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ORIGINAL RESEARCH

miR-1297 Suppresses Osteosarcoma Proliferation and Aerobic Glycolysis by Regulating PFKFB2

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Background: *MiR-1297* is reported to function as a tumor suppressor of various cancers. However, the role of *miR-1297* in the development of osteosarcoma (OS) has not been elaborated. The purpose of this study was to investigate the functional effects of *miR-1297* on OS progression and the underlying mechanism.

Methods: The expression of protein and mRNA in OS cells was evaluated by Western blotting and quantitative real-time polymerase chain reaction. Cellular proliferation was investigated by cell counting kit-8, colony formation and apoptosis assays. Bioinformatics methods were used to predict target genes. The relationship between *PFKFB2* and *miR-1297* was demonstrated by dual-luciferase reporter assay. Metabolic changes in OS cells were monitored using an XF96 metabolic flux analyzer.

Results: We found that *miR-1297* was downregulated in OS and that lower expression of *miR-1297* promoted proliferation and contributed to the Warburg effect in OS cells. Furthermore, we showed that silencing *PFKFB2* inhibited proliferation and reduced aerobic glycolysis while overexpression of *PFKFB2* reduced the anti-tumor function of *miR-1297* in OS cells. Mechanistically, *miR-1297* acted as a tumor suppressor in OS and reduced the expression of *PFKFB2* by directly targeting its 3′UTR.

Conclusion: The *miR-1297*/*PFKFB2* axis regulated OS proliferation by controlling the Warburg effect. Our results revealed a previously undiscovered function of *miR-1297* in OS, which strongly linked metabolic alterations with cancer progression. Targeting *miR-1297* may become a promising therapeutic approach for OS.

Keywords: osteosarcoma, miR-1297, PFKFB2, Warburg effect

Introduction

Osteosarcoma (OS), one of the most common primary bone tumors, arises most often in adolescents and children, with a second peak in incidence among the elderly.[1] Up to now, the main therapies for OS are surgery, immunotherapy, and chemotherapy. Nonetheless, the survival rate of patients has not markedly improved in the last few decades and many mysteries regarding better management still remain.[2] Thus, it is vital to explore the mechanism underlying OS progression as well as to investigate potential effective therapeutic targets.

MicroRNAs (miRNAs) are short single-stranded, non-coding RNAs consisting of about 18–25 nucleotides, which function as regulators of target genes by binding to the target mRNAs.[3,4] The development of OS is a complex process and miRNAs play extensive roles in these processes. As previously described, some miRNAs have the potential to become therapeutic targets and biomarkers for OS patients.[5–7] Recently, *miR-1297* has been reported to play a role as a tumor suppressor in many

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cancers. Gao et al demonstrated that downregulation of *miR-1297* enhanced cell proliferation via regulation of *CREB1* in gastric cancer.[8] Wang et al reported that *miR-1297* inhibited the progression of colorectal cancer by suppressing cyclin D2 expression.[9] However, the biological functions and specific roles of *miR-1297* in OS are as yet unknown.

In this work, we confirmed that the expression level of *miR-1297* in OS cell lines and tissues is downregulated. *MiR-1297* inhibited aerobic glycolysis in OS cells, resulting in the suppression of cell proliferation. Further studies indicated that *miR-1297* inhibited aerobic glycolysis by targeting *PFKFB2* directly. Therefore, these results indicated that *miR-1297* has the potential to be a new anticancer target in OS.

Materials and Methods

Cell Culture and Reagents

The Cell Bank of the Chinese Academy of Sciences (Shanghai, China) provided the human OS cell lines (MNNG-HOS, U-2OS Saos-2 and MG63 cells) and human osteoblast hFOB1.19 cells. All cell lines were cultured according to ATCC instructions. The human OS cells were grown at 37°C in 5% CO_2, while the hFOB1.19 cells were grown at 34.5°C in 5% CO_2. Antibodies against *PFKFB2* (ab234865; Abcam, Cambridge, UK) and *GAPDH* (bsm-33033M; Bioss, Beijing, China) were used.

Transfection Assay

For the experiments, MNNG-HOS and U-2OS cells were transfected with small interfering RNAs (siRNAs); the siRNA oligos were purchased from Gene Pharma (Shanghai, China). Transfection procedures were carried out in accordance with the manufacturer's operating procedures. Sequences of *PFKFB2* siRNAs were as follows: si-1, 5′-AACUAACACGCUACCUCAA-3′ (sense) and 5′-CC AUUUACCUUUGCC GGCA-3′ (antisense); si-2, 5′-AA GAUGCAGCCUACCUGAA-3′ (sense) and 5′-CCUAA CUCAUACGGCGACA-3′ (antisense).

The plasmids containing *PFKFB2* or *miR-1297*-FLAG and the negative control plasmid were purchased from OBiO Technology (Shanghai, China). These plasmids were packaged into virus particles using HEK 293T cells to determine the virus titer. In order to establish stable cells that overexpressed *miR-1297* or *PFKFB2*, target cells were infected with 1×10^8 lentiviral transduction units containing 6 mg/mL of Polybrene (Sigma-Aldrich, St. Louis, MO, USA). After 72 h, infected cells were screened with 2.5 mg/mL puromycin. Western blotting or quantitative real-time polymerase chain reaction (qRT-PCR) verified the efficiency of overexpression.

