CANCER BIOLOGY & THERAPY
2024, VOL. 25, NO. 1, 2399363
https://doi.org/10.1080/15384047.2024.2399363

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RESEARCH PAPER

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CircBIRC6 affects prostate cancer progression by regulating miR-574-5p and DNAJB1

Bin Zhao[a#], Jinye Yang[a#], Fengming Ran[b], Yuanlong Shi[a], Libo Yang[a], Yuanpeng Duan[a], Zhiyu Shi[a], Xin Li[a], Jianpeng Zhang[a], Zhiyao Li[c], and Jiansong Wang[d]

[a]Department of Urology, Yunnan Cancer Hospital, The Third Affiliated Hospital of Kunming Medical University, Peking University Cancer Hospital Yunnan, Kunming, China; [b]Department of Pathology, Yunnan Cancer Hospital, The Third Affiliated Hospital of Kunming Medical University, Peking University Cancer Hospital Yunnan, Kunming, China; [c]Department of Ultrasound Medicine, Yunnan Cancer Hospital, The Third Affiliated Hospital of Kunming Medical University, Peking University Cancer Hospital Yunnan, Kunming, China; [d]Department of Urology, The Second Affiliated Hospital of Kunming Medical University, Kunming, China

ABSTRACT

Background: Prostate cancer (PCa) is among the three main types of cancer. Although prostate-specific antigen (PSA) is routinely tested, it has disadvantages, such as poor prognostic ability. Therefore, finding more PCa markers and therapeutic targets remains a subject of study. CircRNAs have been found to have regulatory roles in various diseases, such as diabetes, Central Nervous System (CNS) neuropathy, etc. where their application in cancer is even more valuable. Therefore, this paper aims to search for differentially expressed circRNAs in PCa and find downstream targeting pathways related to autophagy.
Method: By detecting the expression of circRNA in the samples, hsa_circ_0119816 was finally identified as the research target. The properties of circRNA were verified by RNase R, actinomycin D, and fluorescence in situ hybridization (FISH). The downstream target miRNAs and target proteins were predicted by an online database, and the targeting relationship was verified using dual luciferase and RNA Immunoprecipitation. The effects of circRNAs and their downstream signalling pathways on prostate cancer cell proliferation, migration, EMT and autophagy were examined by CCK-8, Transwell, immunofluorescence and Western blotting.
Results: CircBIRC6 is highly expressed in prostate cancer samples. Knockdown of its expression inhibits cell proliferation, invasion, EMT and autophagy and promotes apoptosis. CircBIRC6/miRNA-574-5p/DNAJB1 is a molecular axis that regulates prostate cancer cells.

ARTICLE HISTORY
Received 3 June 2024
Revised 27 August 2024
Accepted 28 August 2024

KEYWORDS
Prostate cancer; circBIRC6; autophagy; miRNA-574-5p; DNAJB1

Introduction

Prostate cancer (PCa) is one of the top three cancers in terms of incidence, and it has been added as one of the leading cancers worldwide due to the increase in both new cases and deaths in recent years.[1] PCa is second only to lung cancer in the number of new cases of cancer, significantly as age increases, with more than 50% of men over the age of 75 suffering from PCa.[2] According to recent statistics, the incidence of PCa is still increasing, with nearly 300,000 new cases in 2023.[3]

Currently, puncture biopsy, MRI and prostate-specific antigen (PSA) are routine diagnostic modalities, and PSA is the most widely used for PCa.[4] Prostate-specific antigen (PSA) has limited prognostic ability, and conditions, including hyperplasia and inflammation, might cause abnormalities in the testing procedure.[5] Surgery, chemotherapy and radiotherapy are the main clinical options for PCa.[6] Early treatment tends to be more efficient. After metastasis develops in PCa patients, the majority experience a poor prognosis and decreased survival rates. In recent years, there have been many explorations of PCa treatment, such as nanotechnology,[7] immunotherapy,[8] etc. These methods are still inadequate for advanced PCa patients. Therefore, searching for more PCa biomarkers and therapeutic targets is significant.

CircRNAs are non-coding circular RNA molecules produced by reverse splicing of precursor mRNAs and are widely recognized to play regulatory roles in diseases.[9] CircRNA-related research has been a hotspot in several disease areas, such as diabetes, central neuropathy, and cancer. Especially in malignant tumors, circRNAs are considered to have important research value. Review articles point out that circRNAs are potential therapeutic strategies in treating endocrine cancer types, such as thyroid cancer (TC)[10,11] and breast cancer (BC).[12] CircRNA102231 is highly expressed in gastric cancer and promotes the progression of gastric cancer.[13] CircRNA104348 can regulate the progression of hepatocellular carcinoma by sponging on miRNAs and their targeting proteins.[14] In addition, in PCa, circRNAs have been shown to influence its occurrence in various ways. CircARHGAP29 in PCa can influence drug resistance in therapy.[15] CircPDE5A regulates metastasis in PCa through methylation.[16] CircSMARCC1 influences the

CONTACT Jiansong Wang wangjiansong@kmmu.edu.cn Department of Urology, The Second Affiliated Hospital of Kunming Medical University, Kunming, Yunnan 650101, China; Zhiyao Li lizhiyao53@sina.com Department of Ultrasound Medicine, Yunnan Cancer Hospital, The Third Affiliated Hospital of Kunming Medical University, Peking University Cancer Hospital Yunnan, Kunming, Yunnan 650118, China

[#]Contributed equally.

Supplemental data for this article can be accessed online at https://doi.org/10.1080/15384047.2024.2399363

proliferation of PCa cells through crosstalk of its molecular axis on macrophages.[17] Therefore, investigating the regulatory mechanisms of circRNAs in PCa is imperative for discovering additional biomarkers and therapeutic targets.

Autophagy is a major biological process in programmed cell death that maintains homeostasis of the internal environment. Dysregulation of autophagy leads to many diseases, such as Alzheimer's disease, diabetes, and cardiovascular disease.[18] Autophagy has a dual role in cancer.[19] On the one hand, autophagy exerts an inhibitory function on tumors, especially in the early stages. Autophagy can suppress cancer by modifying the tumor microenvironment by inhibiting inflammation and tissue injury.[20] On the other hand, autophagy promotes cancer metastasis. During the late stages of cancer, autophagy serves the cells' metabolic requirements and enhances the viability of tumor cells.[21] In addition to the different regulation of autophagy at various stages of cancer, the molecular mechanisms that autophagy regulates together with circRNAs also have potential. It has been demonstrated that regulating circRNAs and autophagy can affect chemoresistance in gastric cancer treatment.[22] CircRNAs can sponge miRNAs and activate autophagy-related signalling pathways. It can also directly regulate autophagy-related proteins. Therefore, studying the relationship between circRNA and autophagy in PCa can provide a potential scientific basis for therapeutic modalities.

This paper aims to investigate the regulatory mechanisms of circRNAs in PCa, discover their sponge-acting miRNAs and regulatory pathways, and explore their relationship with autophagy. This study hopes to provide new strategies for treating and detecting PCa.

