Research Square

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Long noncoding RNA (lncRNA) MALAT1 regulates glioma via miR-140-5p/VEGFA axis

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Abstract

Background

Gliomas are lethal adult central nervous system tumors. LncRNA MALAT1 is implicated in cancer progression, and VEGFA promotes angiogenesis, but how MALAT1 regulates VEGFA in gliomas remains unclear. This study investigates whether MALAT1 modulates VEGFA via miR-140-5p.

Methods

This study was performed by using quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR), the MTT cell viability assay, dual luciferase reporter gene, western blot, flow cytometry. Further, we performed gene ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis at the cell level to explain the function of MALAT1 in gliomas. Using transcriptome and survival analysis, it was explained that the role of VEGFA gene in glioma.

Results

qRT-PCR analysis showed that the expression level of MALAT1 and VEGFA significantly increased and miR-140-5p obviously decreased in patients compared with the control group. Dual luciferase reporter confirmed that MALAT1 directly binds to the miRNA-140-5p. Knockdown of MALAT1 remarkably inhibited glioma cells proliferation while accelerated cell apoptosis in vitro. Moreover, transfection of miR-140-5p mimics inhibited the up-regulation of VEGFA protein level, while miR-140-5p shRNA prominently promoted the expression of VEGFA. These results demonstrate that MALAT1 play a vital role in glioma via the miR-140-5p/VEGFA axis. MALAT1 might be a potential therapeutic target for the treatment of glioma. Transcriptome and survival analysis showed that compared with the control group, the expression level of VEGFA gene was significantly increased, and VEGFA gene had a significant promoting effect on tumor development. Reducing VEGFA gene expression can prolong survival.

Conclusions

MALAT1 gene may regulate the progression of glioma through miR-140-5p/VEGFA axis in different pathways. The research results provide new ideas for the treatment of glioma.

1 Introduction

Glioma is the most diffused Central Nervous System tumor that accounts for the 75% of all the primary brain cancers [1]. This type of cancer is originated from the brain and spinal cord, and comprises a clinically, histologically and genetically heterogeneous brain tumor category [2]. Patients with glioma exhibited an elevated risk of several severe complications, including headache, cognitive impairment,

epilepsy, dysphasia, and progressive hemiparesis, and were associated with substantial mortality and morbidity. [3–4]. In 2005, the Stupp protocol, which included surgical resection, radiotherapy, and the DNA alkylator temozolomide, was introduced officially as a standard therapy of glioma [5–6].

In recent years, the application of new epigenetic molecules, such as long non-coding RNA (lncRNA) and microRNAs (miRNAs), had emerged in glioma characterization [7]. LncRNAs were functionally defined as a group of transcripts > 200 nucleotides (nt) in length without protein-coding potential. Moreover, LncRNAs were considered as a vital factor for controlling genes' expression by suppressing RNA polymerase II or regulating chromatin and histone modifications [8–10]. It had been proven that LncRNAs emerged as critical mediator in many processes of cell biology, and involved in many cancers, such as colorectal cancer, renal cancer, breast cancer, and esophageal cancer [11–14].

Recently, Metastasis Associated in Lung Adenocarcinoma Transcript 1 (MALAT1), a novel identified lncRNA and also called non-coding nuclear-enriched abundant transcript 2 (NEAT2), gained much attention. It was discovered being associated with metastasis in early-stage of non-small cell lung cancer [15], and revealed to be one of the highly expressed lncRNAs in many cancers, and corresponded to tumor initiation, progression, and recurrence [16–18]. Some studies have demonstrated that MALAT1 was aberrational expressed in glioma tissues and associated with the progression of glioma [16, 19–20]. However, the molecular mechanism underlying the effects of MALAT1 in glioma cells remains unclear.

Cancer-associated miRNAs serve as key modulators that inhibit gene expression through complementary binding to the 3'UTR of their target genes [21]. MiRNAs performed critical functions in the mediation of key biological processes, including cellular metabolism, cell division and death, intracellular signaling, and immunity and cell movement [22–23]. More and more evidences illustrated that several key miRNA exhibited a strong association on a specific groups of cancers. For instance, miR-140-5p associated with the survival outcomes of multiple cancers, including gastric cancer, small cell lung carcinoma and others [24–26]. Recently, some studies reported that microRNA-140 (miR-140) contributed important roles in glioma, including inhibited glioma cell proliferation and invasion, and promoted glioma cell apoptosis [27]. In addition, it was observed that miR-140-5p decreased in several types of cancer, and impairs the proliferation and metastasis of cancer cells by regulating the vascular endothelial growth factor A (VEGFA) gene or VEGFA/matrix metalloproteinase 2 signaling. VEGFA, a main supervisor of angiogenesis, is well known to play a crucial role in tumorigenesis. Moreover, several published works proved that down-regulated VEGFA expression could block the growth of glioma cells [27–30]. Nevertheless, the correlation between miR-140, MALAT1, and VEGF with invasion and angiogenesis in glioma remains to be unclear.

In this study, the binding correlation between MALATI1 with miR-140-5p, and the regulation mechanism on VEGFA were preliminarily investigated. The specific functions of these factors in glioma were assessed to eliminate their effects on apoptosis, proliferation, by which further determine whether the miR-140-5p/VEGFA pathway can be implemented as an alternative therapeutic candidate for glioma.

Results may deepen the understanding of glioma development and provide some promising theoretical and practical evidences for glioma target therapy.

2 Materials and methods

2.1 Cell culture

Glioma cells were cultured in Dulbecco's modified Eagle medium (DMEM) with high glucose, 100 U/mL penicillin, 100 μg/mL streptomycin, and 10% fetal bovine serum (FBS, Gibco, Carlsbad, CA, USA), after mixed, cells were incubated in a humidified incubator at 37 ℃ with 5% CO_2, as the previously described methods [31].

2.2 Real-time quantitative RT-PCR

Total RNA of the samples were extracted using TRIzol reagent assay (Invitrogen). All extracted RNAs were treated with RNase-free DNase I (40 U/mg RNA, Takara), and purified using the RNeasy Mini Kit (Qiagen) with avoiding DNA contamination. cDNAs were synthesized using GoTaq qPCR Master Mix Kit (Promega) after adjusting to the same concentration in the control and treatment groups. RT-PCR was performed with the ABI7500 Real-Time PCR System (Applied Biosystems), and GoTaq qPCR Master Mix Kit (Promega, China). Gene-specific primers were designed using primer 3 online software v.0.4.0 (http://bioinfo.ut.ee/primer3-0.4.0). PCR primers listed below: For MALAT1: Sense 5'-GAATTGCGTCATTTAAAGCCTAGTT-3'; Antisense 5'-GTTTCATCCTACCACTCCCAATTAAT-3'; For miR-140-5p: sense 5'-GCTTAACTGTAAACGCCCTTG-3; Antisense 5'-GGGCATCGTCGAGGGTT-3'; For VEGFA:Sense 5'-CTCACCAAGGCCAGCACATAGG-3'; Antisense 5'-ATCTGGTTCCGAAAACCCTGAG-3'. The RG-3000A real-time PCR system (RoterGene) and SYBRTM Premix EX TaqTM (Perfect Real Time) kit (Takara) were applied. The housekeeping gene were β-Actin (sense 5'-TCGACAACGGCTCCGGCA-3'; Antisense 5'-AAGGTGTGGTGCCAGATT-3') and U6 (sense 5'-CTCGCTTCGGCAGCACA-3'; Antisense 5'-AACGCTTCACGAATTTGCGT-3'). The qRT-PCR protocol included an initial denaturation at 95 ℃ for 10 s, followed by 35 cycles consisting of denaturation at 95 ℃for 5s, annealing at 60℃ for 30s, the data were analyzed with a normalized gene expression method ($2^{-\Delta\Delta Ct}$) [32]. Entire experiment was carried out in triplicate.

