

ORIGINAL ARTICLE

MicroRNA-151 regulates the growth, chemosensitivity and metastasis of human prostate cancer cells by targeting PI3K/AKT

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Summary

Purpose: Prostate cancer causes significant mortality and microRNAs (miRs) have been shown to regulate the growth and metastasis of different cancers. In this context, the present study was designed to investigate the potential of miR-151 in the treatment of prostate cancer.

Methods: The normal and the prostate cancer cell lines (LN-CaP, PC-3 and Du-145) were used in this study. The expression of miR-151 was determined by qRT-PCR. The DAPI and annexin V/propidium iodide (PI) staining were used for the detection of apoptosis. Transwell assay was used for the estimation of cell migration and invasion. Western blot analysis was used for the determination of the protein expression.

Results: miR-151 was downregulated in prostate cancer cells and showed inhibitory effect on cell growth which was

manifested as decline in cell survival and loss of viability of cancer cells. Additionally, the chemosensitivity of prostate cancer cells to 5-FU was enhanced under miR-151 overexpression. Furthermore, miR-151 also inhibited the migration and invasion of cancer cells. The results of western blot analysis showed that miR-151 overexpression blocks the PI3K/AKT signalling pathway in prostate cancer cells

Conclusion: Taken together, miR-151 has growth inhibitory effect against prostate cancer and negatively regulates the cell migration and invasion along with enhancement of chemosensitivity of cancer cells.

Key words: prostate cancer, metastasis, cell proliferation, migration, invasion, chemosensitivity

Introduction

The 4th foremost type of cancer and second frequent cancer in males, prostate cancer, is responsible for significant number of deaths worldwide. More than 1 million men were diagnosed with prostate cancer in 2012 accounting for about 15% of all the malignancies diagnosed in men. Moreover, approximately 0.35 million deaths were reported due to prostate cancer in 2012 [1]. Owing to changing lifestyle, the incidence of prostate cancer is believed to increase significantly in the

coming decade. According to recent reports the number of prostate cancer cases will increase to 1.7 million and the number of deaths will increase approximately to 0.5 million. Prostate cancer is rare in people below the age of 40 years and most of the prostate cancer cases are reported in elderly people above the age of 65 years [2]. Surgery, radio- and chemotherapy and hormone therapy are used separately or in combination for the management of this disease. Nonetheless, the frequent relaps-

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es, poor survival rates and increasing incidence of prostate cancer demands the development of novel and effective treatment strategies [3]. Researchers have proved that there is an active involvement of an important class of RNA molecules, the miRs, in most of the human cancers [8-10]. MiRs are small non-coding regulatory RNAs, usually 20-22 nucleotides in length, which act at post-transcriptional level and repress their target genes by binding to mRNA untranslated (3' and 5') regions [4]. Almost 60% of eukaryotic genes are believed to be regulated by miRs [5]. MiRs are generally repressed in human cancers but this is not true for all cases [6]. They act as tumor suppressors or oncogenes to regulate tumorigenesis and progression of human cancers along with playing a significant role in metastasis to surrounding tissues [7]. Studies have revealed dysregulation of miRs in prostate cancer [8]. In this context the present study was designed to investigate the potential of miR-151 in the treatment of prostate cancer.

Methods

Cell lines culture conditions

The normal prostate epithelium PNT2 cells and the prostate cancer cell lines LNCaP, PC-3 and Du-145 were procured from Type Culture Collection of Chinese Academy of Sciences, Shanghai, China. The cells were cultured in RPMI-1640 medium containing 10% fetal bovine serum (FBS), 100 µg/ml streptomycin and 100 U/ml penicillin in a humidified atmosphere containing 5% CO₂.

Expression analysis

The transfected PC-3 prostate cancer cells and subsequently RNS were isolated from these cells by RNeasy Kit (Qiagen, Hilden, Germany). Following RNA extraction, the Omniscript RT kit (Qiagen, Hilden, Germany) was used to synthesize the cDNA from 1 µg of RNA. Thereafter, qRT-PCR was employed to determine the expression using the Taq PCR Master Mix Kit (Qiagen, Hilden, Germany) as per the guidelines of the manufacturer.

