

Effects of microRNA-210 on the diagnosis and treatment of prostate cancer

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Abstract. The present study aimed to investigate the effects of microRNA-210 (miR-210) in the diagnosis and treatment of prostate cancer. Venous blood was collected from 30 prostate cancer patients, that were treated in the Medical Group of Ping Mei Shenma General Hospital (Pingdingshan, China) from June 2013 to May 2015, and 20 healthy men. The miR-210 expression levels in patients and healthy men was quantified. Primary prostate cancer cells were placed in three treatment groups: i) NC group, untreated; ii) BL group, empty vector; and iii) anti-miR-210 group, miR-210 inhibitor-transfected. Cell proliferation and apoptotic rate were detected by MTT and flow cytometry, respectively. The expression levels of miR-210 and regulator of differentiation 1 (ROD1) were detected by reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and the ROD1 protein expression in each group was detected by western blotting. Cell proliferation rate of the anti-miR-210 group was significantly reduced when compared with the NC and BL groups ($P \leq 0.05$); however, the apoptotic rate of the anti-miR-210 group was significantly increased compared with the NC and BL groups ($P \leq 0.05$). RT-qPCR revealed that the expression level of miR-210 and ROD1 in the anti-miR-210 group was significantly reduced when compared with the NC and BL groups ($P < 0.05$). MiR-210 was overexpressed in the serum of prostate cancer patients and transfection with an miR-210 inhibitor was able to effectively inhibit the proliferation of prostate cancer cells and promote apoptosis.

Introduction

Prostate cancer (PCa) is the second most common cancer among men worldwide (1). Numerous factors are contribute to development of PCa, including genetic mutations and the tumor microenvironment (2,3). Therefore, understanding of the oncogenic pathologies in the progression of PCa may contribute to improved clinical diagnosis and treatment.

MicroRNAs (miRNA) are endogenous, non-coding short chain RNAs, which alter gene expression at the post-transcriptional level affecting angiogenesis, cell cycle, apoptosis, invasion and migration of the cells and tumor-associated gene expression, thereby affecting the tumor proliferation, invasion and metastasis (4-6). Several studies have suggested that miRNAs have been associated with PCa tumorigenesis and the clinical outcome of patients (7-9). Previous studies confirmed that microRNA-210 (miR-210) in renal cell carcinoma, pancreatic cancer, colorectal cancer, and regulation of cancer cell proliferation, migration and invasion in renal cell carcinoma (10-14). Additionally, the present study investigated its function in the regulation of proliferation and apoptosis and determined that the regulator of differentiation 1 (ROD1), a protein also termed polypyrimidine trace binding protein 3 (PTBP3), is a potential the target of miR-210 in cancer cells (15). However, to the best of our knowledge there is limited investigation of the correlation between miR-210 and prostate cancer has been relatively limited.

In this study, we had detected the difference of serum miR-210 expression between health and prostate cancer patients at first, and further study the effect of miR-210 on prostate cancer cells.

Materials and methods

Patients. A total of 30 prostate cancer patients that were treated between June 2013 to May 2015 were selected for the prostate cancer (PCa) group and 20 healthy people (all male; age range, 47-83 years; median age, 58 years), which underwent physical examination in our hospital at the same period were selected for the healthy control group. Venous blood was collected from healthy people and patients with PCa and serum was isolated immediately using centrifugation in serum-gel tubes at 3,500 x g for 10 min at 4°C and stored at -80°C, until further experiments were performed.

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Cell line culture. The cells which have been collected from the PCa patients and used for primary cell culture. The RPMI 1640 medium containing 10% fetal bovine serum was used to culture the cells at 37°C and 5% CO₂.

