Retracted: MicroRNA let-7b Inhibits Proliferation and Induces Apoptosis of Castration-Resistant Prostate Cancer Cells by Blocking the Ras/Rho Signaling Pathway via NRAS

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Castration-resistant prostate cancer (CRPC) is a heterogeneous disease with a high mortality rate. MicroRNA let-7b has been documented to act as a tumor suppressor in various cancers. The present study intends to explore how let-7b affects CRPC by influencing the Ras/Rho signaling pathway. The expression of neuroblastoma RAS viral oncogene homolog (NRAS) and let-7b in CRPC tissues and cells was determined. The binding relationship between let-7b and NRAS was predicted by the Targetscan website and verified by the dual luciferase reporter gene assay. Gain-of-function and loss-of-function approaches were used to investigate the relationship among let-7b, NRAS, and Ras/Rho signaling pathway as well as their effects on the proliferation, invasion, and apoptosis of CRPC cells. The tumor formation ability of nude mice was tested in vivo. Poorly expressed Let-7b and highly expressed NRAS were presented in CRPC tissues and androgen-independent cell line C4-2. NRAS was verified as a target gene of let-7b. Overexpression of let-7b or silencing of NRAS repressed C4-2 cell proliferation and invasion in vitro and tumor growth in vivo as well as induced C4-2 cell apoptosis in vitro through the blockage of the Ras/Rho signaling pathway. Let-7b overexpression or NRAS silencing reduced matrix metalloproteinase-2, matrix metalloproteinase-9, Bcl-2, cyclinD1, and CyclinB expression, but elevated Caspase3 expression in vivo and in vitro. Taken together, in CRPC, let-7b blocks the Ras/Rho signaling pathway by inhibiting NRAS expression, thereby inhibiting cell proliferation and invasion, and promoting cell apoptosis. Thus, let-7b targeting NRAS may be a potential therapeutic target for the repression of CRPC.

Prostate cancer is a kind of noncutaneous cancer with the highest morbidity and the second highest mortality among men.1 In recent years, advanced and metastatic prostate cancer have become a heavy burden that cannot be solved in short term in the healthcare system.2 For metastatic prostate cancer, patients can only receive temporary disease control measures by blockading or castrating androgen.3 Usually, prostate cancer may develop into a lethal form of castration-resistant prostate cancer (CRPC) within 2–3 years as the majority of patients resist to the treatment.4 CRPC is historically considered as a kind of chemoresistant tumor with a palliative treatment only lasting 1–2 years.5 It is a major challenge in the treatment of CRPC for lacking easily available and reliable biomarkers.6 For the same, we conducted a series of experiments in the present study to explore new targets for CRPC treatment.

Let-7b belongs to the microRNA let-7 family and usually acts as a tumor suppressor in different types of...
malignancies. Let-7 presents in various species and changes of let-7 expression in humans are linked to a wide range of cancers. It has been proposed that, in prostate cancer, let-7 may inhibit the formation of tumor by regulating the expression of oncogenes specifically related to self-renewal and growth capacity. The Targetscan website revealed there were special binding regions between let-7b and neuroblastoma RAS viral oncogene homolog (NRAS) prior to our investigation. Genes of the RAS family, including KRAS and NRAS, always play a crucial role in human cancers. Recent evidence has suggested that the majority of colorectal carcinoma cells carry mutant NRAS, and NRAS mutations can be potential biomarkers for surveillance and therapy in bladder cancer and metastatic colorectal cancer. Importantly, it has been suggested that the Ras/Rhodopsin (Rho) signaling pathway plays a irreplaceable role in the biological characteristics of cells and prostate cancer cells. The Rho signaling pathway is one of the Ras homolog gene family members. It is known that Rho-associated coiled-coil forming kinase regulates the formation of cells actin cytoskeleton, then participating in paramyxovirus infection in an association with various malignancies. Moreover, Guanosine-5'-tri-phosphate-binding RAS-like 3 has been found as a downregulated gene that can suppress the development of tumors in the prostate cancer cell line PC-3. Additionally, the Ras/Rho signaling pathway has been identified as a sustained proliferative signaling pathway in the development of prostate cancer. Therefore, we assumed that the interaction among let-7b, NRAS, and the Ras/Rho signaling pathway might exert functions in the occurrence and development of CRPC.

