

MicroRNA-122 regulates docetaxel resistance of prostate cancer cells by regulating *PKM2*

ZHIRONG ZHU*, GUILIANG TANG* and JIAJUN YAN

Department of Urology, Shaoxing People's Hospital, Zhejiang University School of Medicine, Shaoxing, Zhejiang 312000, P.R. China

Received August 22, 2019; Accepted February 25, 2020

DOI: 10.3892/etm.2020.9377

Abstract. Prostate cancer (PCa), an epithelial malignancy that occurs in the prostate, is the second leading cause of cancer death worldwide. MicroRNAs (miRs/miRNAs) are reported to have important applications in the field of cancer diagnosis and treatment. The present study aimed to investigate the function of miRNA-122 in the chemoresistance of PCa cells and the underlying mechanism. Significantly decreased miR-122 and increased pyruvate kinase (*PKM2*) levels were observed in docetaxel-resistant PCa cells, and *PKM2* was negatively correlated with miR-122. MiR-122 mimic transfection in docetaxel-resistant LNCaP cells significantly inhibited cell proliferation, promoted apoptosis and decreased glucose uptake and lactate production, which was counteracted by *PKM2* overexpression. Inhibition of miR-122 in LNCaP cells had an opposite effect to miR-122 mimic transfection. In addition, miR-122 mimic transfection significantly increased the sensitivity of docetaxel-resistant LNCaP cells to docetaxel, while inhibition of miR-122 significantly decreased the sensitivity of LNCaP cells to docetaxel. Luciferase reporter assays showed that miR-122 regulated *PKM2* expression by binding to the 3'-untranslated region of *PKM2*. The results suggest that upregulation of miR-122 could enhance docetaxel sensitivity, inhibit cell proliferation and promote apoptosis in PCa cells, possibly through the downregulation of its target protein *PKM2*.

Introduction

Prostate cancer (PCa) is an epithelial malignancy that occurs in the prostate (1). PCa mainly occurs in men over the age of 50 and is the second leading cause of cancer death worldwide (2,3). Currently, endocrine therapy, including surgical or drug castration and antiandrogen (bicalutamide or flutamide) therapy, is the main treatment for hormone-sensitive advanced PCa patients (4). However, the vast majority of patients are eventually treated with androgen-deprivation therapy and progress to metastatic castration-resistant PCa (CRPC), which is the leading cause of PCa-related mortality (5-7). Novel treatments, such as docetaxel and abiraterone, were shown to improve the survival of patients with metastatic CRPC; however, most patients develop drug resistance (8,9).

While healthy cells rely on carbohydrate molecules oxidized in mitochondria to acquire energy, most tumor cells obtain their energy supply through relatively low-yield glycolysis, which does not involve oxygen or mitochondria (10). Malignant, rapidly growing tumor cells typically have a 200-fold higher rate of glycolysis compared with normal tissues, even under oxygen-sufficient conditions (11). Therefore, it is speculated that this change in metabolism is the root cause of cancer (12). It was reported that glycolysis in cancer cells is characterized by high glucose consumption and lactate production (13). Cancer cells often take up high amounts of glucose and rely on glycolysis for ATP generation, more efficiently converting glucose into macromolecules that are needed for a variety of cellular processes (14-16). Pyruvate kinase (*PKM2*), a key rate-limiting enzyme that catalyzes the final step in glycolysis, was reported to be highly expressed in multiple cancers (17,18) and can promote glucose metabolism and cell growth (19). A number of studies showed that upregulation of *PKM2* can promote malignancy and downregulation of *PKM2* can inhibit cell growth, migration and invasion in various types of cancer (20-24).

MicroRNAs (miRs/miRNAs) are small noncoding, single-stranded RNAs which regulate gene expression by regulating the stability or translation of target mRNAs by binding to their 3'-untranslated regions (UTRs) (25,26). Studies showed that miRNAs have important applications in the field of cancer diagnosis and treatment (27-32). For example, exo-anti-miR-214 can reverse the resistance of gastric cancer cells to cisplatin (33). MiR-122, an abundant liver-specific

Correspondence to: Dr Jiajun Yan or Dr Zhirong Zhu, Department of Urology, Shaoxing People's Hospital, Zhejiang University School of Medicine, 568 Zhongxing North Road, Shaoxing, Zhejiang 312000, P.R. China
E-mail: 403601758@qq.com
E-mail: zxr123z@163.com; zhzi-4485@163.com

*Contributed equally

Key words: prostate cancer, microRNA-122, pyruvate kinase, proliferation, apoptosis, glycolysis

miRNA, was shown to reverse doxorubicin resistance in liver cancer cells by inhibiting glycolysis in tumors via *PKM2* inhibition (34,35). In colon cancer, overexpression of miR-122 can increase the sensitivity of fluorouracil (5-FU)-resistant colon cancer cells to 5-FU by *PKM2* downregulation (36). Previous findings showed that miR-34a and miR-21 play a role in the chemoresistance of PCa cells (37-40). The present study aimed to investigate the function of miRNA-122 in the chemoresistance of PCa cells and the underlying mechanism.

Materials and methods

Cell culture. Prostate cancer docetaxel-resistant (LNCaP/Docetaxel) and docetaxel-sensitive (LNCaP) LNCaP cells were purchased from the Cell Bank of Type Culture Collection of the Chinese Academy of Sciences. Cells were cultured with DMEM (cat. no. SH30243.01; HyClone; GE Healthcare Life Sciences) supplemented with 10% FBS (cat. no. 16000-044; Gibco; Thermo Fisher Scientific, Inc.) and 1% penicillin and streptomycin (100X; cat. no. P1400; Beijing Solarbio Science & Technology Co., Ltd.) in a 37°C incubator (Forma 3111; Thermo Fisher Scientific, Inc.) with 5% CO₂.

Isolation of primary PCa cells. PCa cells were isolated from 20 patients with PCa who were treated in Shaoxing People's Hospital, Shaoxing, China from February 2018 to February 2019 (age range, 60-80 years; mean age, 70.12±8.43 years). The inclusion criteria were as follows: i) patients did not receive any treatment and ii) clinical data of patients were complete. All cases were confirmed by a review by the Shaoxing People's Hospital Pathology Center. The exclusion criteria were cases without complete clinical data. Fresh prostate cancer tissues were washed three times with D-Hank's balanced salt solution containing 500 IU/ml penicillin and streptomycin. Once surrounding inactivated tissues (cloudy appearance, dull and loss of normal tissue elasticity) were removed with ophthalmic scissors, the tissues (normal color and elasticity) were cut into pieces (~300 times) in a sterile 5 ml syringe and incubated with 5 ml trypsin-EDTA (0.125% trypsin and 0.53 mol/l EDTA) for 5 min at 37°C. A total of 10 ml RPMI-1640 medium (cat. no. 88365; Thermo Fisher Scientific, Inc.) containing 10% FBS (Gibco; Thermo Fisher Scientific, Inc.) was added to terminate digestion in a 15 ml centrifuge tube. After centrifugation at 4°C and 800 x g for 5 min, the pellet was resuspended in 5 ml RPMI 1640 containing 5 ng/ml epidermal growth factor (cat. no. PHG0311L; Gibco; Thermo Fisher Scientific, Inc.), 50 µg/ml bovine pituitary extract (cat. no. 13028014; Gibco; Thermo Fisher Scientific, Inc.) and 10% FBS. A total of 5 ml of the suspension was seeded in the T25 cell flasks for incubation in a 37°C and 5% CO₂ incubator. Fibroblasts can adhere growth in the T25 cell flasks after 4-6 days. Medium was replaced every two days, and the cells were passaged when they were reached 80% confluence. Cells that were sub-cultured 3 times were used for subsequent experiments.