Materials. RPMI 1640 culture medium was purchased Gibco; Thermo Fisher Scientific, Inc. (Waltham, MA, USA), fetal bovine serum was purchased from ExCell Bio, Inc. (Shanghai, China), TRIzol Reagent, PrimeScript RT Master Mix reagent kit and SYBR Premix Ex Taq kit were purchased from Takara Bio, Inc. (Otsu, Japan), The miR-210 primer was constructed by the Shanghai GenePharma Co., Ltd. (Shanghai, China), Opti-MEM medium and Lipofectamine™ 2000 were purchased from Invitrogen; Thermo Fisher Scientific, Inc., the miR-210 inhibitor was synthesized by the Invitrogen; Thermo Fisher Scientific, Inc. (cat. no. 4464084), MTT was purchased from Amresco LLC (Solon, OH, USA), fluorescence quantitative PCR instrument purchased from ABI-7500 system employing Primix Ex Taq II SYBR kit, the centrifuge was purchased from Sigma-Aldrich; EMD Millipore (Billerica, MA, USA) and the flow cytometer was purchased from BD Biosciences (Franklin Lakes, NJ, USA).

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA was extracted from the serum of healthy and PCa groups and reverse transcribed to cDNA at 42°C for 60 min and 70°C for 5 min. The following primer sequences were used: miR-210 forward (F), 5'-CTGTGCGTG TGACAGCGGCTGA-3', reverse (R) primers for universal primer real-time PCR Uni-miR primers (Takara Bio, Inc.); ROD1 F, 5'-AAGGAAATGAATGGGCAGCCGTTAG-3' and R, 5'-CATG-TAGTTGAGGTCAATGAAGGGGTC-3'; GAPDH F, 5'-GCTCTCTGCTCCTCCTGTTC-3' and R, 5'-GACTCCGACCTTCACCTTCC-3'. GAPDH was used as the internal control, qPCR was performed according to the manufacturer's instructions of the Primix Ex Taq II SYBR kit. Each sample was analyzed in triplicate. The following thermocycling conditions were used: 95°C for 30 sec, 95°C for 5 sec, 60°C for 31 sec and for 40 cycles. The 2^{-ΔΔC_q} method (16) was used to calculate the expression levels of miR-210 and ROD1.

Transfection. The transfection was performed according to the manufacturer's protocol using Lipofectamine™ 2000 for a 96-well (1x10⁴ cells/per well) transfection. A total of 48 h following transfection, cells divided into the following treatment groups: i) NC group, untreated; ii) BL group, empty vector; and iii) anti-miR-210 group, miR-210 inhibitor-transfected.

Cell apoptosis detection. Apoptosis of cells was detected using Annexin V-fluorescein isothiocyanate/propidium iodide (Nanjing Keygen Biotech, Nanjing, China) double staining kit. Following transfection for 48 h, 1.0x10⁵ cells/well, washed with PBS, 50 μl Annexin buffer, 5 μl 7-aminoactinomycin D, 5 μl Annexin V-phycoerythrin dye were added, following a reaction of 15 min, 200 μl buffer was added to detect apoptosis by flow cytometry using the BD FACSuite™ software (version 1.0; BD Biosciences). The experiment was repeated four times.

Western blot analysis. Cells were lysed in cold radioimmunoprecipitation lysis buffer (Thermo Fisher Scientific, Inc.).

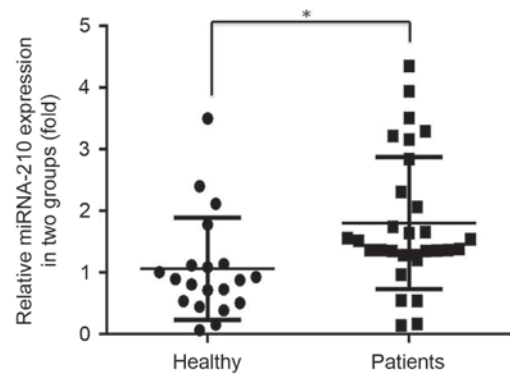


Figure 1. miR-210 expression was lower in samples from healthy individuals compared with and patients with PCa. *P<0.05 vs. healthy samples. PCa, pancreatic cancer; miR, microRNA.