Method and materials

Clinical sample organisation and cell culture

All 23 clinical samples were collected from patients. All patients were those who had not received any treatment, such as chemotherapy or radiotherapy and were pathologically diagnosed with prostate cancer or prostate hyperplasia. The study was authorized by the Ethics Committee of Kunming Medical University. Patient information was detailed in Supplementary Table S1. The cells used in this part of the study were purchased from the Institute of Cell Research, Chinese Academy of Sciences. Human normal prostate epithelial cells (RWPE-1) and human prostate cancer cell lines (22RV1, C4–2, PC-3, DU145) were grown in RPMI-1640 medium containing 10% fetal bovine serum and 1% streptomycin-penicillin double antibody, and cultured in a constant temperature incubator at 37°C.

qPCR

Cells in different groups were centrifuged, and total RNA was extracted using a Trizol reagent. cDNA synthesis kit was used for reverse transcription, synthesized cDNA, and concentration was determined. The assay was performed according to the instructions of the fluorescence quantification kit. The primer sequences are shown in Supplementary Table S2. The amplification conditions were set as follows: pre-denaturation, 95°C, 30 s; denaturation, 95°C, 15 s; and extension, 75°C, 15 s. The reaction was carried out for 40 cycles. At the end of the reaction, the relative expression was calculated using the $2^{-\Delta\Delta t}$ method with GAPDH as the control.

Actinomycin D

After digestion, centrifugation and resuspension treatments, cells were inoculated into six-well plates. Actinomycin D (Sigma, USA) was configured as a stock solution. When the cell confluence reached 80%, 1 ml of PBS was added to clean the cell surface, and then 2ug/ml actinomycin D was added to each well. Cells were collected at 0 h, 4 h, 8 h, 12 h and 24 h, and the expression level was measured at each time point.

RnaseR

After collecting the cells, RNA was extracted with Trizol, the concentration was diluted to 500 ng/ul, and tested using the RNaseR kit (Geneseed, China). The experiment was divided into RNaseR+ and RNaseR- according to the instructions. The two groups were placed in a water bath system and incubated at 37°C for 30 min. After the inactivation of RNaseR, a reverse transcription reaction was carried out, after which the expression level was determined.

Fluorescence in situ hybridisation (FISH)

The probe was designed and synthesized by the company (Ribobio, China). Cells were digested, centrifuged for dilution, and then inoculated into six-well plates. Experiments were performed after the cells reached 60% confluence. The cells were rinsed with 1 ml of PBS and fixed with 4% Paraformaldehyde at room temperature. After washing, the hybridization solution containing the probe was added and stored at 42°C overnight. The cells were washed with PBS and co-incubated with antibodies. Finally, DAPI was added to stain the cells, and the images were observed and captured under a fluorescence microscope.

Cell transfection

The overexpression vectors and their control empty vectors, siRNA and their negative controls involved in this paper were purchased from the company (Ribobio, China). The cells were digested and centrifuged. The cell precipitate was resuspended to prepare a cell suspension. The cell suspension was inoculated into a six-well plate and incubated for 24 h. A cell confluence of 80% is required for transfection. Transfection was performed using 5ul Lipofectamine 3000 (Thermo Fisher, USA). The transfection reagent was added to the cells and incubated in an incubator for 6 h. The transfected cells were collected by centrifugation for subsequent experiments.

CCK-8

Cells were digested, centrifuged, and then inoculated into 96-well plates with 100ul of cell suspension per well to set the time point. After cell apposition, remove the 96-well plate at 24 h time point. After adding CCK8 solution (Yeasen, China), the plate was incubated at 37°C for 2 h at a constant temperature, and the OD value was measured at 450 nm at the end.

Flow cytometry

The cells were digested and processed for centrifugation at 1000rpm/5 min. The cell suspension was prepared by resuspending the cells after aspirating the supernatant. The cell suspension was fixed and centrifuged again. Propidium iodide staining solution was prepared and added to the samples. PBS was added to the samples for 1500rpm/5 min, and after thorough mixing, the samples were placed on a flow cytometer to detect the cell cycle.

Transwell

The cells were digested and placed into a centrifuge tube. The cell suspension was removed and added to the upper chamber of the Transwell chambers (Corning, USA). The FBS medium (Thermo-Fisher Scientific, USA) was added to the lower chamber. The Transwell was incubated in an incubator for 24 h, and the upper chamber was removed. The cells of the upper chamber were gently wiped off using a cotton swab and then washed with PBS. Transwell chambers were fixed using paraformaldehyde for 30 min. After washing again, a crystal violet staining solution was added for staining. The cells were observed under a microscope, and images were collected.

Immunofluorescence

Cells were subjected to plate spreading operation followed by fixation using 4% paraformaldehyde for 30 min and then washed using PBS. After permeabilisation of the cells using PBS diluent, they could be closed with a closure solution for 30 min at room temperature. Dropwise addition of primary antibody was incubated overnight, followed by the addition of secondary antibody and left to stand at room temperature. Nuclei were stained using Dapi, incubated at room temperature, and protected from light. After a final wash with PBS, images were observed under a confocal microscope. The antibody information was shown in Supplementary Table S3.

Western blotting

Cells were digested and centrifuged, the supernatant was aspirated, and PBS was added to resuspend the cells. Cells were lysed using RIPA lysate (Solarbio, China) to extract total proteins. SDS-PAGE electrophoresis was used to separate the protein samples. Gel was made first, and after 15 min of resting, the protein samples were prepared for uploading. After uploading the samples, a protein maker was added, and electrophoresis was performed. Afterward, the membrane was transferred to the PVDF membrane and closed using 5% BSA. After sealing, they were incubated with primary antibodies overnight. After washing, the membrane was incubated with a secondary antibody. After developmental imaging, average gray values were calculated using ImageJ. The antibody information is shown in Supplementary Table S4.

Dual luciferase

The plasmids used were designed and synthesized by the Company (RiboBio, China). Refer to the instructions of the luciferase assay kit for experiments. Cells were digested, centrifuged and left at 50%-60% density, then transfected with mimics and mutant plasmids using Lipofectamine 3000 (Invitrogen, USA). Cells were collected after 48 h, and the luciferase activity was detected by the Dual-Luciferase System.

RNA immunoprecipitation (RIP)

Cells were lysed using RIP buffer. DNase (Sigma, USA) was added to the lysate to remove DNA. Anti-Ago2 and anti-IgG (Sigma, USA) were added to the supernatant from the above steps and incubated at 4°C for 16 h. Assays were performed using the RIP kit. The cell lysate was co-incubated with antibody magnetic beads for anti-Ago2 and anti-IgG. Subsequently, ProteinA/G magnetic beads and polysome lysis buffer were added, shaken well, and the supernatant was discarded. The RNA bound to the beads was eluted, and the RNA was extracted using Trizol for reverse transcription and qPCR.

Statistical analysis

The experimental data were statistically analyzed using GraphPad Prism 9 (GraphPad Prism, USA). Statistical differences between the control and experimental groups were analyzed by t-test or one-way and two-way ANOVA tests.

