H4K, HMGB3, NEK2, CHEK1
	GO:0000775	Chromosome, centromeric region	24	1.01×10^{-12}	MKI67, NUF2, CENPF, NDC80, CENPE
MF	GO:0044427	Chromosomal part	40	9.95×10^{-12}	HIST1H4K, CHEK1, AURKB, RCC1, CITED2
	GO:0003682	Chromatin binding	17	1.32×10^{-5}	CDC6, EZH2, CENPF, GLI2, RCC1
	GO:0004674	Protein serine/threonine kinase activity	29	1.30×10^{-4}	STK33, ZAK, NEK2, TTK, CHEK1
	GO:0001882	Nucleoside binding	75	1.67×10^{-4}	STEAP3, ACOX2, KIF23, STK33, ZAK
	GO:0001883	Purine nucleoside binding	74	2.26×10^{-4}	STEAP3, ACOX2, KIF23, STK33, ZAK
	GO:0030554	Adenyl nucleotide binding	73	2.43×10^{-4}	STEAP3, ACOX2, KIF23, STK33, ZAK

B, Top 5 BP, CC and MF items significantly associated with DEGs that were downregulated in glioma samples compared with controls

Item type	Function ID	Function name	Count	P-value	Gene
BP	GO:0019226	Transmission of nerve impulse	41	4.62×10^{-9}	CAV2, KCNMB4, SCN1B, AGTPBP1, SYT5
	GO:0007268	Synaptic transmission	34	2.25×10^{-7}	KCNMB4, CAV2, SCN1B, AGTPBP1, SYT5
	GO:0007155	Cell adhesion	56	2.89×10^{-6}	DLC1, EDIL3, AZGP1, CD47, TYRO3
	GO:0022610	Biological adhesion	56	2.92×10^{-6}	DLC1, EDIL3, AZGP1, CD47, TYRO3
	GO:0001508	Regulation of action potential	14	3.32×10^{-6}	KLK6, KCNMB4, PLP1, GRIN2A, TAC1
CC	GO:0044459	Plasma membrane part	150	5.97×10^{-10}	DLC1, ATP1B1, SEC31B, SYT5, EFNA1
	GO:0005886	Plasma membrane	217	7.49×10^{-8}	DLC1, ATP1B1, SEC31B, SYT5, EFNA1
	GO:0048471	Perinuclear region of cytoplasm	33	5.51×10^{-7}	CAV2, SH3RF1, PRKCZ, TF, SYT5
	GO:0030054	Cell junction	45	7.75×10^{-6}	DLC1, CAV2, PRKCZ, SYT5, GABBR1
	GO:0016323	Basolateral plasma membrane	22	1.38×10^{-4}	DLC1, TF, CAV2, ATP1B1, ERBB4
MF	GO:0008092	Cytoskeletal protein binding	46	8.21×10^{-7}	NDN, ABLIM3, ALDOB, SNCA, PXX
	GO:0008289	Lipid binding	40	9.23×10^{-6}	PRKCZ, RBP7, SNAP91, PREX1, SNCA
	GO:0019899	Enzyme binding	39	4.89×10^{-4}	GLRX3, CAV2, PRKCZ, PREX1, ALDOB
	GO:0030695	GTPase regulator activity	32	6.50×10^{-4}	DLC1, CYTH1, PREX1, RASGEF1B, RTKN
	GO:0019911	Structural constituent of myelin sheath	4	6.54×10^{-4}	PLP1, MOBP, MAL, MBP

BP, biological process; CC, cellular component; MF, molecular function; count, the number of DEGs in the target item; $P < 0.05$ was considered to indicate statistical significance.

Table II. Pathways that upregulated and downregulated differentially expressed genes (DEGs) were significantly enriched in.

A, Pathways that were significantly associated with DEGs that were upregulated in glioma samples compared with controls in the GSE31262 microarray

