

PFKFB4 Interference Regulates Mitochondrial Membrane Fusion and Affects Glioma Proliferation and Apoptosis

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Abstract

Background: Fructose-2,6-biphosphatase 4 (PFKFB4) is a key enzyme in glucose metabolism, and its differential expression is closely related to the occurrence and development of tumors. However, the related molecular mechanisms in glioblastoma(GBM) remain unclear.

Methods: Firstly, the expression of PFKFB4 in normal and GBM tissues was analyzed by bioinformatics in The Cancer Genome Atlas (TCGA) database, and the relationship between PFKFB4 and survival was analyzed. Then detected the expression of PFKFB4 in tissues and cells of glioma. After PFKFB4 Interference, observed the proliferation by CCK8 and EDU, apoptosis of U87 cells by flow cytometry. the content of ATP and mitochondrial membrane function were detected. The possible interaction mechanism between PFKFB4 and mitochondria was analyzed by bioinformatics and verified by qPCR and Western blotting(WB). Finally, the experiment of subcutaneous tumor formation in nude mice was verified.

Results: Our study found that PFKFB4 is highly expressed in glioma tissues and cell lines. High expression levels are associated with a poor prognosis. In addition, PFKFB4 inhibition attenuates the proliferation and mitochondrial function of cancer cells promotes apoptosis, and can also reduce ATP levels. Further studies showed that PFKFB4 affects the metabolic reprogramming of gliomas by regulating mitochondrial membrane fusion, Regulating the protein expression of mitofusin 1(MFN1), mitofusin 2(MFN2), opticatroph-1(OPA1). Animal studies verify these results.

Conclusion: PFKFB4 may be a potential therapeutic target for glioma.

Background

Glioma has the highest incidence among tumors of the central nervous system, among which GBM is the most common [1]. Glioma is a neuroepithelial tumor with high malignancy, high recurrence rate, and poor prognosis and often exhibits infiltrating rapid growth, proliferation, and strong invasion [2]. Despite advances in molecular diagnosis, surgical treatment, chemotherapy, and radiotherapy in recent years, the overall prognosis remains poor [3]. The average 5-year survival rate of patients with malignant glioma is only 4–29%, and the average survival time is approximately 1 year. It remains a disease that poses a great threat to human health [4].

PFKFB4 is a protein of the PFKFB family and is widely present in various biological cells. The gene is located at 3p21.31 and composed of 14 exons and 13 introns covering 44,332 bases [5]. Previous studies have suggested that [6] the main biological function of PFKFB4 is to use the phosphatase activity in its core structure to promote the formation and degradation of fructose-2,6-diphosphate (F-2,6-BP), which is the allosteric activator of fructose phosphate kinase-1 (PFK-1) and the most effective stimulator of glycolysis, thus regulating cellular glycolysis levels. Increasingly, recent studies have suggested that

PFKFB4 is overexpressed in cancers, including liver, prostate, lung, and colorectal cancer [7, 8]. PFKFB4 is activated by some common oncogenic signaling pathways, such as C-MYC, Ras, p53, and HIF-1 α [9, 10]. Chesney et al. [11] found that selective PFKFB4 inhibition in tumor cells using 5- (N - (8-methoxy-4-quinolyl) amino) pentanoate nitrate (5MPN) can reduce F-2,6-BP, glucose uptake, and tumor growth.

MFN1 and MFN2 are mitochondrial transmembrane GTP enzymes with 60% amino acid homology that can coordinate the regulation of mitochondrial membrane fusion. This fusion and simultaneous mitochondrial division are necessary to maintain the structure and integrity of hereditary mitochondria. OPA1 was initially identified as a genetic cause of autosomal dominant optic nerve atrophy. It is a widely expressed protein located in the inner membranes of mitochondria, which regulates mitochondrial membrane fusion and crest morphology and prevents mitochondrial apoptosis. MFN1, MFN2, and OPA1 regulate mitochondrial outer and intimal membrane fusion, respectively. Mitochondrial fusion is very important for cell growth and development and has evolved into a mechanism that can counteract the harmful effects of mtDNA mutations [12]. The deletion of MFN1 and MFN2 in skeletal muscles leads to severe mitochondrial dysfunction [13]. Recently, a study reported that mitochondrial fusion is an important cause of tumorigenesis and a key factor in inducing tumor metabolic reprogramming. Knocking down the expression of MFN2 or OPA1 can inhibit mitochondrial fusion and tumor growth [14]. PFKFB4 plays an important role in carcinogenesis and participates in the survival and progression of many tumors; however, there are few studies on gliomas. It is still uncertain whether PFKB4 is involved in affecting the function of tumor cell mitochondria. Therefore, it is necessary to explore this aspect in depth.

Methods

Bioinformatics analysis

The PFKFB4 sequencing expression data and correlation analysis were obtained from TCGA (<https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga>). The differential expression analysis used the "EdgeR" package in R software, and the Pearson correlation analysis was also performed in R. Correlation analysis used the GEPIA database (<http://gepia.cancer-pku.cn/>). Survival and prognosis analysis of high and low PFKFB4 expression was performed using the OncoLnc database (<http://www.oncolnc.org/>).

