European Review for Medical and Pharmacological Sciences 2019; 23: 6849-6857

DANCR sponges miR-135a to regulate paclitaxel sensitivity in prostate cancer

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Abstract. – OBJECTIVE: Paclitaxel is one of the most common drugs for cancer treatment. LncRNA DANCR is a regulator of up-regulation in tumors. Our experiment aims to clarify the role of DANCR in paclitaxel sensitivity of prostate cancer.

PATIENTS AND METHODS: We found that the expression of DANCR in prostate cancer tissues and cells was significantly higher than that in normal groups. DANCR knockdown could reduce cell proliferation and induce cell apoptosis in cells. Moreover, DANCR silence enhanced the effect of paclitaxel on cell proliferation and apoptosis in prostate cancer cells.

RESULTS: DANCR targeted and negatively regulated the expression of miR-135a. miR-135a overexpression inhibited cell proliferation and promoted cell apoptosis and paclitaxel sensitivity in prostate cancer cells. miR-135a inhibition reversed the promoting effect of DANCR silence on paclitaxel sensitivity in prostate cancer cells.

CONCLUSIONS: Downregulation of DANCR increased paclitaxel sensitivity in prostate cancer cells by negatively regulating the expression of miR-135a.

Key Words:
DANCR, miR-135a, Paclitaxel, Apoptosis, Prostate Cancer.

Abbreviations

lncRNA = Long-chain non-coding RNA, Ct = cyclic threshold, WT = wild-type, MUT = mutant luciferase reporter vector, ANOVA = analysis of variance, ceRNA = competitive endogenous RNA, si-NC = siRNA control, qRT-PCR = quantitative real-time polymerase chain reaction.

Introduction

Prostate cancer is one of the most common malignant tumors in the urinary system[1]. One of the most common chemotherapeutic drugs for prostate cancer is paclitaxel, which is of great significance to the quality of life and health for cancer patients. However, the development of resistance limits the anti-tumor effect of paclitaxel[2]. Thus, it is important to find effective ways to improve the sensitivity of paclitaxel in prostate cancer cells for cancer treatment.

Long-chain non-coding RNA (lncRNA) is a molecule between 200 and 100 000 nt in length, which does not have the ability to encode proteins, but can regulate gene expression in the form of RNA in various aspects such as epigenetic regulation, transcriptional regulation and post-transcriptional regulation[3,4]. LncRNA DANCR is a recently discovered class of lncRNA, which plays an important role in the occurrence and development of tumors[5]. At present, DANCR has been found highly expressed in hepatocellular and colorectal cancers and could promote the occurrence and development of tumors[6,7]. Previous study[8] in prostate cancer has shown that the high expression of DANCR is related to the pathological stage of prostate cancer. However, the role and potential mechanism of DANCR in paclitaxel sensitivity to prostate cancer remain largely unclear.

Our experiment first detected the expression changes of DANCR in prostate cancer tissues and cell lines, and then down-regulated the expression of DANCR by cell transfection to explore the effects of down-regulated DANCR on paclitaxel sensitivity in prostate cancer cells. Moreover, we also identified its sponge miRNA by bioinformatics software to preliminarily explore its mechanism.

Patients and Methods

Patients and Tissues

Prostate cancer tissues and corresponding adjacent normal samples were collected from 36 patients with prostate cancer at Qilu Hospital of Shandong University. All tissues were imme-

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diately stored at -80°C until used. All patients provided the informed consent and this study was approved by the Ethics Committee of Qilu Hospital of Shandong University.

Cell Culture

Human prostate cancer cells (PC3, C4-2 and DU145) were purchased from the Cell Bank of Cancer Research Institute, Cancer Hospital, Chinese Academy of Sciences, and human normal prostate epithelial cells RWPE-1 were purchased from Shanghai Jining Industrial of China. Prostate cancer cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing penicillin and streptomycin, and 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA) was added before use. Normal prostatic epithelial cells RWPE-1 were cultured in Roswell Park Memorial Institute-1640 (RPMI-1640) containing penicillin and streptomycin, and 10% fetal bovine serum. The cells were cultured at 37°C in an incubator with 5% CO_2. When the growth density was 80%, the cells were passaged according to 1:3.

