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Relationship between circulating miRNA-222-3p and miRNA-136-5p and the efficacy of docetaxel chemotherapy in metastatic castration-resistant prostate cancer patients

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Abstract

Background Metastatic castration-resistant prostate cancer is the most dangerous stage of prostate cancer, with a high mortality rate. Docetaxel chemotherapy is one of the most effective treatment methods currently, but some patients do not respond to chemotherapy. To avoid unnecessary toxicity in non-responders, this study explores the potential of circulating microRNAs as early biomarkers of docetaxel response in patients with metastatic castration-resistant prostate cancer.

Methods PC3 cells and DU145 cells were divided into the control, NC mimics, and miRNA-136-5p-mimics groups. Cell viability was measured using the CCK-8 assay. Cell apoptosis was determined by flow cytometry. Cell migration and invasion abilities were evaluated using the Transwell assay. Real-time quantitative PCR was used to measure the miRNA levels in cells and peripheral blood of patients. The miRNA-136-5p target genes were predicted by using the PITA, TargetScan, and miRanda databases. The target genes were analyzed with KEGG pathway analysis.

Results In both PC3 and DU145 cells, the miRNA-136-5p-mimics group exhibited significantly increased cell survival rates, migration and invasion numbers, and significantly decreased apoptosis rates than the control group ($p < 0.05$). The miRNA-222-3p and miRNA-136-5p levels were significantly increased in docetaxel-resistant PC3 and DU145 cells ($p < 0.05$). The levels of circulating miRNA-222-3p and miRNA-136-5p were significantly associated with docetaxel treatment ($p < 0.05$). Higher levels of miRNA-222-3p were observed in non-responsive patients ($p < 0.05$). The area under the curve for miRNA-222-3p was 0.76 (95%CI: 0.55–0.97), indicating its effectiveness as a predictive factor for non-responsive patients to docetaxel. Patients with high expression of miRNA-34c-5p after docetaxel chemotherapy had shorter overall survival times ($P < 0.05$). Bioinformatics analysis identified 110 potential target genes of miRNA-136-5p. KEGG revealed that these genes were mainly distributed in three pathways. Among them, the PI3K-AKT pathway was closely related to the metastasis of prostate cancer cells.

Conclusion Our study demonstrates that miRNA-136-5p promotes the proliferation and invasion of PC3 and DU145 cells while inhibiting apoptosis. Circulating miRNA-222-3p may serve as a biomarker for early therapeutic response

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to docetaxel, and further clinical investigation is warranted. Additionally, miRNA-136-5p may have anti-cancer effects during docetaxel chemotherapy in metastatic castration-resistant prostate cancer.

Keywords PC3 cells, DU145 cells, Docetaxel-resistant PC3 and DU145 cells, Proliferation, Apoptosis, Migration, Invasion, Metastatic castration-resistant prostate cancer, miRNA-222-3p, miRNA-136-5p, Docetaxel chemotherapy response marker, Bioinformatics analysis

Background

Prostate cancer is the most prevalent malignancy in men and the second most common cause of cancer-related mortality [1]. Nearly all patients with advanced prostate cancer progress to metastatic castration-resistant prostate cancer (mCRPC) after approximately 24 months of androgen deprivation therapy [2]. mCRPC is the most aggressive stage of prostate cancer, and survival time rarely exceeds 3 years [3]. Although numerous drugs have received approval for mCRPC treatment, including abiraterone, enzalutamide, and cabazitaxel, docetaxel remains the first-line chemotherapy for mCRPC [4]. Heterogeneous treatment responses are observed among patients, and over time, responders may acquire resistance. Docetaxel resistance is caused by multiple factors, such as changes in the structure or function of microtubules, alterations in androgen receptor signaling, activation of epithelial-to-mesenchymal transition signals, and drug efflux [5–7]. Prostate-specific antigen (PSA) as a standalone biomarker lacks reliability for mCRPC patients [8]. Due to the lack of effective non-invasive prognostic biomarkers, the optimization of treatment for mCRPC patients is limited. Therefore, it is urgently needed to identify new biomarkers to predict treatment response, avoid unnecessary treatment-related toxicity in non-responders, and facilitate personalized therapy.

MicroRNAs (miRNAs) are a type of non-coding RNA that regulates the expression of post-transcriptional genes by binding to the 3'-untranslated region of specific mRNAs, leading to translation inhibition or mRNA degradation. miRNAs play a crucial role in proliferation, differentiation, and progression by regulating the expression of approximately 70% of human genes [9]. miRNAs modulate the cytotoxic effects of docetaxel by regulating docetaxel-induced apoptosis or epithelial-mesenchymal transition [10, 11]. It has been preliminarily reported that miRNA-136-5p is involved in the resistance of prostate cancer cells to docetaxel [12]. Moreover, miRNA-222-3p might have a significant role in metastatic prostate cancer by negatively regulating the expression of synaptosome-associated protein 91, indicating that miRNA-222-3p could potentially serve as a biomarker and a therapeutic target for metastatic prostate cancer [13]. Abudoubari et al. found that the f/t ratio and the relative expression of miRNA-222 in patients' blood were independent factors influencing prostate cancer [14]. Enwald et al. [15] reported that miRNA-34c-5p negatively correlated with the change in blood PSA levels in patients receiving atorvastatin. Moreover, various miRNAs have been implicated in drug resistance [16–18]. The expression of circulating miRNAs is dysregulated in tumor patients and can remain stable under various storage conditions [18]. Therefore, miRNAs may serve as biomarkers for predicting treatment response.

According to our knowledge, so far only three articles have reported the association between circulating miRNAs and the efficacy of docetaxel treatment [19–21]. In 2014, Lin et al. [19] reported significant differences in miRNA-222 levels after docetaxel chemotherapy between responders and non-responders. In 2017, they validated this difference in a larger independent mCRPC cohort and found it to be non-significant [20]. Two consecutive studies by the same team yielded different conclusions. Therefore, further research is still needed to confirm the previous findings. Zedan et al. found a significant correlation between higher plasma baseline levels of miRNA-141-3p and miRNA-375-3p in docetaxel-treated patients and shorter radiographic progression-free survival time [21]. Currently, the relationship between circulating miRNAs and docetaxel treatment response remains to be further determined.

Here, our study aims to explore the potential of circulating miRNAs (i.e. miRNA-222-3p, miRNA-136-5p, and miRNA-34c-5p) as biomarkers for predicting the efficacy of docetaxel treatment in mCRPC patients. Our study results can assist patients in minimizing avoidable adverse reactions to chemotherapy, facilitating the formulation of tailored treatment plans, and preventing any unnecessary delay in treatment.

Methods

Study participants

A total of 30 patients with mCRPC were enrolled in this study. They received docetaxel chemotherapy (regimen: docetaxel 75mg/m^2 intravenous infusion every 3 weeks) for mCRPC at the Tumor Hospital, Xinjiang Medical University, from October 2016 to December 2019. Inclusion criteria were as follows: (1) Pathologically confirmed prostate cancer; (2) Serum testosterone < 1.7 nmol/L or 50 ng/dL and PSA progression or radiographic progression; (3) No history of other prostate-related diseases; (4) Docetaxel-based chemotherapy was performed based on the patient's condition. Exclusion criteria were as follows: (1) Previous radiotherapy, targeted therapy, or

other interventions before chemotherapy; (2) Pathological diagnosis of prostate cancer with mixed tumor components of other types. All patients had signed informed consent. All methods were performed following the Declaration of Helsinki. This study was approved by the Ethics Committee of the Affiliated Tumor Hospital of Xinjiang Medical University (approval no. S-2017131).