Pathway ID	Pathway name	Count	P-value	Gene
hsa04110	Cell cycle	24	2.18×10^{-12}	CDC6, CDK1, DBF4, SKP2, TTK
hsa04115	p53 signaling pathway	11	3.88×10^{-5}	STEAP3, CCNB1, CDK1, CCNE1, CCNB2
hsa04510	Focal adhesion	18	1.41×10^{-4}	EGFR, COL4A2, VAV3, COL4A1, TNXB
hsa05200	Pathways in cancer	24	1.68×10^{-4}	EGFR, CEBPA, COL4A2, COL4A1, SKP2
hsa05222	Small cell lung cancer	10	1.08×10^{-3}	LAMA1, CCNE1, COL4A2, COL4A1, SKP2
hsa04512	ECM-receptor interaction	10	1.08×10^{-3}	LAMA1, COL4A2, TNXB, COL4A1, TNC
hsa04914	Progesterone-mediated oocyte maturation	10	1.27×10^{-3}	CCNB1, CDK1, MAD2L1, CCNB2, PLK1
hsa04114	Oocyte meiosis	11	2.04×10^{-3}	CCNB1, CDK1, CCNE1, MAD2L1, CCNB2
hsa04810	Regulation of actin cytoskeleton	14	1.56×10^{-2}	EGFR, VAV3, DIAPH1, BAIAP2, DIAPH3
hsa05212	Pancreatic cancer	7	2.36×10^{-2}	EGFR, VEGFA, PIK3R3, EGF, TGFB1
hsa04670	Leukocyte transendothelial migration	9	2.97×10^{-2}	VCAM1, CYBB, VAV3, ACTN1, RAPGEF4
hsa05219	Bladder cancer	5	4.02×10^{-2}	EGFR, VEGFA, EGF, MMP2, DAPK1
hsa05210	Colorectal cancer	7	4.55×10^{-2}	EGFR, BIRC5, FZD3, PIK3R3, FZD7

B, Pathways that were significantly enriched with DEGs that were downregulated in glioma samples compared with controls in the GSE31262 microarray

Pathway ID	Pathway name	Count	P-value	Gene
hsa04360	Axon guidance	13	1.16×10^{-2}	PLXNB1, GNAI1, EFNA1, ABLIM3, PLXNB3
hsa04514	Cell adhesion molecules (CAMs)	13	1.38×10^{-2}	MAG, PTPRM, SELL, NRXN3, NFASC
hsa04144	Endocytosis	15	3.34×10^{-2}	FGFR2, STAMBP, SH3GL3, PRKCZ, PLD1

Count, the number of DEGs in the target item. $P < 0.05$ was considered to indicate statistical significance.

Meanwhile, downregulated DEGs were primarily enriched in functions such as transmission of nerve impulse ($P = 4.62 \times 10^{-9}$), plasma membrane part ($P = 5.97 \times 10^{-10}$) and cytoskeletal protein binding ($P = 8.21 \times 10^{-7}$; Table I). Furthermore, the KEGG pathway enrichment analysis indicated that upregulated DEGs were enriched in pathways such as cell cycle ($P = 2.18 \times 10^{-12}$), while downregulated genes were enriched in axon guidance pathway ($P = 1.16 \times 10^{-2}$; Table II). The DEGs listed in Tables I and II were selected based on P-value and $-\log_2$ FCI value of DEGs.

PPI network and module networks. A PPI network was constructed based on the aforementioned selection criterion, which contained a total of 2422 protein interactions and 418 DEGs. Subsequently, the top 15 nodes, including the upregulated CDK1 (degree=88) and downregulated CDH1 (degree=41), were highlighted in the PPI network (Table III). The MCODE tool in Cytoscape software subsequently revealed 5 upregulated modules (Fig. 2) and 2 downregulated modules (Fig. 3). The results demonstrated that in total, 78 upregulated genes, including angiotensinogen (AGT), PLK1 and CDK1, were involved in the 5 upregulated modules, while 24

downregulated genes, including CDH1, GNAI1 and G-protein subunit $\alpha 01$, were involved in the 2 downregulated modules.

miRNA-target regulatory network analysis. The miRNA-target regulatory network was constructed with miRNA that were associated with upregulated and downregulated DEGs (top 15 according to the degree). The results demonstrated that in total, 15 miRNA and 11 downregulated genes were present in the network (26 nodes and 32 interactions; Fig. 4). The genes in the transcriptional regulatory network were all downregulated. Discs large MAGUK scaffold protein 2 (DLG2; targeted by miRNAs including miR-218, miR-135A and miR-135B), FOXO1 (targeted by miRNAs including miR-135A, miR-135B and miR-493) and GNAI1 (targeted by miRNAs including miR-218, miR-524 and miR-506) were 3 notable genes with the highest number of interactions (Fig. 4).