Lentivirus construction. The coding sequence (CDS) of *PKM2* (AY352517.1) was synthesized and validated by DNA sequencing. Following the insertion of the *PKM2* CDS into

the pLVX-Puro vector (Clontech Laboratories, Inc.) at the *EcoRI-BamHI* site, the pLVX-Puro-*PKM2* plasmid was co-transfected into 293T cells (American Type Culture Collection) cultured with DMEM containing 10% FBS and 1% penicillin-streptomycin, with the viral packaging plasmids psPAX2 and pMD2.G (Addgene, Inc.) using Lipofectamine™ 2000 (Invitrogen; Thermo Fisher Scientific, Inc). After 48 h of transfection, the supernatant was collected by centrifugation for 5 min at 1,000 x g and 4°C.

Cell transfection. LNCaP and LNCaP/Docetaxel cells in the logarithmic growth phase were suspended to 1x10⁶ cells/ml after trypsinization. Subsequently, 2 ml cell suspension was inoculated into six-well plates for overnight culture at 37°C with 5% CO₂. Once the cells grew to 60-70% confluence, LNCaP and LNCaP/Docetaxel cells were transfected with 5 µl negative control (NC)-miRNA (100 pmol; 5'-CAGUACUUUUGUGUAGUACAA-3'), 5 µl hsa-miR-122-5p inhibitor (100 pmol; 5'-CAAACACCATTGTCACACTCCA-3') or 5 µl of hsa-miR-122-5p mimic (100 pmol; 5'-UGGAGUGUGACAAUGGUGUUUG-3') using Lipofectamine™ 2000 (cat. no. 11668-019; Invitrogen; Thermo Fisher Scientific, Inc.). Following 24 h of transfection, serum-free transfer solution was replaced with complete medium to culture for a further 48 h.

Docetaxel treatment. LNCaP and LNCaP/Docetaxel cells were treated with gradient concentrations of docetaxel (0.25, 0.5, 1, 2, 4, 8, 16 and 32 µg/ml; cat. no. 114977-28-5; Shanghai Aladdin Biochemical Technology Co., Ltd.), followed by the detection of cell inhibition rate. After treatment with docetaxel, the apoptosis levels of primary PCa cells, treated LNCaP or treated LNCaP/Docetaxel cells were detected.

Reverse transcription-quantitative PCR (RT-qPCR). Total RNA was isolated from PCa cells (LNCaP, LNCaP/Docetaxel or primary PCa cells) with or without miR-122 inhibitor or mimic, and/or combined with *PKM2* overexpression (*oePKM2*) lentivirus using TRIzol® reagent (cat. no. 15996026; Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. Following RNA quantification and integrity confirmation, ~1 µg of RNA was reversed transcribed into cDNA using the RevertAid First Strand cDNA Synthesis kit (cat. no. K1622; Thermo Fisher Scientific, Inc.) using the following heat cycle: 37°C for 5 min; 55°C for 15 min and 85°C for 5 min. qPCR was performed on a 7300 Real-Time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.) with a miRNA RT-PCR Detection Kit (cat. no. AOMD-Q020; GeneCopoeia, Inc.) or a Maxima SYBR Green/ROX qPCR master mix (cat. no. K0223; Thermo Fisher Scientific, Inc.). The following thermocycling conditions were used for the qPCR: Initial denaturation at 95°C for 10 min, 40 cycles of 95°C for 15 sec and 60°C for 45 sec and a final extension step at 95°C for 15 sec. miR-122 expression were normalized to U6 levels and *PKM2* expression were normalized to GAPDH levels using the 2^{-ΔΔC_q} method (41). The primer pairs used for the qPCR are listed in Table I.

Western blot analysis. Total protein was isolated from PCa cells (LNCaP, LNCaP/Docetaxel or primary PCa cells) with

Table I. Primer sequences used for reverse transcription-quantitative PCR.

Gene	Forward sequence	Reverse sequence
RT primer for miR-122		5'-GTCGTATCCAGTGCAGGGTCCGAGG TATTCGCACTGGATACGACGCCTAG-3'
miR-122	5'-CGCCATTATCACAATAAGCTACTG-3'	5'-AGTGCAGGGTCCGAGGTATT-3'
<i>PKM2</i>	5'-TCCAGGTGAAGCAGAAAG-3'	5'-CGGATGAATGACGCAAAC-3'
U6	5'-CTCGCTTCGGCAGCAC-3'	5'-AACGCTTCACGAATTTGCGT-3'
GAPDH	5'-AATCCCATCACCATCTTC-3'	5'-AGGCTGTTGTCACTTTC-3'

miR-122, microRNA-122; *PKM2*, pyruvate kinase; RT, reverse transcription.

or without treatment of miR-122 inhibitor or mimic, and/or combined with *oePKM2* lentivirus using RIPA buffer supplemented with protease and phosphatase inhibitors (cat. no. R0010; Beijing Solarbio Science & Technology Co., Ltd.). Following protein quantification using a BCA protein quantification kit (Thermo Fisher Scientific, Inc.), 25 μ g of protein/lane was separated via 10% SDS PAGE followed by semi-dry transfer onto PVDF membranes (cat. no. HATF00010; EMD Millipore). After blocking with 5% skimmed milk for 1 h at room temperature, membranes were incubated overnight with primary antibodies against *PKM2* (1:500; cat. no. ab137852; Abcam) and GAPDH (1:2,000; cat. no. 5174; Cell Signaling Technology, Inc.) at 4°C with gentle agitation. Following six washes with TBS-Tween-20, membranes were incubated for 2 h at room temperature with goat anti-rabbit horseradish peroxidase (HRP)-labeled secondary antibodies (1:1,000; cat. no. A0208; Beyotime Institute of Biotechnology). Protein bands were visualized using an Tanon 5200 chemiluminescent imaging system (Tanon Science and Technology Co., Ltd.) after 5 min of development with the Immobilon Western Chemiluminescent HRP substrate (cat. no. WBKLS0100; EMD Millipore) in the dark. *PKM2* protein expression was quantified using ImageJ (version 1.47; National Institutes of Health) with GAPDH as the loading control.

Cell proliferation assay. PCa cells (LNCaP or LNCaP/Docetaxel) in the logarithmic growth phase were trypsinized and a 3x10⁴ cells/ml suspension was prepared by counting the cells under an inverted microscope at x40 magnification (XDS-500C; Shanghai Caikon Optical Instrument Co., Ltd.). Subsequently, in a 96-well culture plate, 100 μ l of the suspension was inoculated and cultured at 37°C overnight. A total of 100 μ l DMEM was used as the blank control. Cell Counting Kit-8 solution (CCK-8; cat. no. ab228554; Abcam) and serum-free DMEM were mixed at a volume ratio of 1:10. Following 0, 24, 48 and 72 h of treatment with miR-122 inhibitor or miR-122 mimic and *oePKM2* lentivirus, 100 μ l of the above CCK-8 mixture was added to the cells and incubated in a 5% CO₂ incubator at 37°C for 1 h. The absorbance was read at a wavelength of 450 nm using a microplate reader (cat. no. DNM-9602; Perlong Medical Equipment Co., Ltd.).