2.3 The MTT cell viability assay

The cell viability rates of glioma cells were tested according to the method described by Nan et.al [33]. Glioma cells in the logarithmic phase of growth were seeded onto 96-well plates at a density of 2×10^4 cells for each well. When apricate, fifty microliters of the MTT solution (5 mg/mL, KeyGEN) was added to each well of the experimental group, cells were incubated at 37℃ for 4h. subsequenntly, the supernatant was discarded, and 200 μl of dimethyl sulfoxide was added to each well to dissolve the pellets. Finally, the optical density (OD) of each well was measured at 570 nm with an automated microplate reader, and cell proliferation was analyzed in each experimental group using GraphPad software. Each experimental group was tested in triplicate times.

2.4 Dual Luciferase Reporter Gene

Target genes of miR-140-5p and the putative miR-140-5p binding sites in MALAT1 were predicted using experiment Bioinformatics software. The wild-type and mutant sequence containing the putative binding region of miR-140-5p were synthesized and cloned into the psiCheck2 reporter plasmid. Plasmid DNA and miR-140-5p mimic or miR-140-5p negative controls were co-transfected into HEK293T cells. When appropriate, the luciferase activity was tested using a Double-Luciferase Reporter Assay Kit (Promega, China), firefly luciferase activity was standardized by Renilla luciferase activity.

2.5 Western blot assay

Cells were lysed with 0.1 mL of strong RIPA lysate (Beyotime Institute of Biotechnology, China) to obtain total protein. The protein concentration were determined with a BCA kit (Beyotime). The plate was incubated for 30 min at 37℃ and then the absorbance was read against the blank at 562 nm. The corresponding volume of protein was added to the loading buffer (Beyotime). The sample was mixed and placed on a boiling water condition for 5 min to denature the cells. The cell lysates with 25μg total protein per well were separated via the appropriate concentration of SDS-PAGE electrophoresis gel. Proteins were transferred to nitrocellulose filter membrane and blocked with 5% bovine serum albumin in TBST for 2h at room temperature (RT), and then incubated overnight at 4˚C with primary antibodies. After blocking, the membranes were incubated with the primary antibodies against GAPDH (5174S, 1:1000, Cell Signaling, Boston, USA), and anti-VEGFA (ab51745, 1:1,000, Abcam) overnight at 4℃ on a shaker. Following incubation, the membranes were washed 3 times with Tris-buffered saline Tween-20 (TBST) for 10 min before incubation with HRP-conjugated homologous goat anti-rabbit secondary antibodies at RT for 1 h. The membranes were washed 3 times for 10 min. Then chemiluminescence imaging analysis system was used to observation.

2.6 Apoptosis analysis via flow cytometry.

Cell apoptosis was performed via apoptotic rate using the Annexin V- FITC/propidium iodide (PI) assay kit (ab14085; Abcam). $5x10^5$ cells were planted in 6-well plates and cultured for 48 h. Cell suspension was collected, washed, and stained with 5 μl Annexin V- FITC and PI in the dark at RT for 10 min, then executed flow cytometry (FACSCalibur; BD Biosciences). Apoptotic cells were examined using CellQuest Pro software (BD Biosciences). Annexin V- FITC- positive cells were considered to be apoptotic cells.

2.7 Wilcoxon differential analysis and survival curve analysis.

GSE147352 data was downloaded from the GEO database, which includes 15 normal samples, 18 LGG samples, and 85 glioblastoma samples. Next, LGG and normal group, as well as glioblastoma and normal group, were used for Wilcoxon differential analysis.515 samples from LGG were selected from the TCGA database (500 samples were screened for missing values) for near prognostic analysis of core genes, and survival curve analysis was performed using R package survivalROC and R package survivminer.

2.8 Statistical analyses.

Each experiment was carried out in triplicate as mentioned in each figure legend performed using GraphPad Prism. (La Jolla, CA, USA). Student's t test was performed to compare two groups for independent samples. Mean values were shown in the figures. Without stated otherwise, statistical significance was established at $^{**}p < 0.01$ and $^{*}p < 0.05$. Data were presented as mean ± SD or SEM.

3 Results

3.1 The expression level of MALAT1, VEGFA, and miR-140-5p in glioma cells

To evaluate the correlation between MALAT1, VEGFA and miR-140-5p, RT-PCR experiments were conducted to ascertain the expression levels of MALAT1, VEGFA and miR-140-5p in U87 and U251 cell lines. The results showed that compared with the control group, the expression levels of MALAT1 and VEGFA in U87 and U251 cell lines were significantly increased ($P < 0.05$) (Fig. 1a, c). However, compared with the control group, the expression level of miR-140-5p was significantly reduced ($P < 0.05$) (Fig. 1b).

3.2 Downregulation of MALAT1 promoted apoptosis and inhibited proliferation in glioma cells.

Flow cytometry analysis showed that knockdown of MALAT1 gene in U87 and U251 cell lines significantly increased apoptosis of glioma cells compared to the corresponding controls (Fig. 2a-b and Fig. 3a-b). MTT assay results illustrated that knockdown of MALAT1 in U87 and U251 cell lines significantly inhibited cell viability compared to controls in a time-dependent manner, with a gradual decrease in cell viability as time increased (Fig. 2c and Fig. 3c).WB results showed that Bcl-2 protein expression in glioma cells was significantly lower than that in the control group, while the levels of Bax and active cystatinase 3 were significantly higher in the siRNA-MALAT1 group ($P < 0.01$) (Fig. 2d-g and Fig. 3d-g).

3.3 MiR-140-5p regulated VEGFA expression.

To investigate potential target genes and the mechanism research of miR-140-5p. The miRDB (http://www.mirdb.org/) and starbase (http://starbase.sysu.edu.cn/) database were scanned, and then identified miR-140-5p as the most potential target miRNA of MALAT1 (Fig. 4a and Fig. 5a). To investigate the relationship between MALAT1 and miR-140-5p, a dual luciferase reporter assay to clone MALAT1 fragments containing predicted binding sites into the luciferase gene and performed luciferase assays were conducted (Fig. 4a and Fig. 5a). Results showed that MALAT1 and miR-140-5p could bind through the predicted binding sites, further confirming that the interaction between MALAT1 occurs through the predicted miR-140-5p binding sites.