Results

Prediction and validation of BIRC6-related CircRNAs

CircRNAs related to BIRC6 were screened in the CircBase database(Supplementary Table S5). Expression was verified in clinical samples and differentially expressed circBIRC6 with statistically significant differences were screened (Supplementary Table S6, Figure S1). The result found that hsa_circ_0119816 was the most significantly differentially expressed circRNA (***$p < .001$), named circBIRC6 for subsequent experiments. (Figure 1a). We detected the expression of circBIRC6 in human normal prostate epithelial cells (RWPE-1) and human prostate cancer cells (22RV1, C4–2, PC-3, and DU145), respectively, and chose the highest- and lowest-expressing PC cells for the further experiments (Figure 1b). To explore the tolerance of circ_0119816 and its cognate linear RNA BIRC6 to RNase R, we conducted RNase R experiments. RNase R results illustrated that circRNA expression was not significantly changed ($p > .05$), whereas the expression of mRNA (BIRC6) was dramatically reduced after RNase R treatment (Figure 1c). To explore the stability of circRNA, we performed Actinomycin D experiments. The expression of BIRC6 was significantly reduced by the addition of Actinomycin

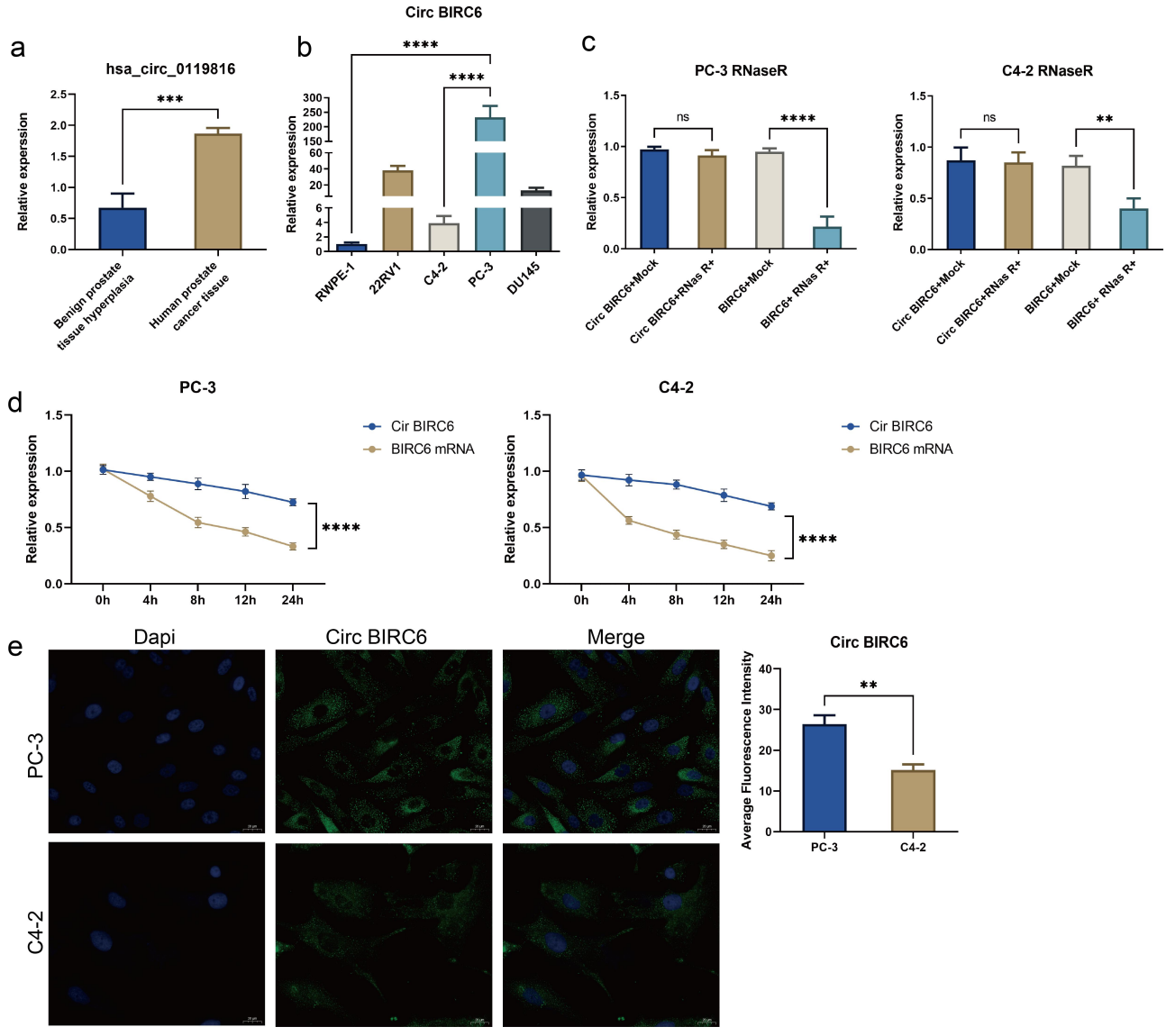

Figure 1. Identification of circBIRC6 (has_circ_0119816) from clinical samples and experimental validation of circBIRC6 properties. (a) qPCR of the expression of different circRNAs in the clinical samples, *n*=3. (b) qPCR of different circRNABIRC6 expression in different cell lines, *n*=3. (c) RNase R experiments verified the properties of circBIRC6, *n*=3 (d) Actinomycin D experiments validate the properties of circBIRC6, *n*=3 (e) FISH experiments validate the localization of circBIRC6 in cells, *n*=5, scale bar=20um n.s, no significant, ****p<.0001, ***p < .001, **p < .01 and *p < .05

D (Figure 1d). These results showed that circBIRC6 is more stable than BIRC6 mRNA. Finally, it was illustrated by fluorescence in situ hybridization (FISH) that circBIRC6 was mainly localized in the cytoplasm of PC-3 and C4–2 (Figure 1e).

Effect of CircBIRC6 on the malignant phenotype of PC cells

To verify the effects of knockdown and overexpression of CircBIRC6 on PC cells, we transfected sh-CircBIRC6 and pcDNA3.1-circ BIRC6 in PC-3 and C4–2, respectively. qPCR was used to verify the transfection efficiency, and CircBIRC6 expression was decreased after the transfection of sh-CircBIRC6. Transfection of pcDNA3.1-circ BIRC6 increased the expression of CircBIRC6 after transfection, and the results indicated that the transfection was successful (Figure 2a). Subsequently, we illustrated the specific effects of CircBIRC6 on the phenotype of PC cells through experiments. First, we used CCK-8 assay and Transwell assay, and the knockdown of CircBIRC6 inhibits the cell viability and invasion cell counts of PC-3 cells. In C4–2 cells, overexpression promoted these (Figure 2b,c). Detecting apoptosis by flow cytometry, we found that the apoptosis rate increased in PC-3 cells after transfection with sh-CircBIRC6 and decreased in C4–2 cells after transfection with pcDNA3.1-CircBIRC6 (Figure 2e). Subsequently, we investigated the EMT of the cells by fluorescent staining. The results showed that reduced CircBIRC6 expression inhibited EMT in PC-3 cells, as evidenced by an increase in E-Cadherin and a decrease in N-Cadherin and Vimentin. The opposite result was observed after overexpression of CircBIRC6 in C4–2, which inhibited the development of EMT, as evidenced by a reduction in E-Cadherin and an increase in N-Cadherin and Vimentin. In addition, we examined the expression of autophagy markers (Figure 2e, Figure S2). By fluorescence staining of LC3, it was found that inhibition of CircBIRC6 expression led to a decrease in