Patients and samples

This study was approved by the Ethics Committee of the Second Affiliated Hospital of Kunming Medical University. Written informed consent from all donors or close relatives was required before using patient samples in this study. Four glioblastomas and adjacent tissues were collected. Patients underwent primary surgery and did not receive radiotherapy or chemotherapy before surgery, and tissues were stored in liquid nitrogen within 2 h of surgical resection.

Cell lines

Human glioma cell lines (U87, U251, and T98G) and human cerebellar astrocytes (HAC) were cultured in Dulbecco's modified eagle medium (DMEM) (Hyclone, Logan, Utah, USA), sh-PFKFB4 U87, and sh-control U87 cells were cultured in MEM (Biosharp, Anhui province, China), 10% fetal bovine serum (Gibco, California, USA), 1% penicillin/streptomycin (Gibco, California, USA) Cells were incubated at 37°C, in a 5% CO₂ atmosphere.

Western Blotting (WB)

The antibodies used for western blotting included Anti-PFKFB4 (Abcam, 1:3,000, rabbit), Anti-MFN1 (1:1,000; rabbit), Anti-MFN2 (1:1,000; rabbit), Anti-OPA1 (1:1,000; rabbit), anti- β -actin (Cell Signaling, 1:1,000; rabbit), and standard procedures for western blotting. ImageJ (v1.5.2, National Institutes of Health) was used to analyze the bands.

PFKFB4 Interference stabilizes the U87 cell line

The four short hairpins PFKFB4 RNAs (shRNA) were cloned into the lentiviral vector pGMLV-SC5-puro constructed using a high-purity plasmid cassette (DP107, Tiangong, China) and KOD-Plus-Neo (Toyobo, Osaka, Japan). The sequencing results of the PFKFB4 interference vector are shown in Table 1. pGMLV-SC5-puro served as a negative control. The constructed lentivirus vector and auxiliary packaging vector plasmid were co-transfected into 293T cells with the HG transgene reagent (Qiagen, Frankfurt, Germany). The virus-rich supernatant was collected, U87 cells were infected with the packaged lentivirus and the negative lentivirus control, and stable cells were selected with puromycin. The infected cells expressing green fluorescence were detected using flow cytometry, and the infection efficiency was evaluated. qRT-PCR and western blotting were used to further screen for the greatest shRNA3 interference.

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

Primers designed by DNAMAN software were synthesized by Sangon Biotech (**Table 2**). RNAisoPlus (Takara) was added to the cells to extract total RNA. Thermo scientific RevertAid First Strand cDNA Synthesis synthetic cDNA. FastStart Universal SYBR Green Master (Roche) \times ROX \times amplified. Applied biosystems by Life Technologies To complete the following steps. Three auxiliary wells were used for each sample.

Cell Proliferation Assay

Cell Counting Kit-8 (Beyotime, China) was used to detect cell proliferation. Cells (sh-PFKFB4, sh-control) were seeded in 96-well culture plates (2,500/well, five plates), and the results were recorded for 5 days. One hundred μ L of cell culture medium was added as a blank control. absorbed the original medium and adding 100 μ L to the ordinary medium containing CCK8 detection solution. After a 2 hour incubation, the absorbance of each well (wavelength 450 nm) was detected by spectrophotometry, and the final value was recorded. The final value = measured absorbance-blank hole absorbance.

EDU

BeyoClick™ EdU-555 (Beyotime, China) was used to detect cell proliferation, and a density of 4×10^5 cells (sh-PFKFB4, sh-control) was inoculated into 6-well culture plates. EdU-labeled cells were fixed with 4% paraformaldehyde, and permeabilized with 1 ml 0.3% Triton X-100 solution, and stained with Hoechst 33342. EdU staining was detected using an inverted fluorescence microscope (20x).

Mito-Tracker Red CMXRos

Mitochondrial labeling was performed using MitoTracker Red CMXRos (Beyotime, China). Cells (sh-PFKFB4, sh-control) were seeded in 6-well culture plates at a 70% cell density, and the MitoTracker working solution was prepared and added to the medium at a ratio of 1: 1,000. The cells were cultured in an incubator for 30 min maintained at 37 °C and 5% CO₂, then fixed with 4% paraformaldehyde. The cells were permeabilized, stained with Hoechst 33342, and observed using an inverted fluorescence microscope (20x).

ATP

Collect the cells (sh-PFKFB4, sh-control) in a centrifuge tube and discard the supernatant, add the ATP extraction solution, centrifuge the supernatant to another EP tube after sonication, add chloroform to mix, and then centrifuge to collect the supernatant for testing. The working and standard solutions were prepared according to the kit's instructions. An ultraviolet spectrophotometer was used according to the manufacturer's instructions, and ATP was calculated after the values were measured.

