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MicroRNA-212 participates in the development of prostate cancer by upregulating BMI1 via NF-κB pathway

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Abstract. – OBJECTIVE: To investigate the role of microRNA-212 in prostate cancer (PCa) and its underlying mechanism.

PATIENTS AND METHODS: MicroRNA-212 expressions in 72 PCa tissues and paracancerous tissues were detected by qRT-PCR (quantitative real-time polymerase chain reaction). The relationship between microRNA-212 expression and clinical characteristics of PCa patients was analyzed. Target genes of microRNA-212 were predicted by TargetScan and verified by luciferase reporter gene assay. Proliferation, cell cycle, and apoptosis of PCa cells were detected after transfection with corresponding plasmids of microRNA-212 in PCa cells, respectively. The effect of microRNA-212 on BMI1 and NF-κB pathway was detected by Western blot.

RESULTS: MicroRNA-212 was downregulated in PCa patients. The survival rate of PCa patients with lower expression of microRNA-212 was remarkably lower than those with a higher level. After overexpression of microRNA-212, we observed inhibited proliferation and arrested cell cycle of PCa cells. Increased apoptosis was found after PCa cells were transfected with microRNA-212 mimic. Luciferase reporter gene assay showed that microRNA-212 was bound to BMI1, which further promoted PCa development via NF-κB pathway.

CONCLUSIONS: MicroRNA-212 was downregulated in PCa tissues, which could promote the PCa development by targeting BMI1 via NF-κB pathway.

Key Words:
MicroRNA-212, Prostate cancer, BMI1, NF-κB pathway.

Introduction

Prostate cancer (PCa) is a malignant tumor, which accounts for the second highest incidence of malignancy in males. In 2008, PCa accounted for 14% of all male cancers and 6% of all cancer deaths[1]. PCa ranked the second tumor death in males from Europe and the United States. 1/7 of Australian men are diagnosed with PCa each year, with an average age of 75 years[2]. The incidence of PCa has been annually increased[3,4], which severely threatens male health. In China, PCa is also the leading cause of death in middle-aged men[5]. PCa is a heterogeneous tumor that can exhibit progression-free inertia and aggressively transfer to other organs[6,7]. Local infiltration and distant metastasis are frequently observed in PCa patients since this disease is very occult without evident clinical symptoms. The mortality rate of advanced PCa is very high[8,9]. Hence, it is urgent to develop new methods so as to better diagnose and treat PCa.

MicroRNA is a non-coding, short-chain RNA with 18-25 nt in length. MicroRNA can't encode proteins. However, it has been shown that microRNA is involved in the regulation of multiple functions of gene transcription[10]. MicroRNAs were initially found in nematodes and are highly conserved among humans, plants, and viruses[11]. Over the past decades, researches have focused on the role of protein-coding genes in tumorigenesis. The possible effects of microRNAs on tumorigenesis, however, have not been recognized. In recent years, differentially expressed microRNAs in various tumors have become the biomarkers in diagnosing and treating tumors[12-14].

It is reported that microRNAs participate in the carcinogenic process of PCa. Szczyrba et al[15] found that microRNA-375 expression is positively correlated with tumor size of PCa. Moreover, microRNA-375 could promote proliferation of PCa cells without affecting cell apoptosis. Giangreco et al[16] found that microRNA-100 and microRNA-125b are downregulated in PCa tissues than

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those of benign prostate tissues. Pre-microRNA-100 and pre-microRNA-125b could reduce the proliferation of primary PCa cells. Knockdown of microRNA-125b in normal cells enhances cell migration. Kontos et al[17] discovered that microRNAs can be served as biomarkers for diagnosing PCa. In addition, Stuopelyte et al[18] pointed out that expression levels of microRNA-148a and microRNA-375 in the urinary circulation can be used as non-invasive indicators for diagnosing PCa. However, the specific effect of microRNAs on treating PCa still remains unclear, which requires for further in-depth studies.

Patients and Methods

Sample Collection

A total of 72 cases of PCa tissues and 72 cases of normal benign prostatic hyperplasia or paracancerous tissues were surgically resected from radical prostatectomy and transurethral treatment of benign prostatic hyperplasia, respectively. Samples were immediately rinsed with DEPC (diethyl pyrocarbonate) water and preserved into liquid nitrogen for the following experiments. Subsequently, general characteristics of enrolled patients were collected. This experiment was approved by the Hospital Ethics Committee and all patients signed the informed consent. The general characteristics of patients were listed in Table I.

Cell Culture and Transfection

RWPE-2, 22RV1, DU145, PC-3M and VCaP cells were cultured in RPMI-1640 (Roswell Park Memorial Institute-1640, Gibco, Grand Island, NY, USA) containing 10% FBS (fetal bovine serum), 100 U/mL penicillin and 100 μg/mL streptomycin (Hyclone, South Logan, UT, USA). Cells were incubated in a 5% CO_2 incubator at 37°C.

Cell transfection was performed when the cell confluence was up to 50%-60% according to the instructions of Lipofectamine2000 (Invitrogen, Carlsbad, CA, USA). Transfection efficacy was then verified by quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR). Transfection plasmids used in this experiments were obtained from Gene Pharma, (Shanghai, China).