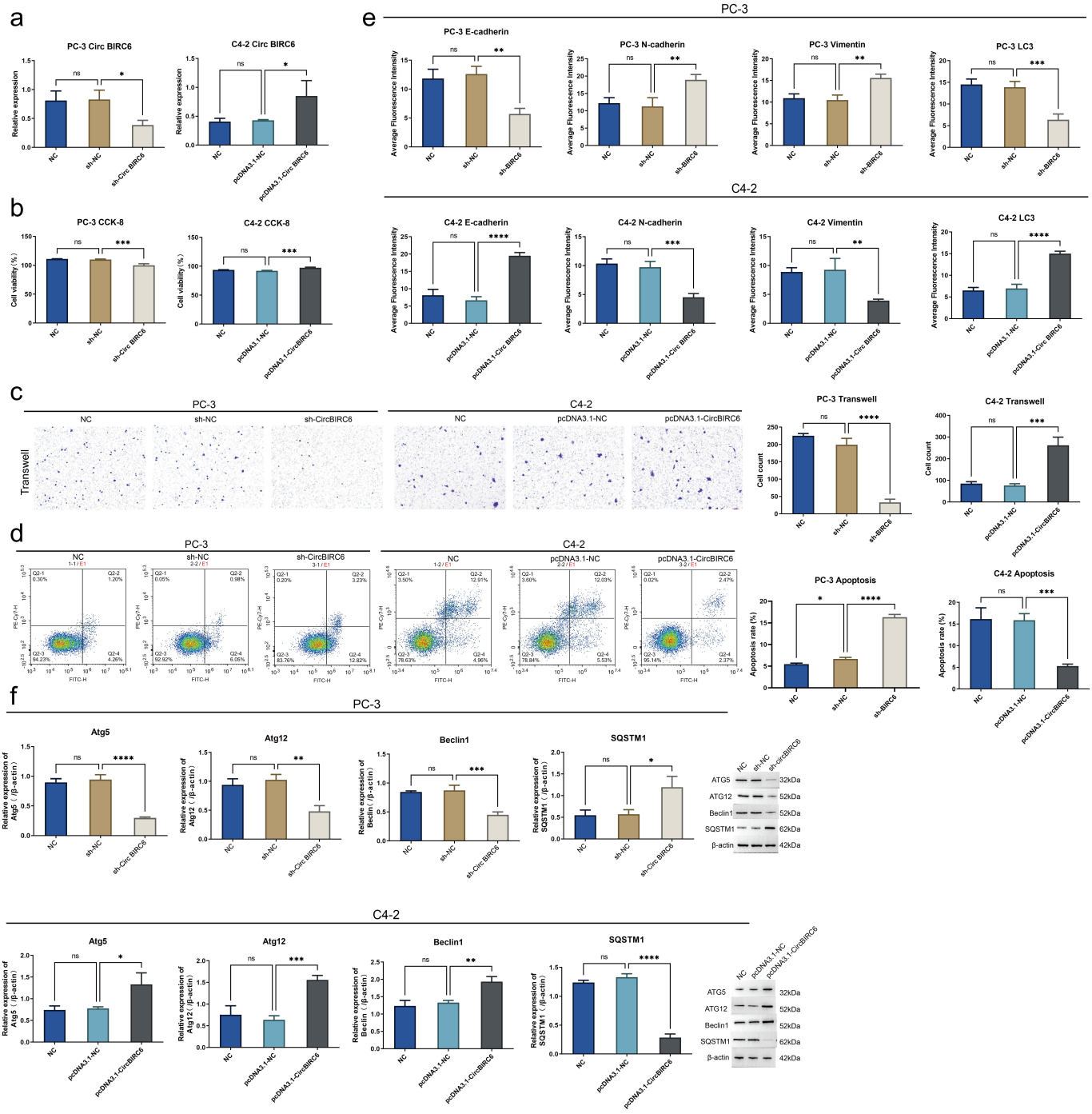

Figure 2. Effects of circBIRC6 on prostate cancer cell lines. (a) Transfection efficiency of circBIRC6-related plasmids, n=3 (b) CCK-8 experiments illustrate the effect of circBIRC6 on cell proliferation, n=3 (c) Transwell assay for the effect of circBIRC6 on cell migration, n=3 (d) Effect of circBIRC6 on cellular regulation detected by flow cytometry, n=3 (e) Statistical results of immunofluorescence (EMT markers and LC3), n=5 (f) Western blotting was used to detect the expression of key proteins of autophagy, n=3 n.s, no significant, ****p<.0001, ***p < .001, **p < .01 and *p < .05

LC3 expression, while overexpression showed the opposite result (Figure 2e, Figure S2). Western blot results showed a reduction in the expression of Beclin1, Atg5, and Atg12 and an increase in the expression of SQSTM1 after the decrease in CircBIRC6 (Figure 2f). These results suggested that the knockdown of CircBIRC6 inhibits proliferation, invasion, EMT and autophagy and promotes apoptosis. However, overexpression of CircBIRC6 resulted in the opposite.

Prediction and validation of CircBIRC6 downstream targets

First, we predicted Circ BIRC6 downstream miRNA through the CircInteractome database. We predicted the target mRNA of downstream miRNA through Starbase. The binding sites are shown in the figure (Figure 3a), downloaded autophagy-related genes from the HADb database and took the intersection with miRNA-574-5p to get 21 intersected genes, and DNAJB1 was selected as the target site for

