Long non-coding RNA *DLEU1* promotes cell proliferation of glioblastoma multiforme

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Abstract. Glioblastoma multiforme (GBM) is the most common malignant tumor with high morbidity and mortality. This study investigated the role of long non-coding RNAs (lncRNAs) in glioblastomagenesis progression. Using the GSE2223 and GSE59612 datasets, and RNA sequencing data of GBM from The Cancer Genome Atlas, differentially expressed (DE) genes including DE messenger RNAs (DEmRNAs) and DElncRNAs between GBM and normal controls were identified. Based on the competing endogenous RNA hypothesis, DElncRNA-micro RNA (miRNA)-DEmRNA interactions were obtained by target gene prediction. Gene Ontology (GO) and Kyoto Encyclopedia of Gene and Genomes pathway analysis of DEmRNAs in the DElncRNA-miRNA-DEmRNA network was performed. Expression and function analyses of DElncRNAs were performed by reverse transcription-polymerase chain reaction (RT-PCR) and an established viability assay, respectively. In total, 712 DE genes were identified. Significant upregulation of lncRNA deleted in lymphocytic leukemia 1 *(DLEU1)* was revealed in GBM and a number of other types of cancer. DLEU1 interacted with 315 miRNAs and 105 DEmRNAs. The DEmRNAs were mainly enriched in tumorigenesis-associated GO terms (angiogenesis, positive regulation of cell proliferation, positive regulation of fibroblast apoptotic processes and regulation of neutrophil migration) and pathways (Hippo signaling pathway, cancer pathways, and Wnt signaling pathway). Correlation analysis revealed that mRNA TNF receptor associated factor 4 (TRAF4) was associated with DLEU1 expression. RT-PCR demonstrated that the expression levels of DLEU1 and TRAF4 were increased in GBM tissues. Small interfering RNA demonstrated that silencing DLEU1 downregulated TRAF4. The viability of GBM cells was significantly decreased following RNA interference with DLEU1 and TRAF4 production. The results demonstrate that DLEU1 and TRAF4 is highly expressed in GBM tissues and promotes proliferation of GBM cells. It may act as a competing endogenous RNA and influence tumorigenesis of GBM.

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Key words: lncRNA, glioblastoma, deleted in lymphocytic leukemia 1, tumorigenesis, cell proliferation

Introduction

Gliomas are the most common primary brain tumors. They arise from cancerous brain and spinal cord glial cells (1,2). Glioblastoma multiforme (GBM) is a World Health Organization grade IV glioma (3). It accounts for 56.1% of all gliomas and has a five-year survival rate of only 5.5% (4). Despite a number of treatment methods, including chemotherapeutic, radiological and surgical interventions, there has been no significant change in the therapeutic effect. Novel therapeutic approaches that are more effective are needed and require more knowledge of the pathogenesis of GBM.

Long non-coding RNA (lncRNA) is a type of RNA that contains over 200 nucleotides and that does not encode protein (5,6). LncRNAs serve important roles in human diseases, especially involving tumors (7,8). In gliomas, lncRNAs are involved in the regulation of various biological processes (9). For example, lncRNA nuclear paraspeckle assembly transcript 1 (NEAT1) is highly expressed in GBM tissues and perturbed expression of NEAT1 suppresses cell proliferation and invasion (10). High expression of lncRNA H19 promotes cell migration and enhances angiogenesis (11,12). The expression of HOX transcript antisense RNA lncRNA is increased in GBM and is significantly associated with high grade brain tumors (13). Additionally, as competitive endogenous RNAs (ceRNAs), lncRNAs and messenger RNAs (mRNAs) can regulate one other by competing for the shared microRNAs (miRNAs/miRs) (14,15). LncHERG is an lncRNA that acts as a competing RNA of miR-940. When lncHERG is highly expressed, cell multiplication, invasion and migration are inhibited in GBM (16).

In this study, the gene expression profile in GBM was analyzed using a microarray dataset and RNA-sequencing (RNA-Seq) datasets. Key lncRNAs in GBM were screened. Based on the ceRNA hypothesis (15), the lncRNA-miRNA-mRNA network was constructed and Gene Ontology (GO) and Kyoto Encyclopedia of Gene and Genomes (KEGG) pathway analysis of mRNAs in the network were performed. Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and cell proliferation

assay were used to explore the functional role of the key lncRNAs in GBM.

Materials and methods

Data, sample and cell information. Gene expression profile datasets GSE2223 (17,18) and GSE59612 (19) were obtained from the Gene Expression Omnibus database (https://www.ncbi.nlm.nih.gov/geo/) (20). Gene expression profile of GBM determined by RNA-Seq of the Cancer Genome Atlas (TCGA-GBM) was extracted from the Genomic Data Commons Data Portal (https://portal.gdc.cancer.gov/). The GSE2223 dataset included microarray data of 54 samples. Of these, four GBM adjacent normal brain tissues and 27 GBM tissues were included in the present study. A total of 17 GBM adjacent normal brain tissues and 39 GBM tissues of 92 samples in the GSE59612 dataset were selected in the present study. For the TCGA dataset, five GBM adjacent normal brain tissues and 156 GBM tissues were included.

A total of 10 GBM tissues were collected from GBM patients who were treated surgically at the People's Hospital of Zhangjiajie (Zhangjiajie, China) between April 2015 and March 2017. The 10 GBM adjacent normal brain tissues were obtained from patients with head trauma who underwent surgical treatment in the same hospital. The age of the participants was 30-60 years old, with a mean age of 49.6±6.7 years, and the male:female ratio was 8:6. All samples were collected immediately following surgical resection and frozen rapidly in liquid nitrogen at -70˚C. The experimental study was approved by the hospital's ethics committee and written informed consent was obtained from all participants.