All patients were initially diagnosed with metastatic hormone-sensitive prostate cancer and received androgen deprivation therapy. After disease progression, they switched to docetaxel chemotherapy. Due to geographical and economic limitations, genetic testing was not feasible for all patients. Therefore, empirical docetaxel chemotherapy was administered to all patients. During systemic chemotherapy, luteinizing hormone-releasing hormone analogs were still used. Patients were hospitalized every 3 weeks and the peripheral blood sample (5 mL) was collected from each patient before each cycle of docetaxel chemotherapy for serum PSA testing. Imaging examination, including whole-body bone scan and contrast-enhanced CT scan of the chest and abdomen, was performed every three cycles of chemotherapy. Response Evaluation Criteria In Solid Tumors (RECIST1.1) were used for tumor assessment. The therapeutic efficacy was evaluated based on PSA and imaging examination. A partial response was defined as the PSA levels decreased by at least 50% following chemotherapy. Disease progression was determined when the PSA levels increased by at least 25% following chemotherapy. Disease stabilization was defined when the PSA levels decreased by less than 50% or increased by less than 25% following chemotherapy [22, 23]. Radiographic progression indicated disease progression, regardless of changes in PSA levels. Patients with partial response were considered responders to chemotherapy, while patients with disease progression and disease stabilization were considered non-responders to chemotherapy.

Cell culture and transfection

The PC3 bone marrow metastatic prostate cancer cells (cat# CL-0185) and DU145 (cat# CL-0075) human prostate cancer cells were obtained from Procell Life Science & Technology Co., Ltd (Wuhan, China). The docetaxel-resistant cell lines, DTX-R-PC3 and DTX-R-DU145, were gifted by the Central Hospital of Wuhan, China. These cells were cultured in Ham's F-12 K medium and MEM (containing NEAA) (Procell Life Science & Technology Co., Ltd, Wuhan, China), respectively, at 37 °C, 5% CO_2, and saturated humidity. The medium was supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin. Fresh culture medium was replaced every 1–2 days, and cells were subcultured twice a week. The cells were cultured between passages 4 and 10 before the experiments. The experimental groups were as follows: (1) control group: normal culture for 48 h; (2) mimics control group: transfection of miRNA negative control (GenePharma, Shanghai, China) at a concentration of 0.1 μM and incubation for 48 h; and, (3) miRNA-136-5p mimics group: transfection of hsa-miRNA-136-5p mimics (GenePharma) at a concentration of 0.1 μM and incubation for 48 h. The transfection efficiency was optimal at 48 h and thus this time point was selected. Fluorescence images were taken after transfection to determine the optimal transfection conditions.

CCK-8 assay

PC3 and DU145 cells were seeded in a 96-well plate at a volume of 100 μL per well (5×10^3 cells per well). The plate was incubated at 37 °C with 5% CO_2 for 24 h until adherence. Subsequently, the cells were treated as above described, with 5 replicates per group. Following the intervention, each well was added with 100 μL of 10% CCK-8 solution (TransGen Biotech, Beijing, China), The plate was then incubated in a CO_2 incubator. After 1 h, the OD value at 450 nm was measured using a microplate reader.

Flow cytometry

After the intervention, the cells and the culture medium (containing apoptotic or necrotic cells that had been suspended) in each group were collected and centrifuged at 1000 rpm for 5 min. The cells in the precipitate were washed twice with pre-chilled PBS and resuspended in 500 μL of 1×Binding Buffer (Elabscience Biotechnology, Wuhan, China). The suspension was passed through a 200-mesh sieve to obtain a single-cell suspension, which was then incubated with 5 μL of Annexin V-PE and 10 μL of 7-AAD (BD Biosciences, San Jose, CA, USA) at 4 °C in the dark for 10 min. Flow cytometry analysis was performed within 30 min.

Transwell cell migration assay

The Transwell cell migration assay was performed according to the previous description [24]. Briefly, 600 μL of Ham's F-12 K or MEM (containing NEAA) medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin was added to the lower chamber (pore size 8.0 μm; Corning, One Riverfront Plaza, NY, USA) (i.e., the bottom of the 24-well plate), while 100 μL of the intervened PC3 and DU145 cells (1×10^6 cells/mL) was added to the upper chamber (pore size 8.0 μm; Corning). Each group consisted of 3 replicate wells. The cells were then incubated for 24 h. Subsequently, the chambers were carefully extracted using forceps and fixed with 4% paraformaldehyde at room temperature for 20 min. The cells on the upper surface were gently wiped off, and the chambers were left to air-dry with the bottom surface facing upwards. Then, Giemsa staining was performed.

The cells were then observed under a microscope and 5 random fields were selected for cell counting and statistical analysis.

Transwell cell invasion assay

As previously described [24], the Transwell chamber was pre-coated with 100 μL of matrix gel (Corning, NY, USA). The gel was allowed to solidify at 37 °C for 5 h. In the lower chamber (i.e. the bottom of a 24-well plate), 600 μL of Ham's F-12 K or MEM (containing NEAA) medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin was added. In the upper chamber, 100 μL of cell suspension (1×10^6 cells/mL), which had been treated, was added. Three replicate wells were used for each group. After incubation for 24 h, the chambers were carefully removed using forceps, and any non-invasive cells on the upper chamber surface were gently wiped away. The chambers were fixed with 4% formaldehyde and stained with Giemsa. Three random fields were selected and the invaded cells were counted under a microscope.

Real-time quantitative PCR

Total RNA was extracted from the peripheral blood of patients and PC3 and DU145 cells of each group, as well as DTX-R-PC3 and DTX-R-DU145 cells, using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA). The peripheral blood was collected into anticoagulant tubes containing EDTA, thoroughly mixed, and centrifuged at 1500 rpm for 10 min at 4 °C. The supernatant containing circulating miRNAs was collected. To minimize exosome contamination, an additional centrifugation step at a higher speed was employed to pellet the exosomes. Then, the RNA was reverse-transcribed into cDNA. The expression levels of miRNA were detected using the SYBR® PremixExTaq™ (Thermo Fisher Scientific Inc, San Jose, CA, USA). The primer sequences are presented in Table 1. The amplification was carried out on the ABI QuantStudio 6 Flex RT-PCR system (Applied Biosystems) (Thermo Fisher Scientific Inc). The PCR system (20 μL) included 10 μL of 2XmiRNA qPCR MasterMix, 0.5 μL of the forward and reverse primer each, 2 μL of cDNA, 1 μL of ROX Reference dye(L), and RNase-free Water. The PCR procedures were 95 °C for 10 min and 40 cycles of 95 °C for 15 s and 60 °C for 60 s. U6 was used as a reference gene for miRNAs in cell samples, while Cel-miRNA-39 served as an internal reference for circulating miRNAs in peripheral blood samples. The expression of the corresponding miRNA was calculated using the $2^{-\Delta\Delta CT}$ method [25].