Cell Counting Kit-8 (CCK-8) Assay

Transfected cells were seeded into 96-well plates at a density of 1×10^6 per well. 10 μL of the CCK-8 solution (cell counting kit-8, Dojindo, Kumamoto, Japan) was added in each well after cell culture for 6, 24, 48, 72 and 96 h, respectively. The absorbance at 450 nm of each sample was measured by a microplate reader (Bio-Rad, Hercules, CA, USA).

Transwell Assay

The upper transwell chamber was previously coated with 100 μL of Matrigel (BD Biosciences, San Jose, CA, USA) and maintained in an incubator for 2 h. After cell density was adjusted to 2×10^5/mL, 100 μL of cell supernatant and 600 μL of culture medium containing 10% FBS were then added in the upper and lower chamber, respectively. Transwell chamber was removed after incubation for 24 h, and the non-migrated cells in the chamber were gently wiped off with a cotton swab. The chamber was fixed with methanol for 15 min, washed with PBS twice and stained in

Table I. The general characteristics of enrolled patients.

Clinicopathologic features	Number of cases	miR-212 expression		*p*-value
		Low (n=36)	High (n=36)	
Age (years)		36	36	0.7717
≤46	15	8	7	
>46	57	28	29	
Tumor size				0.0022*
T1-T2	37	12	25	
T3-T4	35	24	11	
N stages				0.0141*
N0	26	8	18	
N0-N3	46	28	18	
Metastasis				0.0978
YES	39	23	16	
NO	33	13	20	

Note: *p<0.05, compared between low and high expression of miR-212.

1% crystal violet for 30 min. Finally, 5 randomly selected fields were captured for cell count.

Cell Cycle Detection

The cell suspension was prepared, washed with Hanks buffer and centrifuged at 800 rpm for 5 min. After re-suspended with culture medium, cells were incubated with 70% pre-cooled ethanol overnight at 4°C. Finally, cells were stained with propidium iodide (PI) for 30 min, followed by cell cycle detection using flow cytometry.

Cell Apoptosis Detection

The cell suspension was collected into marked flow tubes, and digested with EDTA-free (ethylenediaminetetraacetic acid) trypsin. After centrifugation, the cells were washed with PBS (phosphate-buffered saline) twice. We then added 380 μl of binding buffer containing calcium ions (Invitrogen, Carlsbad, CA, USA), 10 μl of Annexin V-FITC fluorescent probe and 5 μL of PI to the cell precipitation, followed by incubation without light for 20 min. Subsequently, cell cycle was analyzed by flow cytometry (Partec AG, Arlesheim, Switzerland).

Luciferase Reporter Gene Assay

The binding sites of microRNA-212 and BMI1 were predicted by TargetScan. The corresponding wild-type and mutant-type sequences of BMI1 were constructed by Ruizhen (Nanjing, China). The microRNA-212 mimic and microRNA-212 NC were constructed by Gene Pharma (Shanghai, China). Luciferase activity was detected and the average value was calculated.

QRT-PCR

We used TRIzol to extract total RNA for reverse transcription according to the instructions of PrimeScript RT reagent Kit (TaKaRa, Otsu, Shiga, Japan). The expression level of the target gene was calculated using the $2^{-\Delta\Delta CT}$ method. Primers used in this experiments were listed as the follows: BMI1, F: 5'-CCACCTGATGTGTGTGCTTTG-3', R: 5'-TTCAGTAGTGGTCTGGTCTTGT-3'; IL-6, F: 5'-CCTCTGGTCTTCTGGAGTACC-3'; TGF-β1, F: 5'-GGTTCATGTCATGGATGGTGC3', R: 5'-TGACGTCACTGGAGTTGTACGG-3'.

Colony Formation Assay

Transfected cells were washed with PBS, digested with trypsin and centrifuged at 100 rpm/min for 3 min. Cells were then seeded in the 6-well plates at a density of 500 cells per well, with 3 replicates in each group. Cells were fixed with methanol and stained with 0.1% crystal violet (Sigma-Aldrich, St. Louis, MO, USA), followed by the detection of colony formation.

Western Blot

Total protein was extracted from treated cells by RIPA (radioimmunoprecipitation assay) solution. The protein sample was separated by electrophoresis on 10% SDS-PAGE (sodium dodecyl sulphate-polyacrylamide gel electrophoresis) and then transferred to PVDF (polyvinylidene difluoride) membrane. After membranes were blocked with skimmed milk, they were incubated with primary antibodies (Cell Signaling Technology, Danvers, MA, USA) overnight at 4°C. The membranes were then washed with TBST (Tris-buffered Saline with Tween 20) and followed by the incubation of secondary antibody. The protein blot on the membrane was exposed by chemiluminescence.

Statistical Analysis

Statistical Product and Service Solutions (SPSS20.0, IBM Co., Armonk, NY, USA) statistical software was used for data analysis and GraphPad Prism (Version X, La Jolla, CA, USA) was introduced for image editing. Measurement data were expressed as mean ± standard deviation ($\bar{x} \pm s$) and compared using the *t*-test. $p<0.05$ was considered to be statistically significant.