Proliferation assay

The estimation of proliferation of prostate cancer cells was made through MTT assay. In brief, the PC-3 cancer cells were stably transfected with miR-NC and miR-151 mimics for 48h. Transfected cells were subsequently cultured in 96-well plates for 24, 28, 72, 96h at 37°C and 5% CO₂. For chemosensitivity assessments, miR-151 cancer cells and non-transfected cells were administered 0.5 µM 5-FU. Around 10µl of Dulbecco's Modified Eagle's Medium (DMEM) from each well was replaced with 10µl of 5% 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, Thermo Fisher Scientific (Weltham, MASS, USA) and again re-

peat incubation of 4h at 37°C was performed. Then, 150 µl of DMSO was added to each well for dissolving the formazan crystals. Subsequently, absorbance at 450nm was recorded with a microplate reader (BioTek, Synergy2, Northern Vermont, USA).

Apoptosis assay

The transfected PC-3 cells were used for the assessment of the nuclear morphology after staining with DAPI stain for 25 min at 20°C. After fixation with 70% methanol, the nuclear morphology was investigated by fluorescence microscopy. Five different random fields were selected for fluorescence microscopy. The annexin V-FITC/PI assay was performed as determined previously [9].

Migration and invasion assay

Transwell chamber without or with Matrigel coating was used to assess the migration and invasion of transfected cancer cells respectively. Briefly, 100µl cell culture containing 6000 cells was added to the upper chamber of the transwell and in the lower chamber 750µl of DMEM medium were placed, supplemented with 10% FBS. After 48h incubation at 37°C/5%CO₂, cells from the surface of membrane's upper side were removed carefully with cotton swabs, while those stuck to lower side of membrane were fixed with 70% ethyl alcohol and stained with 0.1% crystal violet. Light microscope (x100) was used for visualization of cells and photographs were taken. At least seven random fields were used for counting the migratory or invasive cells.

Western blotting

Transfected PC-3 cells were subjected to mechanical lysis with ice-cold hypotonic buffer. The buffer contained protease inhibitors. The protein content of the PC-3 cell lysates were evaluated by bicinchoninic acid (BCA) assay. Similar quantities of the proteins from each sample were loaded and subsequently separated on SDS-PAGE. After transferring the gels to the nitrocellulose membranes, the membranes were treated with primary antibodies for 55 min for at 23°C. This was followed by incubation with secondary antibody. The visualisation of the bands was carried out by chemiluminescence reagent.

Statistics

Three independent experiments were performed to confirm the present data. The values are shown as mean ± SD. Student's t-test and one-way ANOVA were used for comparisons between two samples. P<0.05 was considered statistically significant.

Results

miR-151 is downregulated in prostate cancer cells

The qRT-PCR analysis was performed to examine the relative expression levels of miR-151 in prostate cancer cell lines (LNCaP, PC-3 and Du-145) and normal prostate cells. The expression of

miR-151 was up to 5-fold lower in prostate cancer cell lines (Figure 1). The miR-151 was seen to have significantly lower expression in all three cancer cell lines, being lowest in PC-3 cell line (Figure 1). This suggested a probable regulatory role of miR-151 in prostate cancer.

miR-151 inhibits the growth of PC3- cells

To decipher miR-151 role in the regulation of prostate cancer growth, miR-151 mimics and miR-NC were transfected into PC-3 cancer cells for 48 h and stable transfection was confirmed by RT-PCR (Figure 2A). MTT assay was performed following the culturing of transfected cells for 0, 12, 24, 48 and 96 h. At all the time points, the cell growth was significantly lower under miR-151 overexpression (Figure 2B).

miR-151 induces apoptosis in PC-3 cells

Assessment of nuclear morphology by DAPI staining revealed that transfection of miR-151

mimics induces changes in the nuclear morphology of the PC-3 cells suggestive of apoptosis (Figure 3A). To confirm apoptosis, annexin V/PI staining assay was performed which revealed increase in the apoptotic cell percentage (Figure 3B). The apoptotic cell percentage was found to be 32.81 in miR-151 mimics transfected in comparison to 2.01% in miR-NC transfected cells (Figure 3B).