Cell apoptosis assay. Following 48 h of treatment, each group of cells (LNCaP, LNCaP/Docetaxel or primary PCa cells) was collected using 0.05% trypsin to detect apoptosis using an

Annexin V-FITC cell apoptosis detection kit (cat. no. C1062L; Beyotime Institute of Biotechnology). In brief, ~1x10⁶ cells were centrifuged at 1,000 x g for 5 min at 4°C. After discarding the supernatant, the cells were gently resuspended in 195 μ l Annexin V-FITC binding solution, followed by a 15-min incubation with 5 μ l Annexin V-FITC at 4°C in the dark. Subsequently, 5 μ l of propidium iodide (PI) staining solution was added and the cells were incubated for 5 min at 4°C in the dark. A tube without Annexin V-FITC and PI was used as a negative control. Flow cytometry was performed and apoptosis percentages were assessed with BD Accuri C6 software (version 1.0.264.21; BD Biosciences).

Detection of glucose uptake and lactate production. PCa cells (LNCaP or LNCaP/Docetaxel) were seeded in 24-well plates and cultured overnight and treated with miR-122 inhibitor or miR-122 mimic and *oePKM2* lentivirus. A 2-NBDG Glucose Uptake Assay kit (cat. no. K682-50; BioVision, Inc.) was used for glucose uptake detection. Following 72 h of treatment, the cells were incubated with 100 μ M 2-NBDG for 1 h. After two washes with PBS, cells were trypsinized and resuspended in DMEM containing 10% FBS, followed by incubation with 5 μ g/ml PI for staining. Subsequently, flow cytometry was performed to measure the proportion of PI-negative and 2-NBDG-positive cells, and glucose uptake was calculated. Lactate production was measured using a lactate test kit (cat. no. A019-2; Nanjing Jiancheng Bioengineering Institute) according to the manufacturer's instructions. The absorbance was measured at a wavelength of 530 nm using a spectrophotometer and lactate production was calculated.

Luciferase reporter assay. TargetScanHuman 7.2 (http://www.targetsca.com/vert_72/) was used to predict miR-122 target sites on *PKM2*. PCa cells (LNCaP or LNCaP/Docetaxel) in the logarithmic growth phase were trypsinized and centrifuged at 800 x g for 5 min at room temperature. After discarding the supernatant, cells were gently resuspended in 1 ml DMEM and counted under an inverted microscope at x40 magnification (XDS-500C; Shanghai Caikon Optical Instrument Co., Ltd.). The cell suspension was inoculated into a six-well plate at a density of 5x10⁵ cells/well and cultured in an incubator at 37°C. Following 24 h of culture, the cells were co-transfected with 1.5 μ g luciferase plasmid (pGL3-Promoter-PKM; Promega Corporation) and miR-122-5p inhibitor or mimic

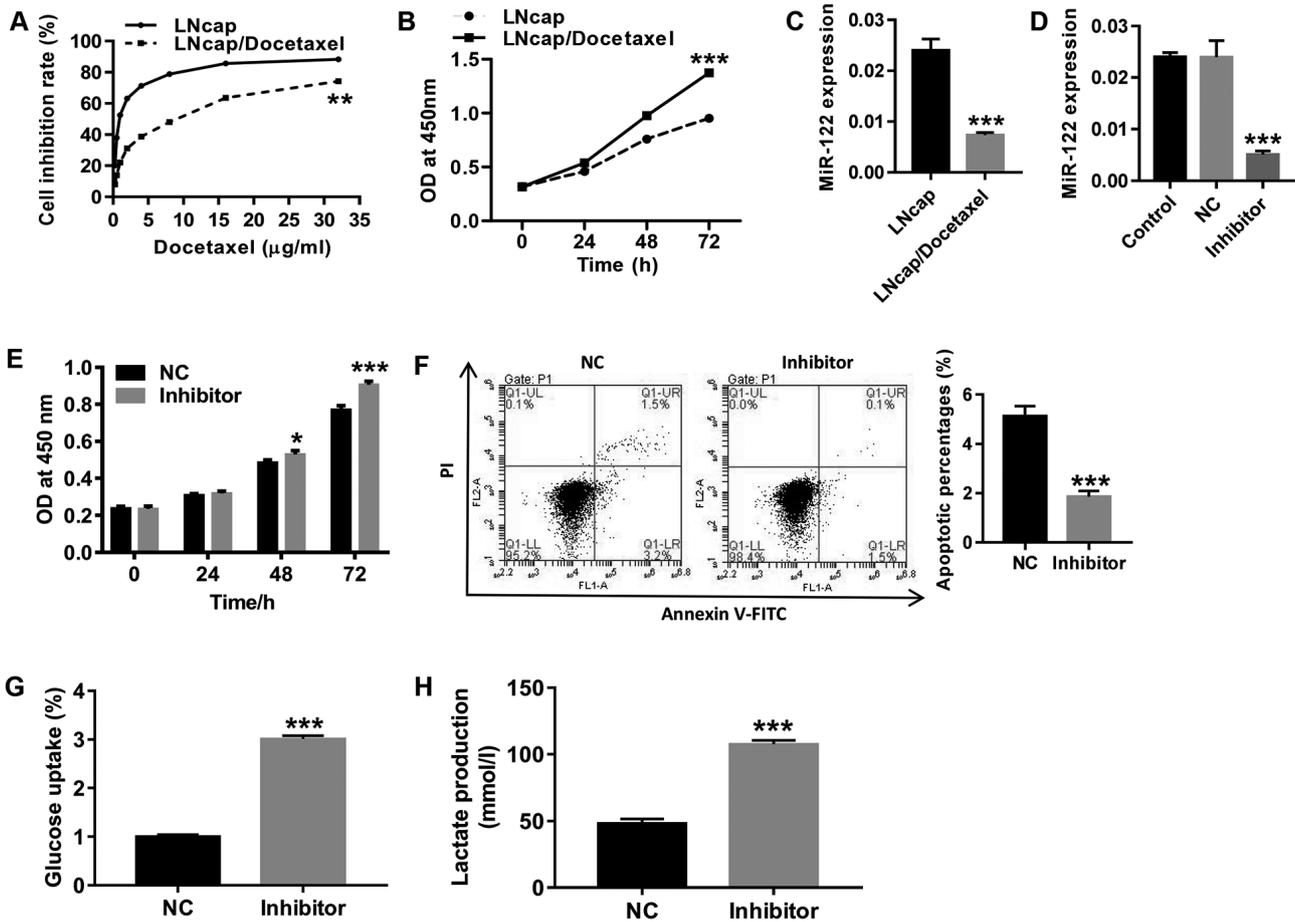


Figure 1. Expression of miR-122 significantly decreased in LNCaP/Docetaxel cells and inhibition of miR-122 in LNCaP cells significantly promotes proliferation and glycolysis and inhibits apoptosis. (A) Following treatment with gradient concentrations of docetaxel (0.25, 0.5, 1, 2, 4, 8, 16 and 32 $\mu\text{g/ml}$), cell proliferation was assessed to determine resistance to docetaxel in LNCaP and LNCaP/Docetaxel cells. $^{**}P<0.01$. (B) Baseline proliferation levels of LNCaP and LNCaP/Docetaxel cells were detected. $^{***}P<0.001$. (C) Expression of miR-122 in LNCaP and LNCaP/Docetaxel cells was detected. $^{***}P<0.001$. (D) The expression of miR-122 in miR-122-treated LNCaP cells was detected. $^{***}P<0.001$ vs. NC. (E) Cell proliferation was detected at 0, 24, 48 and 72 h. $^{*}P<0.05$ and $^{***}P<0.001$. (F) Cell apoptosis was detected at 48 h. The ordinate of the histogram is the sum of early and late apoptosis. $^{***}P<0.001$. (G) Glucose uptake and (H) lactate production were detected. $^{***}P<0.001$. miR-122, microRNA-122; OD, optical density; NC, negative control; PI, propidium iodide.

using Lipofectamine™ 2000 (cat. no. 11668-019; Invitrogen; Thermo Fisher Scientific, Inc.). Following transfection, the cells were washed with PBS, and then incubated in 500 μl PLB for 15 mins with gentle agitation at room temperature. The luciferase activity of the *PKM2* reporter was determined using a Dual-Luciferase Reporter Assay system (cat. no. E1910; Promega Corporation) on a GloMax®-Multi+ Microplate Multimode reader (Promega Corporation). Firefly luciferase activity was detected following addition of 100 μl LAR II and 20 μl sample lysate in 96-well plates. *Renilla* luciferase activity was detected following addition of 100 μl Stop & Glo reagent.