The SHG-44 and U251 GBM cell lines were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA) and maintained in RPMI 1640 medium (Thermo Fisher Scientific, Inc., Waltham, MA, USA) containing 10% fetal bovine serum (GE Healthcare Life Sciences, Logan, UT, USA), penicillin (100 units/ml), and streptomycin (100 ug/ml) and incubated in a 5% CO_2 incubator at 37˚C.

Small interfering si-NC, (si)-DLEU1 and si-tumor necrosis factor receptor-associated factor 4 (TRAF4) was purchased from Shanghai GenePharma Co., Ltd. (Shanghai, China). The si-NC, si-DLEU1, si-TRAF4 and the empty vectors (10 nM) were transfected into SHG-44 and U251 cell line using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.). Silencing was confirmed 12 h after transfection, by reverse transcription-polymerase chain reaction (RT-PCR) and western blot analysis. The specific primer sequences are presented in Table I.

Data analyses. Differentially expressed genes (DEGs) including differentially expressed mRNAs (DEmRNAs) and differentially expressed lncRNAs (DElncRNAs) between GBM and GBM adjacent normal brain tissues of the GSE2223 and GSE59612 datasets with normalized expression were analyzed using the Limma package (version 3.34.6; http://www.bioconductor.org/packages/release/bioc/html/limma.html). DEGs between GBM and GBM adjacent normal brain tissues of the TCGA-GBM were screened using the DESeq package (version 3.34.6, http://www.bioconductor.org/packages/release/bioc/html/DESeq.html). Count data were used to normalize data in the DESeq analysis. The fold-change (FC) of the DEGs, $\log_2$FC and false discovery rate (FDR) were obtained following testing. The cutoff thresholds were set to a $|\log_2 FC| \geq 1$ and FDR <0.05. The overlapping genes of the three datasets and genes displaying consistent regulation were included. Following annotation with Blast2GO5.0 (https://www.blast2go.com/), the lncRNA gene-type DEGs were selected as candidate lncRNAs.

Identification of cancer-associated lncRNAs. Gene Expression Profiling Interactive Analysis (GEPIA; http://gepia.cancer-pku.cn/) was used to analyze expression levels, survival analysis and correlation of genes between tumors, and corresponding normal tissues. The expression levels of candidate lncRNAs between cancer and normal control were analyzed on the GEPIA website, which demonstrated 31 tumors types which have been tested. The parameters for expression levels screening are P-value Cutoff (0.01) and log2FC Cutoff (1) on this site.

Construction of lncRNA-miRNA-mRNA network and correlation analysis of DElncRNAs and DEmRNAs. The integrated miRNA-DEmRNA and miRNA-DElncRNA pairs were simultaneously predicted by miRanda (http://www.microrna.org/microrna/home.do) and RNAhybrid (https://bibiserv.cebitec.uni-bielefeld.de/rnahybrid/), respectively. The parameters were set to energy <-20 and score >150 in miRanda and energy <-25 in RNAhybrid. Only the overlap results of miRanda and RNAhybrid were selected as the miRNA-DEmRNA and miRNA-DElncRNA pairs. The DElncRNA-miRNA-DEmRNA networks were constructed by miRNA-bridges using Cytoscape software (version 3.4.0) (21,22). Correlation analysis of DElncRNAs and DEmRNAs was performed using the GEPIA website.

GO and KEGG pathway analysis. To investigate the underlying functional role of lncRNAs, GO biological processes and KEGG pathway analysis were performed for the mRNAs in lncRNA-miRNA-mRNA interactions with DAVID 6.8 software (https://david.ncifcrf.gov/summary.jsp).

RT-PCR. Total RNA from GBM and normal brain samples was isolated using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. After DNase digestion, RNA quantification and purity were measured by the ratio of 260/280 nm. RNA integrity was measured by 1.2% agarose gel electrophoresis. The reverse transcriptase reaction kit (Applied Biosystems; Thermo Fisher Scientific, Inc.) was used to reverse-transcribe RNA samples at 50˚C for 60 min. RT-PCR was performed in triplicate according to the manufacturer's protocol of SYBR Green PCR Master Mix and reactions were carried using in a PCR Thermal Cycler (Takara Bio, Inc., Otsu, Japan). The conditions were: Initial denaturation at 95˚C for 10 min, followed by 40 cycles of denaturation at 95˚C for 5 sec, and annealing and extension at 55-58˚C for 30 sec. The relative expression levels of genes were calculated as relative quantification, calculated as $2^{-\Delta\Delta Cq}$ (18).

Western blotting. Total proteins were extracted from cells using Radio Immunoprecipitation Assay Lysis Buffer (Beyotime Institute of Biotechnology, Shanghai, China). The

Table I. Sequences for si-NC, si-DLEU1#1, si-DLEU1#2, si-TRAF4#1 and si-TRAF4#2.