Moreover, the effects of miR-140-5p mimetics at different concentrations on the proliferation and survival rates of U87 and U251 cell lines were tested. The results of qRT PCR were shown in Figs. 4b and 5b. When the concentration of miR-140-5p mimic was below 200nM, it had no effect on the cell survival rate of glioma cells. When the concentration of miR-140-5p mimic reached 200nM, the survival rate of glioma cells significantly increased ($P < 0.05$). When the concentrations of miR-140-5p mimetics were 40, 80, and 160 nM, the expression of miR-140-5p was significantly upregulated in U87 cell line ($P < 0.05$), but only the last two concentrations significantly upregulated the expression of miR-140-5p in U251 cell line ($P < 0.05$). Considering the cost, an 80nM miR-140-5p mimic was used in the following experiment (Fig. 4c and Fig. 5c).

The upregulation of miR-140-5p expression reduced the activity of psiCheck2-MALAT1 (Fig. 4d and Fig. 5d). In order to further explore the mechanism of miR-140-5p, it was found through experimental bioinformatics software analysis that VEGFA may be a potential target gene of miR-140-5p. The relationship between miR-140-5p and VEGFA in glioma was detected by WB method. Transfect miR-140-5pshRNAp/mimetics in glioma cells to determine VEGFA levels. Results showed that compared with the control group, downregulation of miR-140-5p increased VEGFA protein levels, while overexpression of miR-140-5 decreased VEGFA protein levels (Fig. 4e-f and Fig. 5e-f). In addition, MALAT1 levels in glioma cells were negatively correlated with miR-140-5p levels.

3.4 Wilcoxon differential analysis and efficacy analysis of VEGFA single gene prognostic model

To further explore the relationship between MALAT1 and VEGFA in gliomas, Wilcoxon differential analysis was performed between LGG and normal groups, and glioblastoma and normal groups. As shown in Figs. 6a and b, there were no difference in the expression of MALAT1 and VEGFA between LGG and normal group. Furthermore, there was no difference in MALAT1 expression between glioblastoma and normal, while VEGFA expression in glioblastoma was significantly higher than in Normal ($P < 0.05$).

1. To further evaluate the effectiveness of the VEGFA single gene model, 515 samples from LGG were selected from the TCGA database (500 samples were selected after screening for missing values) for prognostic analysis of core genes. The median expression of a single gene was applied as the optimal threshold for risk scoring, and patients were divided into high-risk scoring group and low-risk scoring group. Using Kaplan Meier survival curves to display risk scores, the results showed that the prognosis of low-risk patients was higher than that of high-risk patients, and the difference was statistically significant ($P < 0.05$). besides, the VEGFA gene had a significant promoting effect on tumor development, and low expression of VEGFA gene can prolong survival (Fig. 7).

3.5 GO and pathway analysis for MALAT1

In order to obtain a comprehensive understanding of the biological functions of MALAT1. We interfered with MALAT1, a total of 12,839 protein-coding genes were found to be differentially expressed with the threshold of $|logFC| > 1$ and $FDR < 0.05$ between glioma cells and control group. The volcano plot of

DEGs in the glioma cells was presented in Fig. 8a. Among these DEGs, 1,308 DEGs were up-regulated while 1,353 DEGs were down-regulated. Furthermore, GO and KEGG pathway enrichment analyses were carried out. According to GO analysis results, it was observed that up-regulated gene were most enriched in structural constituent of ribosome, structural molecule activity, extracellular region, and ribosome (Fig. 8b). In contrast, down-regulated gene were enriched in nucleic acid binding, nucleolus, regulation of biosynthetic process and positive regulation of biosynthetic process (Fig. 8c). According to KEGG analysis, DEmRNA genes in the glioma cells were most enriched in pathways associated with Ribosome, Ribosome biogenesis in eukaryotes, FoxO signaling pathway, Longevity regulating pathway-worm, and Glycolysis / Gluconeogenesis (Fig. 8d).

4 Discussion

In the present study, the carcinogenetic effect MALAT1 working on glioma especially on cell proliferation and apoptosis were investigated. Our results showed that MALAT1 was upregulated in glioma and that an inhibition of MALAT1 suppressed proliferation but promoted apoptosis in glioma cells, demonstrated by in vitro cellular experiments. Meanwhile, increased MALAT1 expression was related with decreased miR-140-5p expression. In addition, it was clarified that MALAT1 connected with miR-140-5p as a miRNA sponge and that miR-140-5p/VEGFA signaling playe a key role in glioma.

LncRNAs have been proven to be associated with numerous biological processes, such as cell proliferation, migration, cell cycle, cell apoptosis and angiogenesis [33]. Recent evidences indicated that MALAT1, a highly abundant and evolutionarily conserved lncRNA, is correlated with angiogenesis [34]. MALAT1 had been suggested to determine the overall survival of glioma patients as a novel glioma prognostic biomarker and therapeutic target [19, 35]. Furthermore, Li, et.al revealed that the upregulation of MALAT1 and MALAT1 knockdown could inhibit proliferation and induce apoptosis in glioma cell lines effectively [36]. We used bioinformatic analysis to find potential miRNAs which may be co-expressed with MALAT1 during glioma proliferation. Results demonstrated that knockdown of MALAT1 resulted in an increased in gliobe cell apoptosis via promotion of apoptotic protein Bax and active-caspase-3, inhibition of anti-apoptotic protein Bcl-2, and inhibited gliobe cell proliferation in vitro. Over the past decade, aberrantly expressed miRNAs have been demonstrated to act as important oncogenic factors or tumor suppressors in human carcinomas. For example, MALAT1 promoted malignant melanoma metastasis and growth by sponging miR-22 and suppressed its expression via bounding to miR-129 [31, 37]. Moreover, previous works also illustrated that miR-140-5p was an antitumor factor in gastric carcinoma, hepatocellular carcinoma, lung carcinoma, and ovarian carcinoma [38]. The functions and molecular mechanisms of miR-140-5p had been found in various biological processes and pathogenic conditions. Additionally, miR-140-5p has been reported to play important regulatory role in other human disorders, such as colorectal cancer, muscle invasive bladder cancer, nephroblastoma, and lung cancer [39–42]. miR-140-5p was also identified as a miRNA target of MALAT1. Many reports have revealed that miR-140-5p participates in the malignant behavior of glioma and could be as cancer suppressor [43]. But the potential relation between MALAT1 and miR-140-5p has not yet been clarified.