MATERIALS AND METHODS

Ethics statement

The study protocol was approved by the Ethics Committee of The Affiliated Huai’an Hospital of Xuzhou Medical University and the Second People’s Hospital of Huai’an. Informed written consent was obtained from each patient. All experimental animal operating procedures were in line with the relevant provisions of the Guiding Opinions on Treating Laboratory Animals (Guo Ke Fa Cai Zi (2006) No. 398) issued by Ministry of Science and Technology of the People’s Republic of China, and the Guide for the Care and Use of Laboratory Animals issued by National Institutes of Health (NIH; No. 85-23, revised in 1996). The principle of using the least number of animals and minimizing the pain of laboratory animals was strictly followed.

Study subject

From 2014 to 2018, 122 clinical specimens were resected from patients diagnosed as CRPC (n = 57) through transurethral resection of the prostate and patients diagnosed as androgen-sensitive prostate cancer (ASPC; n = 65) through laparoscopic radical prostatectomy. All specimens were obtained from the Department of Urology of The Affiliated Huai’an Hospital of Xuzhou Medical University and the Second People’s Hospital of Huai’an. According to the guidelines of the European Association of Urology (2010), patients were re-diagnosed as CRPC and treated with bicalutamide or flutamide before transurethral resection of the prostate to relieve symptomatic obstruction. All specimens were cut into small pieces and stored in liquid nitrogen in a freezer at –80°C.

The culture of cancer cells

The ASPC cell line (LNCaP) and the CRPC cell lines (C4-2, CWR22Rv1, PC-3, and DU-145) were preserved in our laboratory. The cells were cultured in an incubator at 37°C with 5% CO2 by Roswell Park Memorial Institute-1640 medium (Nitrogen, Carlsbad, CA) containing 10% fetal bovine serum (HyClone, Logan, UT), 10 mmol/L 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, and 0.2% sodium bicarbonate. When the cell confluence reached 90%, the cell passage was performed. The expression of let-7b in cells was detected by reverse transcription quantitative polymerase chain reaction (RT-qPCR). The C4-2 cells with the lowest expression of let-7b were used for subsequent experiments.

Plasmid construction, cell transfection, and Ras signaling pathway intervention

According to the known sequences of let-7b and NRAS from the National Center for Biotechnology Information, Sangon Biotechnology (Shanghai, China) was commissioned to construct plasmids of let-7b mimic, let-7b mimic negative control (NC), let-7b inhibitor, let-7b inhibitor-NC, NRAS, NRAS-NC, si-NRAS, and si-NRAS-NC using pCMV-Flag-N/C vectors. The cells at passage 3 were trypsinized and seeded into a 24-well plate, and the culture medium was discarded when the cells grew into a single layer. Then the transfection was conducted according to the instructions of a Lipofectamine 2000 kit (Invitrogen, Carlsbad, CA). Farnesylthiosalicylic acid (FTS) was used as the inhibitor of Ras/Rho signaling pathway.

Dual luciferase reporter gene assay

NRAS was screened out as the potential target gene of let-7 by the bioinformatics website, and the dual luciferase assay was performed for verification. C4-2 cells were seeded in 24-well plates and cultured for 24 hours. The wild-type (WT) NRAS fluourescein reporter vector (NRAS-WT-Luc) and the mutant (Mut) NRAS fluourescein reporter vector (NRAS-Mut-Luc) were sequenced for the subsequent transfection. The pmiR-RB-REPORTTM plasmid (Guangzhou RiboBio, Guangzhou, Guangdong, China) was digested with restriction endonucleases, whereas the synthetic target gene fragments NRAS-WT-Luc and NRAS-Mut-Luc were co-transfected into C4-2 cells with let-7b mimic or let-7b mimic NC, respectively. Lipofectamine 2000 (Invitrogen) was used for cell transfection according to the instructions. The medium was renewed at the 6-hour post-transfection, and the cells were collected at the 24th hour. The luciferase activity was detected at 560 nm using a Dual-Luciferase Reporter Assay Kit (Promega, Madison, WI) and a microplate reader (MK3; Thermo Scientific, Rockford, IL). Each reaction was run in triplicate to obtain the mean value.