Name	Guide	Passenger
si-NC	5'-UUCUCCGAACGUGUCACGUTT-3'	5'-ACGUGACACGUUCGGAGAATT-3'
si-DLEU1#1	5'-UUUUUUGUGCAGUUUCAGCAA-3'	5'GCUGAAACUGCACAAAAAAUC-3'
si-DLEU1#2	5'-UUCCUUUUUGAUAGUAUUCAA-3'	5'GAAUACUAUCAAAAAGGAAAA-3'
si-TRAF4#1	5'-UUAAUAAAUACAAUUCCGGAU-3'	5'CCGGAAUUGUAUUUAUUAAUU-3'
si-TRAF4#2	5'-UUUCAUAGGUGAAACGUGGAU-3'	5'CCACGUUUCACCUAUGAAACA-3'

TRAF4, TNF receptor associated factor 4; DLEU1, deleted in lymphocytic leukemia 1; si, small interfering.

proteins were quantified using the bicinchoninic acid protein assay kit (Shanghai Solarbio Bioscience & Technology Co., Ltd., Shanghai, China). Cell lysates were separated by 10% SDS-PAGE, transferred to polyvinylidene fluoride membranes, and membranes were blocked at room temperature with 5% skimmed milk in TSB Tween-20 (0.05% v/v; TBS-T) for 1 h and incubated with specific antibodies at 4˚C overnight. The primary antibody was a 1:1,000 dilution of rabbit anti-TRAF4 (cat. no. Ab108991; Abcam, Cambridge, UK) or mouse anti-GAPDH (cat. no. AG019; Beyotime Institute of Biotechnology). The membranes were washed three times for 10 min every time with TBS-T, followed by incubation with secondary antibodies goat anti-mouse immunoglobulin (Ig)G (1:1,000; cat. no. A0216; Beyotime Institute of Biotechnology) and goat anti-rabbit IgG (1:2,000; cat. no. Ab6721; Abcam) for 1 h at room temperature. The intensities of the immunoreactivity were detected with an enhanced chemiluminescence kit (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The images were developed on X-ray film. The experiments were repeated ≥3 times.

Cell proliferation assay. A MTT kit (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) was used to analyze cell proliferation according to the manufacturer's protocol. All the cells were cultured in 96-well plates in the dark, the formazan was dissolved with dimethyl sulfoxide (DMSO) and the absorbance value at 570 nm was detected every 24 h.

Statistical analyses. SPSS 17.0 software (SPSS, Inc., Chicago, IL, USA) was used to analyze statistical significance. All the experiments were independently performed three times. The data are expressed as the mean ± standard deviation. The difference between the groups was analyzed with an analysis of variance (ANOVA) or Student's t test. Post hoc tests were performed using a Tukey test following the ANOVA. * and ** refer to the statistically significant difference of expression ($P<0.05$) and extremely significant difference of expression ($P<0.01$), respectively. $P<0.005$ was considered to indicate a statistically significant difference.

Results

Analysis of DEGs. A total of 730 overlapping DEGs were screened from the GSE2223, GSE59612 and TCGA-GBM datasets (Fig. 1A). Among these, 712 DEGs displaying consistent regulation in all the three datasets were selected for further study. The annotation analysis revealed three lncRNAs, 36 miscellaneous RNAs (miscRNAs) and 673 mRNAs (Fig. 1B). LncRNA DLEU1 was upregulated, while prostate androgen-regulated transcript 1 (PART1) and miR7-3HG were downregulated.

Identification of cancer-associated lncRNAs in GBM. Among the DElncRNAs, the expression of DLEU1 was upregulated in GBM and a number of other tumor types, including cervical squamous cell carcinoma and endocervical adenocarcinoma, colon adenocarcinoma, lymphoid neoplasm diffuse large B-cell lymphoma, brain lower grade glioma, lung squamous cell carcinoma, ovarian serous cystadenocarcinoma, pancreatic adenocarcinoma, rectum adenocarcinoma, stomach adenocarcinoma, thymoma, and uterine carcinosarcoma (Fig. 2). The results highlighted the important role of DLEU1 in carcinogenesis.

LncRNA-miRNA-mRNA network. The integrated DLEU1-miRNA-DEmRNA interactions were identified with the miRanda and RNAhybrid methods. The network was constructed using Cytoscape software (Fig. 3). In the lncRNA DLEU1-mediated ceRNA network, DLEU1 interacted with as many as 315 miRNAs and 105 DEmRNAs. Among the miRNAs, miR-107, miR-1179, miR-133a, miR-133b and miR-346 have been reported to be downregulated, and are involved in the progression of in GBM.

GO and pathway analysis. To investigate the functional role of genes in the DLEU1-miRNA-DEmRNA network, GO and pathway analysis of mRNAs were performed with DAVID 6.8 software (23,24). The top 10 GO terms and pathway terms are presented in Fig. 4. The mRNAs mainly enriched in the tumorigenesis associated GO terms were angiogenesis, positive regulation of cell proliferation, positive regulation of fibroblast apoptotic process, regulation of neutrophil migration and others. The pathway analysis revealed mRNAs primarily enriched in important pathways associated with tumorigenesis (Hippo signaling pathway, pathways in cancer and Wnt signaling pathway). The genes involved in the significant GO terms and pathway terms are presented in Table II. Wnt family member (WNT)5A, frizzled class receptor 7 (FZD7), transcription factor 7 like 1 (TCF7L1), WW domain containing transcription regulator 1 (WWTR1) and cluster of differentiation (CD)44 were enriched in at least four significant GO or pathway terms.