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

The extraction of total RNA and reverse transcription of total RNA were performed as previously described.[10] A SYBR® Premix Ex Taq™ kit (Takara Bio, Otsu, Japan) was used to quantify the *PFKFB2* transcripts. The setup of the thermal cycler program was as previously described.[11] For miRNA qRT-PCR, 1 mg of total RNA was reverse-transcribed using stem-loop primers from a Bio-TNT miRNA qRT-PCR Detection Primer Set (BioTNT Biotechnologies, Shanghai, China). The qRT-PCR was performed using SYBR-green Master mix (BioTNT Biotechnologies). The reaction conditions were: 95°C for 5 min, followed by 95°C for 5 s, 60°C for 30 s, for a total of 40 cycles, and fluorescence signals were detected. The endogenous controls were 18S and U48 small-nuclear RNA.

Western Blot Assay

Western blotting was conducted as described previously.[11] In simple terms, whole-cell proteins were extracted and the equivalent protein was subjected to electrophoresis on a 10% sodium dodecyl sulfate polyacrylamide gel. After the protein was electrically transferred to the membrane and blocked, membranes were incubated overnight with the primary antibodies at 4°C and were washed three times with TBST. After incubation with the secondary antibody for 1 h at room temperature, it was detected and combined with a western electrochemical luminescent substrate (Share-Bio, Shanghai, China).

CCK-8 and Colony Formation Assays

The CCK-8 assay was performed according to the vendor's instructions (Dojindo Molecular Technologies, Japan). Briefly, 3×10^3 cells were seeded into 96-well plates. The absorbance at a wavelength of 450 nm was measured at 0, 24, 48, 72, 96, and 120 h using a tablet reader (Thermo Fisher Scientific, Waltham, MA, USA).

The infected OS cells were cultured in a 6-well plate at an initial cell density of 1×10^3 cells/well. After two weeks, ice-cold PBS was used to wash the colonies. Then, the cell pellet was fixed with 4% paraformaldehyde

and stained with 0.1% crystal violet. Finally, photographic images were captured and cell numbers were counted.

Fluorescence in situ Hybridization (FISH)

A microarray containing tissue from 40 OS patients was obtained from Alena Biotechnology Co., Ltd. (Xi'an, China). OS tissue sections were hybridized with the miR-1297 probes (Servicebio, Wuhan, China). Fluorescence in situ hybridization was performed as described previously.[12]

Measurement of Oxidative Phosphorylation and Glycolysis

The extracellular acidification rate (ECAR) and oxygen consumption rate (OCR) of cultured cells were tested using an XF96 metabolic flux analyzer (Seahorse Biosciences, Billerica, MA, USA) in accordance with the manufacturer's instructions. In brief, 80 μL of a suspension of the target cells (3×10^4) were plated into each well of an XF96 96-well plate (Seahorse Biosciences) and incubated overnight at 37°C. XF calibration solution (Seahorse Biosciences) was added to the XF sensor box (seahorse bioscience company) and incubated at 37°C without adding CO_2 overnight. On the second day, DMEM (1 g/mL glucose, pH 7.4; Seahorse Biosciences) modified by XF assay was used to replace the complete medium, and the cells were incubated for 1 h at 37°C without CO_2, while 10 mM glucose, 1 mM oligomycin (Sigma-Aldrich) and 80 mM 2-deoxyglucose (D8375; Sigma-Aldrich) were injected continuously to assess the ECAP and the OCR. XFe Wave software (Seahorse Biosciences) was used to analyze the results.

Cell Apoptosis

In order to evaluate cell apoptosis, the adherent cells were separated after culturing for 24 h in serum-free medium. Cells were stained using an Annexin V/FITC Kit (BD Biosciences, San Jose, CA, USA) following the manufacturer's instructions. Finally, flow cytometry was used to analyze the results.

Measurement of Cellular ATP Level and Lactate Production

An ATP assay kit (Promega, Madison, WI, USA) was used to detect cellular ATP level according to the manufacturer's instructions. A fluorescence luminometer (Perkin Elmer, Waltham, MA, USA) was used to determine bioluminescence. The ATP level was calculated from a standard curve. A lactate assay kit (BioVision, Zurich, Switzerland) was used to detect extracellular lactate levels according to the manufacturer's protocols. All values were normalized to the cellular protein level. Three independent experiments were performed.

Dual-Luciferase Reporter Assay

Luciferase reporter plasmids were cloned into the pMIR vector, which contained both mutant 3′-UTR and wild-type *PFKFB2*. The cells were transfected with luciferase reporters, either wild-type or mutant *PFKFB2* 3′-UTR, combined with *miR-1297* mimics or negative control (NC) for miRNA mimics using Roche X-tremeGENE HP DNA Transfection Reagent (Roche, Basel, Switzerland). Luciferase activity was analyzed using the Dual-Glo Luciferase Assay System (Promega) after 48 h.

Database Analysis

TargetScan (http://www.targetscan.org) and miRDB (http://www.mirdb.org) were used to predict potential target genes of *miR-1297* and potential target sites of *miR-1297* on *PFKFB2*.

Statistical Analyses

Graphpad software was used for statistical analyses. All data are represented as mean ± SD. The chi-square test was used for proportion comparison. The comparison between different groups was performed by Student's *t*-test. A P value < 0.05 was considered to show a statistically-significant difference.