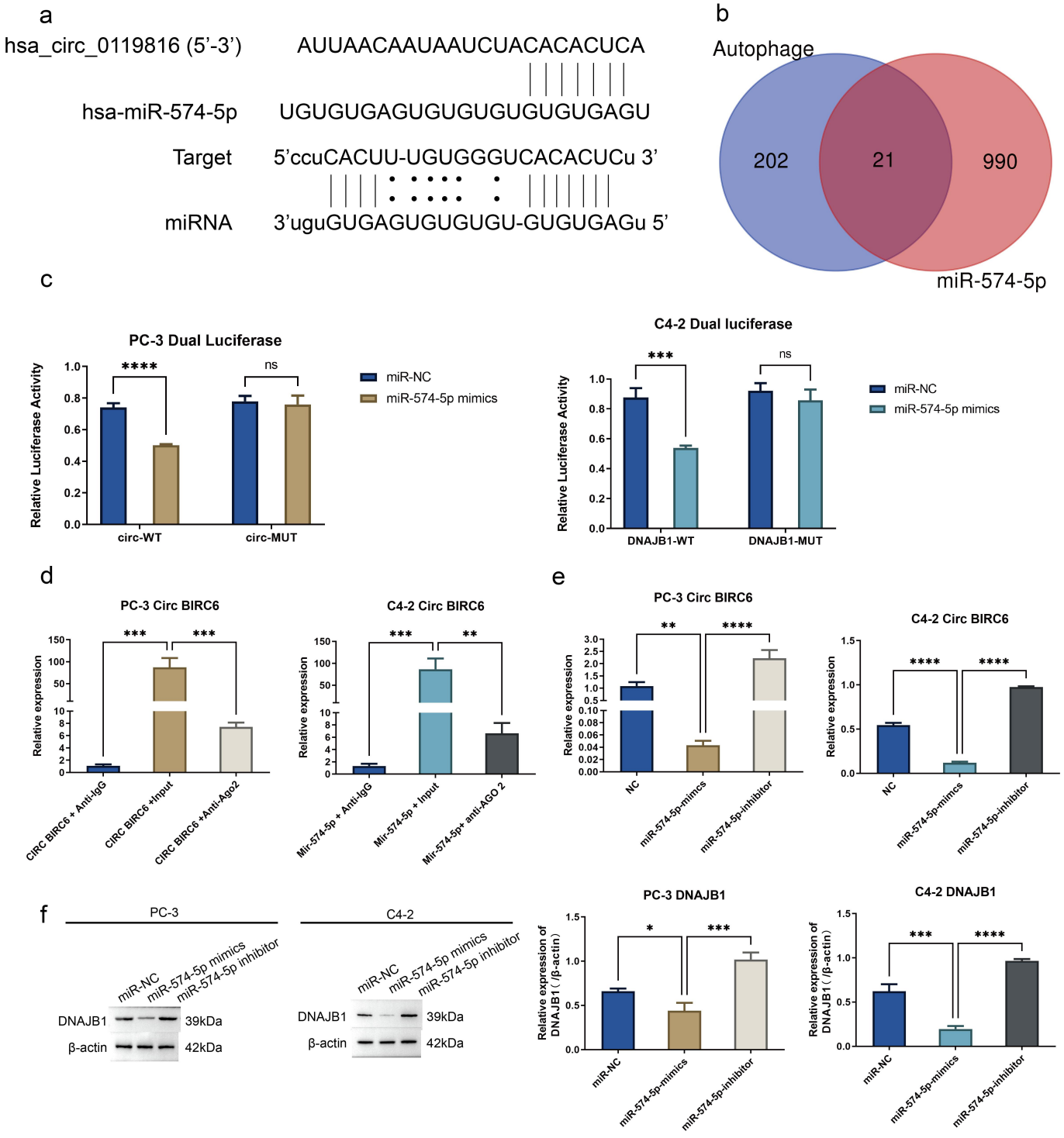

Figure 3. Prediction and validation of circBIRC6 targeting downstream. (a) Binding target prediction downstream of circBIRC6 targeting (b) Intersection of circBIRC6-targeted miRNAs with autophagy-related genes (c) Dual luciferase validates targeting relationships (d) RNA Immunoprecipitation (RIP) validates targeting relationships (e) Expression of circBIRC6 after transfection with miRNA mimics and inhibitors by qPCR (f) Expression of DNAJB1 after transfection with miRNA mimics and inhibitors by western blotting n.s, no significant, ****p<.0001, ***p < .001, **p < .01 and *p < .05, n=3

subsequent study (Figure 3b, Supplementary Table S7). The associated fluorescence activities of wild-type circBIRC6 and DNAJB1 were found to be suppressed after transfection of miR-574-5p mimics by dual luciferase, which verified that the targeting relationship between them was established (Figure 3c). RNA Immunoprecipitation (RIP) results illustrated that CircBIRC6 and miR-574-5p were significantly enriched in the anti-AGO2 group, verifying their relationship between interactions (Figure 3d). The expression of miR-574-5p regulated CircBIRC6 was detected by qPCR (Figure 3e). The results showed that the expression of CircBIRC6 was decreased and increased after transfection of miR-574-5p inhibitor and mimics in PC-3 and C4–2 cells, respectively. Western blotting showed a decrease in DNAJB1

expression after increasing miR-574-5p expression; the results were reversed after transfection with an inhibitor (Figure 3f). These results consistently demonstrated the targeting relationship between Circ BIRC6/miRNA-574-5p/DNAJB1.

Effect of Circ BIRC6/miRNA-574-5p/DNAJB1 for PC cells

After discovering and validating the targeting relationships of CircBIRC6, miR-574-5p and DNAJB1, we further investigated their effects on PC cells. First, transfection efficiency was detected by qPCR and Western blotting (Figure 4a). The expression of miRNA-574-5p was increased after transfection of miRNA-574-5p mimic in PC-3 cells but decreased by transfection of pcDNA3.1-Circ BIRC6. The expression of DNAJB1 was increased after transfection of miRNA-574-5p inhibitor in C4–2 cells and restored after transfection of sh-DNAJB1. These results indicated that transfection was successful. After transfection with miR-574-5p mimic, cell viability

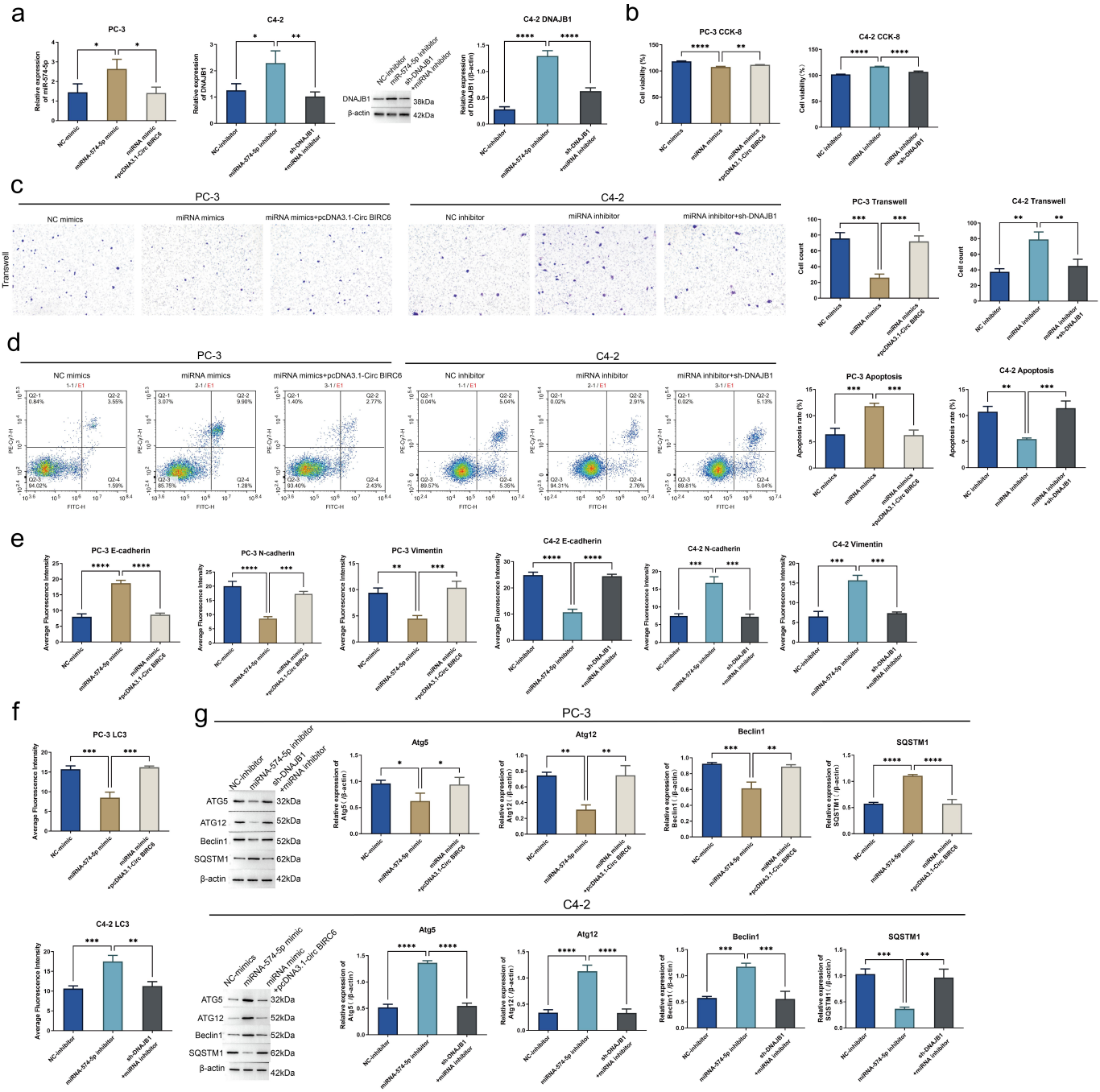

Figure 4. Effect of CircBIRC6/miRNA-574-5p/DNAJB1 manipulation on PC cells. (a) Transfection efficiency of plasmids by qPCR and Western blotting, n=3 (b) CCK-8 experiments illustrate the effect of Circ BIRC6/miRNA-574-5p/DNAJB1 on cell proliferation, n=3 (c) Transwell assay for the effect of Circ BIRC6/miRNA-574-5p/DNAJB1 on cell migration, n=3 (d) Effect of Circ BIRC6/miRNA-574-5p/DNAJB1 on cellular regulation detected by flow cytometry, n=3 (e) Statistical results of immunofluorescence (EMT markers and LC3), n=5 (f) Western blotting to detect the expression of key proteins of autophagy, n=3 n.s, no significant, ****p<.0001, ***p < .001, **p < .01 and *p < .05