miR-151 regulates chemosensitivity of PC-3 cells

The effects of miR-151 overexpression on chemosensitivity of PC-3 cells was determined with 5-FU. Assessment of proliferation rate was made through MTT assay for miR-NC, miR-151 mimics-transfected cells along with 5-FU (0.5 μ M) administered miR-NC and miR-151 mimics-transfected cancer cells. Cancer cell proliferation was lowest under miR-151 overexpression plus 5-FU as compared to 5-FU or miR-151 overexpression separately (Figure 4). Together, the results are indicative that miR-151 has a potential to increase the chemosensitivity of prostate cancer cells to drug treatment and thus strongly advocate the application of combinatorial molecular and chemotherapeutic against human prostate cancer.

miR-151 inhibits the migration and invasion of the PC-3 cells

Migratory and invasive potential of PC-3 cancer cells under miR-151 overexpression and downregulation was determined through transwell chamber assay using miR-NC transfected cells as negative control. Both the migration and invasion of cancer cells decreased significantly under miR-151 overexpression but the downregulation of miR-151 was seen to enhance the cell migration and invasion (Figure 5A and B). The migration and

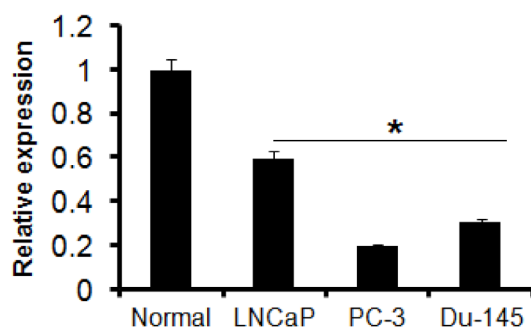


Figure 1. qRT-PCR analysis showing the expression of miR-151 in normal and prostate cancer cells. The experiments were performed in triplicate and the values are expressed as mean $\pm$ SD (* p <0.05).

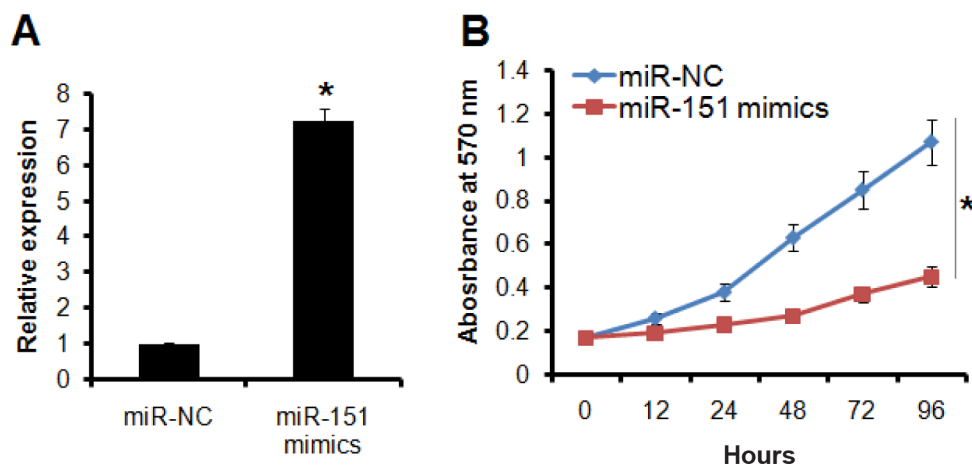


Figure 2. A: The qRT-PCR analysis showing the expression of miR-151 in miR-NC and miR-151 mimics transfected PC-3 prostate cancer cells. **B:** Cell viability of the miR-NC and miR-151 mimics transfected PC-3 cells. The experiments were performed in triplicate and the values are expressed as mean $\pm$ SD (* p <0.05).