Table 1 Primer sequences

Gene	Gene ID	Sequences 5′ to 3′
hsa-miRNA-136-5p	406,927	ACUCCAUUUGUUUUGAUGAUGGA
		CAUCAUCAAAACAAAUGGAGUUU
hsa-miRNA-222-3p	723,814	GCAGAGCTACATCTGGCTAC
		CTCAACTGGTGTCGTGGAGT
U6	26,827	CTCGCTTCGGCAGCACA
Cel-miRNA-39	266,867	TCACCGGGTGTAAATCAGCTTG

Bioinformatics analysis of miRNA-136-5p target genes

The miRNA-136-5p target genes were predicted by using the PITA (http://genie.weizmann.ac.il/pubs/mir07/mir07_data.html; accession date: August 9, 2023), TargetScan (http://www.targetscan.org/; accession date: August 9, 2023), and miRanda databases (http://www.microrna.org/microrna/home.do; accession date: August 9, 2023). The identified target genes were subjected to KEGG pathway analysis on the "DAVID" database (https://david.ncifcrf.gov/). Cytoscape software was used to construct a miRNA-target gene interaction network to determine the gene regulatory relationship associated with miRNA. GEPIA (gene expression profiling interactive analysis) (http://gepia.cancer-pku.cn/) was employed to analyze differential gene expression in prostate cancer tissues compared to normal tissues. PROGgeneV2 (http://www.progtools.net/gene/) was used to evaluate the relationship between genes and prognosis.

Statistical analysis

Data are presented as mean ± SD or median (interquartile range). We used GraphPad Prism5 software (La Jolla, CA, USA) to conduct one-way ANOVA, T-tests, Log-rank tests, and Kaplan-Meier analysis. For receiver operating characteristic (ROC) analysis, we employed IBM SPSS software (version 26.0; Chicago, IL, USA). A p-value less than 0.05 was considered statistically significant.

Results

The level of miRNA-136-5p is upregulated by miRNA-136-5p mimics

Real-time quantitative PCR showed that in both PC3 (Fig. 1A) and DU145 cells (Fig. 1B), the expression level of miRNA-136-5p in the miRNA-136-5p-mimics group was significantly higher than the control and mimics control groups ($P<0.05$). However, there was no significant difference in the expression level of miRNA-136-5p between the control group and the mimics control group ($P>0.05$).

MiRNA-136-5p mimics can promote cell proliferation

CCK-8 assessed the cell proliferation of PC3 and DU145 cells. The cell morphology is presented in Fig. 2A. The CCK-8 results revealed that in both PC3 and DU145 cells, the OD450 values between the control group and the mimics control group were not significantly different (Fig. 2B). However, the OD450 value in the miRNA-136-5p mimics group was significantly higher than that

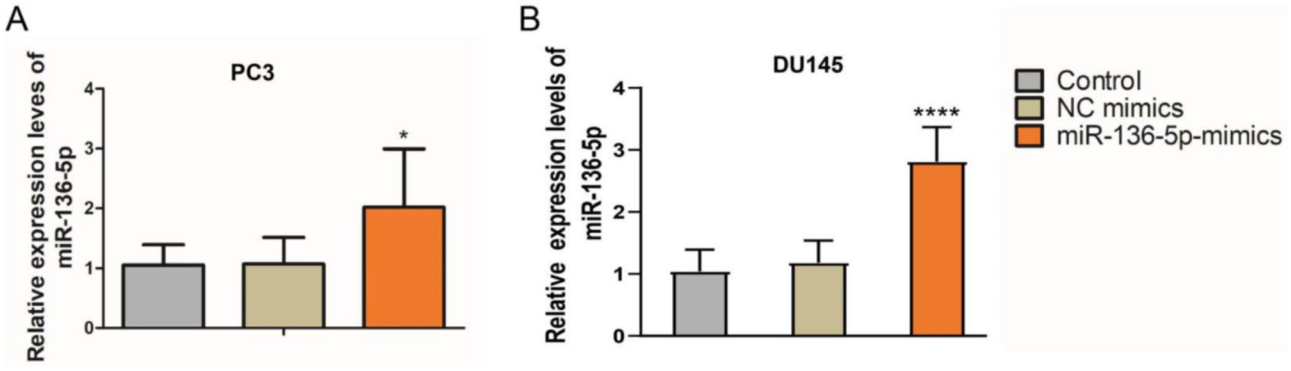

Fig. 1 Level of miRNA-136-5p in each group. Real-time quantitative PCR measured the miRNA-136-5p in each group. (**A**) Relative miRNA-136-5p level in DU145 cells. (**B**) Relative miRNA-136-5p level in PC3 cells. Compared with control, $^{*}P < 0.05$

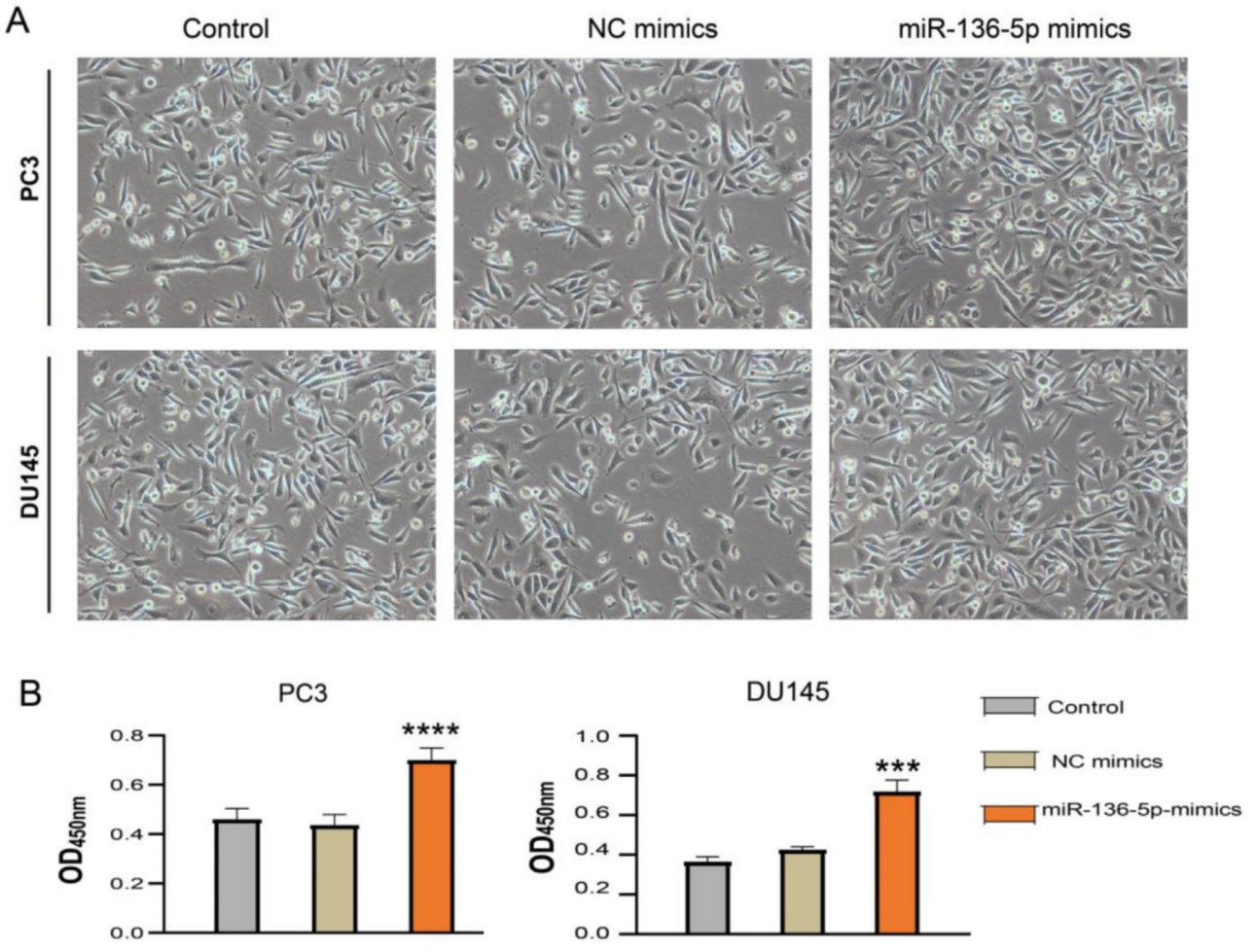

Fig. 2 Effect of miRNA-136-5p on cell proliferation. (**A**) Cell morphology of PC3 and DU145 cells in each group. (**B**) OD450 value of PC3 and DU145 cells in each group, as detected by CCK-8 assay. Compared with control, $^{*}P < 0.05$

in the control group and mimics control group ($P<0.05$). This indicates that cell proliferation is upregulated by miRNA-136-5p mimics.