RT-qPCR

Total RNA was isolated from clinical samples, collected tumor tissues of nude mice and cultured cells by TRizol
(Invitrogen) with reference to the instructions, and the RNA concentration and purity were measured by a Nano-Drop ND-1000 spectrophotometer. Then, RNA was reversely transcribed into cDNA using a PrimeScript RT reagent Kit (Takara Biotechnology, Dalian, Liaoning, China). Primers for let-7b, NRAS, U6, and glyceraldehyde-3-phosphate dehydrogenase were designed and synthesized by Sangon Biotechnology (Shanghai, China; Table 1). The product was subjected to real-time fluorescent qPCR (SYBR Premix Ex Taq II, Takara Biotechnology) by a 7.500 fluorescent qPCR instrument (ABI Company, Oyster Bay, NY). Relative expression of genes was determined by a relative quantitative 2−ΔΔCt method. Each reaction was run in triplicate.²⁴,²⁵

Western blot analysis

Tissues or cells were lysed with Radio-Immunoprecipitation assay lysis buffer (P0013B; Beyotime Biotechnology, Shanghai, China) containing phenylmethylsulfonyl fluoride and phosphatase inhibitor for 30 minutes on ice and centrifuged at 16,099.2 g at 4°C for 10 minutes. The bicinchoninic acid Protein Quantitation Kit (Beyotime Biotechnology) was used to measure the protein concentration. A total of 30 μg cell total protein was taken for sodium dodecyl sulfate-polyacrylamide gel electrophoresis, then transferred to a nitrocellulose membrane and blocked with Tris-buffered saline with Tween 20-prepared 5% skim milk powder for 1.5 hours. The membrane was then incubated with primary antibodies at 4°C overnight, including rabbit anti-human antibodies to NRAS (ab129162, 1:25,000), Ras (ab52939, 1:5,000), Rho (ab40673, 1:2,500), Rho-kinase 1 (ROCK1; ab45171, 1:2,000), matrix metalloproteinase (MMP)-2 (ab92536, 1:1,000), MMP-9 (ab73734, 1:1,000), Caspase3 (ab13487, 1:500), B-cell lymphoma-2 (Bcl-2; ab32124, 1:1,000), cyclinD1 (ab134175, 1:25,000), CyclinB (ab32053, 1:50,000), and glyceraldehyde-3-phosphate dehydrogenase (ab9485, 1:2,500). Horseradish peroxidase-labeled secondary goat anti-rabbit antibody to immunoglobulin G (ab205718, 1:2,000–1:50,000) was incubated with the membrane for 2 hours at room temperature. All above-mentioned antibodies were purchased from Abcam (Cambridge, UK). The color was developed by enhanced chemiluminescence and photographed by SmartView Pro 2000 (UVCI-2100; Major Science, Saratoga, CA). Quantity One software was used to analyze the gray value of protein bands.²⁶

EdU assay

Cells were cultured in a 96-well plate at a density of 1.6 × 10⁵ cells/well for 48 hours, reacted with 50 mM 5-ethynyl-2'-deoxyuridine (EdU) solution (Cell-Light EdU Apollo488 In Vitro Imaging Kit; Guangzhou RiboBio) at 37°C for 4 hours. Cells were fixed with 4% formaldehyde for 15 minutes and treated with 0.5% Triton X-100 for 20 minutes for permeabilization at room temperature. The cells were then stained with 100 mL Apollo for 30 minutes at room temperature and with 100 mL Hoechst 33342 for another 30 minutes. A fluorescence microscope (Olympus Optical, Tokyo, Japan) was introduced for observation and photography. The number of EdU-positive cells (red-stained cells) was calculated by an Image-Pro Plus 6.0 software (Media Cybernetics, Bethesda, MD). EdU incorporation rate = the number of EdU-positive cells/the number of C4-2 cells (blue-stained cells). Each reaction was run in triplicate.