Results

MiR-1297 is Downregulated in OS Cell Lines and Inhibits the Proliferation and Promotes the Apoptosis of OS Cells

To investigate the expression of *miR-1297* in OS, we initially made an assessment of the expression level of *miR-1297* in hFOB1.19 cells and OS cell lines (Saos-2, MG63, MNNG-HOS, and U-2OS) by qRT-PCR. The results suggested that, compared to the expression level in normal human osteoblast cell lines, *miR-1297* was significantly downregulated in OS cell lines, especially in MNNG-HOS and U-2OS cell lines (Figure 1A). Furthermore, we detected differences in the expression of *miR-1297* in OS (n = 40) tissue samples through fluorescence in situ hybridization (FISH). We found that the lower expression of *miR-1297* was related to the advanced pathological staging in OS

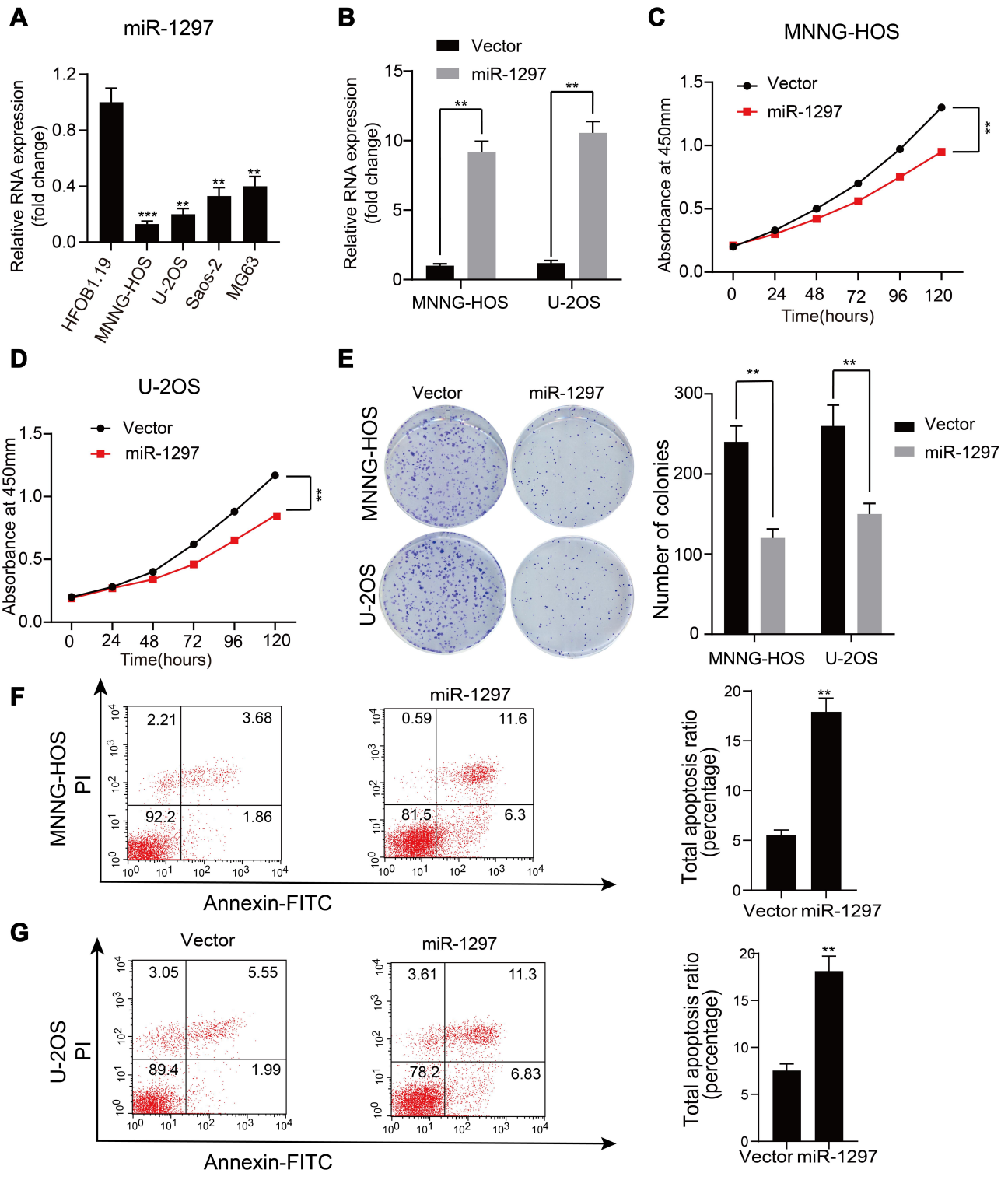

Figure 1 *miR-1297* is downregulated in OS cell lines and closely related to apoptosis and proliferation of the OS cells. (**A**) The representative expression level of *miR-1297* in hFOB1.19 (normal osteoblast cell line) and Saos-2, U-2OS, MNNG-HOS and MG-63 (OS cell lines). $^{**}p < 0.01$, $^{***}p < 0.001$. (**B**) The overexpression efficacy of *miR-1297* in MNNG-HOS and U-2OS (wild OS cells) was verified by qRT-PCR, $^{**}p < 0.01$. (**C** and **D**) Overexpression of *miR-1297* inhibited the proliferation of MNNG-HOS and U-2OS cells using the cell counting kit (CCK)-8 assay, Values are means ± SD, $^{**}p < 0.01$. (**E**) Upregulated of *miR-1297* suppressed the proliferation of MNNG-HOS and U-2OS cells (OS cells lines verified determined by colony formation assay, Values are means ± SD, $^{**}p < 0.01$). (**F** and **G**) Upregulated of *miR-1297* significantly induces apoptosis of MNNG-HOS and U-2OS (OS cells lines), Values are means ± SD, $^{**}p<0.01$.

(Supplementary Figure 1A and B). In order to explore the exact function of *miR-1297* in the progression of OS, we developed *miR-1297*-overexpressing cell lines and confirmed the efficiency of overexpression by qRT-PCR (Figure 1B). According to the CCK-8 proliferation assay (Figure 1C and D) and colony formation assay (Figure 1E), the results showed that overexpression of *miR-1297* inhibited the proliferation of MNNG-HOS and U-2OS cells. Next, we explored the effect of *miR-1297* overexpression on the apoptosis of OS cells. As shown in Figure 1F and G, compared with the control cells, overexpression of *miR-1297* significantly increased the apoptosis of OS cells. These results suggested that *miR-1297* inhibited the proliferation of OS cells and promoted their apoptosis.