It was observed from carefully searching of miR target database that a putative binding sites of miR-140-5p were contained in MALAT1. Subsequently, the relationship between MALAT1 and miR-140-5p were verified using a DLR assay. Results revealed that overexpression of miR-140-5p could decrease the expression of MALAT1, and MALAT1 could specifically bind to miR-140-5p and had negative regulation with the expression of miR-140-5p. In addition, several studies have reported that different pathways may exist between miRNA-140-5p and VEGFA [44]. It was reported that the MALAT1-microRNA-gene regulatory network had been involved in the occurrence and development of different diseases. For instance, some researchers had illustrated that miR-140-5p strongly regulated the transcription of the growth factor (VEGFA), which plays a vital role in ULM cell growth and has a higher-levels in ULM tissue [45]. Moreover, miR-140-5p directly targeted VEGFA and inhibited the expression of 14-3-3γ [46]. In the results of this study, downregulation of miRNA-140-5p dramatically increased the expression level of VEGFA, while upregulation of miRNA-140-5p significantly decreased the expression level of VEGFA. We further evaluated the expression of MALAT1 and VEGFA in RNA seq, as well as the model efficacy of individual VEGFA genes. Results showed that VEGFA expression in glioblastoma was significantly higher than that in normal individuals, and the expression level of VEGAF gene could affect the future survival of glioma patients. This further revealed the impact of VEGFA gene on glioma.

In summary, MALAT1 could directly target miRNA-140-5p, which in turn targets VEGFA, and their interactions may influence the progression of glioma. Our findings provide novel insights into the MALAT1/miR-140-5p/VEGFA axis in glioma and may facilitate the development of therapeutic strategies for this disease.

Declarations

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Competing interests

The authors declare no competing interests.

Authors' contribution

Jianglong Yu: Conceived and designed the analysis, Wrote the paper. Abudula . Aisha, Shutao Ma: Collected the data.Performed the analysis. Xiaofei Duan, Xinli Liu, Yiderisi . Aosiman: Contributed data or analysis tools

Data Availability Statement

The datasets generated during analyzed the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable. This study did not involve human or animal experiments.

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Consent for publication

Not applicable.

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Figures

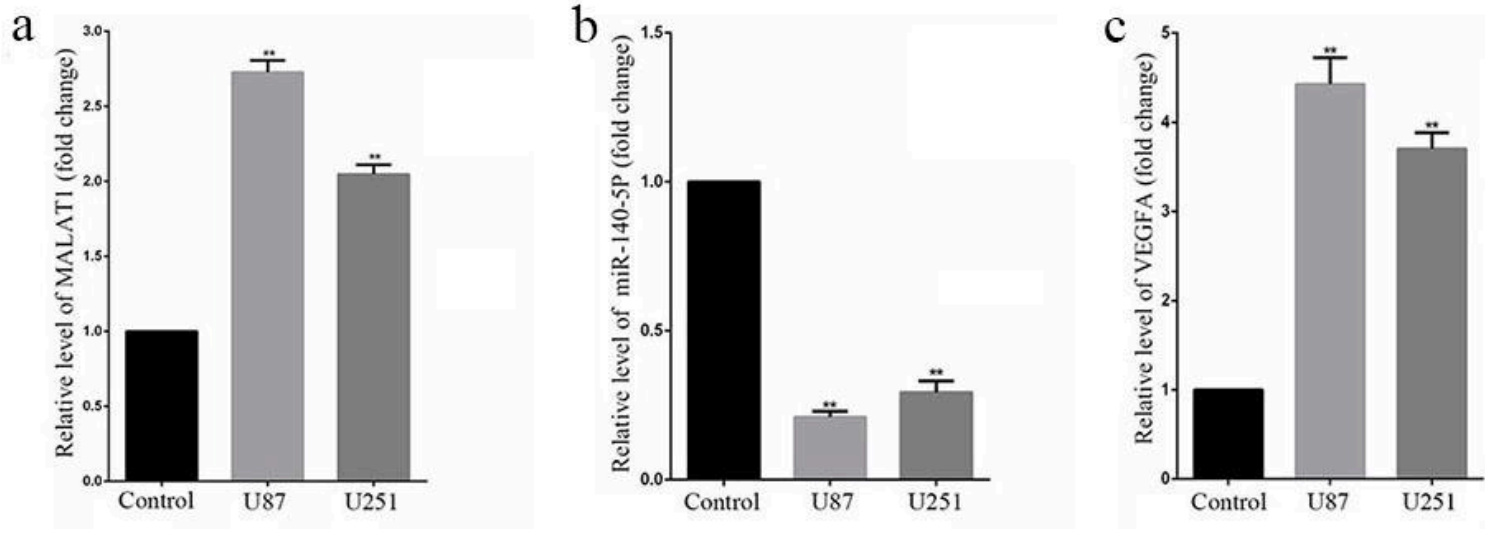


Figure 1

The relative level of RNA in different glioma cells. (A-C) RT-PCR analysis results showed the expression of MALAT1, miR-140-5p, and VEGFA in U87 and U251 cells. **p<0.01 vs control group. All experiments were performed at least thrice independently.