Transwell assay

Single cell suspension was made from C4-2 cells that had been transfected for 24 hours. The cells were resuspended in serum-free medium (cell density of 8 × 10⁴ cells/mL). A total of 200 μL cell suspension from each group was added to the Matrigel-coated apical chamber, and the basolateral chamber was added with medium containing 100 mL/L fetal bovine serum. After culture for 48 hours, remaining cells were fixed with ethanol and stained by crystal violet. The basement membrane of the chamber was air-dried and examined under a microscope, and six fields of view were randomly selected to obtain mean value with pictures captured.

Terminal deoxynucleotidyl transferase-mediated 2'-deoxyuridine 5'-triphosphate-biotin nick end-labeling staining

A in situ apoptosis assay kit (Chemicon International, Temecula, CA) was used for terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL) staining. C4-2 cells after transfection were pretreated with 10 nmol/L docetaxel for 24 hours, washed, and stained according to the instructions of the kit. The cells were observed and photographed by an Axiovert 200 fluorescence microscope (Carl Zeiss, Oberkochen, Germany). The number of positively stained cells was counted by an EVOS FL microscope (Thermo Fisher Science, Waltham, MA). Each reaction was run in triplicate.

Flow cytometry

After transfection for 24 hours, cells were detached with ethylene diamine tetraacetate acid-free trypsin, and centrifuged at 698.75 g for 5 minutes. The cells were collected through centrifugation and then fixed by pre-cooled 3 mL 70% ethanol overnight at 4°C. The cells were centrifuged again the next day and stained with 0.5 mg/mL propidium iodide staining solution. Next, the cells were filtered through a
cell strainer and treated by a flow cytometer (FACS Calibur; BD Biosciences, Franklin Lakes, NJ). Propidium iodide was detected at a wavelength > 575 nm, and the cell apoptosis was detected by the percentage of cell cycle distribution.

Tumor xenograft in nude mice
A total of 36 specific-pathogen-free male nude mice (age: 6 weeks, weight: 14–16 g; SLAC Laboratory Animal, Shanghai, China, Shanghai Laboratory Animal Center, Chinese Academy of Sciences, Shanghai, China) were housed under constant temperature (25–27°C) and constant humidity (45–50%). The cells in the logarithmic growth phase were resuspended in 50% Matrigel (BD Biosciences), and the cell concentration was adjusted to 1 × 10⁷ cells/mL. Then, 0.5 mL single cell suspension (5 × 10⁶ cells) treated with plasmids of blank, let-7b mimic, let-7b mimic NC, NRAS, NRAS NC, and let-7b mimic + NRAS was injected subcutaneously into the left axilla of nude mice after anesthesia (6 mice for each). The tumor size (length and width) was measured by digital caliper on the 5th, 10th, 15th, 20th, 25th, and 30th days after inoculation and calculated by the formula, tumor volume (mm³) = (length × width²)/2. After 30 days of inoculation, nude mice were euthanized using CO₂ with the tumor resected, fixed by formalin, and weighed. Let-7b expression and mRNA expression of NRAS were detected by RT-qPCR in collected tumor tissues, and the protein expression of NRAS, MMP-2, MMP-9, Cleaved Caspase3, Bcl-2, cyclinD1, and CyclinB was detected by Western blot analysis.

Statistical analysis
All data were processed by SPSS version 21.0 statistical software (IBM, Armonk, NY). All data were verified to follow normal distribution and homogeneity of variance. The measurement data were expressed as mean ± SD. Unpaired Student’s t-test was used for comparison between two groups, and one-way analysis of variance was used for comparison among multiple groups, followed by Tukey’s post hoc test. Data from each group at different time points were analyzed by repeated measures analysis of variance, followed by Tukey’s post hoc test. The difference was regarded as statistically significant when P < 0.05.

RESULTS
Let-7b is Poorly Expressed and NRAS is Highly Expressed in CRPC Tissues and Cells
Initial investigation was conducted considering the expression profile of let-7b and NRAS in CRPC tissues and cells by means of RT-qPCR and Western blot analysis. Results showed that the expression of let-7b was lower and that of NRAS was higher in CRPC tissues than in ASPC tissues (P < 0.05; Figure 1a,b). The determination of let-7b and NRAS expression in ASPC cell line LNCap and CRPC cell lines (C4-2, CWR22Rv1, PC-3, and DU145) demonstrated that compared with the ASPC cell line LNCap, let-7b
expression was significantly decreased in four CRPC cell lines ($P < 0.05$), among which the lowest expression was detected in C4-2 cell line (Figure 1c). Besides, there was a significant increase of NRAS expression in CRPC cell lines ($P < 0.05$; Figure 1d). These results supported that the expression of let-7b was poor and that of NRAS was high in CRPC.