MiR-1297 is Involved in the Regulation of the Warburg Effect in OS Cells

It is well known that the Warburg effect plays a vital role in tumor development, contributing to the growth of cancer cells.[13] We used a metabolic flux analyzer to explore whether *miR-1297* has any relationship with the Warburg effect in the development of OS by measuring the glycolytic rate (ECAR) and mitochondrial respiration (OCR). Overexpression of *miR-1297* significantly suppressed the ECAR in MNNG-HOS and U-2OS cells (Figure 2A and B) but enhanced OCR in these cells (Figure 2C and D). Furthermore, the overexpression of *miR-1297* in OS cells also augmented the level of ATP produced by oxidative phosphorylation and decreased the production of lactate by aerobic glycolysis (Figure 2E and F). Collectively, these data suggested that *miR-1297* inhibited aerobic glycolysis in OS cells.

PFKFB2 is Identified as a Direct Target of miR-1297

In order to predict the latent target genes of *miR-1297*, we utilized two widely-applied prediction algorithms, TargetScan (http://www.targetscan.org) and miRDB (http://www.mirdb.org). We found that *PFKFB2*, *CHST2* (carbohydrate sulfotransferase 2), *DEPDC1* (DEP domain containing 1), *B4GALT1* (beta-1,4-galactosyltransferase 1), *MXI1* (MAX interactor 1, dimerization protein), *PGM2* phosphoglucomutase 2), and *PCK1* (phosphoenolpyruvate carboxykinase 1) were related to *miR-1297* and the Warburg effect (Figure 3A). We analyzed the expression level of these genes when *miR-1297* was overexpressed in OS cell lines. We found that the expression level of *PFKFB2* was notably downregulated in the *miR-1297*-overexpressing cell lines (MNNG-HOS and U-2OS Figure 3B–C). As shown in Figure 3D, there are two possible target sites of *miR-1297* in *PFKFB2*. We found that *miR-1297* significantly suppressed the luciferase activity of the wild-type *PFKFB2* 3′-UTR (WT), but not the Mut 3′-UTR of *PFKFB2* in MNNG-HOS and U-2OS cells (Figure 3E and F). In summary, all these data suggested that *PFKFB2* was a target of *miR-1297* in OS cells.

PFKFB2 Promotes the Proliferation of OS Cells and Contributes to the Warburg Effect in OS Cells

PFKFB2 belongs to the *PFKFB* family, and has been found to be overexpressed in many tumors such as glioma, prostate cancer and acute lymphoblastic leukemia.[14–16] However, further study is necessary to explore the mechanism underlying its actions. We knocked down *PFKFB2* using siRNA and used Western blotting to confirm the efficiency of the knockdown (Figure 4A). CCK-8 proliferation assay (Figure 4B and C) and colony formation assay (Figure 4D and E) indicated that knocking down of *PFKFB2* significantly suppressed the multiplication of the OS cells, while an apoptosis assay indicated that silencing of *PFKFB2* induced OS cell apoptosis (Figure 4F and G). In OS cells, knockdown of *PFKFB2* also increased the OCR and reduced the ECAR (Figure 4H and I). In conclusion, all these experimental results indicated that *PFKFB2* promoted the Warburg effect and contributed to the multiplication of OS cells.

MiR-1297 Suppresses Proliferation and Aerobic Glycolysis by Inhibiting PFKFB2 Expression in OS Cells

In our research, once we had identified that *miR-1297* inhibited the multiplication of OS cells (Figure 1), we next further probed whether or not *miR-1297* suppressed proliferation and aerobic glycolysis by targeting *PFKFB2* in OS cells. We first overexpressed *miR-1297* in wild-type and stable *PFKFB2*-overexpressing cell lines. Western blotting was used to confirm the overexpression efficiency (Figure 5A). As shown in Figure 5B–G, colony formation assays, CCK8 assays and apoptosis assays indicated that overexpression of *miR-1297* inhibited cell growth while overexpression of *PFKFB2* reduced the anti-tumor function of *miR-1297*. Furthermore, the aerobic glycolysis which was previously suppressed by *miR-1297*, was partly restored by overexpression of *PFKFB2* (Figure 5H–K). All these results indicated the *miR-1297* suppressed