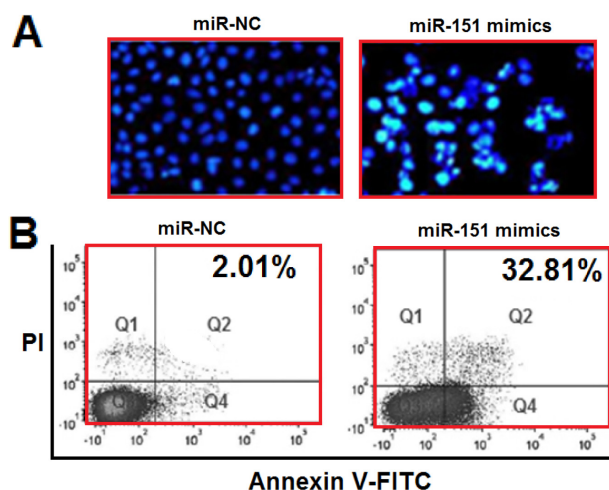


Figure 3. A: DAPI staining showing miR-151 overexpression induces apoptosis in the miR-151 overexpressing PC-3 cells. **B:** Annexin V/PI staining showing that miR-151 increases apoptosis in the PC-3 cells. The experiments were performed in triplicate.

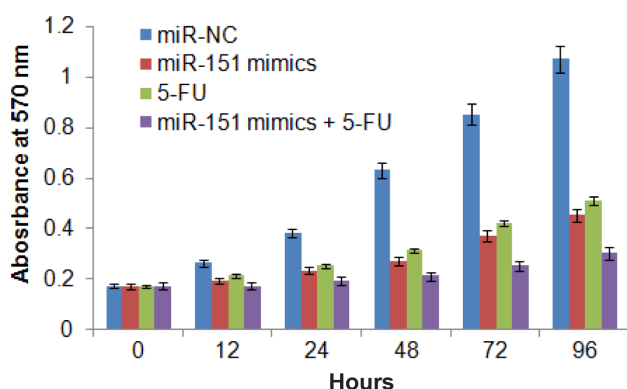


Figure 4. The cell viability assay showing miR-151 overexpression increases the sensitivity of the PC-3 cells to 5-FU. The experiments were performed in triplicate and expressed as mean $\pm$ SD ($p < 0.05$).

invasion of cancer cells was reduced by 63 and 68% under miR-151 overexpression. Thus it is concluded that miR-151 negatively regulates the migration and invasion of prostate cancer cells.

miR-151 effects the PI3K/AKT signalling pathway

The effects of miR-151 overexpression were also examined on the PI3K/AKT signalling pathway in PC-3 cells. The results clearly showed that overexpression of miR-151 suppresses the phosphorylation of PI3K and AKT. Nonetheless, no visible effect was observed on the total PI3K and AKT content.

Discussion

MiRs regulate the expression of a number of human genes and are thus involved in diverse cellular and physiological processes. For the first time it was found in *Caenorhabditis elegans* that miRs regulate the expression of genes. Since then lots of research has been made in the field of miRs. They have been reported to control not only the overall development of animals but also exhibit vital roles in fine designing of cellular fate and differentiation [10]. Disease development in animals is one of the prime aspects of animal biology falling under the regulation of this important group of regulatory RNAs. Dysregulation of miRs has been shown to influence the onset and proliferation of almost all human cancers and researchers are actively involved in elucidating the role of miRs in different human cancers, henceforth to understand the molecular mechanisms which lead to deviation of controlled cellular plan and initiate the development of malignant growth. Studies on prostate cancer have enlightened the involvement of a number of miRs