Validation of gene expression. Based on the results of bioinformatics analysis, CDK1, CDC20, PLK1, AURKA, CDH1, FOXO1 and GNAI1 were selected to confirm the mRNA expression in U87 glioma cells and HA1800 by RT-qPCR. The mRNA expression of CDC20 ($P < 0.01$), FOXO1 ($P < 0.01$)

Table III. Top 15 gene nodes in the protein-protein interaction network.

A, Top nodes among genes that were significantly upregulated in glioma samples compared with controls in the GSE31262 microarray

Gene	Degree
CDK1	88
CDC20, PLK1, TOP2A	77
CDK2	74
CCNB1	73
AURKB	72
CCNB2	71
BIRC5	69
PCNA, MAD2L1	67
BUB1	66
AURKA	65
CCNA2	60
KIF11	56

B, Top nodes among genes that were downregulated in glioma samples compared with controls in the GSE31262 microarray

Gene	Degree
CDH1	41
DLG2	30
RHOG, ERBB4	25
ERBB3	23
MBP	22
GNAI1, SOX10, NGFR	21
RHOU, FOXO1	20
TUBB4A, TJP1	19
PRKCZ	18
PRKCB	17

and PLK1 ($P<0.05$) was significantly upregulated in the U87 glioma cells compared with control cells (Fig. 5). Meanwhile, the expression of CDK1, AURKA, CDH1 and GNAI1 were detected in HA1800 cells and not in U87 glioma cells, which indicated that CDK1, AURKA, CDH1 and GNAI1 were downregulated in U87 glioma cells (data not shown). The upregulation of CDC20, PLK1 and FOXO1 detected by RT-qPCR in the present study was consistent with the results of the bioinformatics analysis.

Discussion

Glioma is responsible for a substantial number of mortalities worldwide, however, the potential molecular mechanism is not fully understood and effective therapeutic strategies are limited (40). The present bioinformatics study revealed 1377 DEGs between the control and the glioma groups. The upregulated and downregulated DEGs were primarily associated

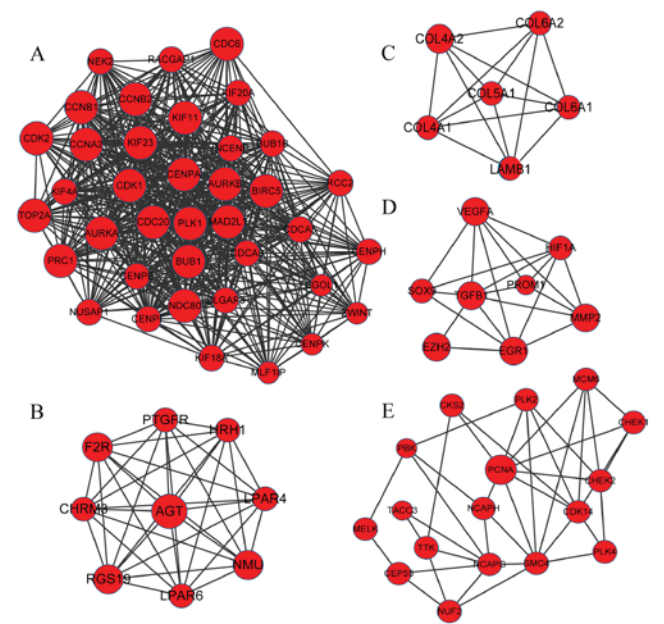


Figure 2. Upregulated modules in the PPI network. A total of 5 upregulated modules were identified in the PPI network using the MCODE tool in Cytoscape software. Module (A) had 38 nodes and 557 interactions. Module (B) had 9 nodes and 36 interactions. Module (C) had 6 nodes and 15 interactions. Module (D) contained 8 nodes and 20 interactions, and Module (E) contained 17 nodes and 39 interactions. Red circles represent upregulated genes. PPI, protein-protein interaction.