**Let-7b Inhibits C4-2 Cell Proliferation and Invasion While Inducing Apoptosis by Targeting NRAS**

It was predicted in the Targetscan website that there were binding sites between let-7b and NRAS (Figure 2a). Dual luciferase reporter gene assay presented that the luciferase activity of NRAS-WT in the let-7b mimic group was significantly lower than that in the NC group ($P < 0.05$), whereas the luciferase activity of NRAS-Mut showed no significant change ($P > 0.05$; Figure 2b). It was verified that there was a targeting relationship between let-7b and NRAS.

In order to explore the relationship between let-7b and NRAS, RT-qPCR and Western blot analysis were performed (Figure 2c,d). The results showed that there was no significant difference for NRAS expression in cells treated with let-7b mimic NC, let-7b inhibitor NC, NRAS NC, and let-7b mimic + NRAS ($P > 0.05$), whereas the NRAS expression was significantly decreased in cells introduced with let-7b mimic and markedly increased in cells introduced with let-7b inhibitor or NRAS ($P < 0.05$). The results elucidated that let-7b could negatively regulate NRAS expression.

The focus was then shifted to the effects of let-7b on the biological behaviors of C4-2 cells from perspectives...
of cell proliferation, invasion, and apoptosis. The results of EdU, Transwell, TUNEL, flow cytometry, and Western blot analysis (Figure 2e–n) demonstrated that there was no significant difference in cell proliferation, invasion, and apoptosis induced by delivery of let-7b mimic NC, let-7b inhibitor NC, NRAS NC, and let-7b inhibitor + NRAS (P > 0.05). Besides, the expression of the proliferation-related factors (cyclinD1 and CyclinB), invasion-related factors (MMP-2 and MMP-9) and apoptosis-related factors (Caspase3 and Bcl-2) had no marked change either (P > 0.05). In cells introduced with let-7 mimic, cell proliferation and invasion were inhibited and the apoptosis was promoted with more cells distributed at the G0/G1 phase and fewer at the S phase (P < 0.05), and there was no significant difference in the number of cells distributed at G2/M phase (P > 0.05). The cell cycle was arrested, and the expression of MMP-2, MMP-9, Bcl-2, cyclinD1, and CyclinB protein was significantly lower, whereas the expression of Caspase3 was higher (P < 0.05). However, opposite changing tendency was observed in cells introduced with let-7b inhibitor or NRAS (P < 0.05). These results verified that let-7b could suppress cell proliferation and invasion as well as promote the apoptosis of C4-2 cells.

NRAS Promotes C4-2 Cell Proliferation and Invasion While Inhibiting Apoptosis by Activating the Ras/Rho Signaling Pathway

In order to study the relationship between NRAS and the Ras/Rho signaling pathway, Western blot analysis was conducted for quantification of related factors. As shown in Figure 3a,b, there was no significant difference with regard to the protein expression of NRAS, Ras, Rho, and ROCK1 in C4-2 cells treated with NRAS NC, si-NRAS NC, and FTS + NRAS (P > 0.05). However, si-NRAS treatment led to diminished NRAS expression, whereas the transduction of FTS and si-NRAS significantly reduced the protein expression of Ras, Rho, and ROCK1 protein (P < 0.05). On the contrary, the transduction of NRAS induced opposite results (P < 0.05). The results demonstrated that NRAS could activate the Ras/Rho signaling pathway.

Further investigation was conducted considering the cellular biological behavior changes with help of EdU assay, Transwell assay, TUNEL staining, flow cytometry, and Western blot analysis (Figure 3c–n). Protein expression of the proliferation-related factors (cyclinD1 and CyclinB), invasion-related factors (MMP-2 and MMP-9) and apoptosis-related factors (Caspase3 and Bcl-2) did not differ significantly among the treatment groups.