Statistical analysis. GraphPad Prism 7.0 (GraphPad Software, Inc.) was used for statistical analysis. Data are presented as the mean \pm SD from triplicate experiments. Unpaired Student's t-test was used to determine the significance between two groups, while multiple groups were compared by one-way ANOVA and Tukey's post hoc test. The Pearson correlation coefficient was used to analyze the correlation between

miR-122 and *PKM2*. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Expression of miR-122 significantly decreased in LNCaP/Docetaxel cells, and inhibition of miR-122 in LNCaP cells significantly promotes proliferation and glycolysis and inhibits apoptosis. Following treatment with gradient concentrations of docetaxel, cell proliferation was detected to determine the drug resistance of LNCaP/Docetaxel cells to docetaxel. As shown in Fig. 1A, the half-maximal inhibitory concentration (IC_{50}) in LNCaP/docetaxel cells was significantly higher compared with LNCaP cells, indicating that LNCaP/Docetaxel cells were docetaxel-resistant. Consistent with a previous report (42), 10 $\mu\text{g/ml}$ of docetaxel was used for subsequent experiments. Baseline proliferation levels of LNCaP/Docetaxel cells were significantly higher compared with LNCaP cells (Fig. 1B). RT-qPCR was performed to detect the expression of miR-122 in human PCa docetaxel-resistant

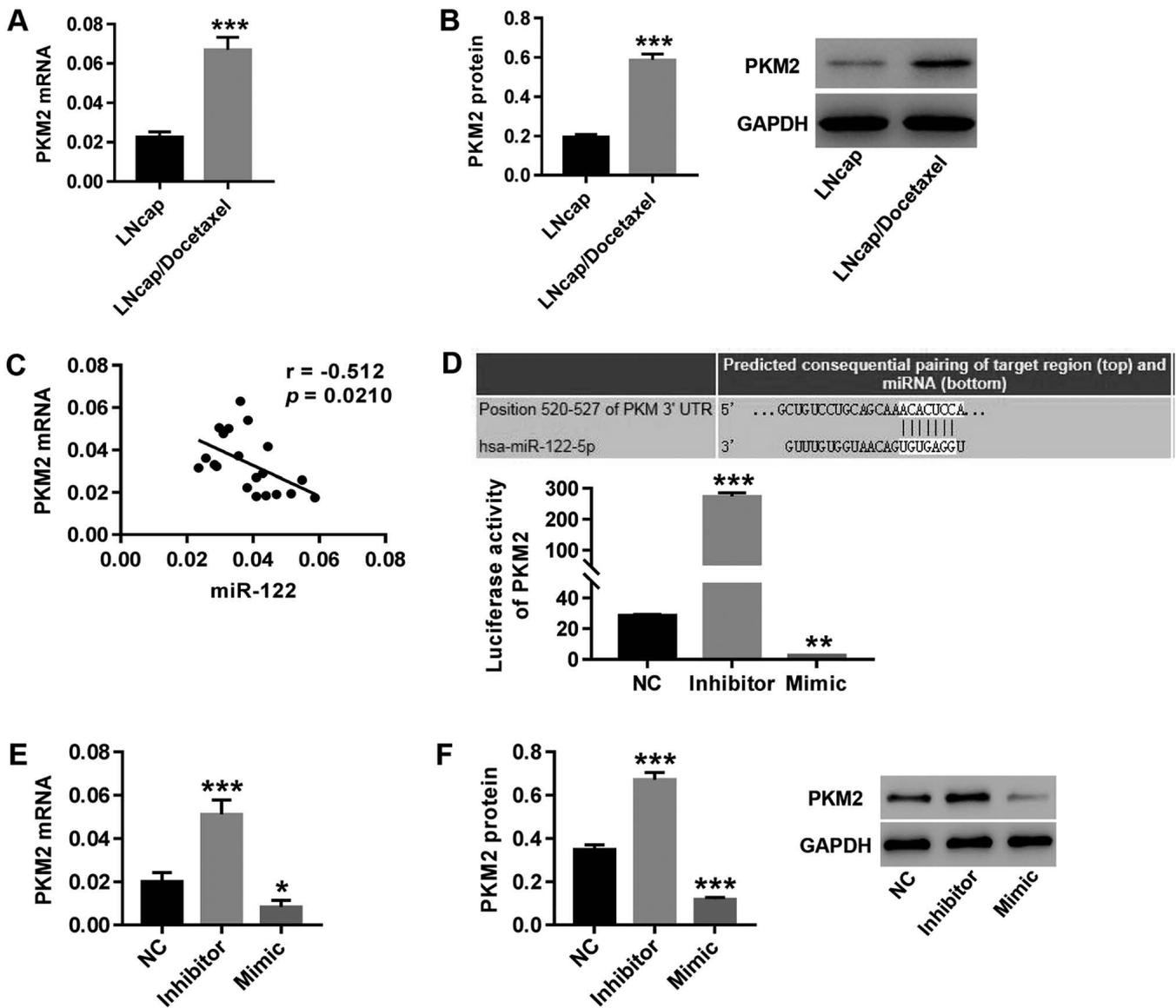


Figure 2. *PKM2* may be a target gene for miR-122 in regulating PCa. The expression of *PKM2* (A) mRNA and (B) protein in LNCaP and LNCaP/Docetaxel cells was detected. *** $P < 0.001$. (C) The correlation between miR-122 and *PKM2* in primary PCa cells was analyzed by Pearson's correlation coefficient analysis. (D) The binding site between the 3'-UTR of *PKM2* and miR-122 was predicted by TargetScan. Following miR-122 inhibitor or mimic treatment, the luciferase activity of the *PKM2* reporter was detected, and *PKM2* (E) mRNA and (F) protein expression was detected. * $P < 0.05$; ** $P < 0.01$ and *** $P < 0.001$ vs. NC. miR-122, microRNA-122; NC, negative control; 3'-UTR, 3'-untranslated region.

(LNCaP/Docetaxel) and -sensitive (LNCaP) cell strains. The results in Fig. 1C show that compared with LNCaP cells, miR-122 levels significantly decreased in LNCaP/Docetaxel cells. Furthermore, LNCaP cells were treated with miR-122 inhibitor (Fig. 1D). Following miR-122 inhibition, the proliferation of LNCaP cells significantly increased (Fig. 1E), and apoptosis was significantly decreased (Fig. 1F), which was accompanied by significantly increased glucose uptake (Fig. 1G) and lactate production (Fig. 1H) compared with the NC group. The findings suggested that miR-122 expression may be associated with docetaxel resistance in PCa.