MiRNA-136-5p mimics can inhibit cell apoptosis

Flow cytometry revealed that the apoptosis rates of PC3 cells in the control group and the mimics control group were not significantly different (Fig. 3A). However, the apoptosis rate of the miRNA-136-5p mimics group was significantly lower than that of the control group and the mimics control group ($P<0.05$). Notably, similar results were obtained in DU145 cells (Fig. 3B). Thus, cell apoptosis is inhibited by miRNA-136-5p mimics.

MiRNA-136-5p mimics can promote cell migration and invasion

To determine the effect of miRNA-136-5p mimics on cell migration and invasion of PC3 and DU145 cells, the Transwell assay was performed. The results demonstrated that the cell migration counts of PC3 (Fig. 4A) and DU145 cells (Fig. 4B) were not significantly different between the control and mimics control groups ($P>0.05$). In contrast, the cell migration count for the miRNA-136-5p mimics group was significantly higher than that of the control group and the mimics control group ($P<0.05$). Similarly, there was no significant difference in cell invasion counts of PC3 (Fig. 4C) and DU145 cells (Fig. 4D) between the control and mimics control groups ($P>0.05$). On the other hand, the cell invasion counts of the miRNA-136-5p mimics group were significantly higher than that of the control group and the mimics control group ($P<0.05$). Therefore, cell migration and invasion are promoted by miRNA-136-5p mimics.

Baseline characteristics of mCRPC patients

This study collected blood samples from 30 patients with mCRPC (Table 2). Their baseline data are listed in Table 2. Patients with stable disease and disease progression were considered non-responders to docetaxel.

Circulating miRNAs are associated with the efficacy of docetaxel chemotherapy

The results of real-time quantitative PCR revealed that the level of circulating miRNA-136-5p in the peripheral blood of mCRPC patients was significantly increased (Fig. 5A and B) after docetaxel chemotherapy ($P<0.05$). Consistently, the level of miRNA-136-5p in the docetaxel-resistant cell lines, DTX-R-PC3 (Fig. 5C) and DTX-R-DU145 (Fig. 5D), was also significantly elevated compared to that in the non-resistant parent cells ($P<0.05$). However, the expression level of miRNA-222-3p significantly decreased (Fig. 5E and F, and 5G) after docetaxel chemotherapy ($P<0.05$). Compared with the non-resistant parent cells, the DTX-R-PC3 (Fig. 5H) and DTX-R-DU145 cells (Fig. 5I) had significantly increased miRNA-222-3p levels ($P<0.05$). The expression level of miRNA-222-3p before docetaxel chemotherapy in non-responsive patients was significantly higher than that in responsive patients (Fig. 5J) ($P<0.05$). The expression of miRNA-222-3p before docetaxel chemotherapy showed significant differences among different ISUP (International Society of Urological Pathology) groups (Fig. 5K) ($P<0.05$). This indicates that circulating

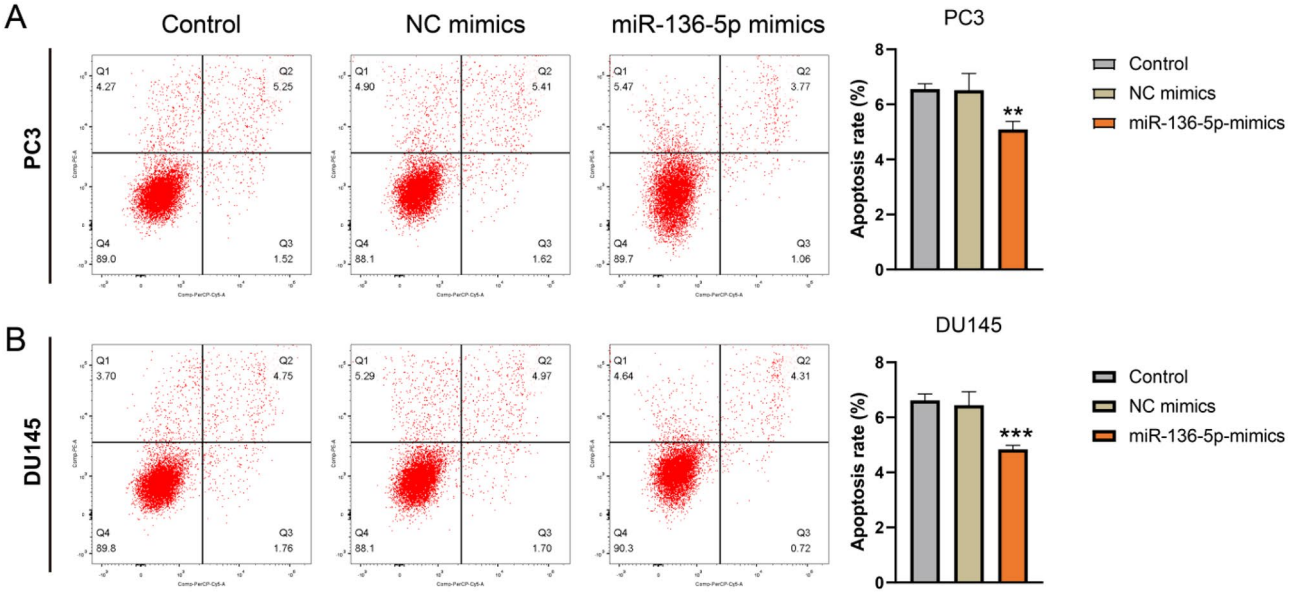

Fig. 3 Effect of miRNA-136-5p on cell apoptosis. Cell apoptosis was detected with flow cytometry. (**A**) Representative flow cytometry results and cell apoptosis rate of PC3 cells. (**B**) Representative flow cytometry results and cell apoptosis rate of DU145 cells. Compared with control, *$P<0.05$