Figure 3 The proliferation and invasion of C4-2 cells are promoted and the apoptosis is inhibited by neuroblastoma RAS viral oncogene homolog (NRAS) overexpression in a Ras/Rho signaling pathway-dependent manner. The protein expression of NRAS, Ras, Rho, and ROCK1 normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) measured by Western blot analysis (a, b). The cell proliferation detected by 5-ethynyl-2'-deoxyuridine staining (200×) (c, d). The cell invasion detected by Transwell assay (200×) (e, f). The cell apoptosis detected by terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling staining (200×) (g, h). The cell cycle distribution detected by flow cytometry (i, j). The protein expression of cyclinD1, CyclinB, matrix metalloproteinase (MMP)-2, MMP-9, Caspase3, Bcl-2 normalized to GAPDH measured by Western blot analysis (k, l). *P < 0.05 vs. the blank group (C4-2 cells without any treatment). The above data were all measurement data and expressed as mean ± SE. Unpaired t test was used for comparison between two groups and one-way analysis of variance with Tukey’s post hoc test was used for comparison among multiple groups. The experiment was repeated three times. FTS, farnesylthiosalicylic acid; NC, negative control.
Highly Expressed let-7b Inhibits the Formation of Xenograft Tumor in Nude Mice

To further verify the effect of let-7b on CRPC, an in vivo experiment was performed. Mice were injected with cells carrying let-7b mimic, NRAS NC, let-7b mimic NC, NRAS, and let-7b mimic + NRAS. The tumor sizes of all mice were recorded every 5 days (Figure 4b). Compared with mice without injection, the size of the tumor had no significant difference in mice injected with cells carrying let-7b mimic NC, NRAS NC, or let-7b mimic + NRAS (P > 0.05). Besides, the size of tumor in mice injected with cells carrying NRAS increased rapidly, while the formation of tumors was inhibited markedly in mice injected with cells carrying let-7b mimic (P < 0.05). The tumor was isolated, photographed, and weighed 30 days after injection (Figure 4a,c). The results demonstrated that the tumor weight had no difference in tumors from mice without injection and from mice injected with cells carrying let-7b mimic NC, NRAS NC, or let-7b mimic + NRAS (P > 0.05). The tumors from mice injected with cells carrying NRAS were heaviest, whereas those from mice injected with cells carrying let-7b mimic were lightest (P < 0.05).

The let-7b expression and the mRNA expression of NRAS in nude mice was detected by RT-qPCR (Figure 4d), and the relative expression of NRAS, MMP-2, MMP-9, Caspase3, Bcl-2, cyclinD1, and CyclinB were isolated, photographed, and weighed. A one-way analysis of variance with Tukey’s post hoc test was used for comparison among multiple groups. n = 6. NC, negative control.
protein expression of NRAS, MMP-2, MMP-9, Caspase3, Bcl-2, cyclinD1, and CyclinB in nude mice was measured by Western blot analysis (Figure 4e). The results showed that compared with nude mice without injection, there was no significant change in the protein expression of MMP-2, MMP-9, Caspase3, Bcl-2, cyclinD1, and CyclinB among tumor tissues from nude mice carrying let-7b mimic NC, NRAS NC, and let-7b mimic + NRAS (P > 0.05). In nude mice harboring let-7b mimic, the expression of let-7b was increased, NRAS expression and protein expression of MMP-2, MMP-9, Bcl-2, cyclinD1, and CyclinB was reduced significantly, and Caspase3 protein expression was elevated (all P < 0.05). Besides, NRAS injection contributed to promoted NRAS expression and protein expression of MMP-2, MMP-9, Bcl-2, cyclinD1, and CyclinB, as well as reduced Caspase3 protein expression (P < 0.05). Thus, these data complemented the studies of the functions of let-7b in vitro, suggesting that let-7b has the potency to downregulate NRAS to retard the formation of xenograft tumors in nude mice.