and the number of invasion cells were reduced, and overexpression of circBIRC6 reversed the changes they caused, suggesting a regulatory relationship between circBIRC6 and miRNA-574-5p. Next, cell viability and invasive cell number increased after transfection with miR-574-5p inhibitor, and the knockdown of DNAJB1 was able to reverse these effects (Figure 4b,c). As miR-574-5p increased, apoptosis also increased, and overexpression of circBIRC6 reduced apoptosis. As miR-574-5p decreased, the apoptosis rate decreased, and the knockdown of DNAJB1 reverted the apoptosis rate of the cells (Figure 4d). Subsequently, we tested the cells for EMT and autophagy (Figure 4e,f, Figure S3). The experimental results of immunofluorescence and western blotting (Figure 4g) suggested that overexpression of miR-574-5p reduced cellular EMT and autophagy, and the addition of pcDNA3.1-CircBIRC6 was able to revert these abilities. Reduction of miR-574-5p could promote cellular EMT and autophagy, and these effects were inhibited after the knockdown of DNAJB1. These results suggested that miR-574-5p negatively regulates circBIRC6 and DNAJB1.

Discussion

PCa is mainly found in elderly males, and the number of people affected by PCa has been increasing worldwide. Although multiple diagnostic and therapeutic tools for PCa are available. However, the 5-year survival rate after treatment remains poor.[23] Once a patient develops metastasis, it implies a poor prognosis. Therefore, exploring new biomarkers and therapeutic targets in PCa is beneficial in interfering with its regulatory mechanisms.

CircRNA is a closed-loop, non-coding RNA molecule. With the continuous research on circRNAs, increasing studies illustrate that cirRNAs influence the development of diseases, such as autoimmune diseases, cardiovascular diseases, and tumors. A retrospective study reported that patients with rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) showed many aberrant expressions of circRNAs.[24] They can sponge on miRNAs and thus regulate apoptosis. Protein binding of circRNAs and lncRNAs is a promising therapeutic target in cardiovascular diseases. CircRNAs alleviate myocardial infarction by regulating oxidative stress.[25] Growing research has demonstrated that circRNAs contribute to the progression of cancer.[26–28]

Baculoviral IAP Repeat Containing 6 (BIRC6) protein is a potent predictor of prognosis in prostate cancer.[29] BIRC6 acts as an inhibitor of apoptosis and is a novel target for treating PCa.[30] This paper identified several circRNAs related to BIRC6 and differentially expressed in PCa. Among them, has_circ_0119816 was statistically most significantly different in PCa. We named it circBIRC6 and verified that it affects the development of PCa cells. CircBIRC6 has been shown to regulate chemoresistance in pancreatic[31] and ovarian cancers,[32] and circBIRC6 promotes migration and invasion of non-small-cell lung cancer cells by sponging its miRNAs.[33] Our article found that circBIRC6 (has_circ_0119816) expression was upregulated in PCa. Experimental validation revealed that knockdown of circBIRC6 inhibited PCa cell proliferation, invasion, autophagy, Epithelial-Mesenchymal Transition (EMT), and promoted apoptosis.

CircBIRC6 has an irreplaceable research value in cancer; therefore, further studies of its regulatory network are still needed. CircRNAs and their regulatory axes have great clinical potential in various cancers. CircBCBM1 is up-regulated in breast cancer and promotes metastasis through the regulation of miR-125a and BRD4.[34] CircRNA_101996 promotes cervical cancer growth and metastasis through miR-1236-3p and TRIM37 to promote cervical cancer growth and metastasis.[35] CircEZH2 promotes colorectal carcinogenesis through miR-133b and IGF2BP2.[36] In this paper, we found and verified the targeting relationship, the negative regulatory relationship between circBIRC6 and miR-574-5p, and the relationship between miRNAs and their target protein, DNAJB1. We found that circBIRC6 affects biological processes such as proliferation, autophagy, migration and invasion of PCa cells through miR-574-5p and DNAJB1. We found that circBIRC6 positively regulates DNAJB1 through miR-574-5p. The promotional function of circnBIRC6 on PCa cells could be restored and reversed by overexpression after the knockdown of miR-574-5p and DNAJB1.

In other studies on PCa, circRNAs were found to promote PCa through miR-515-5p and FKBP1A.[37] CircRNA_100146 was up-regulated in PCa and promoted PCa cell proliferation and metastasis by sponging up-regulation of TRIP13 by miR-615-5p.[38] In addition, some circRNAs have an oncogenic role in cancer. For example, circDHRS3 is lowly expressed in PCa, and overexpression inhibits the proliferation and metastasis of prostate cancer cell lines.[39] CircPHF16 inhibits prostate cancer metastasis through miR-581 and Wnt pathway.[40]

Growing research illustrates that miR-574-5p could be a promising therapeutic target in many human diseases, especially cancer. MiR-574-5p is a biomarker of non-small cell lung cancer in its early stage.[41] It promotes cancer metastasis and invasion through downstream target proteins.[42] In a study illustrating thyroid cancer, miR-574-5p regulates cancer progression through the Wnt protein signalling pathway.[43] A research investigation on gastric cancer demonstrated that miR-574-5p ultimately regulates gastric cancer progression by modulating endothelial growth factor (VEGF), thereby affecting angiogenesis.[44] Based on the two-sided regulatory role of miR-574-5p in multiple cancers. In our article, we investigated its regulatory role in PCa. We found that it inhibits the cancer-promoting effects of DNAJB1 by targeting DNAJB1. DNAJB1 serves as a therapeutic target in multiple cancers. Modulation of DNAJB1 in breast cancer may reverse chemotherapy resistance.[45] DNAJB1 binds to other proteins in hepatocellular carcinoma, enabling its use as a drug target in hepatocellular cancers.[46] A proteomic analysis revealed that DNAJB1 could serve as a novel biomarker for cholangiocarcinoma.[47]

Overall, CircBIRC6 is significantly upregulated in PCa, and knockdown circBIRC6 can inhibit the proliferation, autophagy, migration, invasion, and EMT of PCa cells. Investigating circBIRC6/miR-574-5p/DNAJB1 could help provide a research strategy for treating PCa or discovering new biomarkers.

Acknowledgments

We appreciate our colleagues and the public database for their helpful suggestions.

Authors' contribution

Conceived and designed the experiments: Jiansong Wang, Zhiyao Li
Performed the experiments: Fengming Ran, Yuanlong Shi, Libo Yang
Analyzed the data: Yuanpeng Duan, Zhiyu Shi, Xin Li, Jianpeng Zhang
Writing original draft: Bin Zhao, Jinye Yang
Writing review & editing: Jiansong Wang, Zhiyao Li
All authors read and approved the final manuscript.

Consent for publication

Upon request, the corresponding author can provide some or all of the data, models, or codes generated or used during the study.