PKM2 may be a target gene of miR-122 in regulating PCa. Both mRNA (Fig. 2A) and protein (Fig. 2B) expression of *PKM2* significantly increased in LNCaP/Docetaxel cells compared with LNCaP cells. *PKM2* expression was negatively correlated

with miR-122 expression in primary PCa cells isolated from tumor tissues of 20 patients with PCa (Fig. 2C). Furthermore, TargetScan was used to predict the binding site between the *PKM2* 3'-UTR and miR-122. Compared with the NC group, the luciferase reporter assay showed that following inhibition of miR-122, the luciferase activity of the *PKM2* reporter was significantly increased (Fig. 2D), accompanied by increased expression of *PKM2* (Fig. 2E and F), while miR-122 mimic transfection had the opposite effect. The results indicated that *PKM2* might be a target gene of miR-122 in regulating PCa.

miR-122 possibly regulates the docetaxel resistance of PCa cells via *PKM2* regulation. The human PCa docetaxel-resistant cell strain LNCaP/Docetaxel was treated with both miR-122 mimic and *oePKM2* lentivirus. As shown in Fig. 3A-C, *oePKM2* and miR-122 mimic treatment

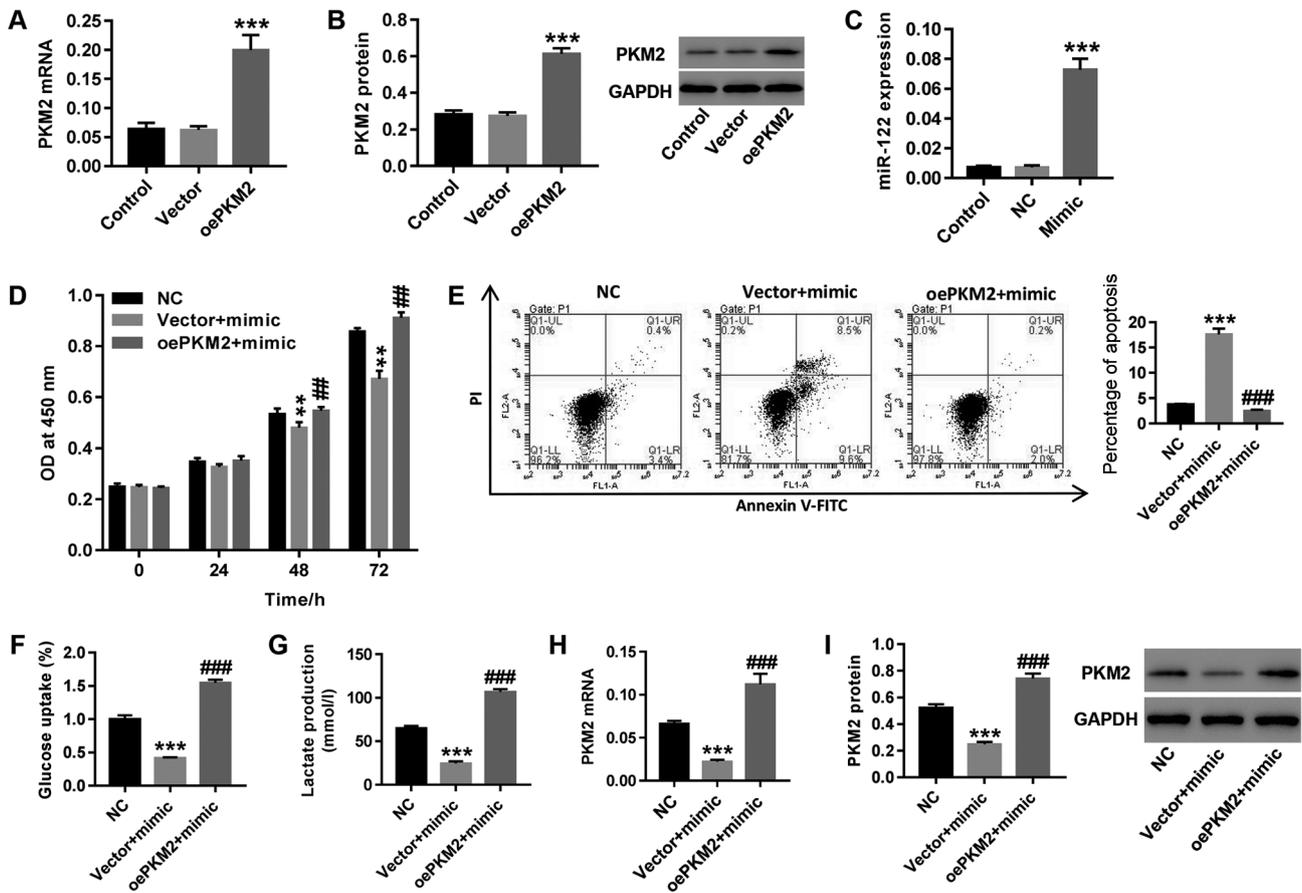


Figure 3. miR-122 regulates docetaxel resistance of PCa cells possibly via *PKM2* regulation. The upregulation efficiency of *oePKM2* lentivirus was detected by (A) reverse transcription-quantitative PCR and (B) western blotting. ****P*<0.001 vs. vector. (C) The efficiency of miR-122 mimic transfection was detected. ****P*<0.001 vs. NC. LNCaP/Docetaxel cells were treated with both *oePKM2* lentivirus and miR-122 mimic. (D) Cell proliferation was detected at 0, 24, 48 and 72 h. ***P*<0.01 vs. NC and ##*P*<0.01 vs. vector + mimic. (E) Cell apoptosis was detected at 48 h. The ordinate of the histogram is the sum of early and late apoptosis. ****P*<0.001 vs. NC and ###*P*<0.001 vs. vector + mimic. (F) Glucose uptake and (G) lactate production were detected. ****P*<0.001 vs. NC and ###*P*<0.001 vs. vector + mimic. *PKM2* (H) mRNA and (I) protein expression was detected. ****P*<0.001 vs. NC and ###*P*<0.001 vs. vector + mimic. miR-122, microRNA-122; OD, optical density; NC, negative control; PI, propidium iodide.

in LNCaP/Docetaxel cells significantly increased *PKM2* and miR-122 expression compared with the vector and NC groups, respectively. Upregulation of miR-122 following miR-122 mimic transfection resulted in significantly increased cell proliferation (Fig. 3D), glucose uptake (Fig. 3F) and lactate production (Fig. 3G) in LNCaP/Docetaxel cells, whereas cell apoptosis (Fig. 3E) increased, concurrent with a decrease in the expression of *PKM2* (Fig. 3H and I). Overexpression of *PKM2* counteracted the effects of miR-122 mimic on LNCaP/Docetaxel cells. The results demonstrated that miR-122 regulated docetaxel resistance in PCa cells, possibly via regulating *PKM2*.

Upregulation of miR-122 could reverse the resistance of LNCaP/Docetaxel cells to docetaxel. The primary cells isolated from 20 patients with PCa were divided into two groups: Low expression and high expression of miR-122 (Fig. 4A). Flow cytometry analysis showed that following treatment with 0.5 μg/ml docetaxel, apoptosis in primary PCa cells with high miR-122 expression significantly increased compared with cells with low miR-122 expression (Fig. 4B). In LNCaP/Docetaxel cells, upregulation of miR-122 expression by miR-122 mimic transfection significantly increased

docetaxel-induced apoptosis, while overexpression of *PKM2* counteracted the effect of miR-122 mimic transfection, and the effect of 10 μg/ml docetaxel was more significant compared with 5 μg/ml docetaxel (Fig. 4C). By contrast, inhibition of miR-122 in LNCaP cells significantly decreased docetaxel-induced apoptosis (Fig. 4D). The results demonstrated that high expression of miR-122 could promote docetaxel-induced apoptosis in PCa cells and upregulation of miR-122 could reverse the resistance of LNCaP/Docetaxel cells to docetaxel.