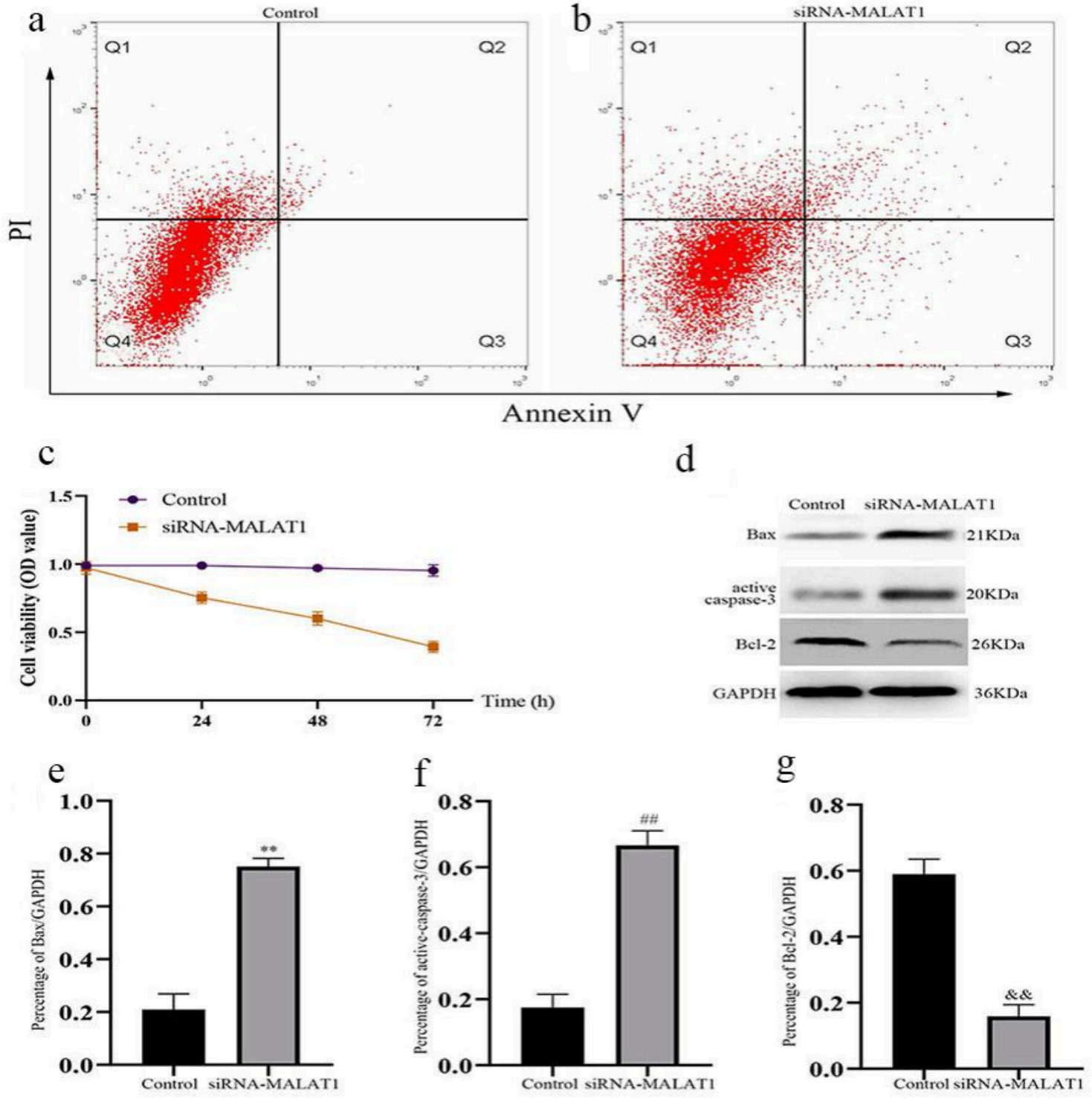


Figure 2

Silencing of MALAT1 inhibited proliferation and promoted apoptosis in U87 cells. (A, B) Representative FACS plots showed Annexin-V FITC (X axis) and propidium iodide (PI; Y axis) stained control and siRNA-MALAT1 group. (C) MMT assay analysis results showed the viability of control and siRNA-MALAT1

group for 0, 24, 48 or 72 h in glioma cells. (D) Representative western blots revealed the levels of Bax, cleaved-caspase3, Bcl-2 proteins in control group and siRNA-MALAT1 group. (E-G) Statistical analysis of (D). All experiments were performed thrice. **p<0.01 vs control group; $^{\#\#}p$<0.01 vs control group; $^{\&\&}p$<0.01 vs control group.

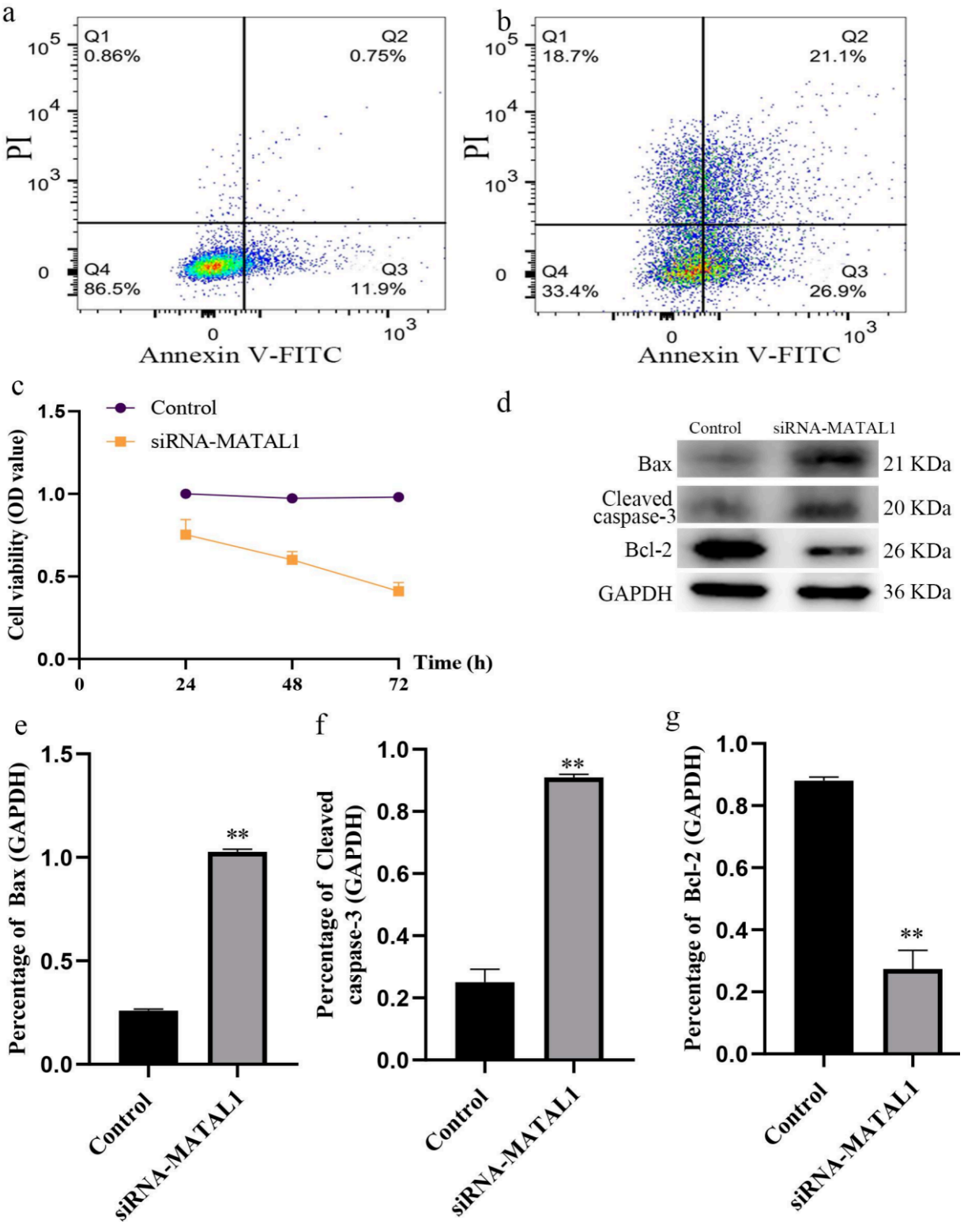


Figure 3

Silencing of MALAT1 inhibited proliferation and promoted apoptosis in U251 cells. (A, B) Representative FACS plots showed Annexin-V FITC (X axis) and propidium iodide (PI; Y axis) stained control and siRNA-MALAT1 group. (C) MMT assay analysis results showed the viability of control and siRNA-MALAT1 group for 0, 24, 48 or 72 h in glioma cells. (D) Representative western blots revealed the levels of Bax, cleaved-caspase3, Bcl-2 proteins in control group and siRNA-MALAT1 group. (E-G) Statistical analysis of (D). All experiments were performed thrice. ***p*<0.01 vs control group.