Data availability statement

Data sets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics statement

Clinical samples were approved by Yunnan Provincial Tumor Hospital (Issue No. KYLX2024–131).

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This study was supported by the Yunnan Provincial Department of Science and Technology - Kunming Medical University Joint Special Fund for Applied Basic Research [202001AY070001-069], Projects funded by the Scientific Research Fund of Yunnan Provincial Department of Education [2020J0207].

ORCID

Jiansong Wang http://orcid.org/0000-0003-2682-609X

References

1. Qi J, Li M, Wang L, Hu Y, Liu W, Long Z, Zhou Z, Yin P, Zhou M. National and subnational trends in cancer burden in China, 2005–20: an analysis of national mortality surveillance data. Lancet Public Health. 2023;8(12):e943–e955. doi:10.1016/S2468-2667(23)00211-6.
2. Houédé N, Rébillard X, Bouvet S, Kabani S, Fabbro-Peray P, Trétarre B, Ménégaux F. Impact on quality of life 3 years after diagnosis of prostate cancer patients below 75 at diagnosis: an observational case-control study. BMC Cancer. 2020;20(1):757. doi:10.1186/s12885-020-07244-y.
3. Zhao J, Guercio BJ, Sahasrabudhe D. Current trends in chemotherapy in the treatment of metastatic prostate cancer. Cancers. 2023;15(15):3969. doi:10.3390/cancers15153969.
4. Rais-Bahrami S, Siddiqui MM, Vourganti S, Turkbey B, Rastinehad AR, Stamatakis L, Truong H, Walton-Diaz A, Hoang AN, Nix JW, et al. Diagnostic value of biparametric magnetic resonance imaging (MRI) as an adjunct to prostate-specific antigen (psa)-based detection of prostate cancer in men without prior biopsies. BJU Int. 2015;115(3):381–388. doi:10.1111/bju.12639.
5. Alberts AR, Schoots IG, Roobol MJ. Prostate-specific antigen-based prostate cancer screening: past and future. Int J Urology: off J Jpn Urol Assoc. 2015;22(6):524–532. doi:10.1111/iju.12750.
6. Evans AJ. Treatment effects in prostate cancer. Mod Pathol: off J U States Can Acad Pathol, Inc. 2018;31(S1):S110–S121. doi:10.1038/modpathol.2017.158.
7. Cherian AM, Nair SV, Lakshmanan VK. The role of nanotechnology in prostate cancer theranostic applications. J Nanosci Nanotechnol. 2014;14(1):841–852. doi:10.1166/jnn.2014.9052.
8. Mitsogiannis I, Tzelves L, Dellis A, Issa H, Papatsoris A, Moussa M. Prostate cancer immunotherapy. Expert Opin Biol Ther. 2022;22(5):577–590. doi:10.1080/14712598.2022.2027904.
9. Verduci L, Tarcitano E, Strano S, Yarden Y, Blandino G. CircRNAs: role in human diseases and potential use as biomarkers. Cell Death & Dis. 2021;12(5):468. doi:10.1038/s41419-021-03743-3.
10. Zhu G, Chang X, Kang Y, Zhao X, Tang X, Ma C, Fu S. CircRNA: a novel potential strategy to treat thyroid cancer (Review). Int J Mol Med. 2021;48(5). doi:10.3892/ijmm.2021.5034.
11. Bai Q, Pan Z, Nabi G, Rashid F, Liu Y, Khan S. Emerging role of competing endogenous RNA and associated noncoding RNAs in thyroid cancer. Am J Cancer Res. 2022;12(3):961–973.
12. Treeck O, Haerteis S, Ortmann O. Non-coding RNAs modulating estrogen signaling and response to endocrine therapy in breast cancer. Cancers. 2023;15(6):1632. doi:10.3390/cancers15061632.
13. Yuan G, Ding W, Sun B, Zhu L, Gao Y, Chen L. Upregulated circRNA_102231 promotes gastric cancer progression and its clinical significance. Bioengineered. 2021;12(1):4936–4945. doi:10.1080/21655979.2021.1960769.
14. Huang G, Liang M, Liu H, Huang J, Li P, Wang C, Zhang Y, Lin Y, Jiang X. CircRNA hsa_circRNA_104348 promotes hepatocellular carcinoma progression through modulating miR-187-3p/RTKN2 axis and activating Wnt/β-catenin pathway. Cell Death & Dis. 2020;11(12):1065. doi:10.1038/s41419-020-03276-1.
15. Jiang X, Guo S, Wang S, Zhang Y, Chen H, Wang Y, Liu R, Niu Y, Xu Y. EIF4A3-induced circARHGAP29 promotes aerobic glycolysis in docetaxel-resistant prostate cancer through IGF2BP2/c-Myc/LDHA signaling. Cancer Res. 2022;82(5):831–845. doi:10.1158/0008-5472.CAN-21-2988.
16. Ding L, Wang R, Zheng Q, Shen D, Wang H, Lu Z, Luo W, Xie H, Ren L, Jiang M, et al. circPDE5A regulates prostate cancer metastasis via controlling wtap-dependent N6-methyladenisine methylation of EIF3C mRNA. J Exp & Clin Cancer Res: cR. 2022;41(1):187. doi:10.1186/s13046-022-02391-5.
17. Xie T, Fu DJ, Li ZM, Lv DJ, Song XL, Yu YZ, Wang C, Li K-J, Zhai B, Wu J, et al. CircSMARCC1 facilitates tumor progression by disrupting the crosstalk between prostate cancer cells and tumor-associated macrophages via miR-1322/CCL20/CCR6 signaling. Mol Cancer. 2022;21(1):173. doi:10.1186/s12943-022-01630-9.
18. Klionsky DJ, Petroni G, Amaravadi RK, Baehrecke EH, Ballabio A, Boya P, Bravo-San Pedro JM, Cadwell K, Cecconi F, Choi AMK, et al. Autophagy in major human diseases. Embo J. 2021;40(19):e108863. doi:10.15252/embj.2021108863.
19. Russell RC, Guan KL. The multifaceted role of autophagy in cancer. Embo J. 2022;41(13):e110031. doi:10.15252/embj.2021110031.
20. Barnard RA, Regan DP, Hansen RJ, Maycotte P, Thorburn A, Gustafson DL. Autophagy inhibition delays early but not late-stage metastatic disease. J Pharmacol Exp Ther. 2016;358(2):282–293. doi:10.1124/jpet.116.233908.
21. Yun CW, Lee SH. The roles of autophagy in cancer. Int J Mol Sci. 2018;19(11):3466. doi:10.3390/ijms19113466.
22. Wang X, Wu WKK, Gao J, Li Z, Dong B, Lin X, Li Y, Li Y, Gong J, Qi C, et al. Autophagy inhibition enhances PD-L1 expression in gastric cancer. J Exp & Clin Cancer Res: cR. 2019;38(1):140. doi:10.1186/s13046-019-1148-5.

