Original Research Article

Krüppel-like factor 8 promotes aerobic glycolysis in prostate cancer cells by regulating AKT/mTOR signaling pathway

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Abstract

Purpose: To investigate the effects of Krüppel-like factor 8 (KLF8) in prostate cancer (PCa) cell viability and glycolysis, and explore its role as a regulatory factor.
Methods: Immunoblot assays were conducted to assess the expression of KLF8 and proteins in AKT/mTOR pathway in PCa cell lines PC-3 and DU145. Cell Counting Kit-8 assays were performed to assess the effect of KLF8 on PCa cell viability. The glycolysis capacity of PCa cells was determined by measuring the levels of glucose intake, lactic acid production, and cellular ATP levels.
Results: Depletion of KLF8 decreased the survival of PCa cells in vitro (p < 0.05). KLF8 depletion also inhibited aerobic glucose metabolism in PCa cells (p < 0.05). Further studies confirmed that KLF8 contributed to the growth and glycolysis of PCa cells via the regulation of AKT/mTOR pathway.
Conclusion: KLF8 regulates glycolysis in PCa cells by regulating AKT/mTOR signaling pathway and is thus a promising therapeutic target for PCa treatment.

Keywords: Krüppel-like factor 8 (KLF8), Prostate cancer (PCa), Aerobic glucose, AKT/mTOR signaling pathway, Therapeutic target

INTRODUCTION

Prostate cancer (PCa) is a malignant tumor caused by excessive proliferation of prostatic epithelial cells [1]. Its morbidity is the second highest among male malignant tumors [2]. As age increases, the morbidity of PCa increases [3]. Prostatic adenocarcinoma originating from prostatic epithelial cells causes over 95 % of all PCa [4]. For early PCa, androgen ablation is a successful treatment method [5]. For the treatment of advanced PCa, chemoradiotherapy has limited efficacy and significant side effects [6]. A variety of targeted therapy drugs are used in the clinic or are in the clinical research stage [7]. However, additional effective therapeutic targets need to be identified.

The Krüppel-like factor family (KLF) are transcription factors with homology in three zinc-finger DNA binding regions of C2-H2 and are involved in tumorigenesis in different types of

cancer [8]. Krüppel-like factor 8 (KLF8) belongs to the KLF family [9]. KLF8 is regulated by NEDD4-mediated ubiquitination [10]. KLF8 is highly expressed in multiple types of cancers and its expression levels are correlated with the cancer's progression [11]. In hepatocellular carcinoma (HCC), KLF8 regulates VEGFA expression and angiogenesis and therefore promotes the development of HCC [12]. It also induces the EMT of pancreatic cancer cells via activating FHL2 [13].

KLF8 regulates the development and metastasis of several types of tumors [14]. However, its possible role in PCa is still unclear. A previous study reported that KLF8 expression is correlated with a poor prognosis in gastric cancer [15]. KLF8 affects glycolysis in cancer cells, activates GLUT4 promoter activity, and further promotes the development of gastric cancer [16]. However, whether KLF8 promotes PCa progression via affecting glucose metabolism in PCa cells needs further experimental evidence.

Herein, we show that KLF8 promotes the survival of PCa cells by regulating the activity of the Akt/mTOR pathway and increasing glycolysis. This study suggests that KLF8 could serve as a novel therapeutic target for the treatment of PCa.

EXPERIMENTAL

Inhibitors and antibodies

The AKT/PI3K inhibitor LY294002 (CAS#: 154447-36-6, R&D Systems, Minneapolis, MN, USA) was administered at a final concentration of 20 µM in PCa cells for 24 hours. The antibodies used in this study are shown in Table 1.

Cell transfection

The PC-3 and DU145 human PCa cells (ATCC, Manassas, VA, USA) were maintained in RPMI-1640 culture medium supplemented with 10% of fetal bovine serum and incubated at 37°C in a 5% CO_2 incubator.

pcDNA3.1-KLF8 plasmids were constructed via RT-PCR amplification (5'-ATTAGAATTCGCCACCATGGTCGATATGGATAAACTCATA-3' and 5'-ACCTCTCGAGTCACATGGTGTCATGGCGAC-3') and cloned into the pcDNA3.1 vector. p-LKO.1-KLF8 shRNA plasmids (sh1#KLF8 and sh2#KLF8) and p-LKO.1-vector shRNA plasmids were obtained from Addgene (Watertown, MA, USA). For the *in vitro* experiments, PC-3 and DU145 cells were transfected with p-LKO.1-KLF8 shRNA plasmids to deplete the expression of KLF8. Scrambled shRNA plasmids were used as a control. The transfection was conducted using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to manufacturer's instructions.