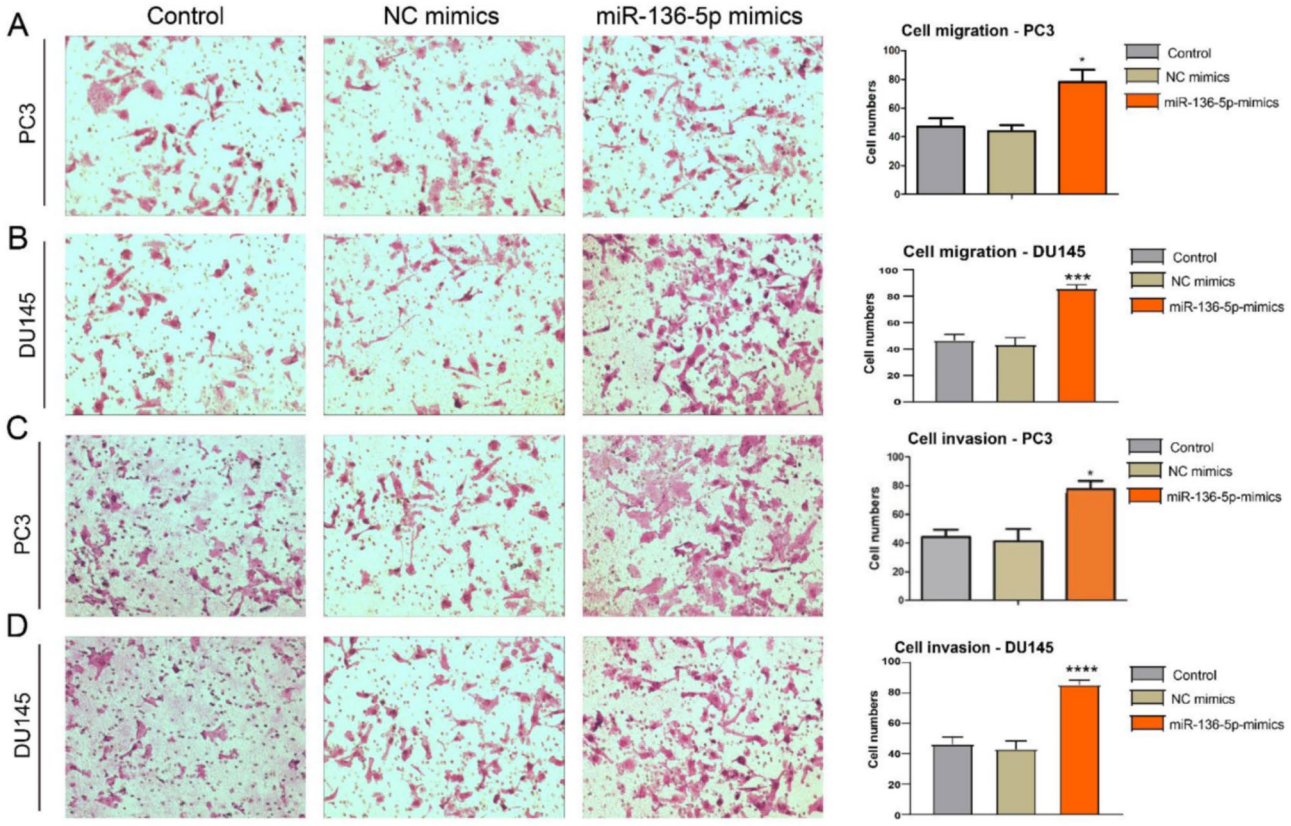

Fig. 4 Effect of miRNA-136-5p on cell migration and invasion. Cell migration and invasion were assessed with Transwell assay. (**A**) Representative Transwell assay results and the number of migrated PC3 cells. (**B**) Representative Transwell assay results and the number of migrated DU145 cells. (**C**) Representative Transwell assay results and the number of invaded PC3 cells. (**D**) Representative Transwell assay results and the number of invaded DU145 cells. Compared with control, $^{*}P < 0.05$

miRNA-136-5p and miRNA-222-3p are associated with the efficacy of docetaxel chemotherapy.

The predictive ability of miRNA-136-5p and miRNA-222-3p in determining chemotherapy response

We assessed the predictive ability of miRNA-136-5p and miRNA-222-3p in determining chemotherapy response using ROC curves. When considered as continuous variables, the high expression of miRNA-222-3p before chemotherapy, as well as the difference in miRNA-136-5p expression before and after chemotherapy, showed potential in predicting non-responsive patients. The respective areas under the curve (AUCs) were 0.76 (95% CI 0.55–0.97) and 0.72 (95% CI 0.51–0.93) (Fig. 6A). When treated as categorical variables, we assigned a value of 0 or 1 to each variable. A value of 0 indicated miRNA-222-3p expression level before chemotherapy below the median or the difference in miRNA-136-5p expression before and after chemotherapy below the median, while a value of 1 indicated the opposite. Both miRNA-222-3p and miRNA-136-5p demonstrated predictive power in identifying non-responsive patients, with respective AUCs of 0.78 (95% CI 0.60–0.96) and 0.67 (95% CI 0.49–0.90) (Fig. 6B). Furthermore, combining miRNA-222-3p and miRNA-136-5p to predict chemotherapy response resulted in an AUC of 0.82 (95% CI 0.64–0.99), displaying superior predictive accuracy compared to the use of either miRNA alone (Fig. 6B).

Additionally, high expression of miRNA-34c-5p after chemotherapy was associated with shorter overall survival of patients according to Kaplan-Meier analysis ($P<0.05$) (Fig. 7).

Target genes of miRNA-136-5p

A total of 3,609 genes were predicted to be associated with miRNA-136-5p. Among them, the PITA database predicted 2,134 target genes, the miRanda database predicted 894 target genes, and Targetscan predicted 196 target genes. Among these, 110 target genes were found to be common to all three databases (Fig. 8A). KEGG pathway analysis revealed that these genes were primarily enriched in three signaling pathways: the PI3K-AKT pathway, metabolic pathways, and MAPK pathway (Fig. 8B). A miRNA-target gene interaction network was created using Cytoscape software (Fig. 8C). According to GEPIA, BZW1 exhibited higher expression in normal

Table 2 Baseline characteristics of mCRPC patients

	No. of cases	Median (interquartile range)
Age (years)		65 (54–81)
Follow-up time (months)		17 (4–44)
Chemotherapy response		
Responders	21	
Non-responders	9	
Patient status		
Live	21	
Deceased	9	
Gleason score		9 (6–10)
6 (3 + 3)	1	
7 (3 + 4)	1	
7 (4 + 3)	1	
8	6	
≥ 9	21	
Serum prostate-specific antigen ($ng \cdot mL^{-1}$)		65.6 (2.2–2512)
Hemoglobin ($g \cdot L^{-1}$)		132 (82–170)
Alkaline phosphatase ($U \cdot L^{-1}$)		94.5 (49–3901)
Metastasis		
Bone	15	
Viscera	5	
Both	10	
Metastasis burden		
High tumor burden	26	
Low tumor burden	4	

tissues compared to prostate cancer tissues (Fig. 8D). Additionally, PROGgeneV2 demonstrated that BZW1 was associated with overall survival in prostate cancer patients (Fig. 8E).

Discussion

So far, our study was the only one that investigated the correlation between circulating miRNA-136-5p and the therapeutic efficacy of docetaxel in treating mCRPC. The results showed a significant increase in the expression level of circulating miRNA-136-5p in mCRPC patients after docetaxel chemotherapy. Similarly, there was a significant elevation in miRNA-136-5p level in docetaxel-resistant PC3 and DU145 cells. Another important finding was the correlation between circulating miRNA-222-3p and the chemotherapy response in these patients. Further analysis classified the patients into two groups based on the ISUP grading system (ISUP 1–3 group and ISUP 4–5 group). The results revealed a significant difference in miRNA-222-3p expression before chemotherapy between the two groups. The ROC curve indicated that, in mCRPC patients, the combined use of miRNA-222-3p expression before docetaxel chemotherapy and the difference in miRNA-136-5p expression before and after chemotherapy had a higher accuracy in predicting chemotherapy response compared to a single miRNA. Therefore, these miRNAs have the potential to become biomarkers for predicting docetaxel treatment response. Additionally, although there was no significant difference in the expression of miRNA-34c-5p before and after chemotherapy (data not shown), patients with relatively higher expression levels of miRNA-34c-5p after chemotherapy had lower overall survival.