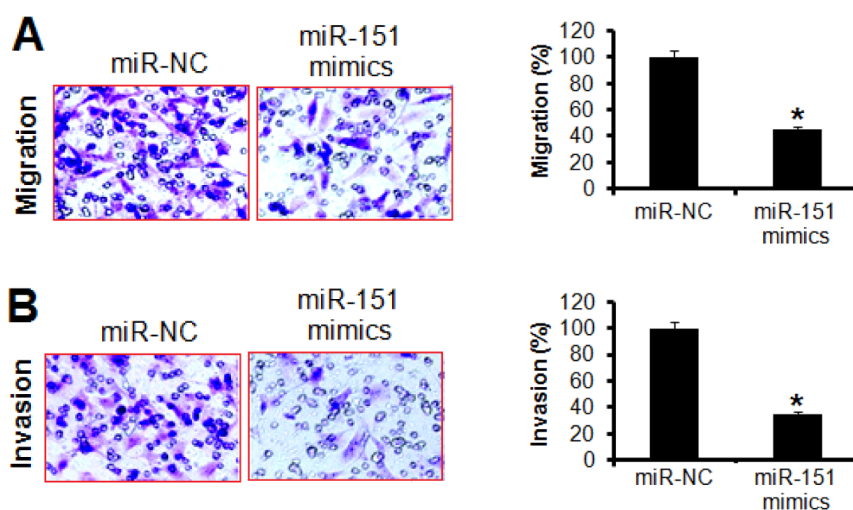


Figure 5. A: Cell migration assay and **B:** cell invasion assay showing that miR-151 decreases the migration and invasion of the PC-3 cells. The experiments were performed in triplicate and expressed as mean $\pm$ SD (* $p < 0.05$).

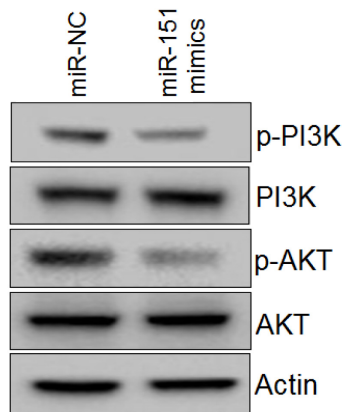


Figure 6. Western blot analysis showing that miR-151 overexpression suppresses the phosphorylation of the PI3K and AKT in PC-3 cells dose-dependently. The experiments were performed in triplicate.

in the growth and development of this malignancy [8,10,11]. The miR-151 has been shown to control the metastasis of the breast cancer [12]. The same miR has been observed to be involved in the development of cholangiocarcinoma [13]. In yet another study, miR-151 has been shown to regulate the migration and invasion of hepatocellular carcinoma [14]. The results of this study also suggest a similar type of regulation in prostate cancer and miR-151 was seen to negatively regulate the prostate cancer cell growth and its overexpression decreased the viability of cancer cells. Drugs such as 5-FU have been used for treating prostate cancer [15]. When prostate cancer cells were administered with 5-FU treatments along with miR-151 overexpression, the cancer cell growth was affected significantly and it was very low in comparison to the drug treatment alone. Hence, our study explored one more candidate miR having the potential to increase the

chemosensitivity of prostate cancer cells towards the administration of anticancer drug molecules. Cell migration and invasion are the two vital processes required for the metastasis of cancer cells [16]. Herein we found that miR-151 overexpression inhibited both the migration and invasion of the cancer cells. Furthermore, miRs have been reported to exert their effects by modulating different signalling pathways [17]. In this study we reported for the first time that miR-151 exerts its effects by suppressing the PI3K/AKT pathway in the PC-3 cells. To sum up, this study explored the anticancer role of miR-151 against the prostate cancer cell proliferation, together with its regulatory potential to enhance the chemosensitivity of cancer cells and to reduce the cancer metastasis through targeting of PI3K/AKT pathway.

Conclusion

Taken together the results of the present study indicated that miR-151 is aberrantly suppressed in prostate cancer. It regulates the growth and metastasis of prostate cancer and may act as a therapeutic target for treatment of this disease.

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Conflict of interests

The authors declare no conflict of interests.