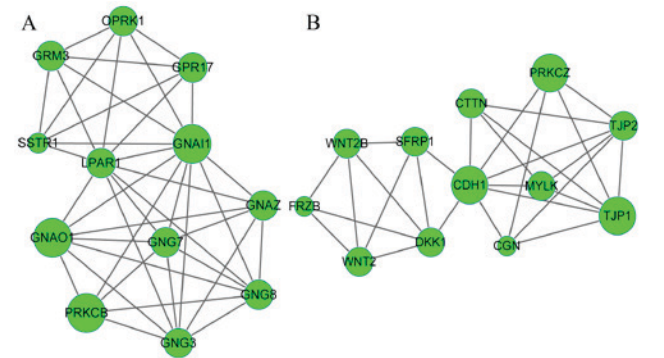


Figure 3. Downregulated modules in the PPI network. A total of 2 downregulated modules were identified in the PPI network using the MCODE tool in Cytoscape software: Module (A) had 12 nodes and 40 interactions; Module (B) had 12 nodes and 28 interactions. Green circles represent downregulated genes. PPI, protein-protein interaction.

with mitotic (M) phase and transmission of nerve impulse functions, respectively, while the major pathways associated with upregulated and downregulated genes were cell cycle and axon guidance, respectively. Certain DEGs, including CDK1 and CDH1, were highly involved in the PPI network. A total of 5 upregulated modules and 2 downregulated modules were identified based on the PPI network. DLG2, FOXO1 and GNAI1 were the three genes with the highest number of interaction in the transcription regulatory network that consisted of DEGs and miRNAs. In addition, CDC20 and FOXO1 were confirmed to be upregulated in U87 glioma cells compared with control cells by RT-qPCR, which was consistent with the results of the bioinformatics analysis.

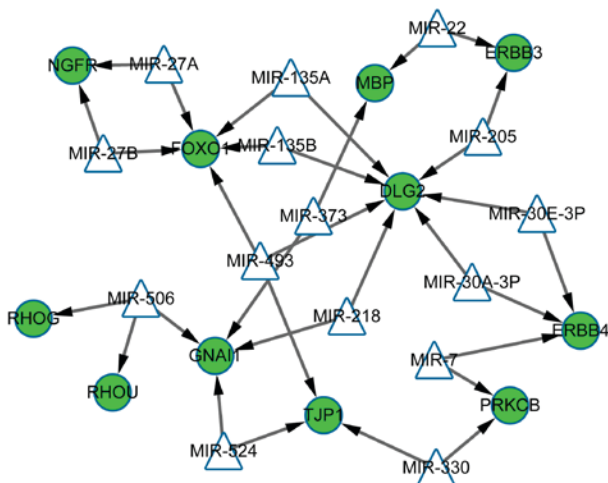


Figure 4. A miRNA-target regulatory network was constructed using the WebGestalt tool. The miRNA-target regulatory network consisted of downregulated genes and their associated miRNAs. Green circles represent downregulated genes and triangles represent miRNAs. miRNA, microRNA; MIR, microRNA.

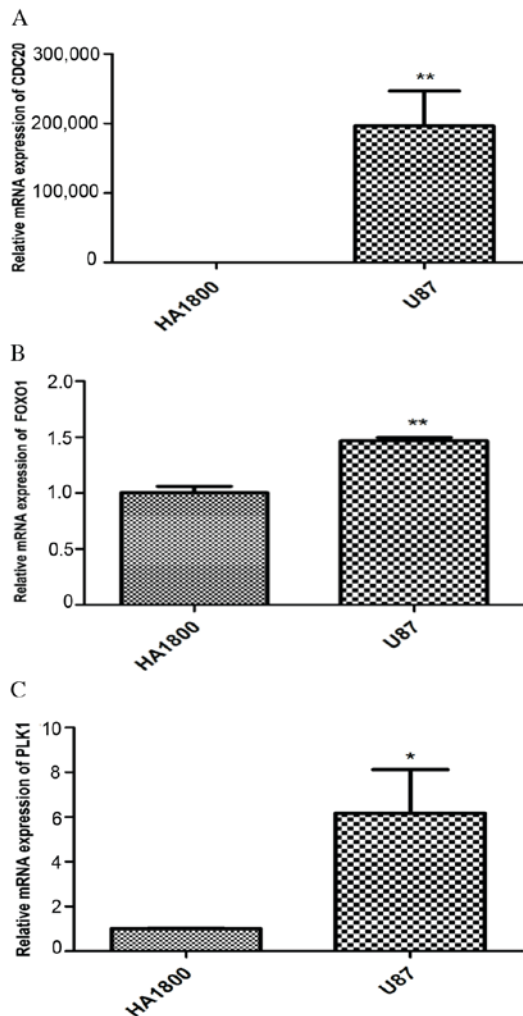


Figure 5. The mRNA expression of CDC20, FOXO1 and PLK1 was investigated in U87 glioma cells and control cells by RT-qPCR. The relative mRNA expression of (A) CDC20, (B) FOXO1 and (C) PLK1, as determined by RT-qPCR. * $P < 0.05$ and ** $P < 0.01$ vs. normal human astrocytes (HA1800). CDC20, cell division cycle 20; FOXO1, forkhead box O1; PLK1, polo-like kinase 1; RT-qPCR, reverse transcription-quantitative polymerase chain reaction.