Immunoblotting

Proteins were separated using SDS-PAGE and transferred onto PVDF membranes (Millipore Corporation, Bedford, MA, USA). The membranes were subsequently blocked in 5% fat-free milk in TBST buffer and incubated with specific primary antibodies against KLF8, p-AKT, AKT, p-mTOR, mTOR, and β-actin at 4°C overnight. After washing to remove non-specific binding, the membrane was treated with HRP-labeled secondary antibody in TBST buffer. Then, the blots were visualized using an ECL kit. The relative protein levels were quantified using ImageJ (NIH, Bethesda, MD, USA).

Cell viability assay

PC-3 and DU145 cells were incubated in 96-well plates with 100 µL culture medium per well. Cell viability was detected using a Cell Counting Kit-8 (CCK-8). CCK-8 solution was added to the cells and incubated at 37°C for 3 hours. Then, absorbance was measured using a Bio-Rad microplate reader (Richmond, CA, USA).

Table 1: Antibodies

Antibody	Information
Anti-KLF8 antibody	1:1000 dilution, ab168527, Abcam, Cambridge, UK
Anti-AKT antibody	1:1000 dilution, ab8805, Abcam, Cambridge, UK
Anti-β-actin antibody	1:1000 dilution, ab8226, Abcam, Cambridge, UK
Anti-p-AKT (phospho T308) antibody	1:1000 dilution, ab38449, Abcam, Cambridge, UK
Anti-mTOR antibody	1:500 dilution, ab32028, Abcam, Cambridge, UK
Anti-p-mTOR (phospho S2448) antibody	1:1000 dilution, ab84400, Abcam, Cambridge, UK

Cell viability was displayed as a percentage of the OD value in the experimental group compared to the control group at 490 nm.

Cell glycolysis test

The glycolysis levels of PCa cells were evaluated according to the glucose intake (ab136955), lactic acid production (ab83429), and cellular ATP levels (ab83355), which were measured using the corresponding kits from Abcam Company (Cambridge, UK). All experiments were conducted according to the respective instructions.

Statistical analysis

Statistical analysis was performed using GraphPad (San Diego, CA, USA). Data were representative of at least three independent experiments and presented as mean ± SD. Statistical analysis was conducted using the Student's t-test and $p < 0.05$ was considered statistically significant.

RESULTS

KLF8 depletion decreased the viability of PCa cells

KLF8 expression decreased after the transfection of its shRNA plasmids in both PC-3 and DU145 cells, confirming effective depletion (Figure 1A).
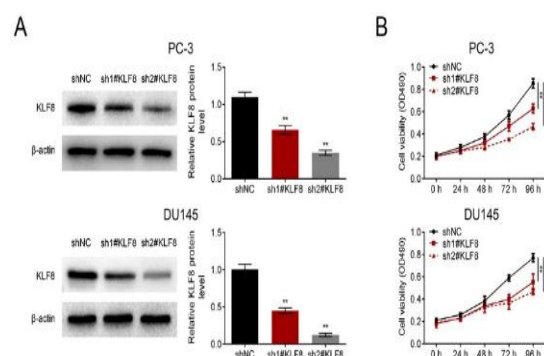

Figure 1: KLF8 depletion decreased the viability of PCa cells *in vitro*. (A) Immunoblot assays showed the expression of KLF8 and β-actin after the transfection of the indicated plasmids in PC-3 and DU145 cells. (B). CCK-8 assays showed the difference of cell viability in PC-3 and DU145 cells after the transfection of the indicated plasmids. Results are presented as mean ± SD; **$p < 0.01$

Subsequently, the effects of KLF8 depletion on the viability of PCa cells was detected using

CCK-8 assays. Interestingly, KLF8 depletion significantly inhibited the viability of PC-3 and DU145 cells *in vitro* (Figure 1B). Therefore, these data revealed the decreased cell viability induced by KLF8 depletion.