23. Alam MU, Kumar J, Norez D, Woolfe J, Tanneru K, Jazayeri SB, Bazargani S, Thomas D, Gautam S, Costa J, et al. Natural history, and impact of surgery and radiation on survival outcomes of men diagnosed with low-grade prostate cancer at ≤ 55 years of age: a 25-year follow-up of > 60,000 men. Int Urology Nephrol. 2023;55(2):295–300. doi:10.1007/s11255-022-03363-6.
24. Mei X, Zhang B, Zhao M, Lu Q. An update on epigenetic regulation in autoimmune diseases. J Transl Autoimmunity. 2022;5:100176. doi:10.1016/j.jtauto.2022.100176.
25. Zheng H, Huang S, Wei G, Sun Y, Li C, Si X, Chen Y, Tang Z, Li X, Chen Y, et al. CircRNA Samd4 induces cardiac repair after myocardial infarction by blocking mitochondria-derived ROS output. Mol Ther: j Am Soc Gene Ther. 2022;30(11):3477–3498. doi:10.1016/j.ymthe.2022.06.016.
26. Kristensen LS, Jakobsen T, Hager H, Kjems J. The emerging roles of circRNAs in cancer and oncology. Nat Rev Clin Oncol. 2022;19(3):188–206. doi:10.1038/s41571-021-00585-y.
27. Yu T, Wang Y, Fan Y, Fang N, Wang T, Xu T, Shu Y. CircRNAs in cancer metabolism: a review. J Hematol & Oncol. 2019;12(1):90. doi:10.1186/s13045-019-0776-8.
28. Yang B, Wang YW, Zhang K. Interactions between circRNA and protein in breast cancer. Gene. 2024;895:148019. doi:10.1016/j.gene.2023.148019.
29. Zhuang W, Zhang C, Hao F, Sun X. Baculoviral IAP repeat containing 6 (BIRC6) is a predictor of prognosis in prostate cancer. Med Sci Monit: int Med J Exp Clin Res. 2018;24:839–45. doi:10.12659/MSM.904052.
30. Luk SU, Xue H, Cheng H, Lin D, Gout PW, Fazli L, Collins CC, Gleave ME, Wang Y. The BIRC6 gene as a novel target for therapy of prostate cancer: dual targeting of inhibitors of apoptosis. Oncotarget. 2014;5(16):6896–908. doi:10.18632/oncotarget.2229.
31. Zheng S, Tian Q, Yuan Y, Sun S, Li T, Xia R, He R, Luo Y, Lin Q, Fu Z, et al. Extracellular vesicle-packaged circBIRC6 from cancer-associated fibroblasts induce platinum resistance via SUMOylation modulation in pancreatic cancer. J Exp & Clin Cancer Res: cR. 2023;42(1):324. doi:10.1186/s13046-023-02854-3.
32. Zhang J, Bai HJ. Effect of circBIRC6 targeting miR-367-3p on cisplatin resistance of ovarian cancer cells. Zhonghua Zhong Liu Za Zhi [Chin J Oncol]. 2021;43(10):1062–1068. doi:10.3760/cma.j.cn112152-20200427-00384.
33. Yang H, Zhao M, Zhao L, Li P, Duan Y, Li G. CircRNA BIRC6 promotes non-small cell lung cancer cell progression by sponging microRNA-145. Cellular Oncol (Dordrecht). 2020;43(3):477–488. doi:10.1007/s13402-020-00503-x.
34. Fu B, Liu W, Zhu C, Li P, Wang L, Pan L, Li K, Cai P, Meng M, Wang Y, et al. Circular RNA circBCBM1 promotes breast cancer brain metastasis by modulating miR-125a/BRD4 axis. Int J Biol Sci. 2021;17(12):3104–3117. doi:10.7150/ijbs.58916.
35. Song TF, Xu AL, Chen XH, Gao JY, Gao F, Kong XC. Circular RNA circRNA_101996 promoted cervical cancer development by regulating miR-1236-3p/TRIM37 axis. Kaohsiung J Med Sci. 2021;37(7):547–561. doi:10.1002/kjm2.12378.
36. Yao B, Zhang Q, Yang Z, An F, Nie H, Wang H, Yang C, Sun J, Chen K, Zhou J, et al. CircEZH2/miR-133b/IGF2BP2 aggravates colorectal cancer progression via enhancing the stability of m(6)A-modified CREB1 mRNA. Mol Cancer. 2022;21(1):140. doi:10.1186/s12943-022-01608-7.
37. Wang G, Zhao H, Duan X, Ren Z. CircRNA pappalysin 1 facilitates prostate cancer development through miR-515-5p/FKBP1A axis. Andrologia. 2021;53(11):e14227. doi:10.1111/and.14227.
38. Zeng L, Liu YM, Yang N, Zhang T, Xie H. Hsa_circRNA_100146 promotes prostate cancer progression by upregulating TRIP13 via sponging miR-615-5p. Front Mol Biosci. 2021;8:693477. doi:10.3389/fmolb.2021.693477.
39. Dai X, Chen X, Chen W, Ou Y, Chen Y, Wu S, Zhou Q, Yang C, Zhang L, Jiang H, et al. CircDHRS3 inhibits prostate cancer cell proliferation and metastasis through the circDhrs3/miR-421/MEIS2 axis. Epigenetics. 2023;18(1):2178802. doi:10.1080/15592294.2023.2178802.
40. Ding L, Lin Y, Chen X, Wang R, Lu H, Wang H, Luo W, Lu Z, Xia L, Zhou X, et al. circPHF16 suppresses prostate cancer metastasis via modulating miR-581/RNF128/Wnt/β-catenin pathway. Cellular Signalling. 2023;102:110557. doi:10.1016/j.cellsig.2022.110557.
41. Foss KM, Sima C, Ugolini D, Neri M, Allen KE, Weiss GJ. miR-1254 and miR-574-5p: serum-based microRNA biomarkers for early-stage non-small cell lung cancer. J Thorac Oncol: off Publ Int Assoc Study Lung Cancer. 2011;6(3):482–488. doi:10.1097/JTO.0b013e318208c785.
42. Zhou R, Zhou X, Yin Z, Guo J, Hu T, Jiang S, Liu L, Dong X, Zhang S, Wu G, et al. MicroRNA-574-5p promotes metastasis of non-small cell lung cancer by targeting PTPRU. Sci Rep. 2016;6(1):35714. doi:10.1038/srep35714.
43. Fan M, Li X, Jiang W, Huang Y, Li J, Wang Z. A long non-coding RNA, PTCSC3, as a tumor suppressor and a target of miRNAs in thyroid cancer cells. Exp Ther Med. 2013;5(4):1143–1146. doi:10.3892/etm.2013.933.
44. Hisano Y, Hla T. Bioactive lysolipids in cancer and angiogenesis. Pharmacol & Ther. 2019;193:91–98. doi:10.1016/j.pharmthera.2018.07.006.
45. Yin Y, Zhang J, Ma T, Chen D, Lu D. miR-1205/DNAJB1 reverses docetaxel chemoresistance in human triple negative breast carcinoma cells via regulation of mutp53/TAp63 signaling. Acta Biochim et Biophys Sin. 2022;54(1):37–46. doi:10.3724/abbs.2021006.
46. Kastenhuber ER, Lalazar G, Houlihan SL, Tschaharganeh DF, Baslan T, Chen CC, Requena D, Tian S, Bosbach B, Wilkinson JE, et al. DNAJB1–PRKACA fusion kinase interacts with β-catenin and the liver regenerative response to drive fibrolamellar hepatocellular carcinoma. Proc Natl Acad Sci USA. 2017;114(50):13076–13084. doi:10.1073/pnas.1716483114.
47. Ren H, Luo M, Chen J, Zhou Y, Li X, Zhan Y, Shen D, Chen B. Identification of TPD52 and DNAJB1 as two novel bile biomarkers for cholangiocarcinoma by iTRAQ-based quantitative proteomics analysis. Oncol Rep. 2019;42(6):2622–2634. doi:10.3892/or.2019.7387.