Discussion

An increasing number of studies have reported that the sensitivity of tumor cells to anticancer drugs can be altered by miRNAs (43-45). miR-34a was reported to enhance the chemosensitivity of PC3 cells to paclitaxel and camptothecin and PCa cells treated with miR-143 showed higher chemosensitivity to docetaxel (37,46). Previous research also showed that miR-122 can reverse drug resistance in several cancers (34,36). In the present study, significantly decreased miR-122 levels were observed in human PCa LNCaP/Docetaxel cells compared with LNCaP cells. miR-122 mimic transfection in LNCaP/Docetaxel PCa cells significantly decreased cell

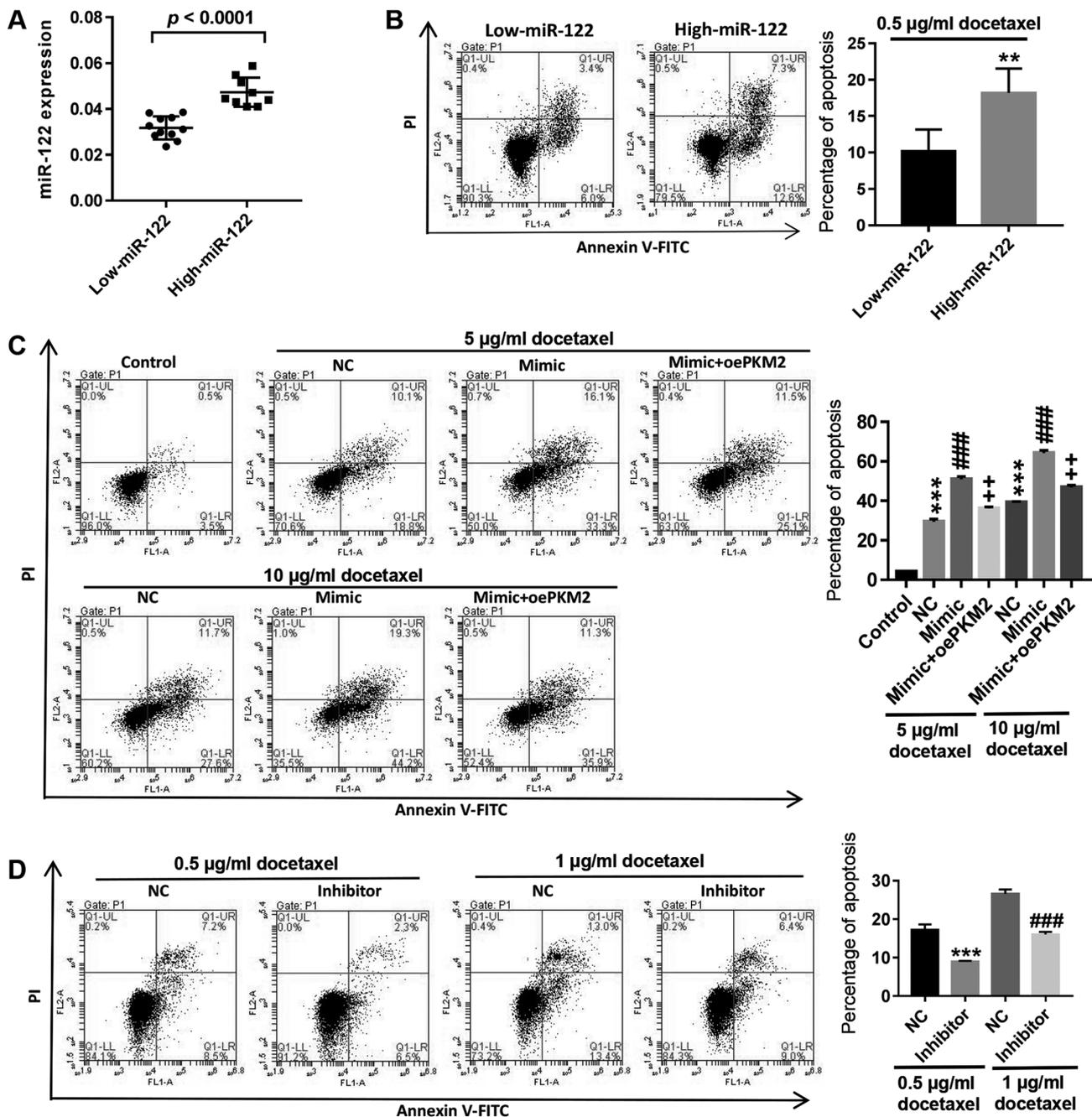


Figure 4. Upregulation of miR-122 could reverse docetaxel resistance of LNCaP/Docetaxel cells. (A) Expression of miR-122 in primary PCa cells isolated from 20 patients with PCa was detected and divided into low-miR-122 and high-miR-122 groups. (B) Cell apoptosis of PCa cells with low miR-122 and high miR-122 expression was detected following 0.5 µg/ml docetaxel treatment. The ordinate of the histogram is the sum of early and late apoptosis. $^{**}P < 0.01$. (C) Following treatment with miR-122 mimic, *oePKM2* or docetaxel (5 and 10 µg/ml), cell apoptosis in LNCaP/Docetaxel cells was detected. The ordinate of the histogram is the sum of early and late apoptosis. $^{***}P < 0.001$ vs. control; $^{###}P < 0.001$ vs. NC and $^{**}P < 0.01$ vs. mimic. (D) Following treatment with miR-122 inhibitor and docetaxel (5 and 10 µg/ml), cell apoptosis in LNCaP cells was detected. The ordinate of the histogram is the sum of early and late apoptosis. $^{***}P < 0.001$ vs. NC + 0.5 µg/ml docetaxel and $^{###}P < 0.001$ vs. NC + 1 µg/ml docetaxel. miR-122, microRNA-122; NC, negative control; PI, propidium iodide.

proliferation, increased apoptosis and inhibited glycolysis, while miR-122 inhibitor transfection in LNCaP showed the opposite effect, suggesting that miR-122 expression may be associated with docetaxel resistance in PCa by regulating cell proliferation, apoptosis and glycolysis. In primary PCa cells, high expression of miR-122 could promote docetaxel-induced apoptosis in PCa cells, and miR-122 mimic transfection significantly increased docetaxel-induced apoptosis in LNCaP/Docetaxel cells, while inhibition of miR-122 showed

the opposite effect. Thus, it was speculated that upregulation of miR-122 could reverse the resistance of LNCaP/Docetaxel cells to docetaxel, which may contribute to the treatment of PCa chemoresistance.

Furthermore, the underlying mechanism of miR-122 in regulating docetaxel resistance in PCa was investigated. In tumors, miRNAs primarily function via regulation of their target genes by targeting specific mRNAs for degradation or translation inhibition (47). A study reported that upregulation

of miR-328 can enhance docetaxel sensitivity, decrease cell proliferation and increase apoptosis in PCa cells by directly targeting p21-activated protein kinase 6 (48). Additionally, the ectopic expression of miR-21 can increase the resistance of PC3 cells to docetaxel by targeting the tumor suppressor programmed cell death protein 4 (40). In addition, a previous study revealed that in human lung cancer xenografts in mice, inhibition of *PKM2* could enhance the efficacy of docetaxel (49). Results of the present study showed that the expression of *PKM2* in PCa cells negatively correlated with miR-122 expression, and the luciferase reporter assay showed that miR-122 regulated *PKM2* expression by binding to the 3'-UTR of *PKM2*. In LNCaP/Docetaxel PCa cells, miR-122 mimic-induced cell proliferation decreased, apoptosis increased and glycolysis inhibition was counteracted by *PKM2* overexpression. Consistent with previous reports on miR-122 in cancer chemoresistance (34-36), it can be inferred that the upregulation of miR-122 expression may reverse the resistance of PCa LNCaP/Docetaxel cells to docetaxel via downregulation of its target gene *PKM2*. However, the present study also had limitations, such as the lack of sequencing data and validation, as well as the lack of studies on other prostate cancer cell types. Mechanisms of miR-122 involved in docetaxel resistance and the function of miR-122 in other prostate cancer cell types can be investigated in future studies to further confirm the current results.