MiRNA-136-5p plays different roles in different types of cancer. For example, miRNA-136-5p regulates colony formation, apoptosis, and sensitivity to docetaxel in docetaxel-resistant non-small cell lung cancer cells through CORO1C [26]. miRNA-136-5p binds to PBX3 and downregulates its expression, further promoting pancreatic tumor proliferation, migration, and invasion [27]. However, miRNA-136-5p exhibits tumor-suppressive functions in other types of cancer, such as triple-negative breast cancer, gastric cancer, and colorectal cancer [28–30]. Interestingly, our results showed that overexpression of miRNA-136-5p promoted cell proliferation and invasion while inhibiting apoptosis. Tan et al. [12] conducted a study on docetaxel-resistant PC3 cells and found that the exosomal circSFMBT2 enhanced prostate cancer resistance to docetaxel through the miRNA-136-5p/TRIB1 axis. This suggests that the miRNA-136-5p/TRIB1 axis could serve as a potential target for the treatment of chemoresistance in prostate cancer, which aligns with the findings from our peripheral blood experiments. It is worth noting that the effects of miRNA-136-5p differ between the two types of prostate cancer cells, which may be attributed to their distinct cellular structures and biological behaviors. Previous research has indicated that docetaxel-resistant PC3 cells exhibit morphological changes, altered proliferation rate, doubling time, and cell cycle distribution compared to PC3 cells [31]. Additionally, docetaxel-resistant PC3 cells overexpress the Rictor and p-AKT (S473) proteins, which are specific subunits or downstream substrates of mTORC2 and play crucial roles in the mechanism of docetaxel resistance [31]. On the contrary, the expression level of circulating miRNA-136-5p significantly increased in mCRPC patients following docetaxel chemotherapy. This inconsistency could be attributed to the lack of tumor microenvironment in the in vitro experiments, as well as the systemic effects of chemotherapy and miRNA produced by non-tumor cells.

We used bioinformatics approaches to explore the potential target genes of miRNA-136-5p in prostate cancer and finally identified 110 target genes, with BZW1 being the most likely candidate among them. KEGG analysis of these target genes identified three related signaling pathways, which may facilitate the understanding of the role of miRNA-136-5p in the development of prostate cancer. One crucial pathway implicated in

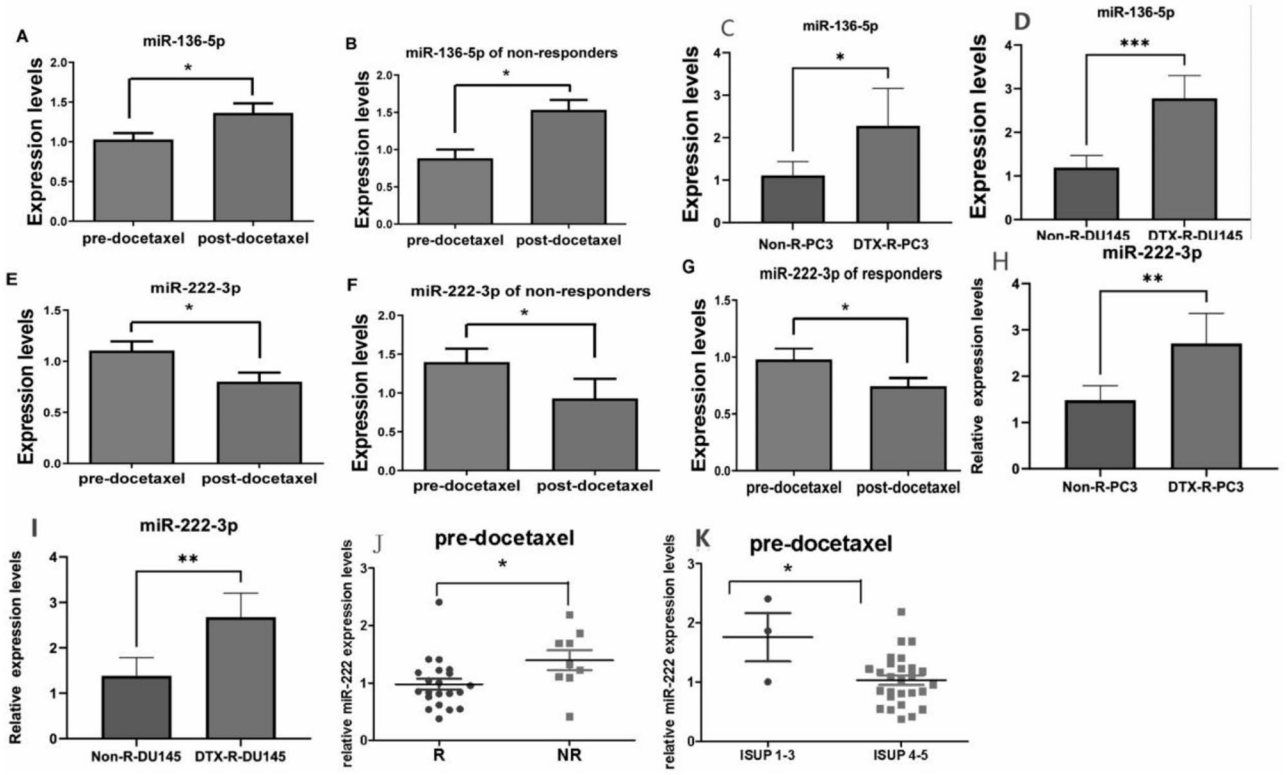

Fig. 5 Analysis of miRNA-136-5p and miRNA-222-3p levels in patients with mCRPC. (**A**) Expression of miRNA-136-5p before and after chemotherapy. (**B**) Expression of miRNA-136-5p before and after chemotherapy in non-responsive patients to docetaxel. (**C**) Expression of miRNA-136-5p in the docetaxel-resistant DTX-R-PC3 cells and the non-resistant parent cells (Non-R-PC3). (**D**) Expression of miRNA-136-5p in the docetaxel-resistant DTX-R-DU145 cells and the non-resistant parent cells (Non-R-DU145). (**E**) Expression of miRNA-222-3p before and after chemotherapy. (**F**) Expression of miRNA-222-3p before and after chemotherapy in non-responsive patients to docetaxel. (**G**) Expression of miRNA-222-3p before and after chemotherapy in responsive patients to docetaxel. (**H**) Expression of miRNA-222-3p in the DTX-R-PC3 and Non-R-PC3 cells. (**I**) Expression of miRNA-222-3p in the DTX-R-DU145 and Non-R-DU145 cells. (**J**) Expression of miRNA-222-3p before chemotherapy in responsive and non-responsive patients. (**K**) Expression of miRNA-222-3p before docetaxel chemotherapy in different ISUP groups. $^{*}P < 0.05$