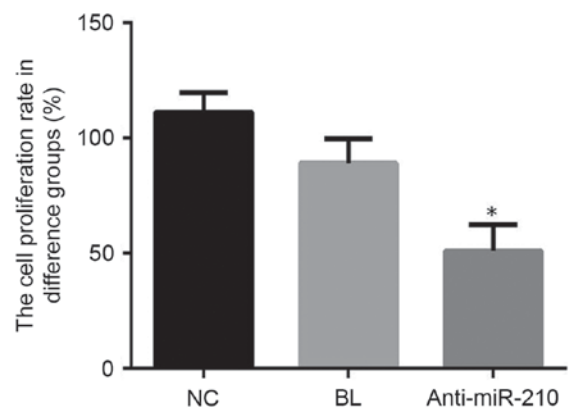


Figure 2. Cell proliferation rate in the three treatment groups. *P<0.05 vs. NC group. NC, untreated control; BL, empty vector.

Protein concentration was measured using a bicinchoninic acid protein assay kit. Proteins (30 μg per lane) were separated on 10% SDS-PAGE and subsequently transferred to nitrocellulose membranes and then incubated with 5% skimmed milk for 2 h at room temperature. Subsequently, the membranes were probed with antibodies specific to ROD1 (cat. no. WH0009991M1; 1:200; Sigma-Aldrich; Merck Millipore) and GAPDH (cat. no. G8795; 1:1,000; Sigma-Aldrich; Merck Millipore) overnight at 4°C. Membranes were then incubated with anti-mouse horseradish peroxidase-conjugated secondary antibodies for 1 h at room temperature (cat. no. A9044; 1:5,000; Sigma-Aldrich; Merck KGaA). The membranes were washed three times in PBS and analyzed using enhanced chemiluminescence film system (GE Healthcare, Chicago, IL, USA).

Statistical analysis. Analysis was performed using SPSS version 19.0 (IBM Corporation, Armonk, NY, USA). Data were expressed as the mean ± standard deviation. One-way analysis of variance followed by a least significant difference post hoc test were used to identify statistical differences groups. P<0.05 was considered to indicate a statistically significant difference.

Results

Expression levels of miR-210 in healthy individuals and patients with PCa. The expression level of miR-210 in the