Flow cytometry

Two groups of cells were collected, and the cell density was at least 1×10^6 cells/ml. Annexin V-dome 633 (lot.NY601, DOJINDO, Japan) detected apoptotic cells,) follow the instructions. flow cytometry analyzer (BD FACSCelesta multicolor cell analyzer) test results.FlowJo-V10 analyzed the data.

Immune-deficient mice experiment

This experiment complied with the ARRIVE guidelines, the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, and EU Directive 2010/63/EU. Six immune-deficient male mice (6 weeks old) were purchased from the animal room of Kunming Medical University (Yunnan, China), raised in a room free of specific pathogens (SPF), and randomly assigned to two groups of nude mice, three each. For subcutaneous tumors, 1.2×10^6 cells (sh-PFKFB4, sh-control) were injected into the left armpit skin (0.2 ml) with an injection time of approximately 1 min. The weights of the mice were recorded weekly. After two weeks, the tumors were dissected, sizes and weights were measured, and western blotting was performed.

Statistics

All the experiments were repeated three times independently. The PRISM 8.0 (GraphPad Software, La Jolla, CA) graphics program and SPSS 21.0 were used for graphical and statistical analysis of the data.

The mean \pm standard deviation represents the measurement data, and the difference between the two groups was determined using Student's *t*-test. Statistical significance was set at $P < 0.05$.

Results

PFKFB4 is highly expressed in GBM, and up-regulation of PFKFB4 expression is associated with poor prognosis.

From the TCGA, PFKFB4 levels in tumor tissues were significantly higher than those in normal tissues ($P < 0.001$) (Fig. 1A). To study the clinical significance of PFKFB4, high and low expression groups were divided into high and low expression groups according to the median risk score of PFKFB4. Using the OncoLnc database, found that patients with high expression of PFKFB4 were usually associated with shorter survival times ($P < 0.001$) (Fig. 1B). These data suggest that PFKFB4 may be a potential poor prognostic factor for glioblastoma.

We further collected fresh surgical samples to detect the expression of PFKFB4 in glioma and adjacent tissues. Western blotting results showed that the expression of PFKFB4 in cancer tissues was significantly higher than that in adjacent tissues (Fig. 1C). In addition, we observed the expression of PFKFB4 in glioblastoma cell lines. The expression of PFKFB4 in glioma cell lines U87, T98G, and U251 was significantly higher than that in normal HAC cells, especially in U87 cells (Fig. 1D).

PFKFB4 interference can inhibit GBM proliferation Promote apoptosis.

We constructed sh-PFKFB4 U87 cells and sh-control U87 cells. The qPCR and Western blotting results showed that sh-PFKFB4 protein and mRNA decreased significantly (Fig. 2A).

CCK8 staining showed that cell proliferation decreased significantly in the sh-PFKFB4 group (Fig. 2B). EDU staining showed that PFKFB4 interference decreased the number of EDU-positive cells (Fig. 2C). The results show that PFKFB4 may be involved in the occurrence and development of GBM. The results of flow cytometry showed that U87 apoptotic cells increased significantly after PFKFB4 Interference(Fig. 2D).

PFKFB4 interference inhibits GBM mitochondrial function.

PFKFB4, as a key enzyme in the glucose metabolic pathway, may be involved in tumor metabolism. We found that the tumor cell ATP content decreased significantly in the sh-PFKFB4 group ($P < 0.01$) (Fig. 2E), The MitoTrackerRed probe experiment showed that in the sh-PFKFB4 group, mitochondrial bioactivity and the mitochondrial membrane potential decreased (Fig. 2F). These results suggest that PFKFB4 may be involved in the regulation of mitochondrial energy metabolism in GBM.

PFKFB4 affects the fusion of the mitochondrial membrane and the metabolism of GBM.

We found that PFKFB4-interfered interfered with the mitochondrial function of U87 cells, and we tried to determine the internal relationship between PFKFB4 and mitochondria. Through the correlation analysis of transcriptome information in the CGA and GEPIA databases, we found a positive correlation between PFKFB4 and OPA1, MFN1, and MFN2. ($P < 0.05$). (OPA1: CGA Cor = 0.529, $P = 1.498 \times 10^{-13}$; GEPIA R = 0.37, $P = 1.3 \times 10^{-6}$) (MFN1: CGA Cor = 0.448, $P = 1.007 \times 10^{-9}$; GEPIA R = 0.24, $P = 0.002$) (MFN2: CGA Cor = 0.535, $P = 6.43 \times 10^{-14}$; GEPIA R = 0.32, $P = 2.5 \times 10^{-5}$) (Fig. 3A, B).

We further verified this finding using qPCR and WB in vitro. The results showed that the expression of MFN1, MFN2, and OPA1 in U87 cells was downregulated after PFKFB4 interference (Fig. 3C, D).

The results of the animal and cell culture experiments are consistent.

Both sh-PFKFB4 and sh-control cells form tumors. After 15 days, there was no erosion in the epidermis, and the tumors were dissected (Fig. 4A), weighed, and the maximum diameter of each tumor was measured. The results are shown in Table 3.