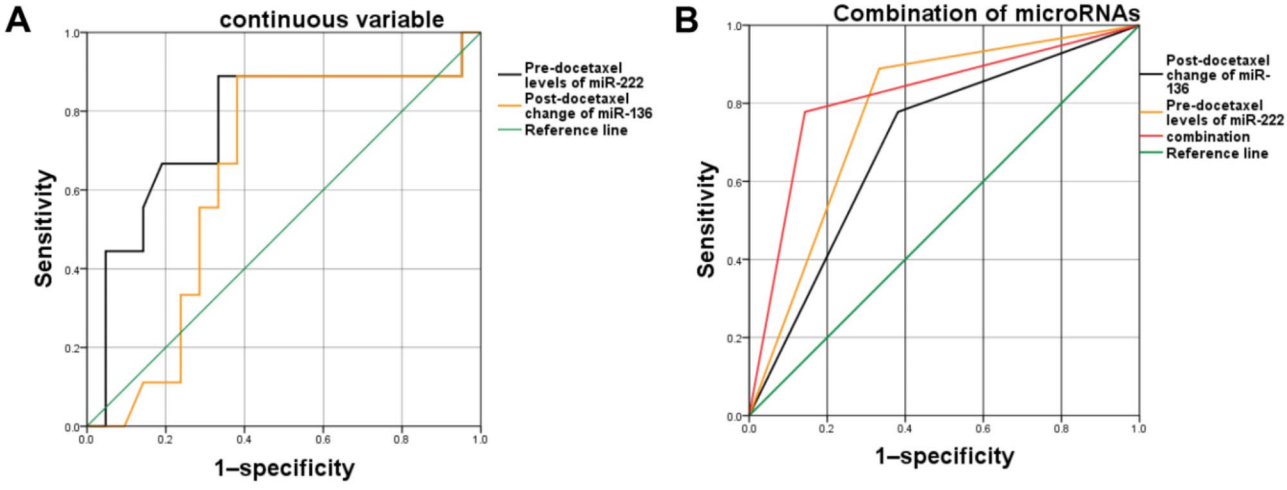

Fig. 6 ROC curve analysis of the predictive ability of miRNA-136-5p and miRNA-222-3p for chemotherapy response. (**A**) When miRNA-136-5p and miRNA-222-3p are treated as continuous variables, the high expression of miRNA-222-3p before chemotherapy and the difference in miRNA-136-5p expression before and after chemotherapy can both predict non-responsive patients. (**B**) The combination of miRNA-222-3p and miRNA-136-5p can predict chemotherapy response

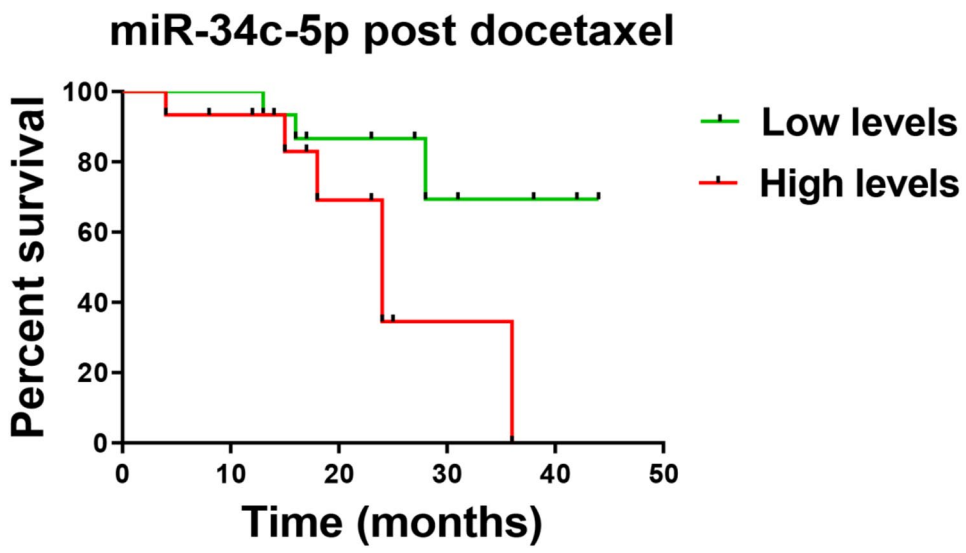

Fig. 7 Kaplan-Meier analysis of the relationship between circulating miRNA-34c-5p and overall survival

various signal transduction and biological processes was the PI3K-AKT-mTOR signaling pathway. This pathway regulates processes such as transcription, protein synthesis, metabolism, autophagy, cell proliferation, apoptosis, angiogenesis, and migration [32, 33]. It is speculated that this pathway plays a significant role in prostate cancer as a potential therapeutic target and/or predictive biomarker for disease onset, progression, and behavior. In addition, TGF-β/ PI3K/Akt mTOR NF-κB transduction pathway is activated in prostate cancer. The high expression of the PI3K/Akt/mTOR pathway in prostate cancer represents its crucial role in prostate cancer progression [34]. Importantly, the tumor appears to become more aggressive after each stage of treatment resistance, ranging from local hormone-sensitive prostate cancer to mCRPC. This aggressiveness is driven by activated adaptive responses, with the tumor relying more on alternative pathways and diminishing its dependence on androgen receptor signaling. Excessive activation and deregulation of the PI3K-AKT-mTOR signal are observed in this context. Notably, it is suggested that approximately 42% of localized PC and 100% of advanced-stage prostate cancer exhibit deregulated PI3K-AKT-mTOR signaling pathways [35].

It has been suggested that quercetin can reverse prostate cancer cell resistance to docetaxel through its effects on the androgen receptor and the PI3K/Akt signaling pathway [36]. Whether miRNA-136 can also act on the androgen receptor through the PI3K/Akt signaling pathway to reduce docetaxel resistance in mCRPC patients remains unclear. This could be a potential direction for future research.

BZW2, a homologous gene of BZW1, is upregulated in bone tissue and promotes tumor development by activating the Akt/mTOR signaling pathway. Shi et al. [37] showed that BZW1 was highly expressed in PCa and was associated with tumor progression and prognosis. Additionally, BZW1 expression promotes cell proliferation through regulation of the TGF-β1/Smad pathway. Here, bioinformatics analysis demonstrated that BZW1 exhibited high expression in prostate cancer tissue compared to normal prostate tissue and was negatively correlated with overall patient survival. BZW1 promotes the progression and metastasis of various cancers, but its research in prostate cancer is scarce, making it an area of great exploration.

Lin et al. [20] reported a significant difference in the levels of miRNA-222 after docetaxel chemotherapy between responders and non-responders. Three years later, they validated this difference in a larger independent mCRPC cohort and found it to be insignificant [21]. Interestingly, our experimental results indicated that the expression level of miRNA-222 before docetaxel chemotherapy was higher in non-responders. In docetaxel-resistant PC3 and DU145 cells, the miRNA-222-3p level was significantly elevated. This difference may be attributed to the difference in cohort size. MiRNA-222 exhibits cancer-promoting or cancer-inhibiting functions that are related to the "environment". Overexpression of miRNA-222 may suppress the expression of KIT, thereby inhibiting the migration and tube formation of endothelial cells [38]. After androgen deprivation therapy, CRPC cells may become dependent on the androgen receptor inhibitory gene, and they require high activity of miRNA-222 to maintain sufficient proliferative capacity. Gui et al. [39] found that miRNA-222 may play a crucial role in promoting prostate cancer cell proliferation in the early stage of CRPC. However, once the androgen receptor activity is restored or excessively expressed in CRPC, this function may weaken. This finding suggests that the carcinogenic