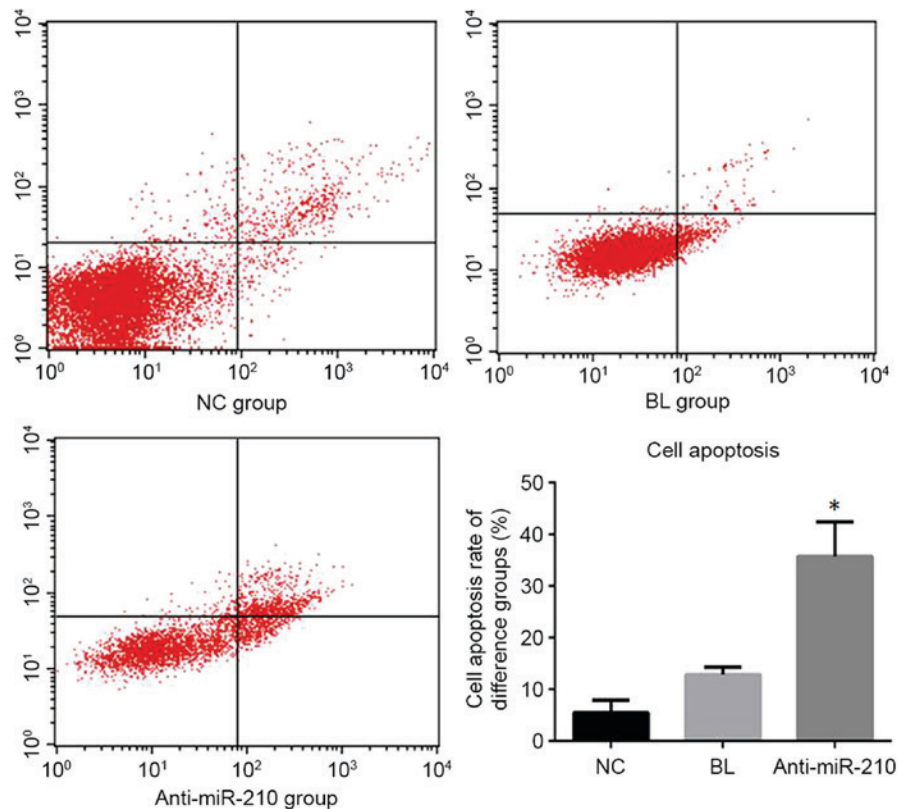


Figure 3. Cell apoptotic rate in the three treatment groups (%). * $P < 0.05$ vs. NC group.

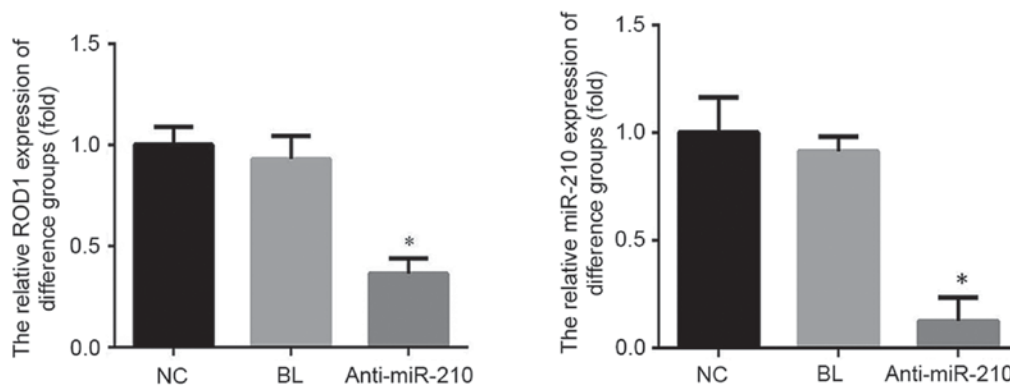


Figure 4. miR-210 and ROD1 gene expression in three groups (fold). * $P < 0.05$ vs. NC group. miR, microRNA.

serum of prostate cancer patients was significantly higher compared with that in healthy individuals ($P < 0.05$; Fig. 1).

Cell proliferation. Cell proliferation in the anti-miR-210 group was significantly lower when compared with the NC group ($P < 0.05$; Fig. 2). These findings suggested that a miR-210 inhibitor may effectively inhibit the proliferation of primary PCa cells.

Cell apoptosis detection. The anti-miR-210 group exhibited an apoptotic rate of $35.75 \pm 6.71\%$, which was significantly higher than the NC group $5.48 \pm 2.47\%$ and BL group $12.83 \pm 1.47\%$ ($P < 0.05$; Fig. 3).

ROD1 mRNA and protein expression level. The expression level of miR-210 and ROD1 in the anti-miR-210 group was

significantly reduced when compared with the NC and BL groups ($P < 0.05$; Fig. 4). The expression level of ROD1 protein in the anti-miR-210 group was reduced compared with the NC group (Fig. 5).