The sizes and weights of the sh-PFKFB4 group were significantly smaller than those of the sh-control group. The expression of OPA1, MFN1, and MFN2 also decreased (Fig. 4B) in the PFKFB4 interference group, which is consistent with the cell culture experimental results.

Discussion

Gliomas originate from glial cells and are the most common primary intracranial tumors. The boundary between invasive growth and normal brain tissue remains unclear [15]. With rapid tumor development, to meet the needs of malignant proliferation for energy and nutrients, tumor cells undergo adaptive metabolic changes to redefine the flow and flux of nutrients in the metabolic network. This process is called metabolic reprogramming of tumors and is considered a new feature of malignant tumors [16]. Previous studies [17–21] found that, even when there was plenty of oxygen, most tumor cells still choose glycolysis rather than oxidative phosphorylation, a feature called the Warburg effect, which was classified as one of the top ten characteristics of tumors in 2011 [22]. Although the Warburg effect has been verified in many tumors, recent studies have shown that mitochondrial-dependent energy synthesis plays a key role in tumor growth and proliferation [23–26]. Not all tumor cells perform glycolysis, as reported in this study, but are divided into glycolysis and oxidative phosphorylation [27]. Ashton explained the potential of oxidative phosphorylation inhibitors in anti-tumor therapy [28].

There is no doubt that mitochondria play a vital role in the oxidative phosphorylation of tumor cells. Mitochondria are highly dynamic organelles that maintain the dynamic balance of frequent fusion and

division, which play a very important role in maintaining the number, morphology, and function of mitochondria. Under normal circumstances, the fusion and division of mitochondria are balanced, and the number and morphology of mitochondria remain stable. With increased fusion, mitochondria exhibit an extended tube network, and energy metabolism is increased, which can protect the cells to a certain extent. With increased division, mitochondria shorten or fragment, which leads to mitochondrial damage [29]. With increasing research, it has been found that MFN1, MFN2, and OPA1 mediate mitochondrial fusion. Mitochondrial division is mediated by dynamin-related protein 1 (Drp1) and fission1 (Fis1) [30]. The mitochondrial function may play a key role in the energy metabolism of some tumors. Increasingly, studies have been conducted on mitochondria in the process of tumorigenesis and development. A study reported that PINK1 and PARK2 inhibit the occurrence of pancreatic tumors by controlling mitochondrial iron-dependent immune metabolism, and the destruction of mitochondrial iron homeostasis may contribute to cancer development [31]. That study also reported that mitochondrial translation elongation factor 4 (mtEF4) is critical for the synthesis of proteins encoded by the mitochondrial genome (mtDNA) and for controlling the quality of mitochondrial respiratory chain biosynthesis.

mtEF4 also plays a key role in tumor progression. Knockdown of mtEF4 in tumor cells can lead to defects in tumor mitochondrial function, leading to cell apoptosis, and mtEF4 overexpression promotes tumor occurrence and development [32].

A new study has suggested that PFKFB4 is a novel protein kinase [33], which can promote tumor growth and metastasis, but its role in transcriptional regulation of tumor glucose metabolism reprogramming is not clear. Our results showed that PFKFB4 expression in glioma cell lines (T98G, U87, U251) was significantly higher than in a healthy cell line (HAC), indicating that PFKFB4 is an oncogene in human glioma. Thus, PFKFB4 interference inhibits glioma cell growth and proliferation. We also found that the mitochondrial membrane potential and ATP content decreased, suggesting that PFKFB4 interference may affect mitochondrial function. We further verified the correlation between PFKFB4 and mitochondrial membrane fusion-related proteins MFN1, MFN2, and OPA1 in the CGA and GEPIA databases.

Interestingly, there was a positive correlation between the two. Furthermore, in the PFKFB4 interference group, we found the mRNA and protein levels of MFN1, MFN2, and OPA1 were down-regulated. Finally, in animal experiments, we found that PFKFB4 interference could inhibit tumor growth in animals, and MFN1, MFN2, and OPA1 also decreased, which may lead to mitochondrial dysfunction. We believe that PFKFB4 plays an important role in the reprogramming of glucose metabolism in GBM cells. Of course, these studies are preliminary, and in future research, we will continue to study the significance of PFKFB4 in tumor metabolism more widely and deeply.

In summary, our results show that PFKFB4, as an oncogene in GBM, plays an important role in metabolic reprogramming by affecting mitochondrial membrane fusion. PFKFB4 may serve as a biomarker for the diagnosis of gliomas and represents a potential therapeutic target.

Abbreviations

GBM glioblastoma multiforme; PFKFB4 fructose-2,6-biphosphatase 4; MFN1 mitofusin 1; Mfn2 mitofusin 2; OPA1 opticatroph-1; TCGA the Cancer Genome Atlas; shRNA short hairpin RNA.

Declarations

Author contributions

Conceptualization: Ninghui Zhao.

Methodology: Kai Zhao, Chaojun Yu, Ji Luo, Minhao Huang.

Data curation: Ji Luo, Qian Wen.

Formal analysis: Kai Zhao, Chaojun Yu, Minhao Huang.

Funding acquisition: Ninghui Zhao.