References

1. Hu Y, Hu D, Li W, Yu X. Neoadjuvant chemotherapy brings more survival benefits than postoperative chemotherapy for resectable gastric cancer: a meta-analysis of randomized controlled trials. *JBUON* 2019;24:201-14.
2. Yu L, Zhou GQ, Li DC. MiR-136 triggers apoptosis in human gastric cancer cells by targeting AEG-1 and BCL2. *Eur Rev Med Pharmacol Sci* 2018;22:7251-6.
3. Tekesin K, Emin GM, Tural D et al. Clinicopathological characteristics, prognosis and survival outcome of gastric cancer in young patients: A large cohort retrospective study. *JBUON* 2019;24:672-8.
4. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. *CA Cancer J Clin* 2016;66:7-30.
5. Huarte M. The emerging role of lncRNAs in cancer. *Nat Med* 2015;21:1253-61.
6. Batista PJ, Chang HY. Long noncoding RNAs: cellular address codes in development and disease. *Cell* 2013;152:1298-1307.
7. Liu S, Mitra R, Zhao MM et al. The Potential Roles of Long Noncoding RNAs (lncRNA) in Glioblastoma Development. *Mol Cancer Ther* 2016;15:2977-86.
8. Xu G, Zhang Y, Li N, Zhang JB, Xu R. LncRNA CCHE1 in the proliferation and apoptosis of gastric cancer cells. *Eur Rev Med Pharmacol Sci* 2018;22:2631-7.
9. Hu Y, Ma Z, He Y, Liu W, Su Y, Tang Z. LncRNA-SN-HG1 contributes to gastric cancer cell proliferation by

- regulating DNMT1. *Biochem Biophys Res Commun* 2017;491:926-31.
10. Wang CL, Wang D, Yan BZ, Fu JW, Qin L. Long non-coding RNA NEAT1 promotes viability and migration of gastric cancer cell lines through up-regulation of microRNA-17. *Eur Rev Med Pharmacol Sci* 2018;22:4128-37.
 11. Liu Y, Yang Y, Li L et al. LncRNA SNHG1 enhances cell proliferation, migration, and invasion in cervical cancer. *Biochem Cell Biol* 2018;96:38-43.
 12. Roth A, Diederichs S. Long Noncoding RNAs in Lung Cancer. *Curr Top Microbiol Immunol* 2016;394:57-110.
 13. Xie W, Yuan S, Sun Z, Li Y. Long noncoding and circular RNAs in lung cancer: advances and perspectives. *Epigenomics-UK* 2016;8:1275-87.
 14. Qiu M, Xu Y, Wang J et al. A novel lncRNA, LUADT1, promotes lung adenocarcinoma proliferation via the epigenetic suppression of p27. *Cell Death Dis* 2015;6:e1858.
 15. Loewen G, Jayawickramarajah J, Zhuo Y, Shan B. Functions of lncRNA HOTAIR in lung cancer. *J Hematol Oncol* 2014;7:90.
 16. Liu XH, Liu ZL, Sun M, Liu J, Wang ZX, De W. The long non-coding RNA HOTAIR indicates a poor prognosis and promotes metastasis in non-small cell lung cancer. *BMC Cancer* 2013;13:464.
 17. Ono H, Motoi N, Nagano H et al. Long noncoding RNA HOTAIR is relevant to cellular proliferation, invasiveness, and clinical relapse in small-cell lung cancer. *Cancer Med* 2014;3:632-42.
 18. Sun X, Wang Z, Yuan W. Down-regulated long non-coding RNA SNHG1 inhibits tumor genesis of colorectal carcinoma. *Cancer Biomark* 2017;20:67-73.
 19. Wang Q, Li Q, Zhou P et al. Upregulation of the long non-coding RNA SNHG1 predicts poor prognosis, promotes cell proliferation and invasion, and reduces apoptosis in glioma. *Biomed Pharmacother* 2017;91:906-11.
 20. Cui Y, Zhang F, Zhu C, Geng L, Tian T, Liu H. Upregulated lncRNA SNHG1 contributes to progression of non-small cell lung cancer through inhibition of miR-101-3p and activation of Wnt/beta-catenin signaling pathway. *Oncotarget* 2017;8:17785-94.
 21. Shi F, Dong Z, Li H, Liu X, Liu H, Dong R. MicroRNA-137 protects neurons against ischemia/reperfusion injury through regulation of the Notch signaling pathway. *Exp Cell Res* 2017;352:1-8.