Results

MicroRNA-212 Was Downregulated in PCa Patients

By analyzing the expression levels of microRNA-212 in 72 PCa tissues, we found that microRNA-212 was downregulated in PCa patients (Figure 1A). Besides, the lower survival rate was found in PCa patients with lower microRNA-212 expression than those with higher level (Figure 1B and 1C). Compared with PCa patients with higher microRNA-212 expression, those with lower level presented higher tumor grade (Table I). We then detected microRNA-212 expression in PCa cell lines and the results showed that 22RV1 cells expressed the lowest level (Figure 1D), which was selected for the following *in vitro* experiments.

Overexpressed MicroRNA-212 Inhibited Proliferation and Migration of PCa Cells

Overexpression plasmid of microRNA-212 was transfected into 22RV1 cells and the trans-

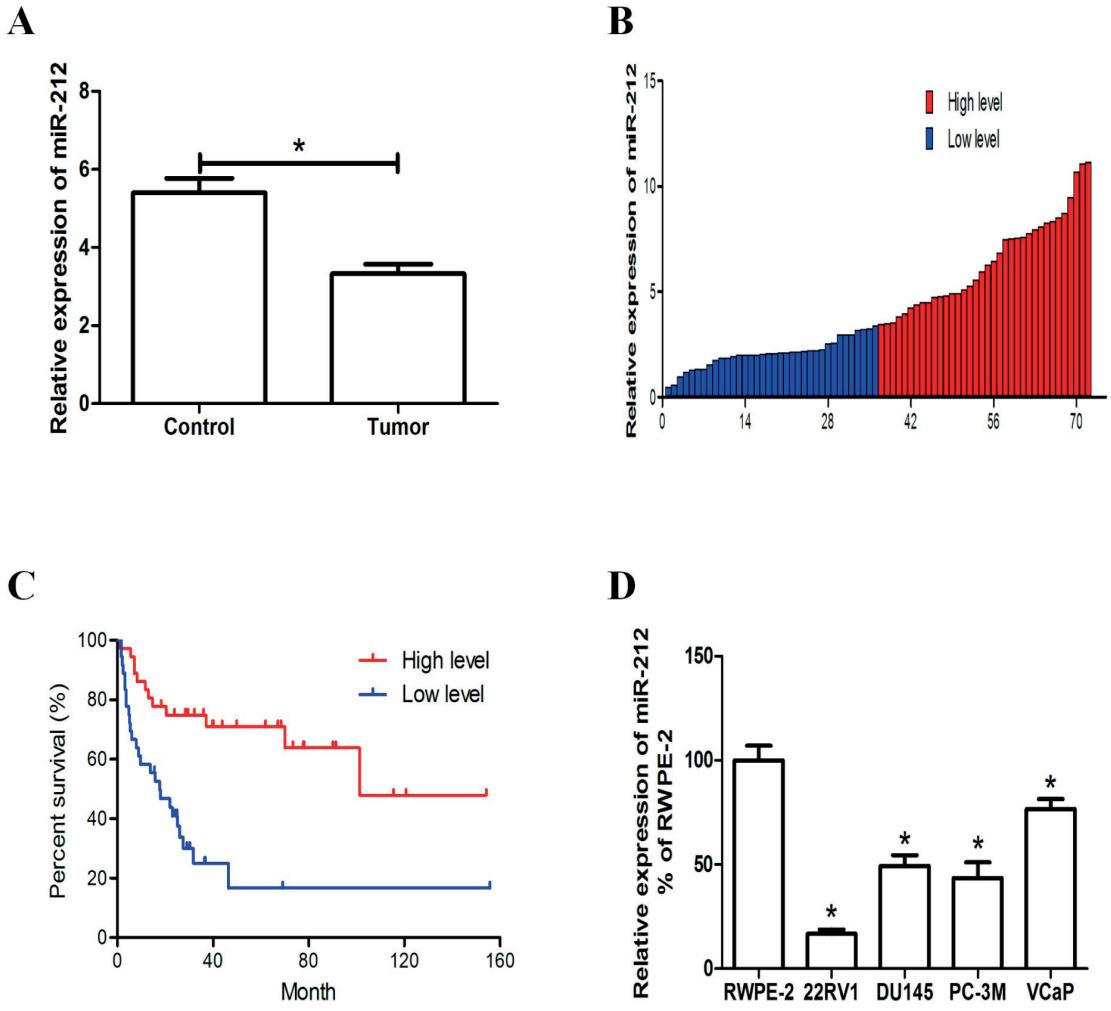

Figure 1. MicroRNA-212 was downregulated in PCa patients and cells. ***A,*** MicroRNA-212 was downregulated in PCa patients. ***B,*** MicroRNA-212 expression in PCa tissues. ***C,*** The survival rate in PCa patients with lower expression of microRNA-212 was lower than those with higher expression of microRNA-212. ***D,*** MicroRNA-212 was downregulated in PCa cells.

fection efficacy was verified by qRT-PCR (Figure 2A). Decreased proliferation (Figure 2B) and migration (Figure 2D) abilities were found after microRNA-212 overexpression in 22RV1 cells. Moreover, overexpressed microRNA-212 also resulted in promoted cell apoptosis (Figure 2D) and arrested cell cycle (Figure 2E). Colony formation assay indicated that the colony formation ability is remarkably inhibited by upregulated microRNA-212 (Figure 2F).