Mitosis and cytokinesis comprise the M phase of an animal cell cycle (41). Through the use of mitosis-specific phosphorylated antibodies, Maeda *et al* (42) demonstrated that morphological assessment is important to identify the development of multinucleated giant cells in glioma. Conde *et al* (43) reported that the overexpression of survivin contributed to chromosomal instability, and the understanding of this biological mechanism may promote further study for anti-cancer therapeutic approaches in glioma. In addition, glioma tumor progression was reduced by blocking mitosis via mitotic spindle catastrophe (44). In the present study, the genes that were upregulated in glioma samples were primarily enriched in M phase. Meanwhile, pathway analysis revealed that the upregulated genes were primarily enriched in cell cycle, which was consistent with the functional analysis. Notably, CDK1 was a common key point gene in M phase and cell cycle. CDK1 is a highly conserved protein that has key functions in cell cycle regulation (45). A previous study demonstrated that the differential expression or silencing of CDK1 was associated with the malignant phenotype of glioma cells (12). Therefore, CDK1 in glioma may be useful in estimating the malignant degree of glioma (46). The PPI network analysis in the present study revealed that CDK1 was the most significantly upregulated gene, with the highest degree, which indicated that it may be a key target gene for further investigation in glioma. Therefore, we hypothesized that the upregulation genes such as CDK1 in M phase function and cell cycle pathway may have important roles in the process of glioma. Furthermore, various neurological disorders are characterized by structural alterations in neuronal connections, which range from presymptomatic synaptic alterations to the loss or rewiring of whole axon bundles (47). A previous study indicated that re-expression of the leucine-rich glioma inactivated 1 gene in glioma cells resulted in the dysregulation of genes implicated in the canonical axon guidance pathway (48). In the current study, the downregulated genes were primarily enriched in axon guidance pathway, which indicates that this pathway may be vital for glioma progression. Although the downregulation of CDH1, which was demonstrated to be important based on the PPI network, was not enriched in the axon guidance pathway, CDH1 is an important tumor suppressor gene (49). A retrospective study concerning the protein expression and epigenetic inactivation of CDH1 in patients with low-grade glioma reported that hypermethylation of the CDH1 promoter was significantly associated with reduced E-cadherin expression and the survival of patients with glioma (50). Furthermore, Yang *et al* (51) demonstrated that alterations in the expression of transforming growth factor- β 1 and E-cadherin were associated with the emergence and development of glioma. As CDH1 was the most significantly downregulated gene in the PPI network, CDH1 may be another key target gene that warrants further investigation in glioma.

FOXO1 has functions in the regulation of gluconeogenesis and glycogenolysis by insulin signaling (52). Cheng *et al* (53) reported that curcumin induced G2/M cell cycle arrest and apoptosis by increasing FOXO1 expression. Negative regulation of glioma stem cells may occur via a FOXO1-associated pathway (54). Meanwhile, DLG2 belongs to the membrane-associated guanylate kinase family (55). In the present study, the miRNA-target gene analysis

demonstrated that FOXO1 and DLG2 were the genes with the highest number of connections in the process of glioma. Furthermore, the mRNA expression of FOXO1 was upregulated in U87 glioma cells compared with control cells, as determined by RT-qPCR. Notably, FOXO1 and DLG2 were both targeted by miR-135A and miR-135B. A previous study reported an important role for miR-135A in glioma etiology (56), and Zhang *et al.* (57) demonstrated that reactive oxygen species-upregulated miR-135a had a pivotal role in glioma cell apoptosis. Although miR-135A has been widely reported in previous studies, studies associating miR-135B with glioma are rare. Based on the results of miRNA-target gene analysis in the current study, we hypothesized that miRNA-target gene regulation is vital for the progression of glioma, including the interactions between FOXO1 and miRNAs such as miR-135A/B. These miRNAs and associated target genes may be potential therapy targets for glioma. However, the identification of the functions of these genes is required to confirm this speculation.