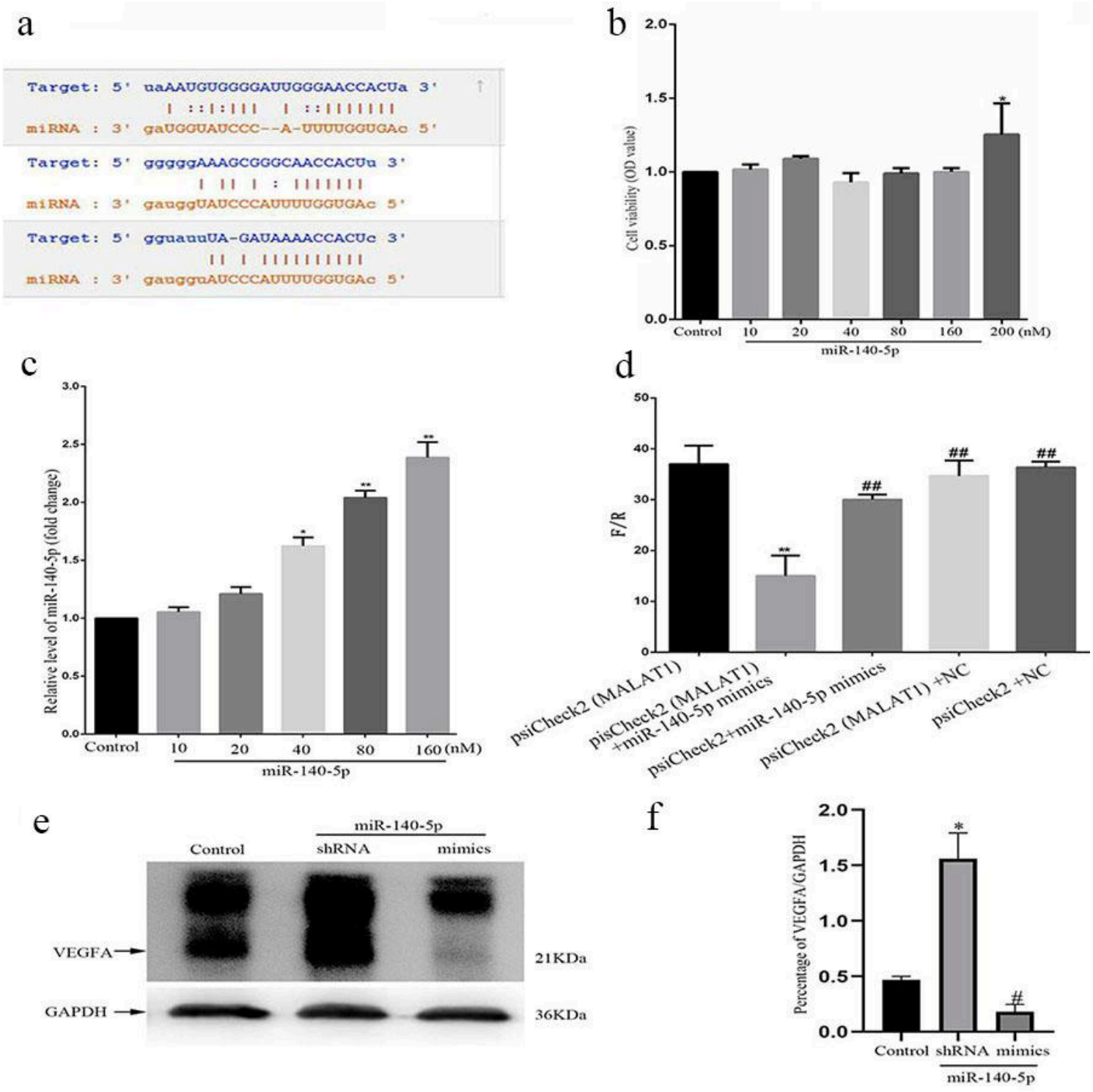


Figure 4

MALAT1 might be negatively associated with miR-140-5p in U87 cells. (A) The predicted target binding site for miR-140-5p in the 3'UTR of lncRNA miR-140-5p based on the miRDB and starbase database analysis. (B) MTT assay results showed that the viability of different concentrations of miR-140-5p (10, 20 40, 80, 160, 200 nM) and control. (C) qRT-PCR analysis showed miR-140-5p in glioma cells transfected with miR-140-5p mimics and the corresponding control glioma cells. (D) Dual luciferase reporter assay results showed the luciferase activity in glioma cells. (E) The expression level of VEGFA changed after miR-140-5p shRNA/mimics. (F) The statistical analysis of (E). *p<0.05 vs. Control, **p<0.01 vs. Control, **p<0.01 vs. psiCheck2 (MALAT2), $^{\#\#}p$<0.01 vs. psiCheck2 (MALAT2) +miR-140-5p mimics. All experiments were performed thrice.