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

After collecting cells, RNA was extracted by TRIzol (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions and 500 ng RNA was used to cDNA synthesis by All-in-one™ Frist-Strand cDNA Synthesis Kit (Fulengen, China). qRT-PCR was performed using SYBR Premix Ex Taq (TaKaRa, Otsu, Shiga, Japan) on CFX96 Real-time PCR Systems (Bio-Rad, Hercules, CA, USA). The primer sequences used in this research were listed as: DANCR (Forward, 5'-CCTATCCCTTTCTCTAAGAA-3'; Reverse, 5'-ACTTCTGCAAAAACGTGCTG-3'); miR-135a (Forward, 5'-AACCCTGCTCGCAGTATTTGAG-3'; Reverse, 5'-GCGGCAGTATGGCTTTTTATTCC-3'); GAPDH (Forward, 5'-CCAAAATCAGATGGGGCAATGCTGG-3'; Reverse, 5'-TGATGGCATGGACTGTGGTCATTCA-3'); U6 (Forward, 5'-CTCGCTTCGGCAGCACA-3'; Reverse, 5'-AACGCTTCACGAATTTGCGT-3'). The internal parameter of DANCR was GAPDH and the internal parameter of miR-135a was U6. Quantitative analysis was compared with cyclic threshold (Ct), and standardized analysis was performed according to Ct values, calculated by $2^{\Delta\Delta Ct}$ method. The conditions of PCR were as follows: 20 s at 95°C, and 40 cycles of 10 s at 95°C and 20 s at 60°C.

Cell Transfection

In our study, Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) was used to transfect cells; the steps of transfection were the same as the standard procedure. DANCR siRNA, siRNA control, miR-135a mimics, mimics control, miR-135a inhibitor and inhibitor control were transfected into cells, respectively (Shanghai Jima Pharmaceutical Technology, Shanghai, China). Cell transfection was performed when the cell growth density reached 60%, and the fluid exchange was performed 6 hours after transfection.

MTT Assay

DU145 cells were seeded in 96-well plate, and 2000 cells were added to each well. After 48 hours of culture, DU145 cells were cultured by conventional method. MTT (2.5 mg/mL, Sigma-Aldrich, St. Louis, MO, USA) was added to each pore, incubated at 37°C for 4 hours, then the pore liquid was absorbed, and dimethyl sulfoxide (DMSO) solution was added (Sigma-Aldrich, St. Louis, MO, USA). After 10 minutes of shaking reaction, the culture plate was placed on the enzyme label to determine OD value at 570 nm.

Flow Cytometry

The cells were washed and suspended in PBS solution for 3 times, centrifuged for 10 minutes at 800 g. After the supernatant was removed, the cells were mixed with Binding Buffer. The cell concentration was 1×10^6/mL. The 100 μL cell culture medium was absorbed and transferred to centrifugal tube of 5 mL. Annexin V-FITC and PI (Solarbio, Beijing, China) were added. After mixing, the cells were incubated for 15 minutes and then mixed with 400 μL Binding Buffer. The apoptotic rate of cells was measured by flow cytometry.

Western Blot

Cells were incubated with RIPA (Sigma-Aldrich, St. Louis, MO, USA) containing PMSF (Sigma-Aldrich, St. Louis, MO, USA) on ice for 20 minutes and then transferred to a 4°C centrifuge for 10 minutes. Carefully, the absorption of protein supernatant solution and the detection of quantitatively protein samples according to BCA method were carried out. The samples were boiled at the same volume of Loading Buffer and boiled for 5 min. After 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was prepared, 40 μg protein samples were added to each swimming lane. The voltage

in concentration gel was 90V, and the voltage in the separation gel was 120V. Proteins were transferred to the polyvinylidene difluoride (PVDF) membrane at a voltage of 90V. PVDF membrane was incubated in 5% BSA (Sigma-Aldrich, St. Louis, MO, USA) blocking solution for 1 hour, and then incubated in the reaction solution of the first antibody (C-Caspase-3 antibody, Abcam, Cambridge, MA, USA; Bax antibody, Biyuntian Institute of Biotechnology, China; Ki-67 antibody, Santa Cruz Biotechnology, Santa Cruz, CA, USA; PCNA antibody, Abcam, Cambridge, MA, USA) and the second antibody (Solarbio Technology, Beijing, China). After the incubation, membranes were washed three times with TBST. Chemiluminescence was carried out according to the ECL luminescence kit (Hangzhou Lianke Biotechnology, Hangzhou, China) and the gray values of the bands were measured by Chemi Doc XRS (Bio-Rad, Hercules, CA, USA). The protein expression level was analyzed in accordance to GAPDH.

Luciferase Reporter Assay

DANCR and miR-135a were found to have complementary binding sites (chr4: 53579679-53579685) by starBase online target gene prediction software. The sequences of DANCR containing miR-135a binding sites (AAGCCAU) were cloned into pGL3-promoter vectors (Promega, Madison, WI, USA) to generate wild-type (WT) DANCR luciferase reporter vectors. The mutant luciferase reporter vector (MUT) was constructed by mutating the CRNDE binding site. MUT or WT DANCR luciferase reporter vectors were co-transfected into DU145 cells with miRNA-135a mimics or mimics control, respectively. Luciferase activity was measured by luciferase activity detection kit (Promega, Madison, WI, USA) after culture 48 hours.