In conclusion, the results demonstrated that high expression of miR-122 could promote docetaxel-induced apoptosis in PCa cells and that the upregulation of miR-122 could reverse the resistance of LNCaP/Docetaxel cells to docetaxel, possibly via the regulation of its target protein *PKM2* by binding to the 3'-UTR. These findings may provide a link between PCa chemoresistance and miRNAs, and targeting miRNA-122 may offer a novel therapy for the chemoresistance of PCa.

Acknowledgements

Not applicable.

Funding

This study was funded by the Shaoxing Municipal Bureau of Science and Technology in China (grant no. 2017B70032).

Availability of data and materials

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

Authors' contributions

ZZ and JY conceived and designed the study. ZZ, JY and GT performed the experiments. ZZ and JY wrote the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All experiments conducted in this study were approved by the Ethics Committee of Shaoxing People's Hospital. Written informed consent was obtained.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

- Han W and Li J: Structure-activity relationship analysis of 3-phenylpyrazole derivatives as androgen receptor antagonists. *J Biomol Struct Dyn*: 1-10, Jul 5, 2019 (Epub ahead of print).
- Siegel RL, Miller KD and Jemal A: Cancer statistics, 2019. *CA Cancer J Clin* 69: 7-34, 2019.
- Fendler A, Jung M, Stephan C, Honey RJ, Stewart RJ, Pace KT, Erbersdobler A, Samaan S, Jung K and Yousef GM: miRNAs can predict prostate cancer biochemical relapse and are involved in tumor progression. *Int J Oncol* 39: 1183-1192, 2011.
- Pereira-Lourenço M, Vieira E, Brito D, Peralta JP, Godinho R, Conceição P, Reis M, Rabaça C and Sismeiro A: Influence of sociodemographic factors on treatment's choice for localized prostate cancer in Portugal. *Arch Ital Urol Androl* 92: 45-49, 2020.
- van Brussel JP and Mickisch GH: Multidrug resistance in prostate cancer. *Onkologie* 26: 175-181, 2003.
- Sturge J, Caley MP and Waxman J: Bone metastasis in prostate cancer: Emerging therapeutic strategies. *Nat Rev Clin Oncol* 8: 357-368, 2011.
- Chi KN, Bjartell A, Dearnaley D, Saad F, Schröder FH, Sternberg C, Tombal B and Visakorpi T: Castration-resistant prostate cancer: From new pathophysiology to new treatment targets. *Eur Urol* 56: 594-605, 2009.
- Cookson MS, Lowrance WT, Murad MH and Kibel AS: Castration-resistant prostate cancer: AUA guideline amendment. *J Urol* 193: 491-499, 2015.
- Tannock IF, de Wit R, Berry WR, Horti J, Pluzanska A, Chi KN, Oudard S, Théodore C, James ND, Turesson I, *et al*: Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer. *N Engl J Med* 351: 1502-1512, 2004.
- Bolten CJ, Heinzle E, Müller R and Wittmann C: Investigation of the central carbon metabolism of *Sorangium cellulosum*: Metabolic network reconstruction and quantification of pathway fluxes. *J Microbiol Biotechnol* 19: 23-36, 2009.
- Tyszka-Czochara M, Konieczny P and Majka M: Recent advances in the role of AMP-activated protein kinase in metabolic reprogramming of metastatic cancer cells: Targeting cellular bioenergetics and biosynthetic pathways for anti-tumor treatment. *J Physiol Pharmacol* 69: 2018.
- Liberti MV and Locasale JW: The warburg effect: How does it benefit cancer cells? *Trends Biochem Sci* 41: 211-218, 2016.
- Chaneton B and Gottlieb E: Rocking cell metabolism: Revised functions of the key glycolytic regulator PKM2 in cancer. *Trends Biochem Sci* 37: 309-316, 2012.
- Wang J, Wang H, Liu A, Fang C, Hao J and Wang Z: Lactate dehydrogenase A negatively regulated by miRNAs promotes aerobic glycolysis and is increased in colorectal cancer. *Oncotarget* 6: 19456-19468, 2015.
- Ha TK, Her NG, Lee MG, Ryu BK, Lee JH, Han J, Jeong SI, Kang MJ, Kim NH, Kim HJ and Chi SG: Caveolin-1 increases aerobic glycolysis in colorectal cancers by stimulating HMG1-mediated GLUT3 transcription. *Cancer Res* 72: 4097-4109, 2012.
- Xu X, Li J, Sun X, Guo Y, Chu D, Wei L, Li X, Yang G, Liu X, Yao L, *et al*: Tumor suppressor NDRG2 inhibits glycolysis and glutaminolysis in colorectal cancer cells by repressing c-Myc expression. *Oncotarget* 6: 26161-26176, 2015.
- Dong G, Mao Q, Xia W, Xu Y, Wang J, Xu L and Jiang F: PKM2 and cancer: The function of PKM2 beyond glycolysis. *Oncol Lett* 11: 1980-1986, 2016.
- Liang J, Cao R, Zhang Y, Xia Y, Zheng Y, Li X, Wang L, Yang W and Lu Z: PKM2 dephosphorylation by Cdc25A promotes the Warburg effect and tumorigenesis. *Nat Commun* 7: 12431, 2016.
- Luan W, Wang Y, Chen X, Shi Y, Wang J, Zhang J, Qian J, Li R, Tao T, Wei W, *et al*: PKM2 promotes glucose metabolism and cell growth in gliomas through a mechanism involving a let-7a/c-Myc/hnRNPA1 feedback loop. *Oncotarget* 6: 13006-130018, 2015.