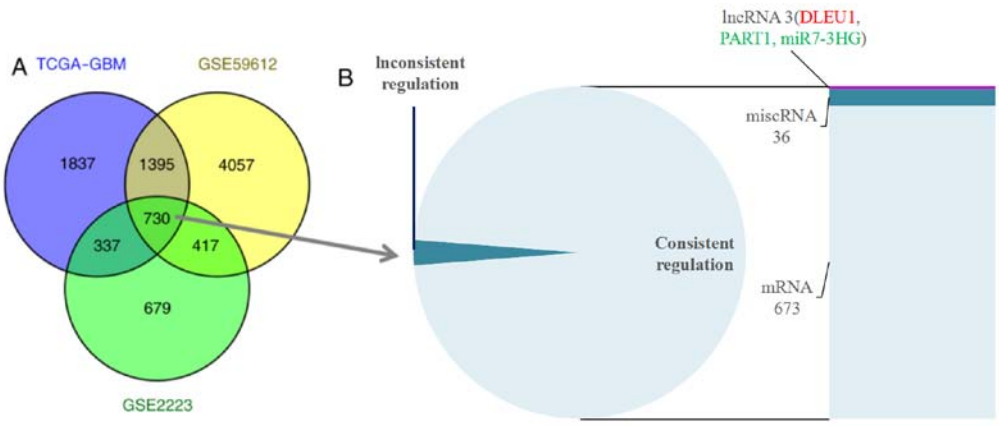

Figure 1. Overlapping DEGs of three datasets (GSE2223, GSE59612 and TCGA GBM RNA-Sequence). (A) Overlapping DEGs of three databases between normal and tumor samples. (B) The regulation association of the DEGs in the three datasets and the gene types of DEGs with a consistent regulation association. Inconsistent regulation is evident as inconsistent upregulation or downregulation of the DEGS in the three databases. DEG, differentially expressed genes, DEGs; TCGA, the cancer genome atlas; GBM, glioblastoma; lnc, long non-coding RNA; misc, miscellaneous.

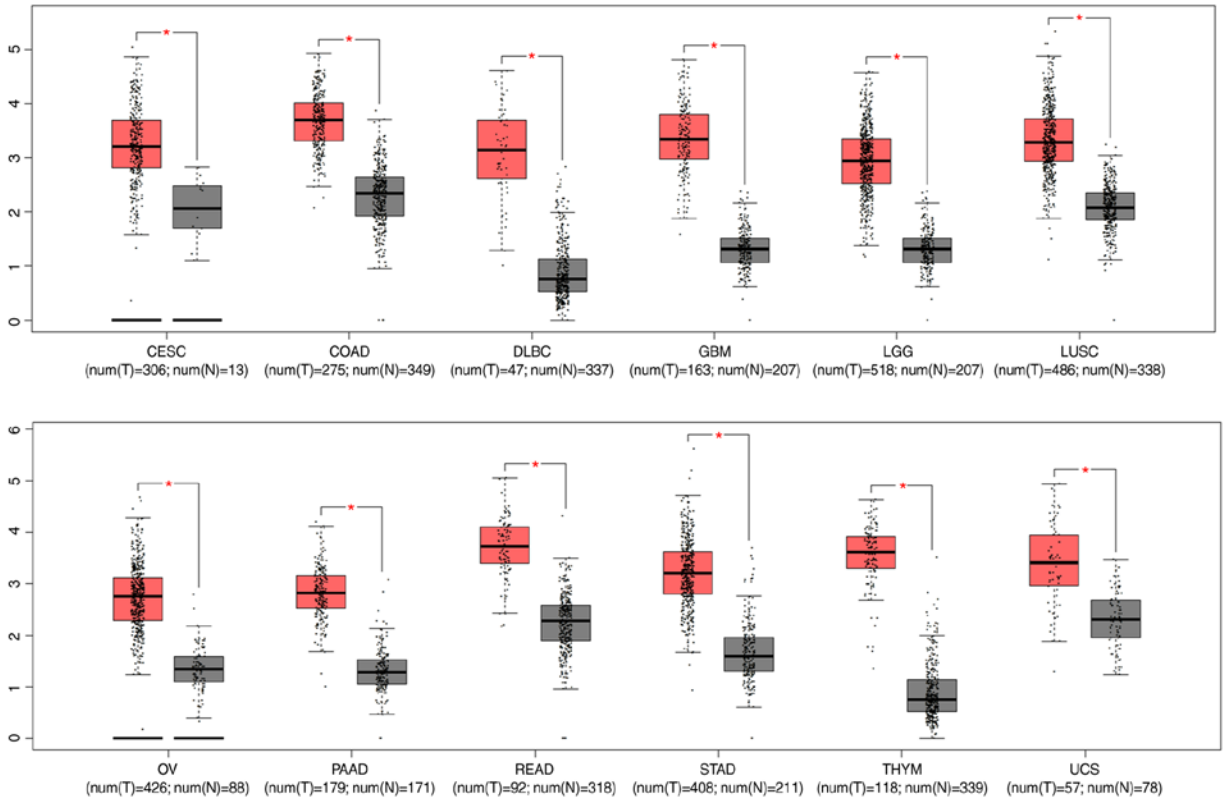

Figure 2. Expression level of lncRNA DLEU1 in multiple types of cancer. *P<0.05. CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; COAD, colon adenocarcinoma; DLBC, lymphoid neoplasm diffuse large B-cell lymphoma; GBM, glioblastoma multiforme; LGG, brain lower grade glioma; LUSC, lung squamous cell carcinoma; OV, ovarian serous cystadenocarcinoma; PAAD, pancreatic adenocarcinoma; READ, rectum adenocarcinoma; STAD, stomach adenocarcinoma; THYM, thymoma; UCS, uterine carcinosarcoma; num, number; T, tumour; N, normal.

DLEU1 is positively associated with TRAF4. TRAF4 serves an important role in other cancers according to previous studies (25-28). Querying GEPIA revealed that the expression of TRAF4 in the DLEU1-miRNA-DEmRNA network was positively associated with DLEU1 (Fig. 5A). To validate the prediction, the relative expression levels of DLEU1 and DLEU1 were analyzed by RT-PCR in 10 and 10 GBM adjacent normal brain tissues. The expression levels of DLEU1 and TRAF4 were both significantly increased in GBM compared with normal control (P<0.01; Fig. 5B and C).