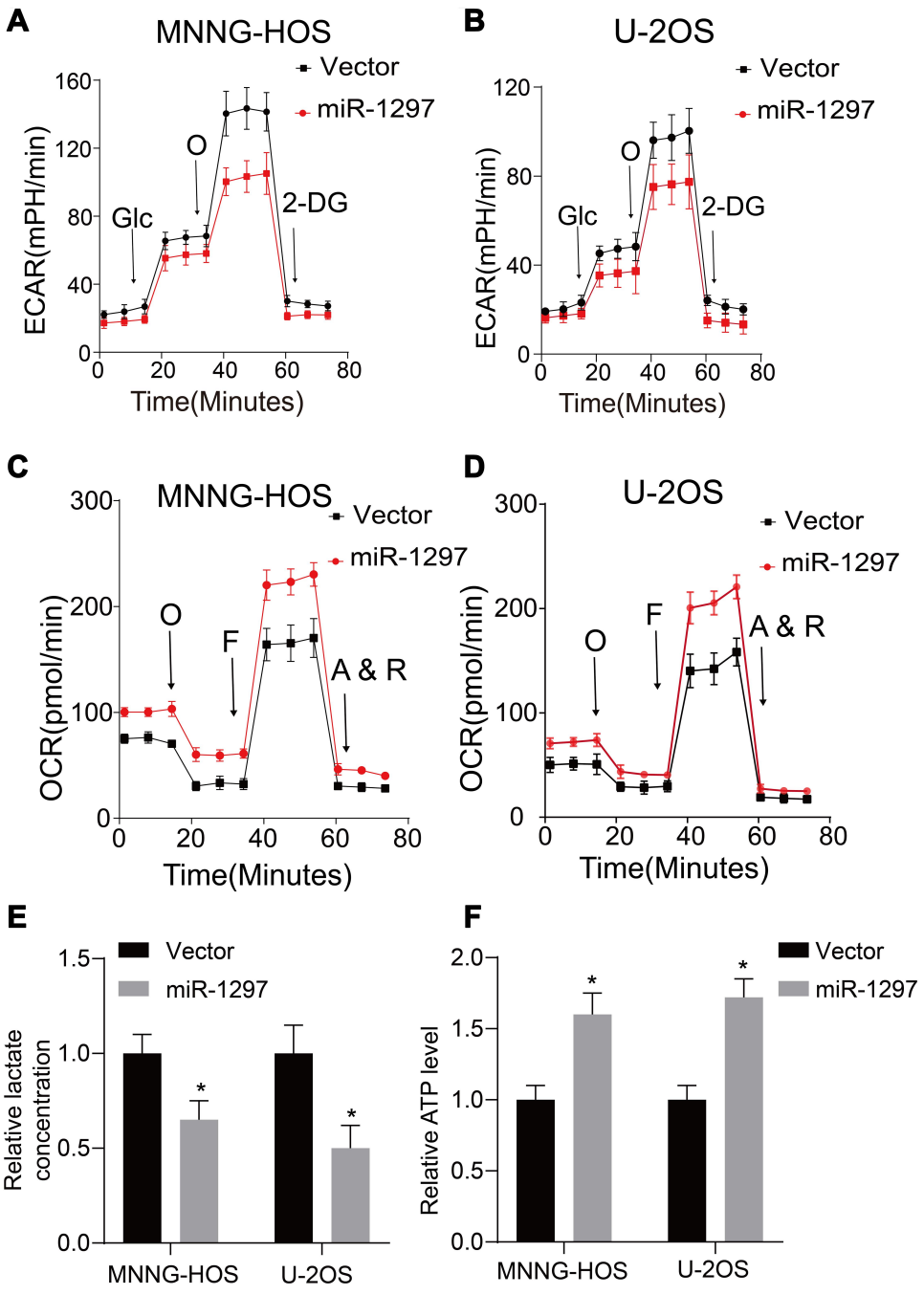

Figure 2 *miR-1297* regulates the aerobic glycolysis of OS cells. (**A** and **B**) Extracellular acidification rate (ECAR) of OS cells lines (MNNG-HOS and U-2OS cells) in control and *miR-1297* overexpression group was varied by using a Seahorse Bioscience XFp analyzer. (**C** and **D**) O_2 consumption rate (OCR) of OS cells lines (MNNG-HOS and U-2OS cells) in control and *miR-1297* overexpression group was detected by using a Seahorse Bioscience XFp analyzer. O: Oligomycin, F: FCCP, A&R: antimycin A/rotenone. (**E** and **F**) Lactate production and ATP level was determined in the OS cells (MNNG-HOS and U-2OS cells) stable knockdown *miR-1297*. Values are means ± SD, **p*<0.05.
Abbreviations: Glc, glucose; Oligo, oligomycin; 2-DG, 2-deoxy-d-glucose.

proliferation and aerobic glycolysis by inhibiting *PFKFB2* expression in OS cells.

Discussion

A large number of studies have indicated that *miR-1297* is closely related to the growth, apoptosis and metastasis of cancer cells. An in vitro study revealed that *miR-1297* suppresses growth of pancreatic cancer by targeting *MTDH*.[17] Wang et al found that *miR-1297* inhibited metastasis by targeting *AEG-1* in cervical cancer.[18] Wang et al reported that serum *miR-1297* is a promising diagnostic biomarker in esophageal squamous cell carcinoma.[19] Therefore, *miR-1297*