Discussion

PCa is a common malignant tumor in men (1). It is the second most common malignant tumor in the United States. Additionally, it is the sixth most common mortality due to cancer (17). Recently, the incidence and mortality of PCa in China has significantly increased and is gradually approaching that of European and American countries, and has become a serious threat to the health of the elderly men (18). Molecular-targeted therapy is a novel therapeutic

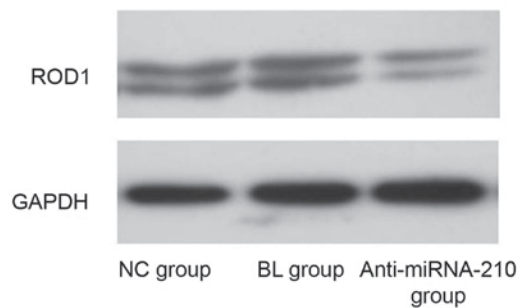


Figure 5. ROD1 protein expression in the different treatment groups. ROD1, regulator of differentiation 1.

method, developed in recent years (19). It has become an important area of research for effective therapeutic targets, and miR-210 appears to provide a new target for the diagnosis and treatment of prostate cancer (20). A previous study revealed that more than half of the miRNAs are located in the region of tumor-associated genes and fragile sites, loss of heterozygosity, amplification area or fault zone, suggesting that miRNAs may be used as oncogenes or tumor suppressor genes (21). At present, miRNAs may regulate gene expression at the post-transcriptional level, which is primarily used as a negative regulator of gene expression. A previous study confirmed that miRNA affected angiogenesis, cell cycle, apoptosis, cell invasion and migration (19). A previous study confirmed that many tumors, including prostate cancer, have many miRNA expression abnormalities, and have a different role in different stages of tumor cell migration, invasion and angiogenesis (20).

Volinia *et al* determined that miR-20a was overexpressed in prostate cancer tissues by using miRNA microarrays (22). Sylvestre *et al* identified that after transfection of an miR-20a inhibitor into PCa cells, the apoptotic rate was significantly increased, which indicated that miR-20a had an anti-apoptotic role in PCa cells (23). Previous studies have confirmed that expression level of miR-210 is increased in a number of solid tumors; however, no uniform trend has been identified, to the best of our knowledge (24-29).

The present study detected the expression of miR-210 in the serum of patients with PCa and healthy individuals. The current findings revealed that miR-210 in the serum of patients with PCa was overexpressed. In order to further clarify the role of miR-210 in PCa, an miR-210 antagonist transfection was used in primary PCa cells. The present findings revealed that the anti-miR-210 group cell proliferation rate was significantly reduced compared with that of the NC and BL groups, and the apoptotic was significantly greater. In order to determine the mechanism of action of miR-210 in prostate cancer, the expression levels of the associated genes and proteins were also determined.

ROD1, a protein also termed PTBP3, is the potential target of miRNA-210 in various types of cancer, including glioblastoma multiforme (30,31). The present study determined that the ROD1 mRNA and protein expression levels in the anti-miR-210 group were significantly reduced and it may be possible that miR-210 inhibitors reduced cell proliferation and increased apoptotic rate of PCa cells by inhibiting ROD1 expression level.

In summary, the present study demonstrated that miR-210 was overexpressed in patients with PCa and that miR-210 may function as an oncogene. Based on the present study, it is possible that miR-210 may be a novel biomarker for prostatic cancer, and it may regulate tumor progression through by targeting ROD1. As PCa is a form of cancer that progresses and spreads quickly, traditional therapies, such as surgery and chemo and radiotherapy have only limited effectiveness in treating this aggressive disease. Therefore, novel approaches, such as molecular targeting would be beneficial in the therapeutic treatment of PCa. Targeting miR-210 may provide a novel therapeutic option in the treatment of this disease. In conclusion, miR-210 may have a key role in PCa diagnosis and treatment by regulating ROD1 miRNA and protein expression levels.

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Availability of data and materials

The analyzed data sets generated during the study are available from the corresponding author on reasonable request.

Authors' contributions

YQ and WH contributed to study design, data collection, data interpretation, preparation of manuscript and literature analysis.

Ethics approval and consent to participate

The present study was approved by the Human Ethics Committee Review Board at the Medical Group of Ping Mei Shenma General Hospital. All participants signed written informed consent forms.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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