Depletion of KLF8 impaired glycolysis in PCa cells *in vitro*

Interestingly, glucose intake levels dramatically decreased after KLF8 depletion in PC-3 and DU145 cells (Figure 2 A). Moreover, lactate production levels and cellular ATP levels also decreased after the transfection of KLF8 shRNA plasmids in PC-3 and DU145 cells (Figure 2 B and C). Therefore, KLF8 depletion impaired glycolysis in PCa cells *in vitro*.
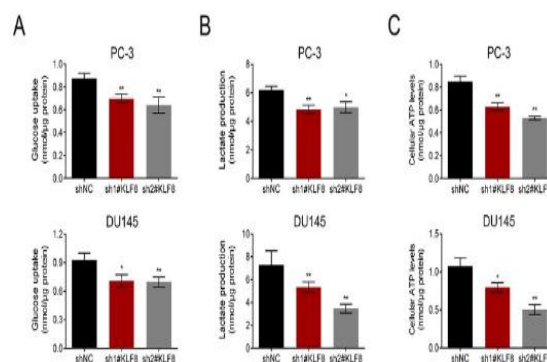

Figure 2: KLF8 depletion impaired glycolysis in PCa cells *in vitro*. (A - C) The levels of glucose uptake (A), lactate production (B), and cellular ATP levels (C) were detected in PC-3 and DU145 cells after transfection of the indicated plasmids. Results are presented as mean ± SD; * $p < 0.05$, **$p < 0.01$

KLF8 regulated AKT/mTOR pathway in PCa cells

The AKT/mTOR pathway is critical in the metabolic regulation of cancer cells. The expression and phosphorylation levels of AKT and mTOR were detected through immunoblot assays. Interestingly, the phosphorylation of AKT was inhibited in both PC-3 and DU145 cells after KLF8 depletion (Figure 3 A and B). Meanwhile, mTOR phosphorylation levels were also decreased in KLF8-depleted PC-3 and DU145 cells (Figures 3A and B). However, the total expression levels of AKT and mTOR in control or KLF8 depleted PCa cells were unchanged (Figures 3A and B). Collectively, KLF8 regulated the AKT/mTOR pathway in PCa cells.

KLF8 contributes to the growth and glycolysis of PCa cells by regulating AKT/mTOR pathway

Immunoblot assays showed that KLF8 overexpression increased the phosphorylation levels of AKT and mTOR in PC-3 cells, consistent with the previous results (Figure 4A). Importantly, LY294002 treatment blocked the increase of the phosphorylation levels of AKT and mTOR caused by KLF8 overexpression in PC-3 cells (Figure 4 A). However, the treatment of LY294002 had no obvious effects on KLF8 expression levels (Figure 4A). Therefore, LY294002 treatment effectively inhibited the AKT/mTOR pathway.
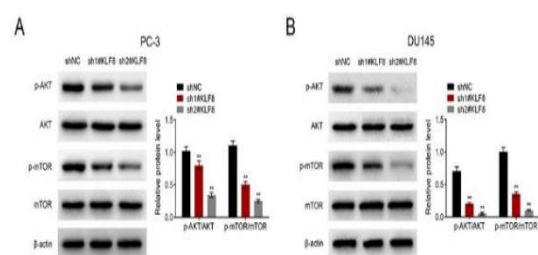
Figure 3: KLF8 regulated the AKT/mTOR pathway in PCa cells. (A, B) Immunoblot assays revealed the expression level of the indicated proteins after transfection of sh1#KLF8, sh2#KLF8, and sh-vector plasmids in PC-3 (A) and DU145 (B) cells. Results are presented as mean ± SD, **$p < 0.01$

Subsequently, the effects of KLF8 overexpression and LY294002 treatment on PC-3 cell growth were assessed. Through CCK-8 assays, KLF8 overexpression dramatically promoted the growth of PC-3 cells, whereas LY294002 treatment significantly suppressed the growth of KLF8 overexpressing PC-3 cells (Figure 4 B).

The effects of KLF8 and LY294002 on PC-3 cell glycolysis were measured. Similarly, the levels of glucose intake, lactic acid production, and cellular ATP were upregulated after KLF8 overexpression (Figure 4 C). However, the LY294002 treatment reversed the increased glucose intake, lactic acid production, and cellular ATP caused by KLF8 overexpression in PC-3 cells (Figure 4 C). Therefore, KLF8 regulated the growth and glycolysis of PCa cells through the AKT/mTOR pathway.