20. Lu W, Cao Y, Zhang Y, Li S, Gao J, Wang XA, Mu J, Hu YP, Jiang L, Dong P, *et al*: Up-regulation of PKM2 promote malignancy and related to adverse prognostic risk factor in human gallbladder cancer. *Sci Rep* 6: 26351, 2016.
21. Zhang HS, Zhang FJ, Li H, Liu Y, Du GY and Huang YH: Tanshinone IIA inhibits human esophageal cancer cell growth through miR-122-mediated PKM2 down-regulation. *Arch Biochem Biophys* 598: 50-56, 2016.
22. Xu Q, Zhang M, Tu J, Pang L, Cai W and Liu X: MicroRNA-122 affects cell aggressiveness and apoptosis by targeting PKM2 in human hepatocellular carcinoma. *Oncol Rep* 34: 2054-2064, 2015.
23. Guo M, Zhao X, Yuan X, Jiang J and Li P: MiR-let-7a inhibits cell proliferation, migration, and invasion by down-regulating PKM2 in cervical cancer. *Oncotarget* 8: 28226-28236, 2017.
24. Taniguchi K, Sugito N, Kumazaki M, Shinohara H, Yamada N, Nakagawa Y, Ito Y, Otsuki Y, Uno B, Uchiyama K and Akao Y: MicroRNA-124 inhibits cancer cell growth through PTB1/PKM1/PKM2 feedback cascade in colorectal cancer. *Cancer Lett* 363: 17-27, 2015.
25. Bartel DP: MicroRNAs: Genomics, biogenesis, mechanism, and function. *Cell* 116: 281-297, 2004.
26. Rigoutsos I: New tricks for animal microRNAs: Targeting of amino acid coding regions at conserved and nonconserved sites. *Cancer Res* 69: 3245-3248, 2009.
27. Gandellini P, Profumo V, Casamichele A, Fenderico N, Borrelli S, Petrovich G, Santilli G, Callari M, Colecchia M, Pozzi S, *et al*: miR-205 regulates basement membrane deposition in human prostate: Implications for cancer development. *Cell Death Differ* 19: 1750-1760, 2012.
28. Hudson RS, Yi M, Esposito D, Glynn SA, Starks AM, Yang Y, Schetter AJ, Watkins SK, Hurwitz AA, Dorsey TH, *et al*: MicroRNA-106b-25 cluster expression is associated with early disease recurrence and targets caspase-7 and focal adhesion in human prostate cancer. *Oncogene* 32: 4139-4147, 2013.
29. Boll K, Reiche K, Kasack K, Mörbt N, Kretzschmar AK, Tomm JM, Verhaegh G, Schalken J, von Bergen M, Horn F and Hackermüller J: MiR-130a, miR-203 and miR-205 jointly repress key oncogenic pathways and are downregulated in prostate carcinoma. *Oncogene* 32: 277-285, 2013.
30. Martens-Uzunova ES, Jalava SE, Dits NF, van Leenders GJ, Møller S, Trapman J, Bangma CH, Litman T, Visakorpi T and Jenster G: Diagnostic and prognostic signatures from the small non-coding RNA transcriptome in prostate cancer. *Oncogene* 31: 978-991, 2011.
31. Musumeci M, Coppola V, Addario A, Patrizii M, Maugeri-Saccà M, Memeo L, Colarossi C, Francescangeli F, Biffoni M, Collura D, *et al*: Control of tumor and microenvironment cross-talk by miR-15a and miR-16 in prostate cancer. *Oncogene* 30: 4231-4242, 2011.
32. Takayama K, Tsutsumi S, Katayama S, Okayama T, Horie-Inoue K, Ikeda K, Urano T, Kawazu C, Hasegawa A, Ikeo K, *et al*: Integration of cap analysis of gene expression and chromatin immunoprecipitation analysis on array reveals genome-wide androgen receptor signaling in prostate cancer cells. *Oncogene* 30: 619-630, 2011.
33. Wang X, Zhang H, Bai M, Ning T, Ge S, Deng T, Liu R, Zhang L, Ying G and Ba Y: Exosomes serve as nanoparticles to deliver anti-miR-214 to reverse chemoresistance to cisplatin in gastric cancer. *Mol Ther* 26: 774-783, 2018.
34. Pan C, Wang X, Shi K, Zheng Y, Li J, Chen Y, Jin L and Pan Z: MiR-122 reverses the doxorubicin-resistance in hepatocellular carcinoma cells through regulating the tumor metabolism. *PLoS One* 11: e0152090, 2016.
35. Kishikawa T, Otsuka M, Tan PS, Ohno M, Sun X, Yoshikawa T, Shibata C, Takata A, Kojima K, Takehana K, *et al*: Decreased miR122 in hepatocellular carcinoma leads to chemoresistance with increased arginine. *Oncotarget* 6: 8339-8252, 2015.
36. He J, Xie G, Tong J, Peng Y, Huang H, Li J, Wang N and Liang H: Overexpression of microRNA-122 re-sensitizes 5-FU-resistant colon cancer cells to 5-FU through the inhibition of PKM2 in vitro and in vivo. *Cell Biochem Biophys* 70: 1343-1350, 2014.
37. Fujita Y, Kojima K, Hamada N, Ohhashi R, Akao Y, Nozawa Y, Deguchi T and Ito M: Effects of miR-34a on cell growth and chemoresistance in prostate cancer PC3 cells. *Biochem Biophys Res Commun* 377: 114-119, 2008.
38. Singh S, Chitkara D, Mehrazin R, Behrman SW, Wake RW and Mahato RI: Chemoresistance in prostate cancer cells is regulated by miRNAs and Hedgehog pathway. *PLoS One* 7: e40021, 2012.
39. Li J, Yang X, Guan H, Mizokami A, Keller ET, Xu X, Liu X, Tan J, Hu L, Lu Y and Zhang J: Exosome-derived microRNAs contribute to prostate cancer chemoresistance. *Int J Oncol* 49: 838-846, 2016.
40. Shi G, Ye D, Yao X, Zhang SL, Dai B, Zhang HL, Shen YJ, Zhu Y, Zhu YP, Xiao WJ and Ma CG: Involvement of microRNA-21 in mediating chemo-resistance to docetaxel in androgen-independent prostate cancer PC3 cells. *Acta Pharmacol Sin* 31: 867-873, 2010.
41. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2^{-ΔΔCT} method. *Methods* 25: 402-408, 2001.
42. Egawa T, Kubota T, Suto A, Otani Y, Furukawa T, Watanabe M, Kumai K and Kitajima M: Docetaxel enhances the cytotoxicity of anthracyclines by increasing intracellular drug accumulation. *Oncol Rep* 9: 777-781, 2002.
43. Lin W, Miao Y, Meng X, Huang Y, Zhao W and Ruan J: miRNA-765 mediates multidrug resistance via targeting BATF2 in gastric cancer cells. *FEBS Open Bio*: Mar 12, 2020 (Epub ahead of print).
44. Hong ST, Lin H, Wang CS, Chang CH, Lin AMY, Yang JCH and Lo YL: Improving the anticancer effect of afatinib and microRNA by using lipid polymeric nanoparticles conjugated with dual pH-responsive and targeting peptides. *J Nanobiotechnology* 17: 89, 2019.
45. Hong BS, Ryu HS, Kim N, Kim J, Lee E, Moon H, Kim KH, Jin MS, Kwon NH, Kim S, *et al*: Tumor Suppressor miRNA-204-5p regulates growth, metastasis, and immune microenvironment remodeling in breast cancer. *Cancer Res* 79: 1520-1534, 2019.
46. Xu B, Niu X, Zhang X, Tao J, Wu D, Wang Z, Li P, Zhang W, Wu H, Feng N, *et al*: miR-143 decreases prostate cancer cells proliferation and migration and enhances their sensitivity to docetaxel through suppression of KRAS. *Mol Cell Biochem* 350: 207-213, 2011.
47. Bian Z, Li L, Tang R, Hou DX, Chen X, Zhang CY and Zen K: Identification of mouse liver mitochondria-associated miRNAs and their potential biological functions. *Cell Res* 20: 1076-1078, 2010.
48. Liu C, Zhang L, Huang Y, Lu K, Tao T, Chen S, Zhang X, Guan H, Chen M and Xu B: MicroRNA-328 directly targets p21-activated protein kinase 6 inhibiting prostate cancer proliferation and enhancing docetaxel sensitivity. *Mol Med Rep* 12: 7389-7395, 2015.
49. Shi HS, Li D, Zhang J, Wang YS, Yang L, Zhang HL, Wang XH, Mu B, Wang W, Ma Y, *et al*: Silencing of pkm2 increases the efficacy of docetaxel in human lung cancer xenografts in mice. *Cancer Sci* 101: 1447-1453, 2010.



This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.