Overexpressed MicroRNA-212 Inhibited BMI1 Expression

Luciferase reporter gene assay verified that microRNA-212 could bind to BMI1 (Figure 3A). Differentially expressed BMI1 has been reported to participate in tumor development. Here, we found decreased protein (Figure 3B) and mRNA (Figure 3C) levels of BMI1 after microRNA-212 overexpression, indicating that microRNA-212 promotes PCa development *via* targeting BMI1.

Overexpressed MicroRNA-212 Inhibited Expressions of Inflammatory Factors

Previous studies have demonstrated that inflammatory factors were abnormally expressed in peripheral blood of PCa patients. After overexpression of microRNA-212, we found a remarkable reduction in IL-6 (Figure 4A) and TGF-β1 (Figure 4B) levels in PCa cells. Our data suggested that overexpressed microRNA-212 inhibits expressions of inflammatory factors.

Overexpressed MicroRNA-212 Inhibited NF-κB Pathway

To further explore the mechanism of microRNA-212 in regulating PCa development, we detected protein expressions of NF-κB pathway-related genes by Western blot. The data demonstrated that protein (Figure 4C) and mRNA (Figure 4D) levels of p50 were decreased after microRNA-212 overexpression. Besides, protein (Figure 4E) and mRNA (Figure 4F) levels of p65 were also decreased after microRNA-212 overexpression. Our results demonstrated that microRNA-212 regulates PCa development by targeting BMI1 *via* NF-κB pathway.

Discussion

Accumulating evidence has shown that microRNAs exert a crucial role in maintaining normal cell growth and function, and differentially expressed microRNAs are closely related to cancers[19]. It has been confirmed that microRNAs could inhibit oncogene expressions[20]. For exam-