Investigation: Kai Zhao, Qian Wen.

Supervision: Ninghui Zhao.

Writing – original draft: Kai Zhao, Chaojun Yu.

Writing – review & editing: Kai Zhao, Ninghui Zhao.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of the Second Affiliated Hospital of Kunming Medical University. Under this supervision, the samples were used with written informed consent from the donor or next of kin.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1. shRNA targets used in the present study.

shRNA	Sequence (5'-3')
PFKFB4-shRNA1	GATCCGCGCGGTGAACTTTCAAAGGTCTCGAGACCTTTGAAAGTTCACCGCGCTTTTTT
PFKFB4-shRNA2	AATTAAAAAAGGCAGGAAGTTCAGCAGAGATCTCGAGATCTCTGCTGAAGTTCCTGCCG
PFKFB4-shRNA3	GATCCGCTGGAGAGGCAAGAGAATGTCTCGAGACATTCTCTTGCCTCTCCAGCTTTTTT
PFKFB4-shRNA4	AATTAAAAAAGCATCGTATATTACCTCATGACTCGAGTCATGAGGTAATATACGATGCG

Note:shRNA=short hairpin RNA; PFKFB4= Fructose-2,6-Biphosphatase 4.

Table2. primer

Gene	Forward primer sequence	Reverse primer sequence
PFKFB4	GTGATGAGGCTACGGAGGACTTC	GTAATATACGATGCGGCTCTGG
GAPDH	GTCTCCTCTGACTTCAACAGCG	ACCACCCTGTTGCTGTAGCCAA
MFN1	GAAATGCTCAAAGGGTGCTC	TCCAAATCACTCCTCCAACA
MFN2	ACAAGGTGAGTGAGCGTCTC	CACAAAGAAGATGCGGTCC
OPA1	CAGTAGAGGTTGCTTGGGAGAC	TATCCTTGAGACGAGCCTGC

Table 3. Size and weight of subcutaneous tumorigenic tissue in Immune-deficient mice

Group	Size(cm)	weight (g)
sh-PFKFB4(1)	0.3	0.06
sh-PFKFB4(2)	0.6	0.03
sh-PFKFB4(3)	0.5	0.03
sh-control(1)	1.5	0.77
sh-control(2)	1.4	0.61
sh-control(3)	1.5	0.85
P Value	0.0004	0.0006

Note: The tumor in the control group was significantly larger than the sh-PFKFB4 group(P=0.0004); The control group was significantly heavier than the sh-PFKFB4 group(P=0.0006).