Silencing DLEU1 downregulates TRAF4 and inhibits cell proliferation. The effects of silencing lncRNA were studied. Following silencing DLEU1 (Fig. 6A), cell viability decreased significantly in SHG-44 and U251 cell (P<0.01; Fig. 6B). However, there was no significant difference between the negative control and blank control concerning

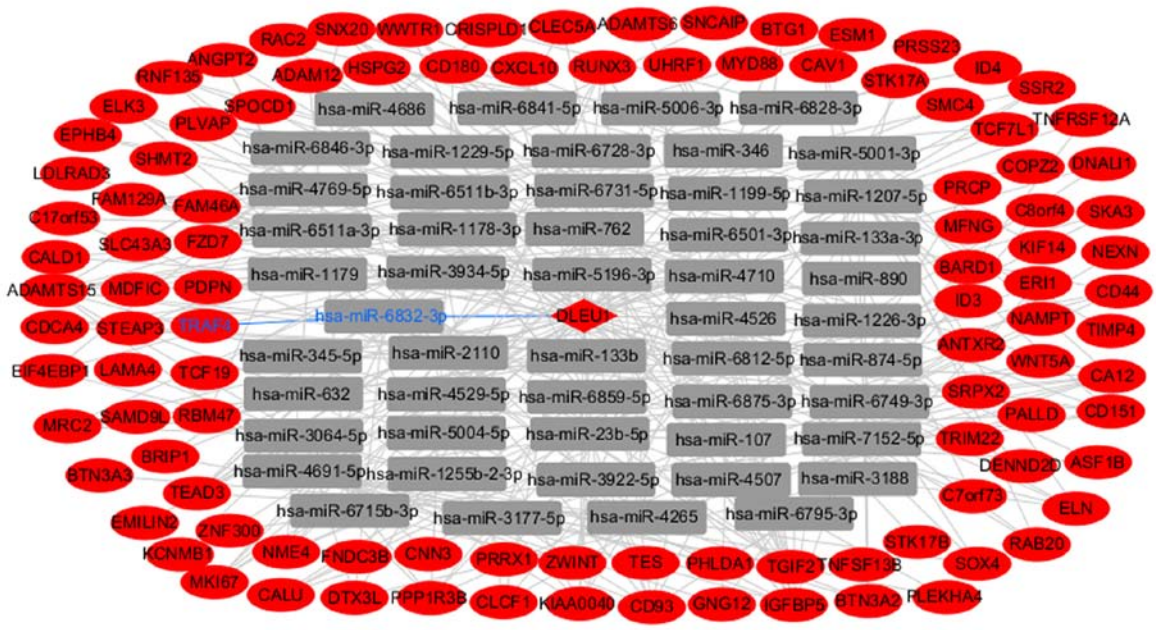

Figure 3. LncRNA DLEU1 mediates the lncRNA-miRNA-mRNA network. Diamonds represent lnRNA, round rectangles denote miRNA, and ovals represent mRNA. Red and gray denote upregulation and unknown regulation, respectively. miRNA/miR, microRNA; lnc, long non-coding RNA.

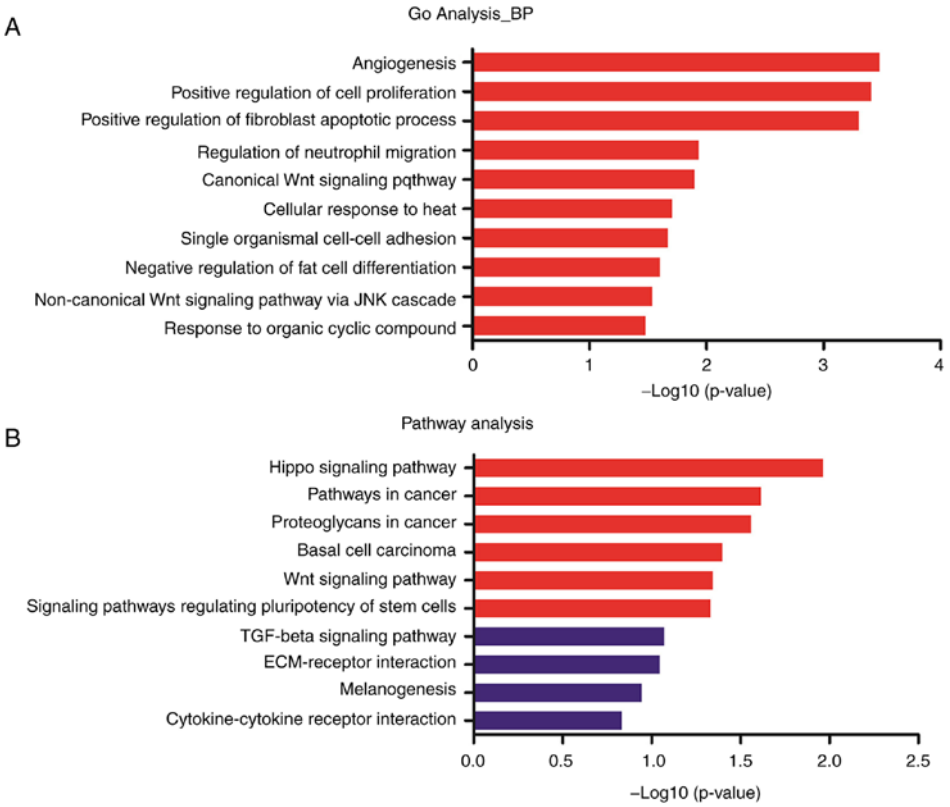

Figure 4. Gene Ontology and pathway analysis. (A) Gene Ontology and (B) pathway analysis of differentially expressed mRNAs in the lncRNA-miRNA-mRNA network. Red and blue denote P<0.05 and P>0.05, respectively. miRNA, microRNA; lnc, long non-coding RNA; TGF, transforming growth factor; ECM, extracellular matrix.

cell viability. Compared with the negative control transfected with the empty vector, the expression of TRAF4 was downregulated in SHG-44 and U251 cell transfected with si-DLEU1 at transcription (Fig. 6C) and the protein level (Fig. 6D).