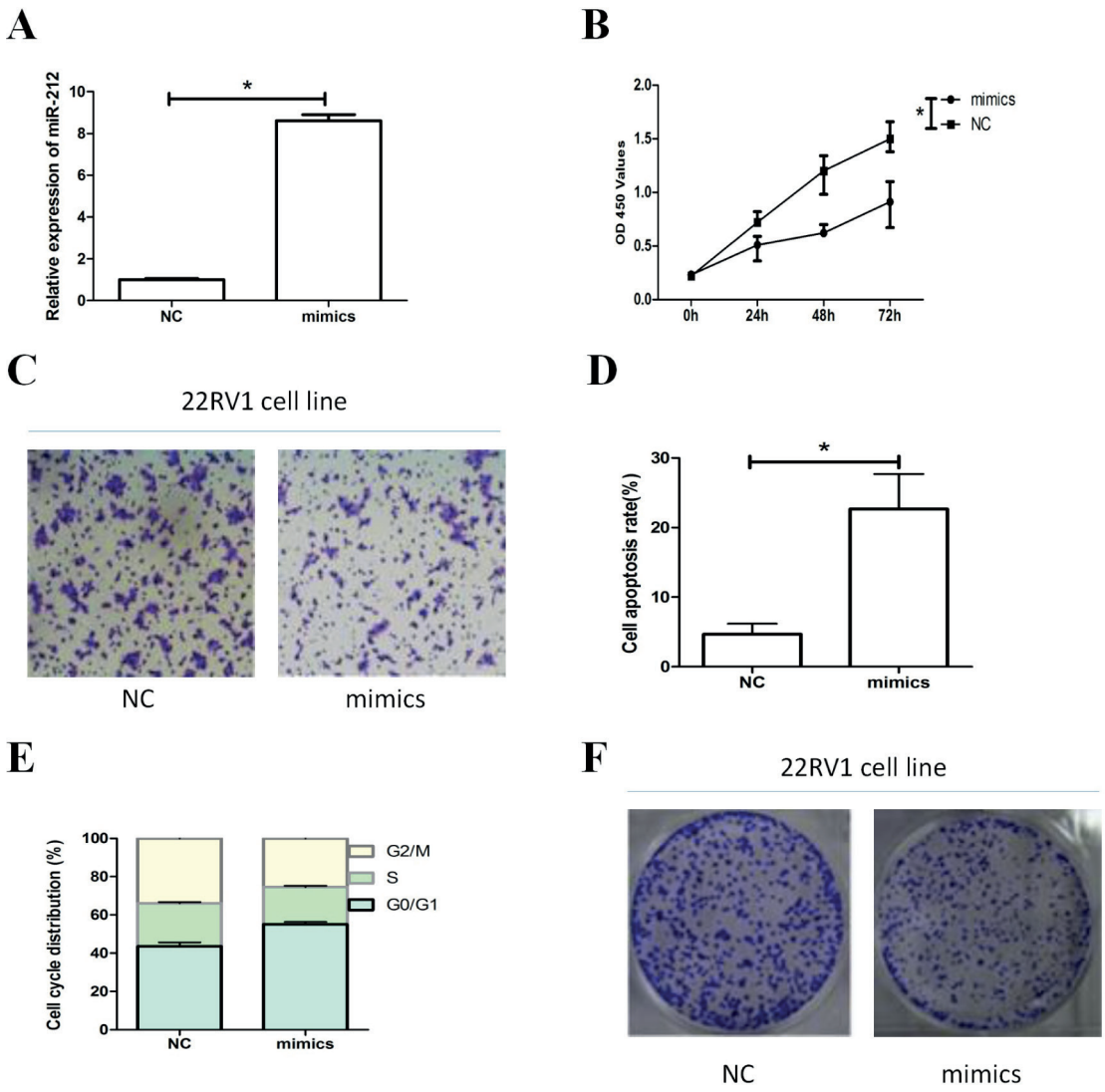

Figure 2. Downregulated microRNA-212 inhibited cell proliferation and migration. ***A,*** MicroRNA-212 expression was increased after microRNA-212 overexpression. ***B,*** Overexpressed microRNA-212 inhibited cell proliferation. ***C,*** Overexpressed microRNA-212 inhibited cell migration. ***D,*** Overexpressed microRNA-212 arrested cell cycle in G0/G1 phase. ***F,*** Overexpressed microRNA-212 inhibited colony formation ability.

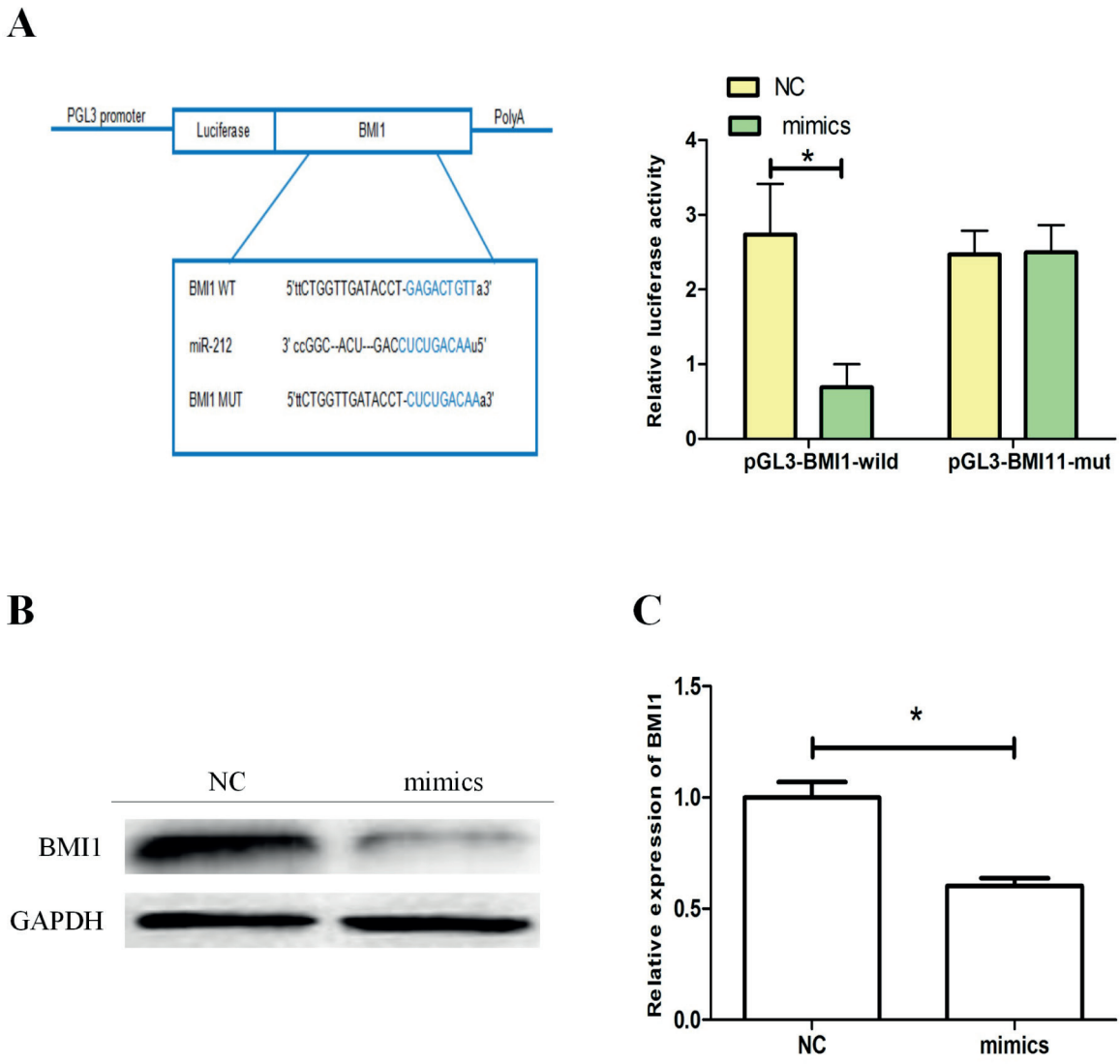

Figure 3. MicroRNA-212regulated BMI1 expression. ***A,*** MicroRNA-212 could bind to BMI1. ***B,*** Overexpressed microRNA-212 reduced protein expression of BMI1. ***C,*** Overexpressed microRNA-212 reduced mRNA expression of BMI1.

ple, knockdown of microRNA let-7 decreases the overexpressed Ras, which further shortens postoperative survival in patients with non-small cell lung cancer[21]. MicroRNA-21 is widely expressed in breast, prostate, lung, colon, pancreas, and stomach, which promotes tumor growth *via* inhibiting cell apoptosis[22]. Disordered microRNAs would severely affect intracellular homeostasis.