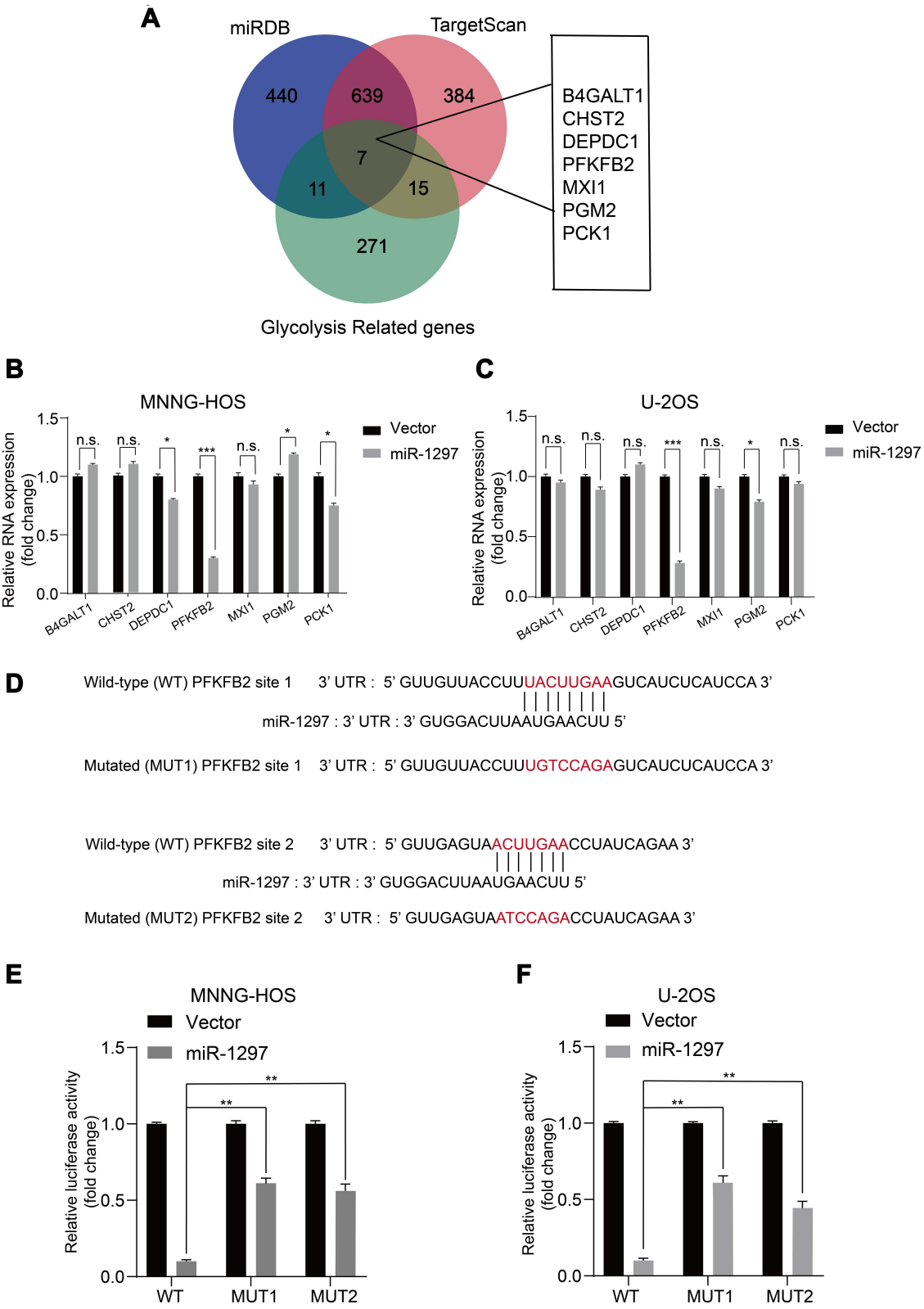

Figure 3 *PFKFB2* is identified as a direct target of *miR-1297*. (**A**) Predicted glycolysis related target genes of *miR-1297* from databases (miRDB and TargetScan) are shown by Venn diagram. (**B** and **C**) Expression of the genes in the glucose metabolic pathway in sh-Control and ov-*miR-1297* groups were determined by qRT–PCR. Values are means ± SD, *** $p < 0.001$ (Student's *t*-test). Values are means ± SD, *p <0.05, ***$p < 0.001$, n.s. p >0.05. (**D**) The wild-type and the mutated sequences of the *PFKFB2* mRNA 3′-UTR (mutation site: red). (**E** and **F**) The luciferase activity of the OS cells (MNNG-HOS and U2OS) in luciferase reporter plasmid containing wild-type *PFKFB2* 3′-UTR (Wt) and mutant PFKFB2 3′-UTR (Mut1 and Mut2) co-transfected with *miR-1297* mimics or a negative control was assessed, Values are means ± SD, **$p < 0.01$.