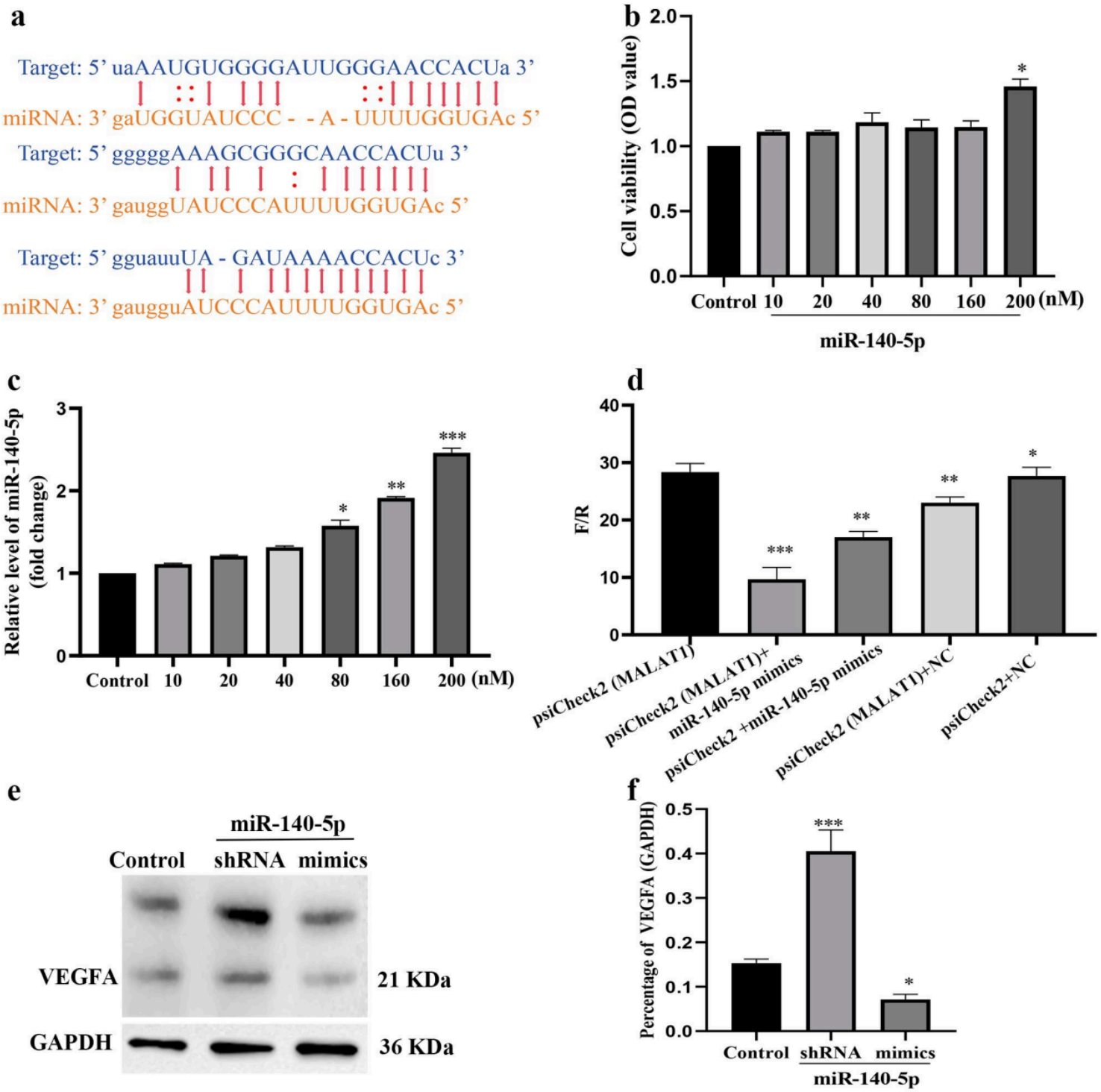


Figure 5

MALAT1 might be negatively associated with miR-140-5p in U251 cells. (A) The predicted target binding site for miR-140-5p in the 3'UTR of lncRNA miR-140-5p based on the miRDB and starbase database analysis. (B) MTT assay results showed that the viability of different concentrations of miR-140-5p (10, 20 40, 80, 160, 200 nM) and control. (C) qRT-PCR analysis showed miR-140-5p in glioma cells transfected with miR-140-5p mimics and the corresponding control glioma cells. (D) Dual luciferase reporter assay results showed the luciferase activity in glioma cells. (E) The expression level of VEGFA changed after miR-140-5p shRNA/mimics. (F) The statistical analysis of (E). *p<0.05 vs. Control, **p<0.01 vs. Control, **p<0.01 vs. psiCheck2 (MALAT2), ***p<0.01 vs. psiCheck2 (MALAT2) +miR-140-5p mimics. All experiments were performed thrice.