In the present study, we found that overexpressed microRNA-212 could inhibit the proliferation of PCa cells. Transwell assay indicated that microRNA-212 overexpression could effectively inhibit migration of PCa cells, suggesting that downregulated microRNA-212 in PCa patients would promote tumor development. Moreover, cell cycle was arrested in G0/G1 phase after overexpression of microRNA-212. Cell apoptosis is an essential biological process in maintaining tissue development and homeostasis. Our data elucidated that overexpression of microRNA-212 remarkably stimulates apoptosis of PCa cells, suggesting that downregulating microRNA-212 in PCa patients would reduce cell apoptosis, thereby promoting tumor development. To sum up, downregulated microRNA-212 in PCa patients could effectively promote cell proliferation, migration and DNA synthesize, whereas it could inhibit cell apoptosis.

To further explore the underlying mechanism of microRNA-212 in regulating PCa, we

predicted the target gene of microRNA-212 by TargetScan and BMI1 was screened out. BMI1 is one of the core members of the PcG family. BMI1 is overexpressed in tumor cells, which is capable of regulating the development and progression of tumors. The BMI1 expression is related to the pathological indicators in tumor patients[23,24]. In addition, scholars[25,26] have shown that BMI1 is post-transcriptionally regulated after binding to microRNA-630. Our work verified that BMI1 is the target gene of microRNA-212 by luciferase reporter gene assay. *In vitro* experiments further confirmed that BMI1 is regulated by microRNA-212.

Studies have shown that serum levels of IL-6 and TGF-β1 are remarkably elevated in PCa patients[27]. In our investigation, overexpressed microRNA-212 resulted in decreased expressions of IL-6 and TGF-β1 in 22RV1 cells. Accumulating evidence has shown that the activation of the NF-κB pathway is closely related to the occurrence of PCa[28]. In the classical NF-κB pathway, NF-κB/Rel is inhibited after binding to IκB[29]. In the non-canonical NF-κB pathway, p100/RelB complex is inactivated in the cytoplasm[30]. The p52/RelB complex with transcriptional ability translocates into the nucleus and activates target gene expressions[31]. We demonstrated that downregu-