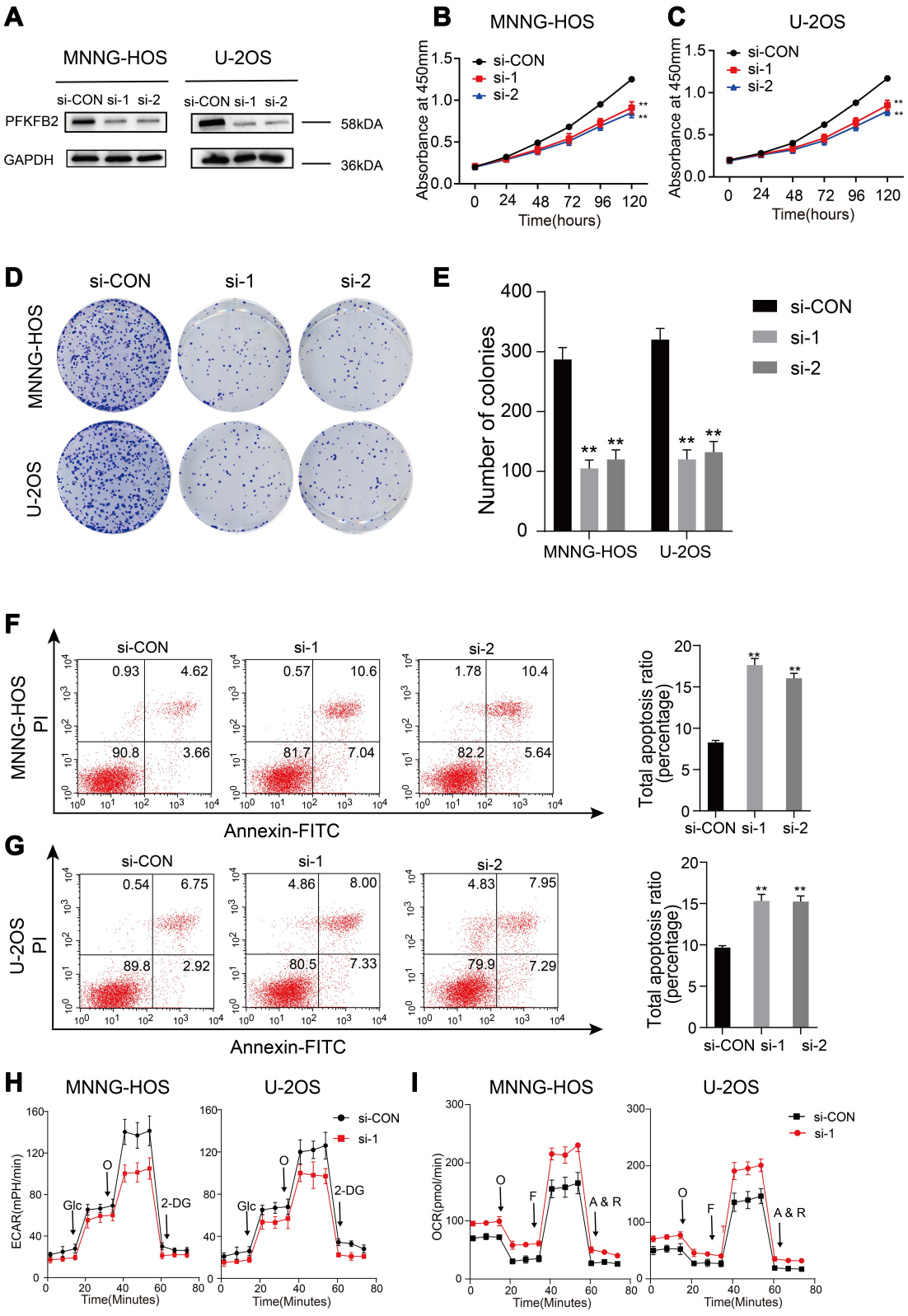

Figure 4 *PFKFB2* makes contribution to the proliferation and the Warburg effect in OS cells. (**A**) The efficacy of si-RNA targeting of *PFKFB2* in the OS cells (MNNG-HOS and U2OS) was detected by using Western blotting. (**B** and **C**) Knockdown of *PFKFB2* inhibited MNNG-HOS and U-2OS cells proliferation using the cell counting kit (CCK)-8 assay. Values are means ± SD, $^{**}p < 0.01$. (**D** and **E**) Knockdown of *PFKFB2* downregulated the proliferation of OS cells (MNNG-HOS and U-2OS) by using colony formation assay. Values are means ± SD, $^{**}p < 0.01$. (**F** and **G**) Silencing of *PFKFB2* has the inhibitory effect on the apoptosis of OS cells (MNNG-HOS and U-2OS). Values are means ± SD, $^{**}p<0.01$. (**H** and **I**) ECAR and OCR of OS cells (MNNG-HOS and U-2OS) in si-Control and si- *PFKFB2* were detected.