DISCUSSION

In recent years, the morbidity of PCa has increased [17]. In 2018, approximately 1,300,000 PCa cases were newly reported worldwide, accounting for 13.5% of the morbidity of malignant tumors in males, and approximately 360,000 deaths occurred, accounting for 6.7% of cancer mortality for male patients [18]. Patients are often in an advanced stage of PCa when they are diagnosed due to the lack of significant early symptoms [6].
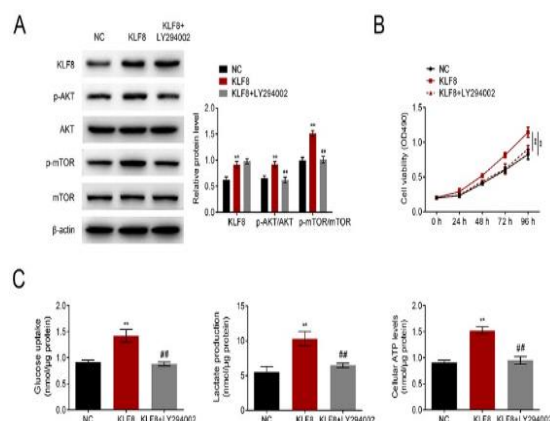
Figure 4: KLF8 contributes to the growth and glycolysis of PCa cells via regulating the AKT/mTOR pathway. (A). Immunoblot assays revealed the expression level of the indicated proteins after LY294002 treatment in control or KLF8 overexpression PC-3 cells. (B). CCK-8 assays showed the cell viability of PC-3 cells after treatment with the indicated plasmids or drugs. (C). Glucose uptake, lactate production, and cellular ATP levels were detected in PC-3 cells after treatment with the indicated plasmids or drugs. Results are presented as mean ± SD; **p < 0.01, ##p < 0.001

By this time, tumor cells spread outside the prostate, and the prognosis is often poor. Finding novel and effective therapeutic targets is urgently needed to combat PCa [19]. In this study, the role of a KLF family transcription factor, KLF8, was examined in the regulation of PCa cell growth and glycolysis. The data confirmed the involvement of KLF8 in the development of PCa and suggested KLF8 could serve as a promising PCa molecular target.

The proteins in the KLF family widely affect the development and metastasis of multiple cancers [20]. KLF8 also regulates the development of cancers and correlates with the prognosis and clinical features of multiple cancers [21]. KLF8 contributes to LUAD metastasis via promoting the EMT process [20]. KLF8 promotes the cancer stem cell-like phenotypes in osteosarcoma via the miR-429-SOX2 axis [22]. KLF8 overexpression also contributes to the growth of lung cancer cells via stimulating JMJD2A expression [21]. Here, the critical role of KLF8 in PCa cells was revealed. These studies, together with this study, suggest KLF8 could serve as an important cancer molecular target.

The regulation of KLF8 on the AKT/mTOR pathway in PCa cells was also revealed. The AKT/mTOR pathway widely affects the progression and metastasis of tumors [23]. Previous studies confirmed that in PCa, microRNA-381 facilitates autophagy and apoptosis via the RELN-mediated AKT/mTOR pathway [23]. EHMT2 promotes the progression of PCa also through the AKT/mTOR pathway [24]. KLF8, as a transcription factor, could promote the growth of PCa cells via this pathway. Thus, it is important to find the proteins transcriptionally regulated by KLF8 in this pathway.

Aerobic glycolysis is a key feature of tumor cells. Several signaling pathways are involved in the regulation of glycolysis and other metabolic activities, such as the Akt/MAPK and Akt/mTOR pathways [16]. A variety of oncogenes regulate cancer glycolysis through these signaling pathways, thereby promoting tumor growth [25]. KLF8 could also regulate Akt/mTOR to promote aerobic glycolysis and cell growth of PCa. Therefore, KLF8 targeted therapy may inhibit aerobic glycolysis in PCa and further inhibit the growth and metastasis of tumor cells.

CONCLUSION

KLF8 regulates the viability and glycolysis of PCa cells. It also regulates the AKT/mTOR pathway in PCa cells, thus confirming that KLF8 promotes the growth and glycolysis of PCa cells via the AKT/mTOR pathway. Therefore, KLF8 is a promising therapeutic target for PCa treatment.

DECLARATIONS

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Cunming Zhang and Song Chen designed the study, supervised the data collection, and analyzed the data. Lide Song interpreted the data and prepared the manuscript for publication. Haibo Ye and Junwei Wang supervised the data collection, analyzed the data, and reviewed the draft of the manuscript. All authors have read and approved the manuscript.

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