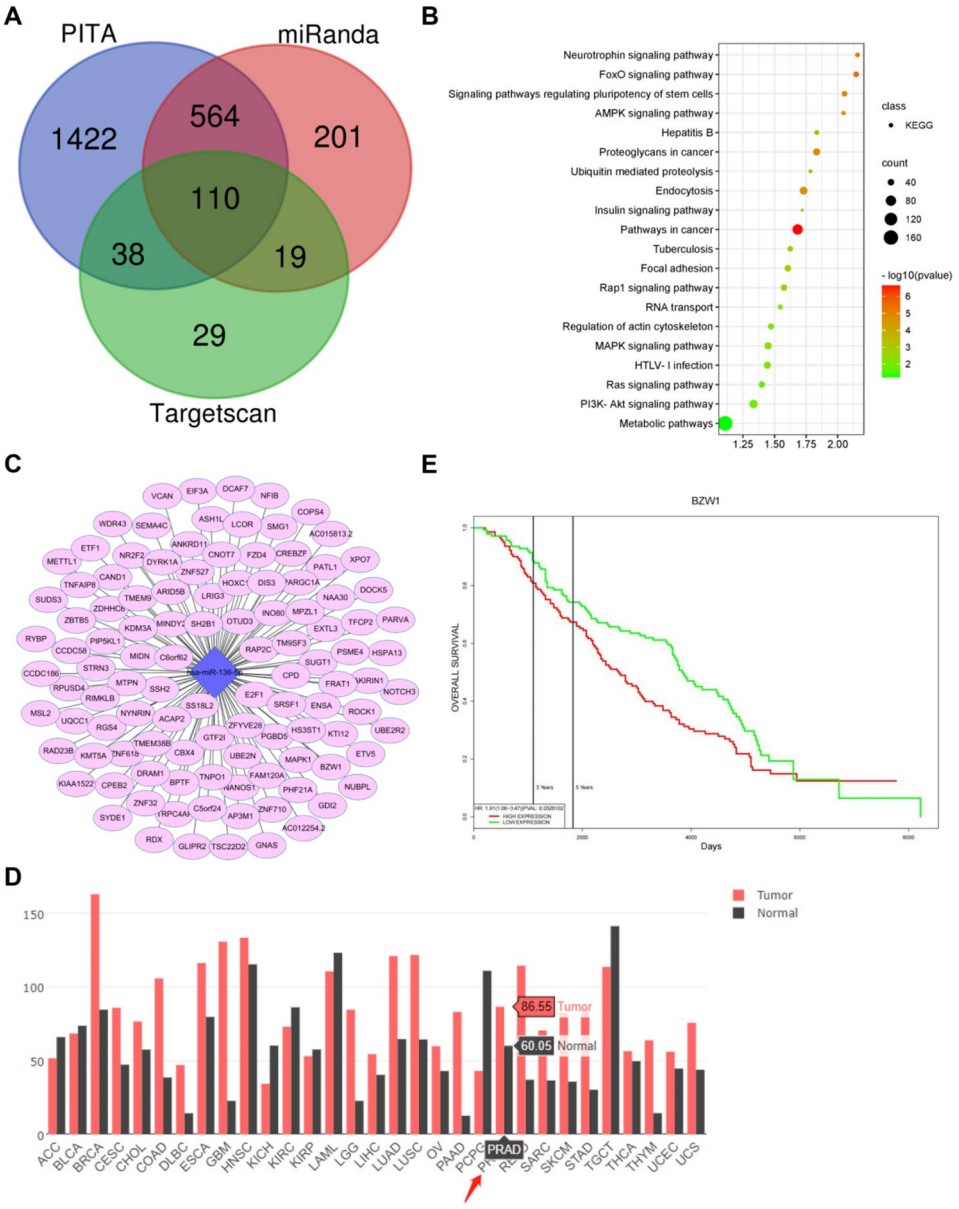

Fig. 8 Analysis of the target genes of miRNA-136-5p. (**A**) Venn diagram of target genes; (**B**) Enrichment of target genes in KEGG pathways; (**C**) miRNA-target gene interaction network; (**D**) Differential expression of BZW1 in prostate cancer tissue and normal prostate tissue (Red represents prostate cancer tissue, while black represents normal prostate tissue); (**E**) Association of BZW1 Gene with overall survival in prostate cancer patients

effect of miRNA-222 may be temporary, and the androgen receptor-mediated activation pathway may sustain the continuous growth of prostate cancer cells [39].

Fredsøe et al. constructed a ratio model consisting of three miRNAs (miRNA-222-3p*/miRNA-24-3p/miRNA-30c-5p), which accurately differentiated between prostate cancer and benign prostatic hyperplasia [40]. They subsequently established a Logistic Regression model, which included five urine miRNAs (miRNA-151a-5p, miRNA-204-5p, miRNA-222-3p, miRNA-23b-3p, and miRNA-331-3p) and serum PSA, and successfully predicted the biochemical recurrence time for 215 prostate cancer patients [41]. The miRNA-222-3p in urine has shown promising applications in the diagnosis and prognosis of prostate cancer, and we have reasons to believe that circulating miRNA-222-3p holds potential value in predicting the chemotherapy response in mCRPC patients.

A large amount of experimental data [42, 43] has shown that miRNA-34 can affect the epithelial-mesenchymal transition, which is an important biological process for prostate cancer cells to acquire invasion, migration, and drug resistance abilities. Overexpression of miRNA-34b or miRNA-34c can significantly inhibit the migration, invasion, and proliferation of prostate cancer cells while

having no significant effect on the apoptosis of prostate cancer cells [44]. Bartoszewska et al. observed that the downregulation of miRNA-34c-5p in prostate cancer cells promoted XBP1s signaling, thereby promoting tumor progression [45]. Most researchers [46, 47] believe that miRNA-34c-5p plays a tumor-suppressive role in prostate cancer. Our study showed that patients with relatively high expression levels of miRNA-34c-5p after chemotherapy had lower overall survival. This difference may be related to the response produced by the body after a combination of chemotherapy with docetaxel and prednisone and requires further research to confirm.

The study has several limitations. First, due to limitations in regional and economic conditions, genetic testing was not feasible for all patients. The empirical usage of docetaxel chemotherapy may not exclude the influence of other relevant pathway gene mutations on the results of the research. Second, the study has a small sample size. Thus, our findings and the potential clinical applications should be validated through further research using larger-scale independent cohorts.

Conclusion

In summary, the circulating miRNA-136-5p and miRNA-222-3p may serve as therapeutic response biomarkers for early prediction of docetaxel chemotherapy. It is expected that, shortly, the therapeutic response of patients with mCRPC to docetaxel chemotherapy can be predicted by detecting circulating miRNAs. This will avoid unnecessary chemotherapy toxicity in patients who do not respond to the treatment, and it will serve as a reference and provide assistance in the selection of more suitable treatment options for these patients.

Abbreviations

mCRPC	Metastatic castration-resistant prostate cancer
PSA	Prostate-specific antigen
miRNAs	MicroRNAs
RECIST1.1	Response Evaluation Criteria In Solid Tumors

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Author contributions

Peng Chen, Yue Niu, and Shuai Yuan designed the study. Shuai Yuan, Xing Bi, and Furhati Shayiti collected and analyzed the data. Peng Chen and Shuai Yuan interpreted the data. Yue Niu searched the literature. Peng Chen wrote the paper. Peng Chen revised the paper. Shuai Yuan collected the funds. All authors read and approved the final manuscript.

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Data availability

The study does not involve the data that needs to be deposited. The other data used to support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

All patients had signed informed consent. All methods were performed following the Declaration of Helsinki. This study was approved by the Ethics Committee of the Affiliated Tumor Hospital of Xinjiang Medical University (approval no. S-2017131).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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