Silencing TRAF4 inhibits cell proliferation. Similarly, the proliferation of cells was studied by silencing TRAF. Compared with the negative control transfected with the empty vector. After silencing *TRAF4* (Fig. 6E), cell viability decreased significantly in SHG-44 and U251 cells (P<0.01;

Table II. Significant GO and pathway analysis of differentially expressed mRNAs in the DLEU1-miRNA-mRNAs network.

Terms	Genes	P-value
GO		
Angiogenesis	CAV1, SRPX2, TNFRSF12A, HSPG2, ESM1, ELK3, ANGPT2, EPHB4	0.0003296
Positive regulation of cell proliferation	KIF14, NAMPT, SHMT2, TNFSF13B, RAC2, CLCF1, SOX4, ID4, ESM1, **WWTR1**, CXCL10	0.0003889
Positive regulation of fibroblast apoptotic process	BTG1, STK17B, STK17A	0.0004981
Regulation of neutrophil migration	MYD88, RAC2	0.0116385
Canonical Wnt signaling pathway	**WNT5A**, SOX4, **TCF7L1, FZD7**	0.0126456
Cellular response to heat	MKI67, C8ORF4, CXCL10	0.0196642
Single organismal cell-cell adhesion	SRPX2, **CD44**, CD93, PDPN	0.0212821
Negative regulation of fat cell differentiation	**WNT5A**, ID4, **WWTR1**	0.0249487
Non-canonical Wnt signaling pathway via JNK cascade	**WNT5A, FZD7**	0.0288453
Response to organic cyclic compound	NAMPT, MKI67, ANGPT2	0.0331943
Mammary gland involution	CAV1, IGFBP5	0.0345148
Signal transduction	NAMPT, PDPN, MRC2, GNG12, ELK3, CXCL10, MYD88, RAC2, TNFSF13B, CLEC5A, ANGPT2, TRAF4, IGFBP5	0.0375587
Negative regulation of protein export from nucleus	SOX4, BARD1	0.0401515
Kidney morphogenesis	SOX4, **WWTR1**	0.0457556
T cell mediated immunity	BTN3A3, BTN3A2	0.0457556
Cartilage development	**WNT5A, CD44**, PRRX1	0.0465266
Positive regulation of protein binding	**WNT5A**, MFNG, CAV1	0.0493935
Pathway		
Hippo signaling pathway	**WNT5A**, TEAD3, **WWTR1, TCF7L1, FZD7**	0.010874
Pathways in cancer	**WNT5A**, LAMA4, RAC2, GNG12, **TCF7L1, FZD7**, TRAF4	0.024194
Proteoglycans in cancer	**WNT5A**, CAV1, **CD44**, HSPG2, **FZD7**	0.027611
Basal cell carcinoma	**WNT5A, TCF7L1, FZD7**	0.040012
Wnt signaling pathway	**WNT5A**, RAC2, **TCF7L1, FZD7**	0.045011
Signaling pathways regulating pluripotency of stem cells	**WNT5A**, ID4, ID3, **FZD7**	0.046643

Genes in bold indicate these genes were enriched in at least four significant GO or pathway terms. GO, Gene Ontology; miRNA, microRNA; DLEU1, deleted in lymphocytic leukemia 1.