Figures

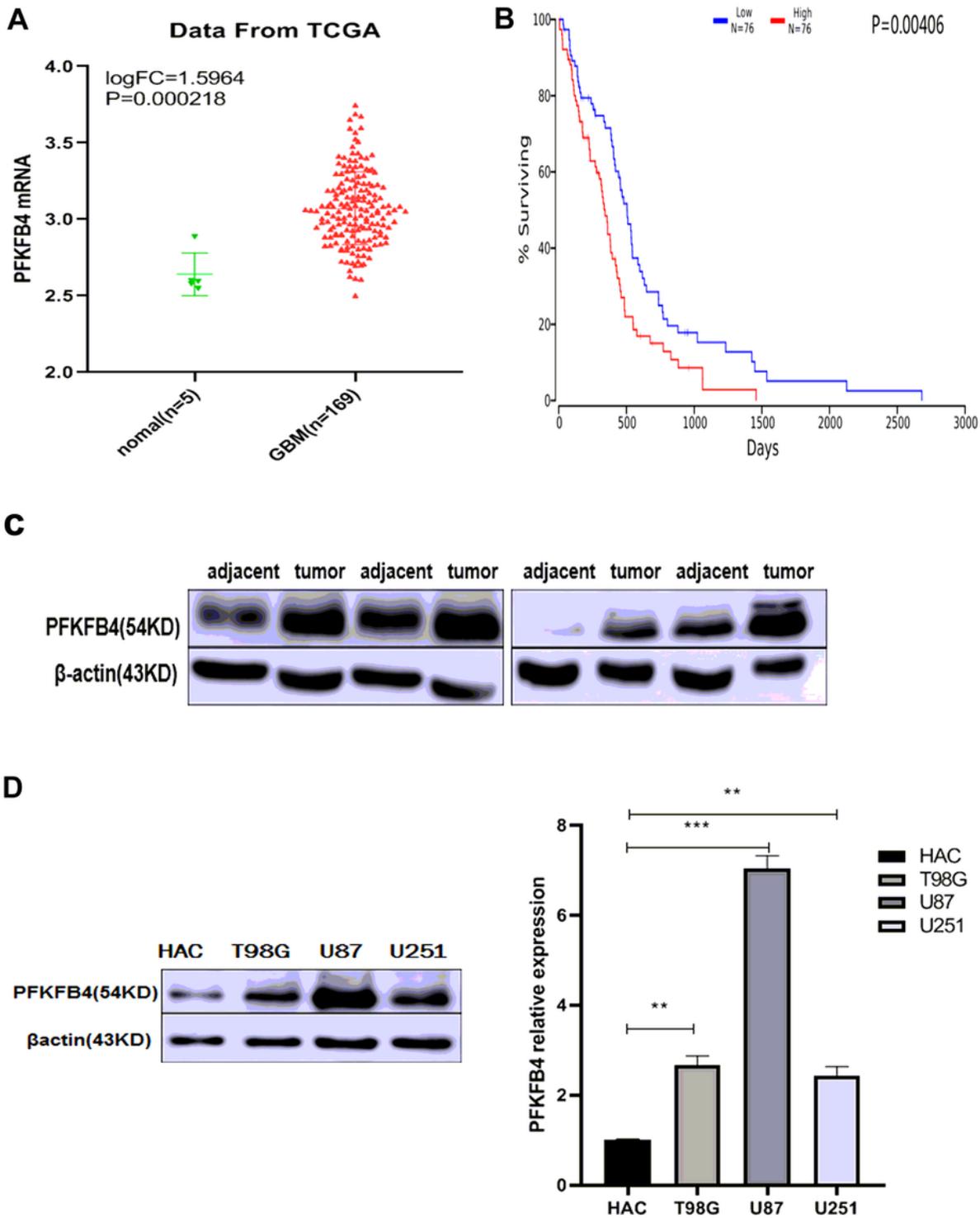


Figure 1

PFKFB4 is highly expressed in glioblastoma multiforme and may be associated with patients' poor prognosis. (A) The expression of PFKFB4 in normal brain tissue and glioblastoma tissue in the CGA database. (B) The effect of high and low expression of PFKFB4 on the survival of patients with glioblastoma in the OncoLnc database. (C) PFKFB4 protein expression in gliomas and their adjacent

tissues. (D) PFKFB4 protein expression in glioma cell lines (T98G, U87, U251) and healthy HAC cells. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