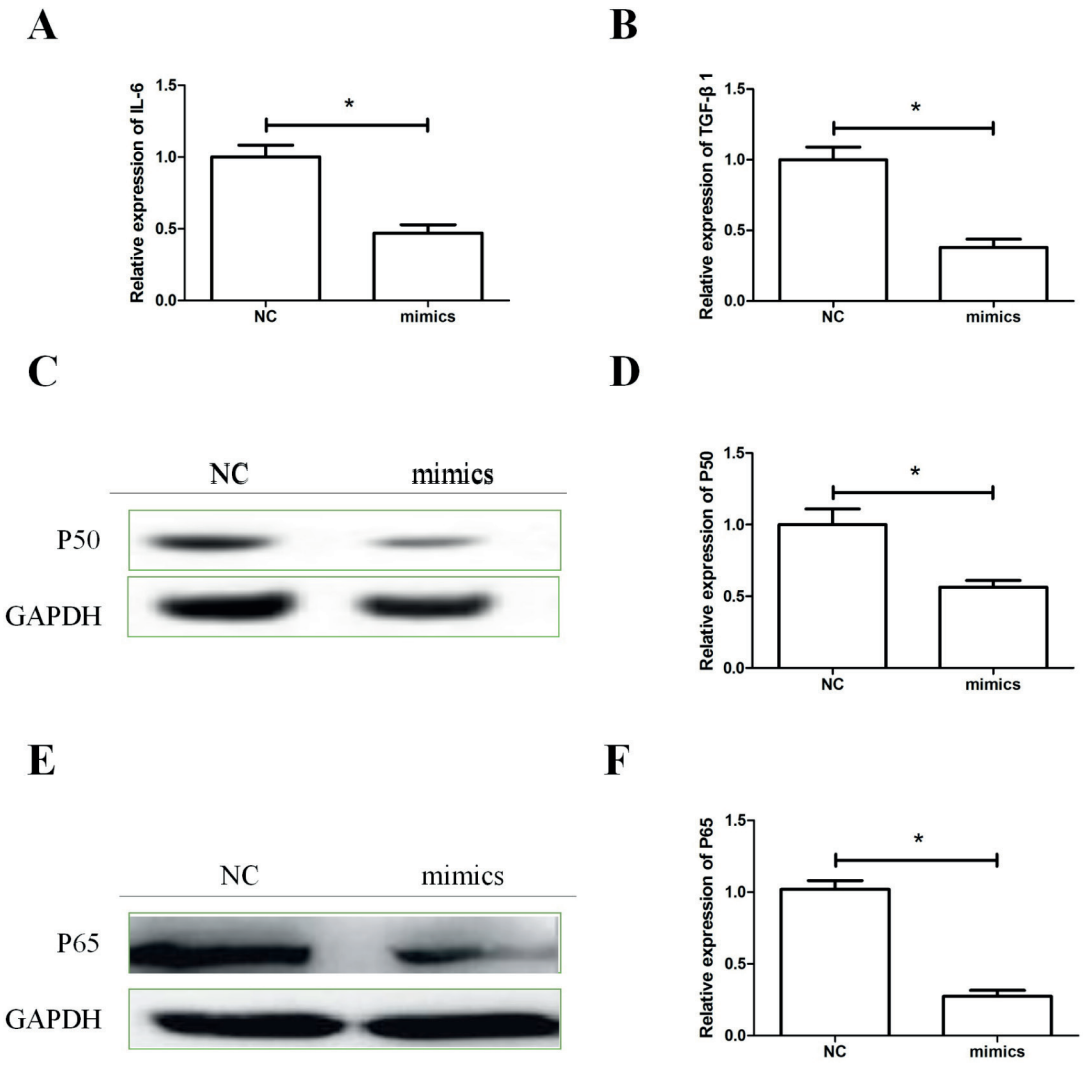

Figure 4. Overexpressed microRNA-212 inhibited NF-κB pathway. ***A-B,*** The mRNA levels of IL-6 (***A***) and TGF-β1 (***B***) after overexpressing microRNA-212 in PCa cells. ***C, D,*** Protein (***C***) and mRNA (***D***) levels of p50 after overexpressing microRNA-212 in PCa cells. ***E, F,*** Protein (***E***) and mRNA (***F***) levels of p65 after overexpressing microRNA-212 in PCa cells.

lated microRNA-212 regulates PCa development through promoting the secretion of inflammatory cytokines *via* NF-κB pathway.

Conclusions

We found that microRNA-212 was downregulated in PCa tissues, which could promote PCa development by targeting BMI1 *via* NF-κB pathway.

Conflict of Interest

The Authors declare that they have no conflict of interest.

Fund acknowledgment

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References

1) Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015; 65: 87-108.
2) Cancer in Australia 2014: Actual incidence data from 1982 to 2011 and mortality data from 1982 to 2012 with projections to 2014. Asia Pac J Clin Oncol 2015; 11: 208-220.
3) Center MM, Jemal A, Lortet-Tieulent J, Ward E, Ferlay J, Brawley O, Bray F. International variation in prostate cancer incidence and mortality rates. Eur Urol 2012; 61: 1079-1092.
4) Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA Cancer J Clin 2015; 65: 5-29.
5) Zhang YF, Guan YB, Yang B, Wu HY, Dai YT, Zhang SJ, Wang JP, Anoopkumar-Dukie S, Davey AK, Sun ZY. Prognostic value of Her-2/neu and clinicopathologic factors for evaluating progression and disease-specific death in Chinese men with prostate cancer. Chin Med J (Engl) 2011; 124: 4345-4349.
6) Veccia A, Caffo O, De Giorgi U, Di Lorenzo G, Ortega C, Scognamiglio F, Aieta M, Facchini G, Mansueto G, Mattioli R, Procopio G, Zagonel V, D'Angelo A, Spizzo G, Bortolus R, Donini M, Lo RG, Massari F, Vicario G, Zucali PA, Alesini D, Bonetti A, Mucciarini C, Nicodemo M, Berruti A, Fratino L, Lodde M, Messina C, Perin A, Santini D, Sava T, Tucci M, Basso U, Maines F, Burgio LS, Galligioni E. Clinical outcomes in octogenarians treated with docetaxel as first-line chemotherapy for castration-resistant prostate cancer. Future Oncol 2016; 12: 493-502.
7) Caffo O, De Giorgi U, Fratino L, Alesini D, Zagonel V, Facchini G, Gasparro D, Ortega C, Tucci M, Verderame F, Campadelli E, Lo RG, Procopio G, Sabbatini R, Donini M, Morelli F, Sartori D, Zucali P, Carrozza F, D'Angelo A, Vicario G, Massari F, Santini D, Sava T, Messina C, Fornarini G, La Torre L, Ricotta R, Aieta M, Mucciarini C, Zustovich F, Macrini S, Burgio SL, Santarossa S, D'Aniello C, Basso U, Tarasconi S, Cortesi E, Buttigliero C, Ruatta F, Veccia A, Conteduca V, Maines F, Galligioni E. Clinical outcomes of castration-resistant prostate cancer treatments administered as third or fourth line following failure of docetaxel and other second-line treatment: results of an italian multicentre study. Eur Urol 2015; 68: 147-153.
8) Tsuzuki T. Intraductal carcinoma of the prostate: a comprehensive and updated review. Int J Urol 2015; 22: 140-145.
9) Robinson B, Magi-Galluzzi C, Zhou M. Intraductal carcinoma of the prostate. Arch Pathol Lab Med 2012; 136: 418-425.
10) Flynt AS, Lai EC. Biological principles of microRNA-mediated regulation: Shared themes amid diversity. Nat Rev Genet 2008; 9: 831-842.
11) Lee RC, Ambros V. An extensive class of small RNAs in Caenorhabditis elegans. Science 2001; 294: 862-864.
12) Zhiping C, Shijun T, Linhui W, Yapei W, Lianxi Q, Qiang D. MiR-181a promotes epithelial to mesenchymal transition of prostate cancer cells by targeting TGIF2. Eur Rev Med Pharmacol Sci 2017; 21: 4835-4843.
13) McGuire A, Brown JA, Kerin MJ. Metastatic breast cancer: The potential of miRNA for diagnosis and treatment monitoring. Cancer Metastasis Rev 2015; 34: 145-155.
14) Shi M, Xie D, Gaod Y, Xie K. Targeting miRNAs for pancreatic cancer therapy. Curr Pharm Des 2014; 20: 5279-5286.
15) Szczyrba J, Nolte E, Wach S, Kremmer E, Stohr R, Hartmann A, Wieland W, Wullich B, Grasser FA. Downregulation of Sec23A protein by miRNA-375 in prostate carcinoma. Mol Cancer Res 2011; 9: 791-800.
16) Giangreco AA, Vaishnav A, Wagner D, Finelli A, Fleshner N, Van der Kwast T, Vieth R, Nonn L. Tumor suppressor microRNAs, miR-100 and -125b, are regulated by 1,25-dihydroxyvitamin D in primary prostate cells and in patient tissue. Cancer Prev Res (Phila) 2013; 6: 483-494.
17) Kontos CK, Adamopoulos PG, Scorilas A. Prognostic and predictive biomarkers in prostate cancer. Expert Rev Mol Diagn 2015; 15: 1567-1576.
18) Stuopelyte K, Daniunaite K, Bakavicius A, Lazutka JR, Jankevicius F, Jarmalaite S. The utility of urine-circulating miRNAs for detection of prostate cancer. Br J Cancer 2016; 115: 707-715.
19) Zhang B, Pan X, Wang Q, Cobb GP, Anderson TA. Computational identification of microRNAs and their targets. Comput Biol Chem 2006; 30: 395-407.