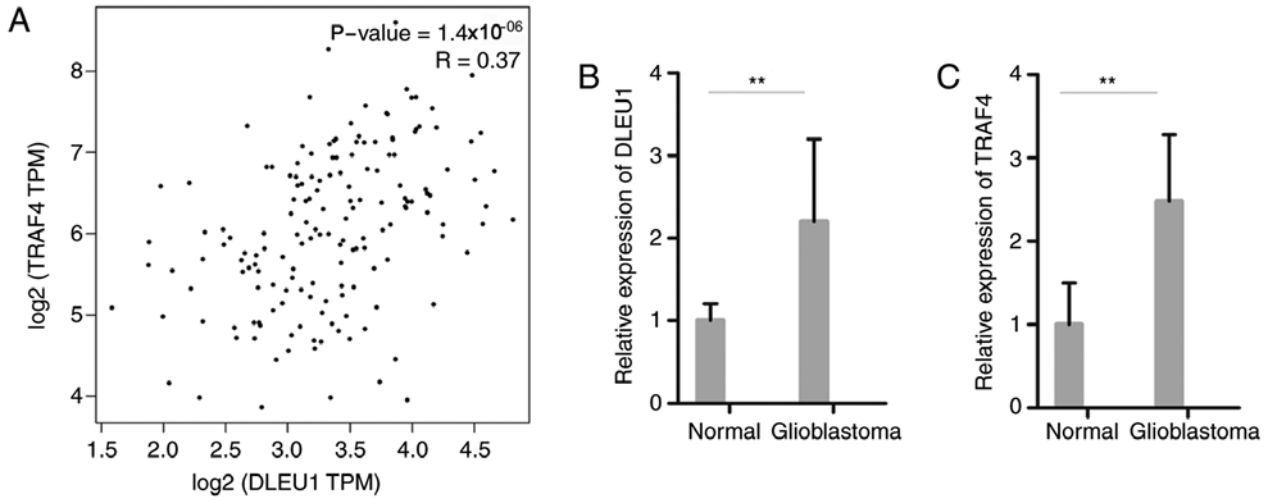

Figure 5. DLEU1 is positively associated with TRAF4. (A) Correlation analysis and relative expression analysis of lncRNA (B) DLEU1 and (C) TRAF4 in glioblastoma tissues. **P<0.01, n=3. Lnc, long noncoding RNA; TRAF4, TNF receptor associated factor 4; DLEU1, deleted in lymphocytic leukemia 1.

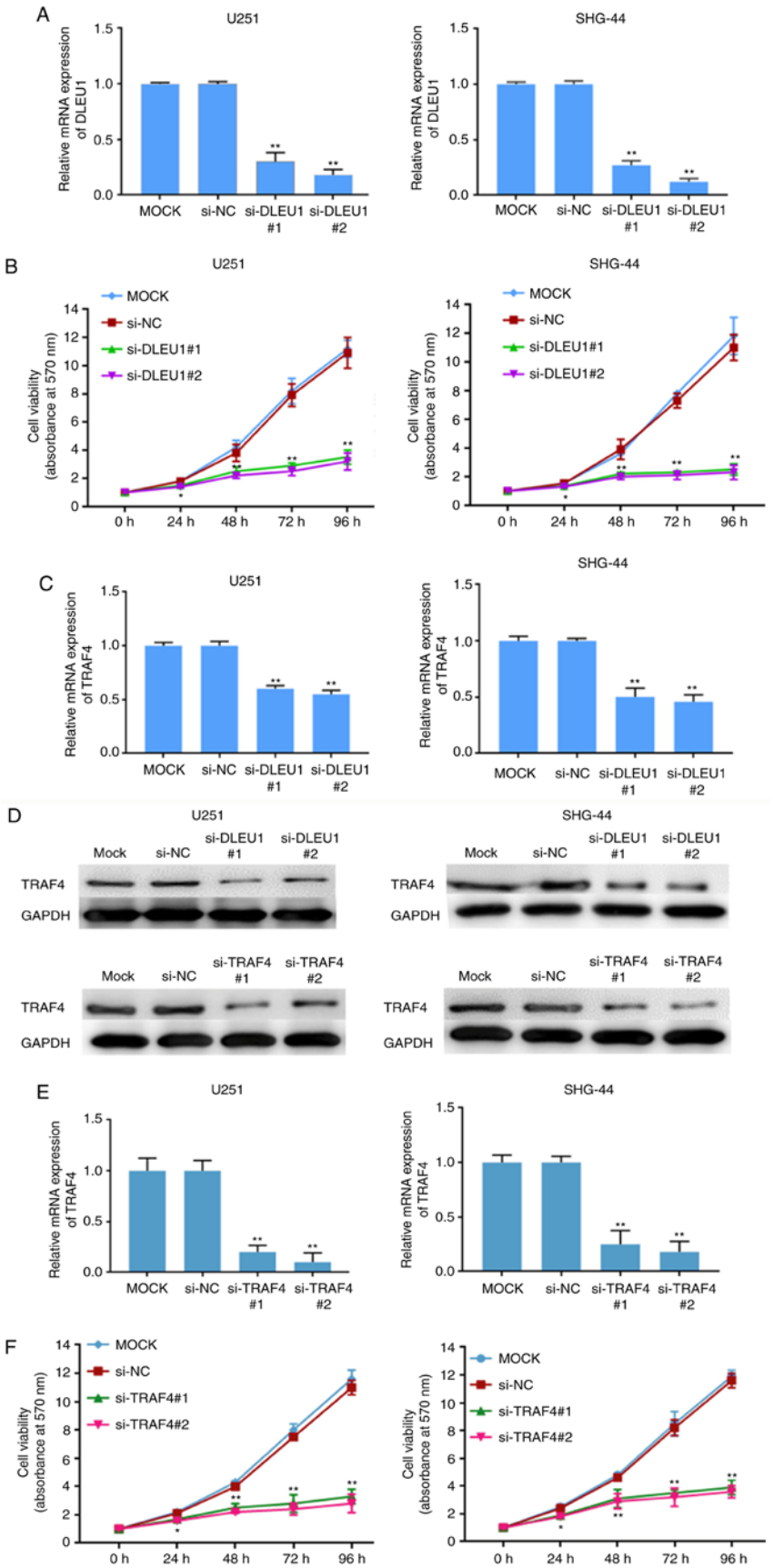

Figure 6. Silencing of lncRNA DLEU1 downregulates TRAF4 and inhibits cell proliferation. Silencing of lncRNA DLEU1 inhibited cell proliferation by MTT assays in (A) SHG-44 and (B) U251 cell. Silencing of lncRNA DLEU1 downregulates TRAF4 in SHG-44 and U251 cell transfected with si-DLEU1 at (C) the transcription and the (D) protein level. Silencing of *TRAF4* inhibited cell proliferation as measured by MTT assays in (E) SHG-44 cells and (F) U251 cells. MOCK, SHG-44 cells and U251 cells transfected empty vector with no any other sequence; NC, SHG-44 cells and U251 cells transfected with non-targeting sequence in humans; si-DLEU1, SHG-44 cells and U251 cells transfected with si-DLEU1; si-TRAF4, SHG-44 cells and U251 cells transfected with si-TRAF4. *P<0.05 and **P<0.01, n=3. Si, small interfering; lnc, long non-coding; NC, negative control; TRAF4, TNF receptor associated factor 4; DLEU1, deleted in lymphocytic leukemia 1.