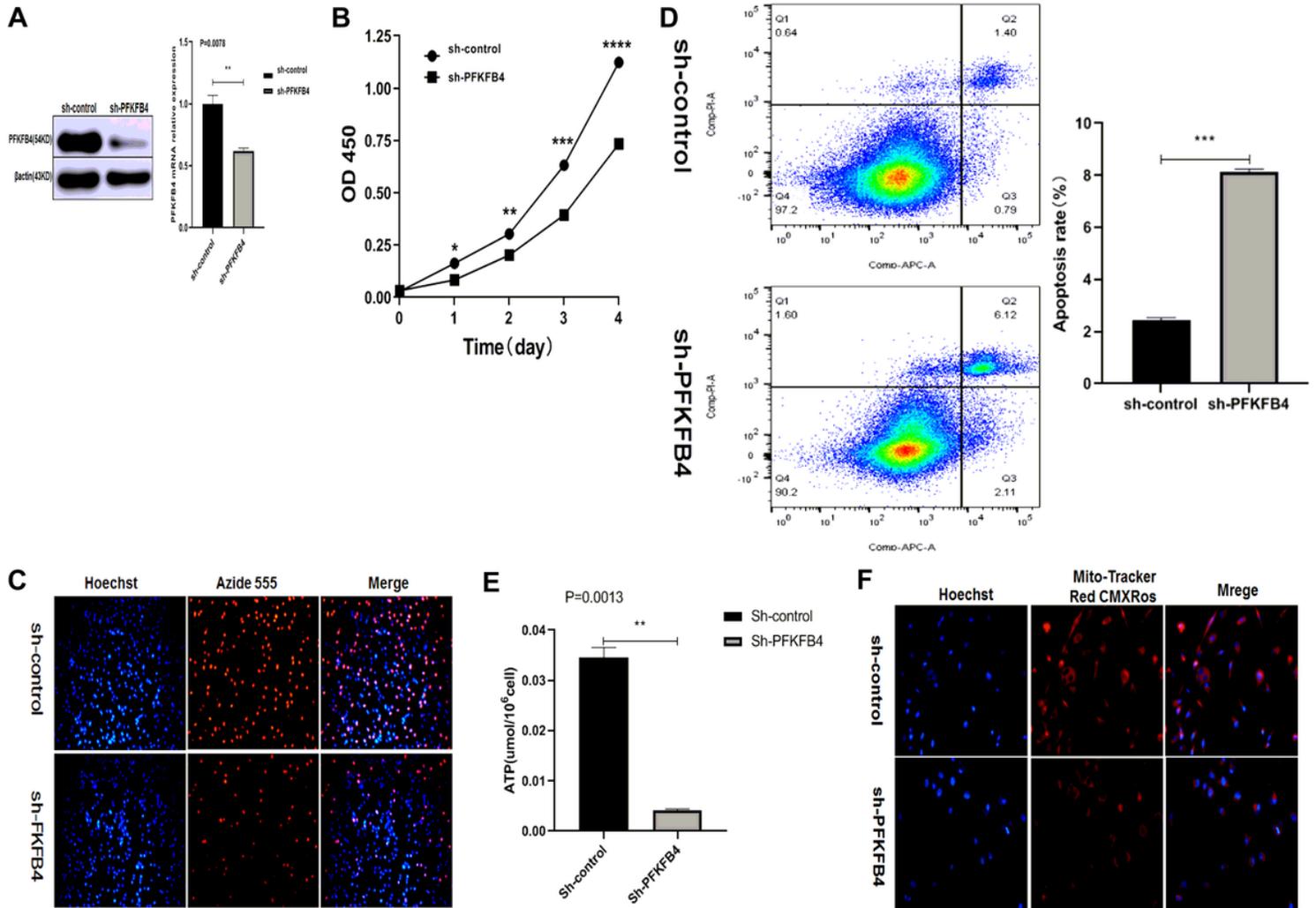


Figure 2

PFKFB4 interference inhibited proliferation, ATP production, promotes apoptosis, and mitochondrial membrane potential in U87 cells (A) WB and qPCR showed that sh-PFKFB4 could significantly down-regulate PFKFB4 expression in U87 cells. (B) PFKFB4 interference significantly inhibited the proliferation of U87 cells. (C) PFKFB4 interference can decrease the percentage of EDU-positive U87 cells. (D) PFKFB4 interference can increase U87 apoptotic cells significantly. *** $P < 0.001$. (E) PFKFB4 interference decreased the ATP content of U87 cells, ** $P < 0.01$. (F) PFKFB4 interference decreased the biological activity and membrane potentials of mitochondria.