**DISCUSSION**

As a more aggressive form of prostate cancer, CRPC is a kind of heterogeneous disease that can finally lead to death. Historically, the survival rate of CRPC is a low median, but a recent study has discovered that miRNAs may be potential targets for new prognostication modalities. Let-7 miRNA family, including let-7b, has been verified to reduce the tumorigenesis, inhibit tumor progression, and

![Diagram depicting let-7b/neuroblastoma RAS viral oncogene homolog (NRAS) regulatory mechanism in castration-resistant prostate cancer. Let-7b blocks the Ras/Rho signaling pathway by inhibiting NRAS expression, thereby suppressing cell proliferation and cell invasion, and promoting cell apoptosis. miRNA, micro RNA; RISC, RNA-induced silencing complex.](Retracted)
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relieve chemotherapy resistance of colorectal cancer. In this study, we performed a series of experiments to investigate the mechanism involving let-7b in the proliferation, invasion, and apoptosis of CRPC cells. Collectively, the data from the present study revealed that let-7b could down-regulate NRAS expression to block the Ras/Rho signaling pathway, by which the progression of CRPC was impeded. One of the important findings in our study was that let-7b presented a low expression, whereas NRAS showed high expression in CRPC tissues and cells. Studies have verified that let-7b is poorly expressed in many different kinds of cancers. For instance, a study has demonstrated that, compared with corresponding normal tissues, the expression of let-7b is downregulated in non-small cell lung cancer tissues. In gastric cancer tissues, let-7b turns out to be decreased significantly in the study of Yu et al. Overexpressed let-7b has been proven to inhibit the proliferation and promote the apoptosis of carcinoma cell lines. Besides, constant proliferation of cancer cells has been indicated to be induced when the RAS protein mutation was amplified or overexpressed. By the above studies, we could reason that upregulated let-7b and downregulated NRAS may inhibit the development of CRPCs.

It was documented in the present study that NRAS, the gene related to Ras/Rho signaling pathway, was downregulated by let-7b and the Ras/Rho signaling pathway was thereby blocked, the mechanism of which inhibited the proliferation and invasion and promoted the apoptosis of CRPC cells. Accumulating evidence has mentioned that let-7b acts as a suppressor in different cancers by targeting genes similar to NRAS. It is believed that let-7b inhibits the expression of the c-Myc gene in a sequence-specific manner both at the mRNA and protein levels. In addition, by directly targeting inhibitor of growth family, member 1, a tumor metastasis-related gene, overexpressed let-7b inhibits the migration and invasion of gastric cancer cells. In human lung squamous cancer and surrounding normal tissues, a reverse expression trend between let-7b and cytochrome P450 2J2 gene has been demonstrated to inhibit tumor development. Those studies verified the validation of let-7b acting as a suppressor in the expression of tumor-promotion-related genes. Besides, the antiproliferative function of let-7 miRNA family members in prostate cancer has been tested in other studies. For example, let-7c may be of potential therapeutic significance for it can suppress cell proliferation in prostate cancer, and by targeting E2F2 and CCND2, let-7a can attenuate the growth of prostate cancer cells. Upregulated let-7c can suppress the clonal expansion, tumor regeneration, and cell proliferation in prostate cancer both in vitro and in vivo. The study of Tripathi et al. has verified that the Rho signaling pathway can be mediated to inhibit highly metastatic prostate cancer cells. Besides, the ROCK signaling pathway is involved in the process of angiogenesis in prostate cancer, and the blocked ROCK signaling pathway can suppress tumor cell growth and angiogenesis. To further confirm our results, we also conducted an in vivo experiment, and the results revealed that highly expressed let-7b inhibited the formation of xenograft tumor in nude mice as expected. Results from another study support our finding that, in gastric cancer, let-7b can suppress the metastasis of cancer cells in nude mice with xenograft models, suggesting the possible application in vivo.

In conclusion, the invasion and proliferation of CRPC cells are inhibited as let-7b blocks the Ras/Rho signaling pathway by inhibiting the expression of NRAS, as summarized in Figure 5. Investigation on the functions of let-7b in CRPC yields a better understanding of the in-depth mechanisms and may have potentially important therapeutic implications in the treatment of CRPC. In the future, further in vivo experiments should be conducted on the correlation of let-7b in tumorigenesis, metastasis, and prognosis, especially in patient samples.

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