Fig. 6F). However, there was no significant difference between the negative control and blank control concerning cell viability.

Discussion

In the present study, microarray data profiling and RNA-Seq profiles of GBM were integrated and re-analyzed. A total of 712 DEGs (673 DEmRNAs, 36 miscellaneous RNA and 3 DElncRNAs) were identified between GBM, and GBM adjacent normal brain tissues. Among the three lncRNAs (DLEU1, PART1 and miR7-3HG), DLEU1 was more expressed in a number of types of cancer, including GBM. The DLEU1-miRNA-DEmRNAs network was constructed. Several miRNAs in the DLEU1-miRNA-mRNAs network have been reported in the progression of tumorigenesis in GBM. For example, miR-107 is downregulated in glioma tissues and cell lines (29). Decreased expression of miRNA-107 promotes cell growth and invasion, inhibits cell apoptosis, and predicts a poor prognosis (30-32). miR-1179 and miR-346 (33) are downregulated in glioma tissues and cell lines, and their high expression inhibits cell proliferation. In GBM (34), miR-133a (35,36), miR-133b (37,38) and miR-346 are downregulated in glioma tissues and cell lines, and high expression inhibits cell proliferation, migration, and invasion. The regulation of the association of these miRNAs in the authors' prediction was in concordance with these previous studies. Therefore, the analysis and prediction were credible.

DEmRNAs in the DLEU1-miRNA-mRNAs network were mainly enriched in the pathway terms of the Hippo signaling pathway, pathways in cancer and Wnt signaling pathway. The Hippo signaling pathway is reportedly a major signaling pathway that regulates cell proliferation and growth, and is important role in the tumorigenesis of GBM (39). CD44 was reported to be upregulated in GBM and protected cancer cells by attenuating the activation of the Hippo signaling pathway (40). Suppression of the activity of the Hippo signaling pathway using Amlexanox was reported to inhibit GBM cell proliferation and induce GBM cell apoptosis (41). The Wnt signaling pathway affects tumor initiation, cell migration and invasion of GBM (42). Multiple factors, including small molecules, including SEN461 (43), small non-coding RNAs, such as miRNA34a (44) and miRNA-577 (45), and mRNAs, such as zinc finger E-box binding homeobox 1 (46), WNT3A (47), and homeobox A13 (48), influence GBM progression via the Wnt signaling pathway. Presently, six DEmRNAs [WNT5A, TEA domain family members (TEAD)3, WWTR1, TCF7L1, FZD7 and Rac family small GTPase 2] in the DLEU1-miRNA-mRNA network were demonstrated to be enriched in the Hippo and Wnt signaling pathways. WNT5A induces GBM cell migration (49). WNT5A is increased in GBM tissues and overexpression of WNT5A promotes the differentiation, proliferation, migration, and invasive growth of GBM cells (49-51). WWTR1, also known as TAZ, is an important regulator of the Hippo signaling pathway. The interaction of WWTR1 with TEAD drives mesenchymal differentiation of malignant glioma and influences tumor progression (52). As a direct target, WWTR1 is upregulated in GBM and promotes cell proliferation (53). Finally, the high expression of FZD7 has been detected in GBM and is associated with poor survival (54,55).

TRAF4 is overexpressed in tissues or cells in osteosarcoma (25), colon cancer (26), oral squamous cell carcinoma (27) and breast cancer (28). Presently, the knockdown of TRAF4 inhibited cell proliferation, migration and invasion, and induced apoptosis. A prior study reported the significantly high expression of DLEU1 in epithelial carcinoma, with associated promotion of cell multiplication, migration, and invasion and suppression of cell apoptosis (56). Other authors reported the intensified expression of DLEU1 in gastric cancer and the association with lymph node metastasis, tumor size, and advanced stage of pathology. Silencing of DLEU1 inhibited cell proliferation (57). In accordance with these findings, the expression level of DLEU1 and TRAF4 was confirmed to be high in GBM tissues by RT-PCR. The expression of DLEU1 was positively associated with TRAF4. RNA interference with DLEU1 downregulated TRAF and inhibited GBM cell proliferation. When TRAF4 knockdown occurs, cell proliferation is inhibited, with similar results to DLEU1. All results indicate that DLEU1 may affect the tumorigenesis of GBM by regulating the expression of TRAF4.

In conclusion, DLEU1 was intensified in GBM tissues and silencing of DLEU1 inhibited cell proliferation. Furthermore, miRNAs and mRNAs in the DLEU1-mediated ceRNA network are involved in cell differentiation, proliferation, migration, and invasive growth of GBM cells. These collective observations support the idea that DLEU1 may serve a pivotal role in the tumorigenesis of GBM. This study identifies a novel lncRNA that may act as a ceRNA in GBM. Further studies are needed to understand the molecular role of DLEU1 in GBM progression.

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Availability of data materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

GH and JW were responsible for the concept and design of the present study. JW and XQ acquired the data. JW and DP performed data analysis and experiments. JW drafted the article. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The experimental study was approved by the ethics committee of People's Hospital of Zhangjiajie and written informed consent was obtained from all participants.

Patient consent for publication

Written informed consent was obtained from all participants.

Competing interests

The authors declare that they have no competing interests.

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