In conclusion, the results of the present study indicate that M phase function and axon guidance pathway may be vital for glioma progression, and CKD1 and CDH1 genes may be associated with the process of glioma. Furthermore, FOXO1, and the miRNAs that target FOXO1, including miR-135A/B, may serve as potential therapy targets for glioma.

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References

- Behin A, Hoang-Xuan K, Carpentier AF and Delattre JY: Primary brain tumours in adults. *Lancet* 361: 323-331, 2003.
- Goodenberger ML and Jenkins RB: Genetics of adult glioma. *Cancer Genet* 205: 613-621, 2012.
- Claus EB, Walsh KM, Wiencke JK, Molinaro AM, Wiemels JL, Schildkraut JM, Bondy ML, Berger M, Jenkins R and Wrensch M: Survival and low-grade glioma: The emergence of genetic information. *Neurosurg Focus* 38: E6, 2015.
- Gage FH: Mammalian neural stem cells. *Science* 287: 1433-1438, 2000.
- Snyder EY and Macklis JD: Multipotent neural progenitor or stem-like cells may be uniquely suited for therapy for some neurodegenerative conditions. *Clin Neurosci* 3: 310-316, 1995.
- Ourednik J, Ourednik V, Lynch WP, Schachner M and Snyder EY: Neural stem cells display an inherent mechanism for rescuing dysfunctional neurons. *Nat Biotechnol* 20: 1103-1110, 2002.
- Shah K, Bureau E, Kim DE, Yang K, Tang Y, Weissleder R and Breakefield XO: Glioma therapy and real-time imaging of neural precursor cell migration and tumor regression. *Ann Neurol* 57: 34-41, 2005.
- Yamanaka R, Yajima N, Abe T, Tsuchiya N, Homma J, Narita M, Takahashi M and Tanaka R: Dendritic cell-based glioma immunotherapy (Review). *Int J Oncol* 23: 5-15, 2003.
- Luo Y, Zhu D, Dang DH, Huang J, Tang Y, Luo X and Wang S: A double-switch cell fusion-inducible transgene expression system for neural stem cell-based anti-glioma gene therapy. *Stem Cell Int* 2015: 649080, 2015.
- Wang L, Wei B, Hu G, Wang L, Jin Y and Sun Z: Gene expression analyses to explore the biomarkers and therapeutic targets for gliomas. *Neurol Sci* 36: 403-409, 2015.
- Gravendeel LA, Kouwenhoven MC, Gevaert O, Rooi JJ, Stubbs AP, Duijm JE, Daemen A, Bleeker FE, Bralten LB, Kloosterhof NK, *et al.*: Intrinsic gene expression profiles of gliomas are a better predictor of survival than histology. *Cancer Res* 69: 9065-9072, 2009.
- Chen H, Huang Q, Zhai DZ, Dong J, Wang AD and Lan Q: CDK1 expression and effects of CDK1 silencing on the malignant phenotype of glioma cells. *Zhonghua Zhong Liu Za Zhi* 29: 484-488, 2007 (In Chinese).
- Xu Y, Wang Z, Wang J, Li J, Wang H and Yue W: Lentivirus-mediated knockdown of cyclin Y (CCNY) inhibits glioma cell proliferation. *Oncol Res* 18: 359-364, 2010.
- Freije WA, Castrovargas FE, Fang Z, Horvath S, Cloughesy T, Liao LM, Mischel PS and Nelson SF: Gene expression profiling of gliomas strongly predicts survival. *Cancer Res* 64: 6503-6510, 2004.
- Bartel DP: MicroRNAs: Genomics, biogenesis, mechanism, and function. *Cell* 116: 281-297, 2004.
- Ebert MS and Sharp PA: Roles for microRNAs in conferring robustness to biological processes. *Cell* 149: 515-524, 2012.