20) Esquela-Kerscher A, Slack FJ. Oncomirs - microRNAs with a role in cancer. Nat Rev Cancer 2006; 6: 259-269.

21) Esquela-Kerscher A, Trang P, Wiggins JF, Patrawala L, Cheng A, Ford L, Weidhaas JB, Brown D, Bader AG, Slack FJ. The let-7 microRNA reduces tumor growth in mouse models of lung cancer. Cell Cycle 2008; 7: 759-764.

22) Volinia S, Calin GA, Liu CG, Ambs S, Cimmino A, Petrocca F, Visone R, Iorio M, Roldo C, Ferracin M, Prueitt RL, Yanaihara N, Lanza G, Scarpa A, Vecchione A, Negrini M, Harris CC, Croce CM. A microRNA expression signature of human solid tumors defines cancer gene targets. Proc Natl Acad Sci U S A 2006; 103: 2257-2261.

23) Shahi MH, York D, Gandour-Edwards R, Withers SS, Holt R, Rebhun RB. BMI1 is expressed in canine osteosarcoma and contributes to cell growth and chemotherapy resistance. PLoS One 2015; 10: e131006.

24) He Q, Liu Z, Zhao T, Zhao L, Zhou X, Wang A. Bmi1 drives stem-like properties and is associated with migration, invasion, and poor prognosis in tongue squamous cell carcinoma. Int J Biol Sci 2015; 11: 1-10.

25) Su Y, Wen Z, Shen Q, Zhang H, Peng L, Chen G, Zhu Z, Du C, Xie H, Li H, Xia Y, Tang W. Long non-coding RNA LOC100507600 functions as a competitive endogenous RNA to regulate BMI1 expression by sponging miR128-1-3p in Hirschsprung's disease. Cell Cycle 2018; 17: 459-467.

26) Gong XF, Yu AL, Tang J, Wang CL, He JR, Chen GQ, Zhao Q, He M, Zhou CX. MicroRNA-630 inhibits breast cancer progression by directly targeting BMI1. Exp Cell Res 2018; 362: 378-385.

27) Adler HL, McCurdy MA, Kattan MW, Timme TL, Scardino PT, Thompson TC. Elevated levels of circulating interleukin-6 and transforming growth factor-beta1 in patients with metastatic prostatic carcinoma. J Urol 1999; 161: 182-187.

28) Jin R, Sterling JA, Edwards JR, DeGraff DJ, Lee C, Park SI, Matusik RJ. Activation of NF-kappa B signaling promotes growth of prostate cancer cells in bone. PLoS One 2013; 8: e60983.

29) Perkins ND. Post-translational modifications regulating the activity and function of the nuclear factor kappa B pathway. Oncogene 2006; 25: 6717-6730.

30) Sun SC. The noncanonical NF-kappaB pathway. Immunol Rev 2012; 246: 125-140.

31) Chen J, Chen ZJ. Regulation of NF-kappaB by ubiquitination. Curr Opin Immunol 2013; 25: 4-12.