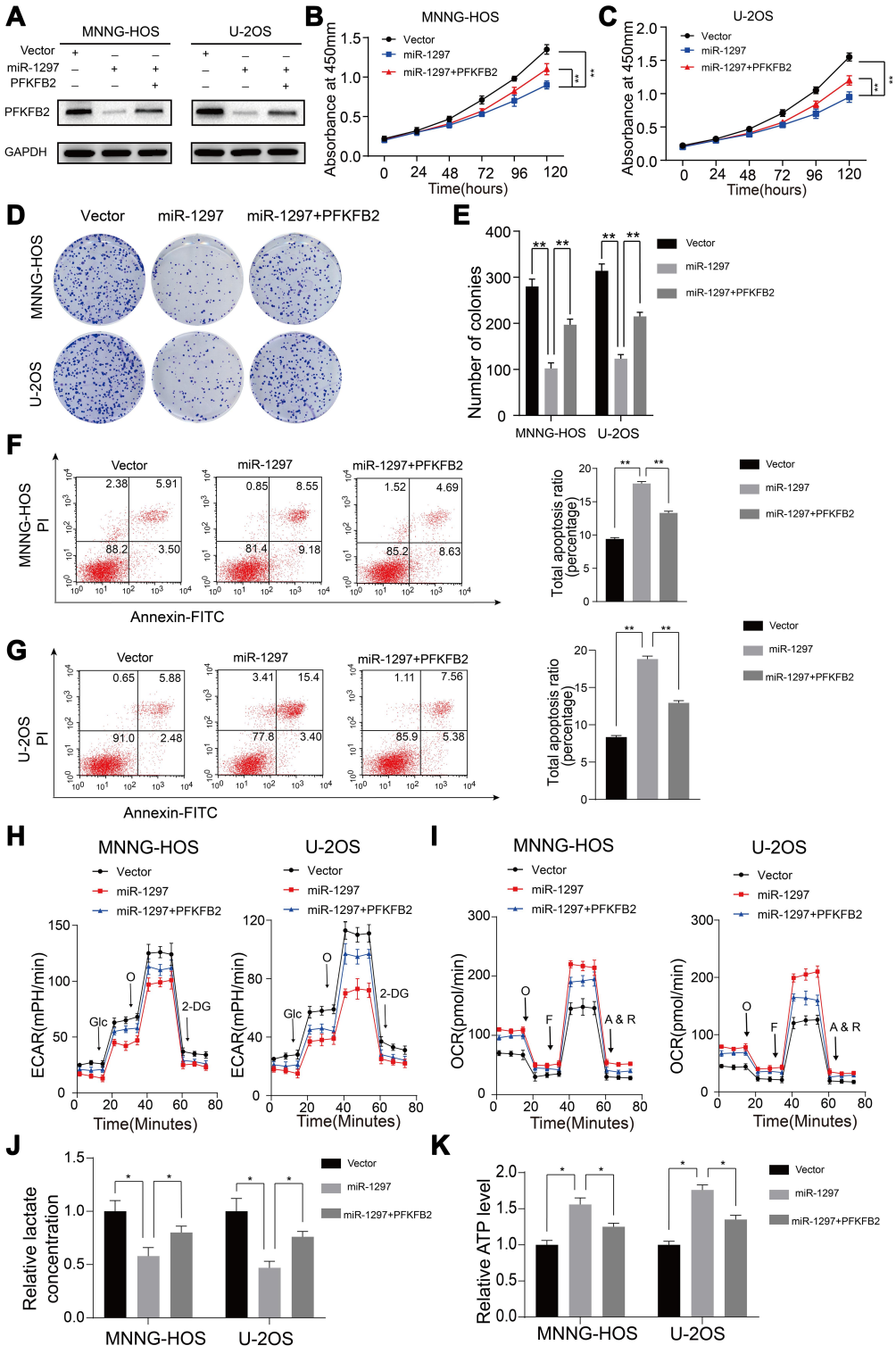

Figure 5 Upregulating of *PFKFB2* partly compensates the effect of silencing of *miR-1297* in OS. (**A**) The efficacy of overexpression of *PFKFB2* in *miR-1297* overexpression OS cells (MNNG-HOS and U-2OS) were confirmed by Western blotting. (**B** and **C**) Overexpression of *PFKFB2*- partly reversed the inhibitory effects of *miR-1297* overexpression on the CCK8 assay of MNNG-HOS and U-2OS cells, values are means ± SD, $^{**}p < 0.01$. (**D** and **E**) *PFKFB2*-overexpression partly reversed the inhibitory effects of *miR-1297* overexpression on the colony formation properties of MNNG-HOS and U-2OS cells, values are means ± SD, $^{**}p < 0.01$. (**F** and **G**) *PFKFB2*-overexpression partly reversed the induce effects of *miR-1297* overexpression on apoptosis of MNNG-HOS and U-2OS cells. Values are means ± SD, $^{**}p<0.01$. (**H** and **I**) Altered level of ECAR and OCR in MNNG-HOS and U-2OS cells in different groups (Control, ov-*miR-1297*, ov-*miR-1297* and ov-*PFKFB2*). Values are means ± SD. (**J** and **K**) Lactate production and ATP level was determined in different groups. Values are means ± SD, $^{*}p<0.05$.

Abbreviations: Glc, glucose; Oligo, oligomycin; 2-DG, 2-deoxy-d-glucose.

is an effective target for the treatment of tumors. In our present study, we ascertained that *miR-1297* was downregulated in OS cell lines and tissues and inhibited the proliferation of OS cells (Figure 1). More importantly, we found that *miR-1297* was involved in metabolic reprogramming and that overexpressing *miR-1297* suppressed aerobic glycolysis in OS cells (Figure 2).

In tumors or other cells under development, the rate of glucose uptake markedly increases and large amounts of lactate are produced, even in the presence of oxygen and fully functioning mitochondria. This process is known as the Warburg effect.[20,21] Previous studies have reported that the Warburg effect plays a vital role in supporting proliferation, aggressiveness and drug resistance of OS cells. Yuan et al reported that *PKM2*, which is a rate-limiting enzyme of the glycolytic process, promotes tumor survival in OS cells both in vitro and in vivo.[22] In addition, lactate dehydrogenase A (*LDHA*) is a glycolytic enzyme in the final step of anaerobic glycolysis and was reported to play a crucial role in promoting OS tumor metastasis.[23] Furthermore, aldolase A (*ALDOA*) which catalyzes the conversion of fructose-1,6-bisphosphate was demonstrated to be involved in the development of OS chemoresistance.[24] Moreover, glycolysis inhibition by 2-deoxy-D-glucose has been shown to significantly delay metastasis and prolong survival in an orthotopic postsurgical OS model.[25] Therefore, inhibition of aerobic glycolysis could be an effective approach for the treatment of OS. Recently, some miRNAs, such as *miR-644a*, *miR-30a-5p* and *miR-214* have been reported to regulate cell growth and the Warburg effect.[26–28] *MiR-1297* has also been reported to be connected with metabolic reprogramming in glioblastoma.[29] In our study, we found that *miR-1297* directly targeted *PFKFB2*, resulting in the impairment of aerobic glycolysis in OS.

The enzyme fructose-2, 6-bisphosphatase 2(*PFKFB2*) is a member of the *PFKFB* family, which is a family of bifunctional enzymes that control the levels of fructose 2,6-bisphosphate (Fru-2,6-P2).[30] Previous research has found that the expression level of *PFKFB2* is evidently increased in a variety of tumors.[31–33] A recent study revealed that *RSK* regulates *PFKFB2* activity in order to promote metabolic rewiring in melanoma.[34] It is also reported that *PFKFB2* regulates glycolysis and proliferation in pancreatic cancer cells.[31] Through database analysis, qRT-PCR, Western blotting and luciferase assays we found that *PFKFB2* was directly regulated by *miR-1297* in OS. Our study provides insight into the biology of OS progression and shows that *miR-1297* is an inhibitor of *PFKFB2* that suppresses the growth of OS cells by inhibiting *PFKFB2*-mediated aerobic glycolysis (Figures 3–5).

In conclusion, our study indicated that the expression level of *miR-1297* was evidently downregulated in OS cells. *MiR-1297* inhibited the proliferation of OS cells by inhibiting aerobic glycolysis via downregulation of *PFKFB2* expression. Our findings provide evidence of the underlying mechanisms of OS development and suggest that *miR-1297* could be a potential target for the treatment of OS.

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Disclosure

The authors declare no conflicts of interest.

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