- Sempere LF, Freemantle S, Pitha-Rowe I, Moss E, Dmitrovsky E and Ambros V: Expression profiling of mammalian microRNAs uncovers a subset of brain-expressed microRNAs with possible roles in murine and human neuronal differentiation. *Genome Biol* 5: R13, 2004.
- Wang W, Kwon EJ and Tsai LH: MicroRNAs in learning, memory and neurological diseases. *Learn Mem* 19: 359-368, 2012.
- Saugstad JA: MicroRNAs as effectors of brain function with roles in ischemia and injury, neuroprotection and neurodegeneration. *J Cereb Blood Flow Metab* 30: 1564-1576, 2010.
- Gabriely G, Wurdinger T, Kesari S, Esau CC, Burchard J, Linsley PS and Krichevsky AM: MicroRNA 21 promotes glioma invasion by targeting matrix metalloproteinase regulators. *Mol Cell Biol* 28: 5369-5380, 2008.
- Wang Q, Li X, Zhu Y and Yang P: MicroRNA-16 suppresses epithelial-mesenchymal transition-related gene expression in human glioma. *Mol Med Rep* 10: 3310-3314, 2014.
- Malzkorn B, Wolter M, Liesenberg F, Grzendowski M, Stühler K, Meyer HE and Reifenberger G: Identification and functional characterization of microRNAs involved in the malignant progression of gliomas. *Brain Pathol* 20: 539-550, 2010.
- Sandberg CJ, Altschuler G, Jeong J, Strömme KK, Stangeland B, Murrell W, Grasmö-Wendler UH, Myklebost O, Helseth E, Vik-Mo EO, *et al.*: Comparison of glioma stem cells to neural stem cells from the adult human brain identifies dysregulated Wnt-signaling and a fingerprint associated with clinical outcome. *Exp Cell Res* 319: 2230-2243, 2013.
- Troyanskaya O, Cantor M, Sherlock G, Brown P, Hastie T, Tibshirani R, Botstein D and Altman RB: Missing value estimation methods for DNA microarrays. *Bioinformatics* 17: 520-525, 2001.
- Fujita A, Sato JR, Rodrigues Lde O, Ferreira CE and Sogayar MC: Evaluating different methods of microarray data normalization. *BMC Bioinformatics* 7: 469, 2006.
- Smyth GK: Limma: Linear models for microarray data. In: *Bioinformatics and computational biology solutions Using R*, Bioconductor, Gentleman R, Carey VJ, Huber W, Irizarry RA and Dudoit S (eds) Springer New York, New York, NY, pp397-420, 2005.
- Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W and Smyth GK: Limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res* 43: e47, 2015.
- Huang W, Sherman BT and Lempicki RA: Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc* 4: 44-57, 2009.
- Huang DW, Sherman BT and Lempicki RA: Bioinformatics enrichment tools: Paths toward the comprehensive functional analysis of large gene lists. *Nucleic Acids Res* 37: 1-13, 2009.
- Dennis G Jr, Sherman BT, Hosack DA, Yang J, Gao W, Lane HC and Lempicki RA: DAVID: Database for annotation, visualization, and integrated discovery. *Genome Biol* 4: P3, 2003.
- Sherlock G: Gene ontology: Tool for the unification of biology. *Canadian Inst Food Sci Technol J* 22: 415, 2009.
- Ogata H, Goto S, Sato K, Fujibuchi W, Bono H and Kanehisa M: KEGG: Kyoto encyclopedia of genes and genomes. *Nucleic Acids Res* 27: 29-34, 1999.
- Giot L, Bader JS, Brouwer C, Chaudhuri A, Kuang B, Li Y, Hao YL, Ooi CE, Godwin B, Vitols E, *et al.*: A protein interaction map of *Drosophila melanogaster*. *Science* 302: 1727-1736, 2003.