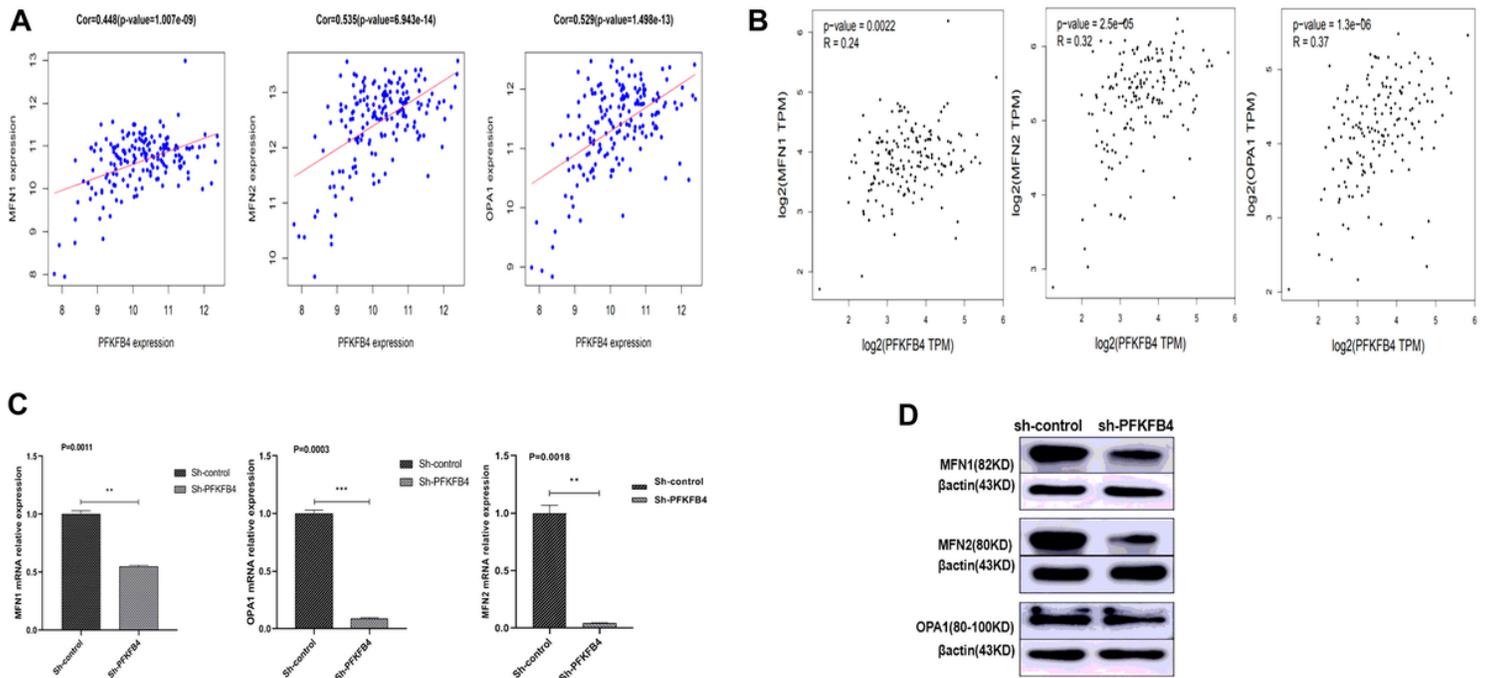


Figure 3

Mitochondrial membrane fusion proteins (MFN1, MFN2, OPA1) were down-regulated after PFKFB4 interference. (A) Pearson correlation analysis of the CGA database found that PFKFB4 in GBM is moderately related to mitochondrial fusion genes OPA1, MFN1, and MFN2 (OPA1: Cor= 0.529, P= 1.498x10⁻¹³), (MFN1: Cor= 0.448, P= 1.007x10⁻⁹), (MFN2: Cor= 0.535, P= 6.43x10⁻¹⁴). (B) GEPIA database analysis showed a positive correlation between PFKFB4 and the mitochondrial fusion genes OPA1, MFN1 and MFN2 in GBM (OPA1: R= 0.37, P= 1.3x10⁻⁶), (MFN1: R= 0.24, P= 0.0022), (MFN2: R=0.32, P= 2.5x10⁻⁵). (C) PFKFB4 interference decreased the relative mRNA expression of MFN1, OPA1, and MFN2 in U87 cells. (D) PFKFB4 interference decreased the protein expression of MFN1, MFN2, and OPA1 in U87 cells.

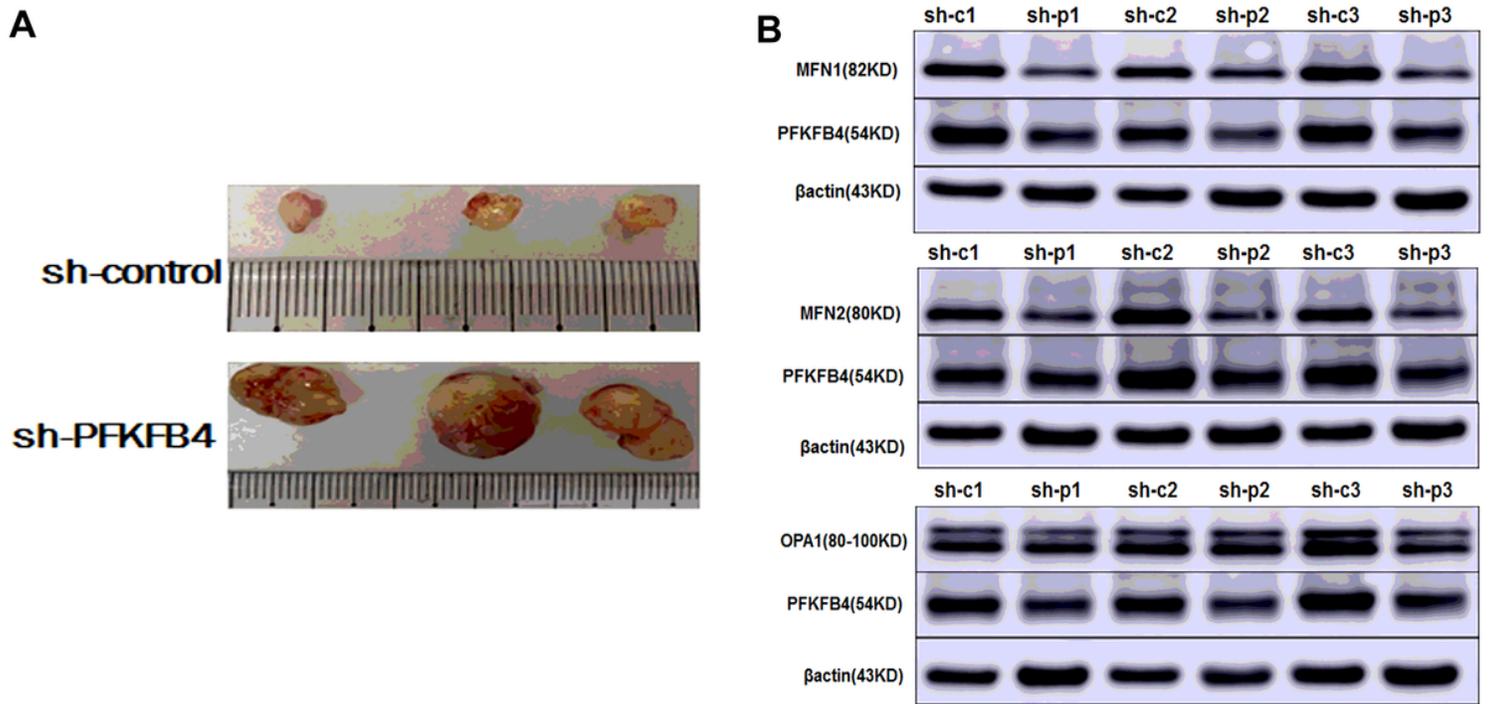


Figure 4

In nude mice, tumors were smaller, and the mitochondrial membrane protein decreased in the PFKFB4 interference group. (A) Tumors were smaller in volume and mass in the PFKFB4 interference groups of immune-deficient mice. (B) PFKFB4 interference also decreased the relative mRNA expression of MFN1, MFN2, and OPA1 in Immune-deficient mice. sh-c1:sh-control (1), sh-c2:sh-control (2), sh-c3:sh-control (3), sh-p1:sh-PFKFB4 (1), sh-p2:sh-PFKFB4 (2), sh-p3:sh-PFKFB4 (3).