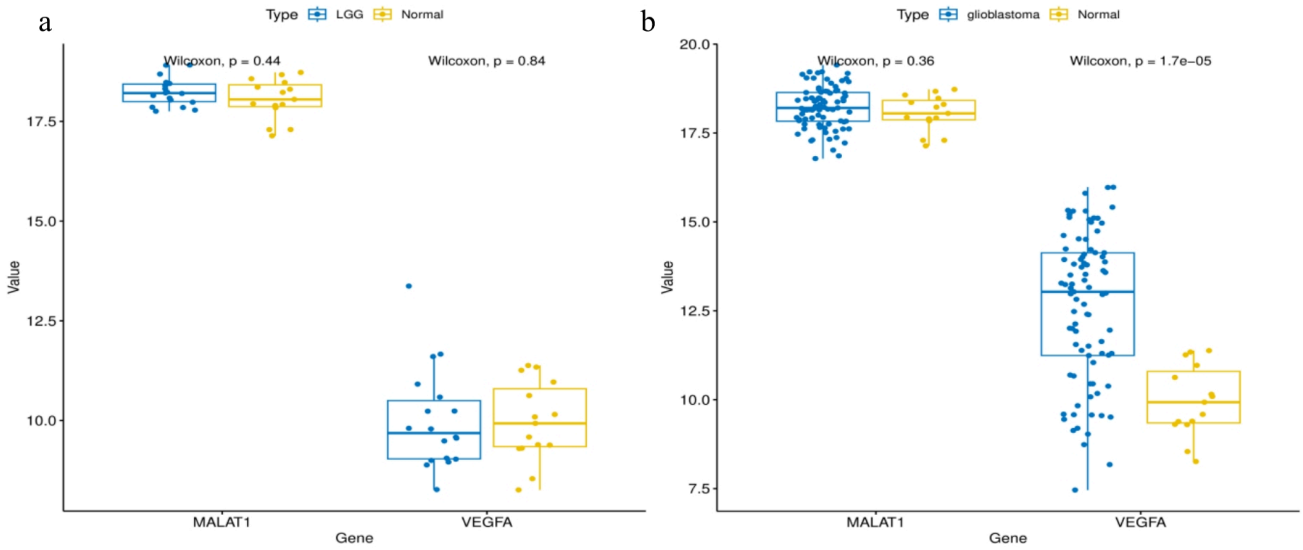


Figure 6: Expression of MALAT1 and VEGFA in RNA seq

Figure 6

Expression of MALAT1 and VEGFA in RNA seq

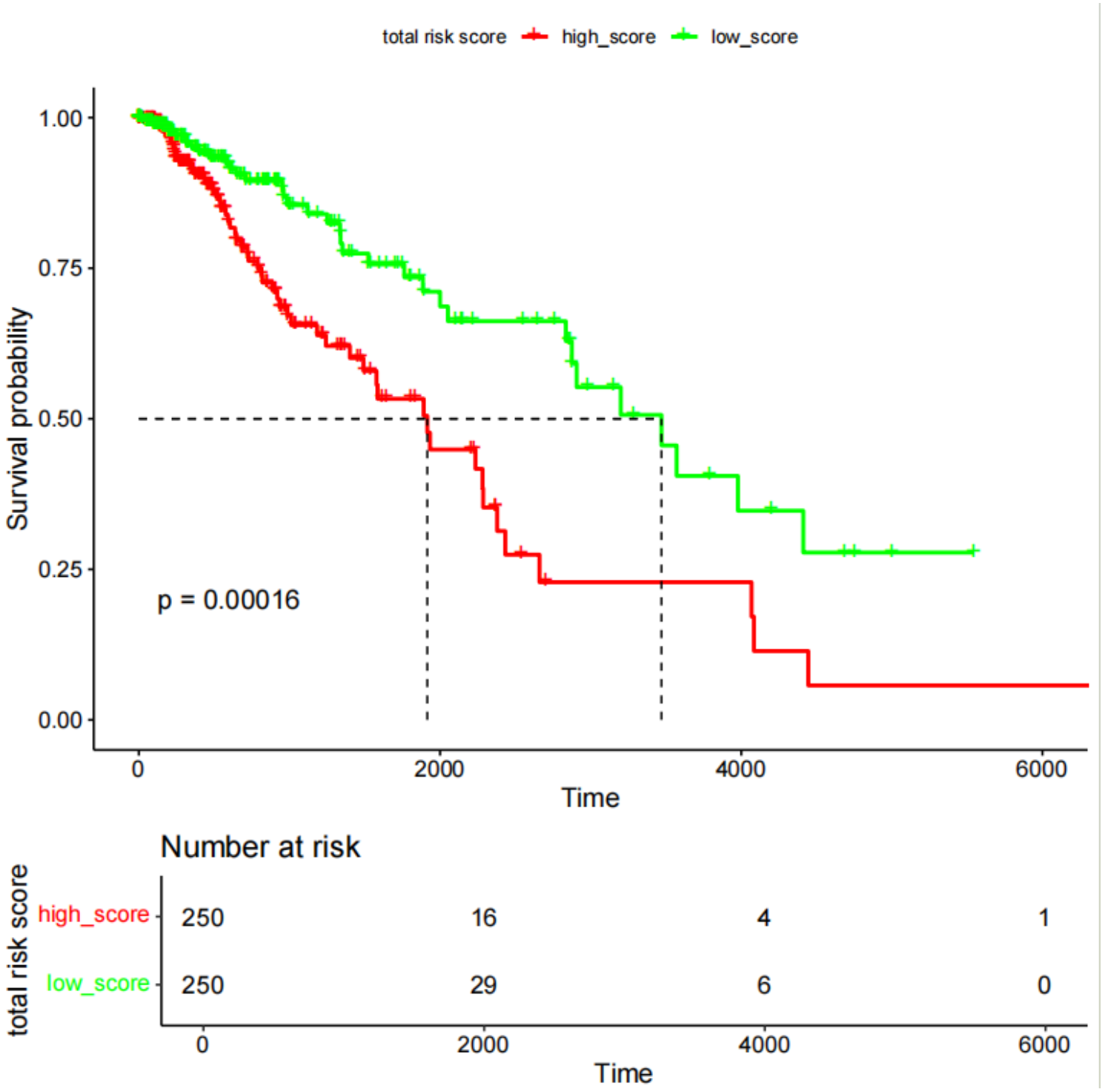


Figure 7

VEGFA gene survival curve

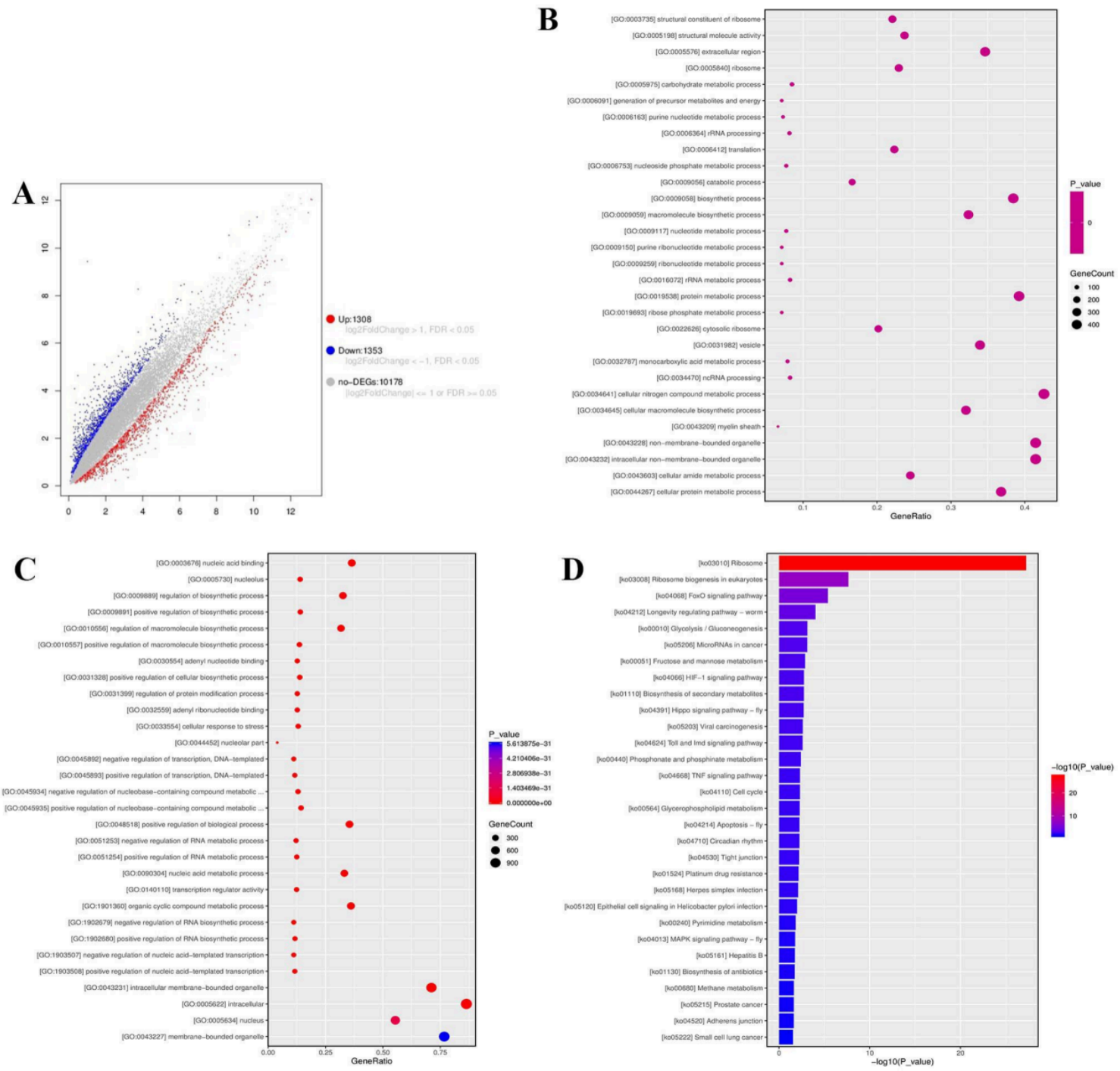


Figure 8

GO and pathway analysis. (A) Volcano map of DEmRNA. (B) Go analysis for up-regulated differentially expressed gene. (C) Go analysis for down-regulated differentially expressed gene. (D) KEGG pathways analysis for DEmRNA.