34. Szklarczyk D, Franceschini A, Kuhn M, Simonovic M, Roth A, Minguéz P, Doerks T, Stark M, Müller J, Bork P, *et al*: The STRING database in 2011: Functional interaction networks of proteins, globally integrated and scored. *Nucleic Acids Res* 39: D561-D568, 2011.
35. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B and Ideker T: Cytoscape: A software environment for integrated models of biomolecular interaction networks. *Genome Res* 13: 2498-2504, 2003.
36. Bader GD and Hogue CW: An automated method for finding molecular complexes in large protein interaction networks. *BMC Bioinformatics* 4: 2, 2003.
37. Wang J, Duncan D, Shi Z and Zhang B: WEB-based GENE SeT analysis toolkit (WebGestalt): Update 2013. *Nucleic Acids Res* 41: W77-W83, 2013.
38. Zhang B, Kirov S and Snoddy J: WebGestalt: An integrated system for exploring gene sets in various biological contexts. *Nucleic Acids Res* 33: W741-W748, 2005.
39. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods* 25: 402-408, 2001.
40. Ostrom QT, Bauchet L, Davis FG, Deltour I, Fisher JL, Langer CE, Pekmezci M, Schwartzbaum JA, Turner MC, Walsh KM, *et al*: The epidemiology of glioma in adults: A 'state of the science' review. *Neuro Oncol* 17: 896-913, 2014.
41. Cattin CJ, Düggelin M, Martinez-Martin D, Gerber C, Müller DJ and Stewart MP: Mechanical control of mitotic progression in single animal cells. *Proc Natl Acad Sci USA* 112: 11258-11263, 2015.
42. Maeda K, Mizuno M, Wakabayashi T, Takasu S, Nagasaka T, Inagaki M and Yoshida J: Morphological assessment of the development of multinucleated giant cells in glioma by using mitosis-specific phosphorylated antibodies. *J Neurosurg* 98: 854-859, 2003.
43. Conde M, Wiedemuth R, Schackert G and Temme A: Overexpression of Survivin causes aneuploidy, DNA damage and defective mitosis in glioma cells. The 65th Annual Meeting of the German Society of Neurosurgery (DGNC): 11-14 May, 2014.
44. Santra M, Santra S and Chopp M: Doublecortin reduces glioma tumor progression via blocking mitosis by mitotic spindle catastrophe and inhibition of glioma cell invasion by depolymerization of actin. *Cell Mol Tum Biol* 16: 354-360, 2007.
45. Castedo M, Perfettini JL, Roumier T and Kroemer G: Cyclin-dependent kinase-1: Linking apoptosis to cell cycle and mitotic catastrophe. *Cell Death Differ* 9: 1287-1293, 2002.
46. Chen QF: Expressions of Cyclin B1, CDK1 and 14-3-3 protein in human gliomas and their significance. *Sichuan Med J*, 2009.
47. Battum EY, Brignani S and Pasterkamp RJ: Axon guidance proteins in neurological disorders. *Lancet Neurol* 14: 532-546, 2015.
48. Kunapuli P, Lo K, Hawthorn L and Cowell JK: Reexpression of LGI1 in glioma cells results in dysregulation of genes implicated in the canonical axon guidance pathway. *Genomics* 95: 93-100, 2010.
49. Wong AS and Gumbiner BM: Adhesion-independent mechanism for suppression of tumor cell invasion by E-cadherin. *J Cell Biol* 161: 1191-1203, 2003.
50. D'Urso PI, D'Urso OF, Storelli C, Catapano G, Gianfreda CD, Montinaro A, Muscella A and Marsigliante S: Retrospective protein expression and epigenetic inactivation studies of CDH1 in patients affected by low-grade glioma. *J Neurooncol* 104: 113-118, 2011.
51. Yang L, Liu M, Deng C, Gu Z and Gao Y: Expression of transforming growth factor- β 1 (TGF- β 1) and E-cadherin in glioma. *Tumour Biol* 33: 1477-1484, 2012.
52. Nakae J, Kitamura T, Kitamura Y, Biggs WH III, Arden KC and Accili D: The forkhead transcription factor foxo1 regulates adipocyte differentiation. *Dev Cell* 4: 119-129, 2003.
53. Cheng C, Jiao JT, Qian Y, Guo XY, Huang J, Dai MC, Zhang L, Ding XP, Zong D and Shao JF: Curcumin induces G2/M arrest and triggers apoptosis via FoxO1 signaling in U87 human glioma cells. *Mol Med Rep* 13: 3763-3770, 2016.
54. Zhao X, Liu Y, Jian Z, Liu X, Chen J, Liu L, Ping W and Xue Y: GAS5 suppresses malignancy of human glioma stem cells via a miR-196a-5p/FOXO1 feedback loop. *Biochimica et biophysica acta* 1864: 1605, 2017.
55. Kuo DH, Robinson KG, Layton AC, Meyers AJ and Sayler GS: Transcription levels (amoA mRNA-based) and population dominance (amoA gene-based) of ammonia-oxidizing bacteria. *J Indust Microbiol Biotechnol* 37: 751-757, 2010.
56. Wu S, Lin Y, Xu D, Chen J, Shu M, Zhou Y, Zhu W, Su X, Zhou Y, Qiu P and Yan G: MiR-135a functions as a selective killer of malignant glioma. *Oncogene* 31: 3866-3874, 2012.
57. Zhang T, Shao Y, Chu TY, Huang Hs, Liou YL, Li Q and Zhou H: Reactive oxygen species-upregulated miR-135a plays a pivotal role in phenethyl isothiocyanate-induced rat C6 glioma cell apoptosis. *Int J Clin Exp Pathol* 9: 11, 2016.



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