Statistical Analysis

The results were presented as the mean ± SD. Statistical analysis was performed using GraphPad Prism 5 software (GraphPad, San Diego, CA, USA). The *p*-values were calculated using the one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test or Student's *t*-test. $p < 0.05$ was considered as a significant difference.

Results

The Expression of DANCR is Increased in Prostate Cancer Tissues and Cells

To explore the role of DANCR in prostate cancer, its expression was measured in 36 patients' tissues and prostate cancer cells. As shown in Figure 1A, the expression of DANCR was significantly enhanced in tumor tissues compared with that in normal samples. Moreover, the expression of DANCR in human normal prostatic epithelial cells RWPE-1 was lower than that in prostate cancer cells PC3, C4-2 and DU145 (Figure 1B). DANCR

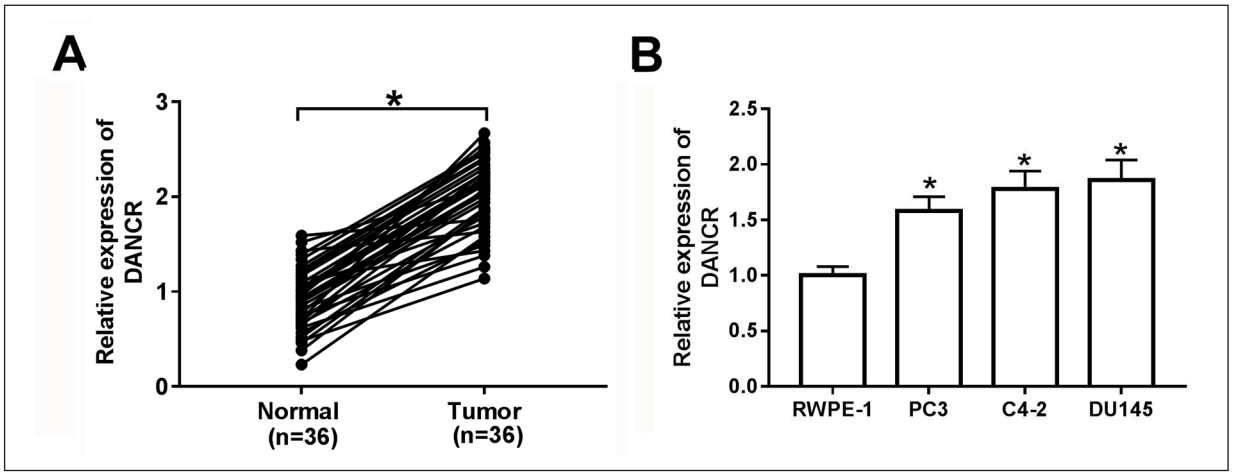

Figure 1. High expression of DANCR in prostate cancer tissues and cells. ***A,*** The expression of DANCR was measured in prostate cancer tissues and adjacent normal samples by qRT-PCR. *Compared with adjacent normal group, $p < 0.05$. ***B,*** The expression levels of DANCR in prostate cancer cell lines (PC3, C4-2 and DU145) and human normal prostatic epithelial cells (RWPE-1) were measured by qRT-PCR. *Compared with RWPE-1 group, $p < 0.05$.

was highly expressed in prostate cancer tissues and cells. We selected DU145 cells with the highest expression level to investigate the role of DANCR.

Knockdown of DANCR Increases Paclitaxel Sensitivity in Prostate Cancer Cells

In order to clarify the role of DANCR in paclitaxel sensitivity to prostate cancer, DANCR siRNA and siRNA control were transfected into DU145 cells and then treated with paclitaxel or not. The results of qRT-PCR showed that the expression of DANCR was decreased in cells transfected with DANCR siRNA (Figure 2A). Moreover, MTT assay showed that down-regulation of DANCR could reduce cell proliferation and aggravated paclitaxel-induced proliferation inhibition in DU145 cells (Figure 2B). To further clarify the effect of down-regulation of DANCR and paclitaxel on cell proliferation, we also used Western blot to detect the expression changes of Ki-67 and PCNA. The results showed that knockdown of DANCR could reduce the levels of Ki-67 and PCNA in cells with or without treatment of paclitaxel (Figure 2C). In addition, the analysis of flow cytometry revealed that silence of DANCR or paclitaxel could induce apoptosis, and silencing DANCR enhanced the apoptotic rate induced by paclitaxel (Figure 2D). Besides, DANCR knockdown promoted the expression levels of C-Caspase-3 and Bax protein in cells with or without treatment of paclitaxel (Figure 2E). These results suggested that down-regulation of DANCR can improve paclitaxel-induced proliferation inhibition and apoptosis promotion in prostate cancer cells.

DANCR is a Decoy of miR-135a

We used bioinformatics software starBase to predict that DANCR and miR-135a have complementary targeting sites (Figure 3A). To validate this prediction, luciferase reporter assay was performed by transfecting WT or MUT DANCR luciferase reporter vectors into DU145 cells. As de-

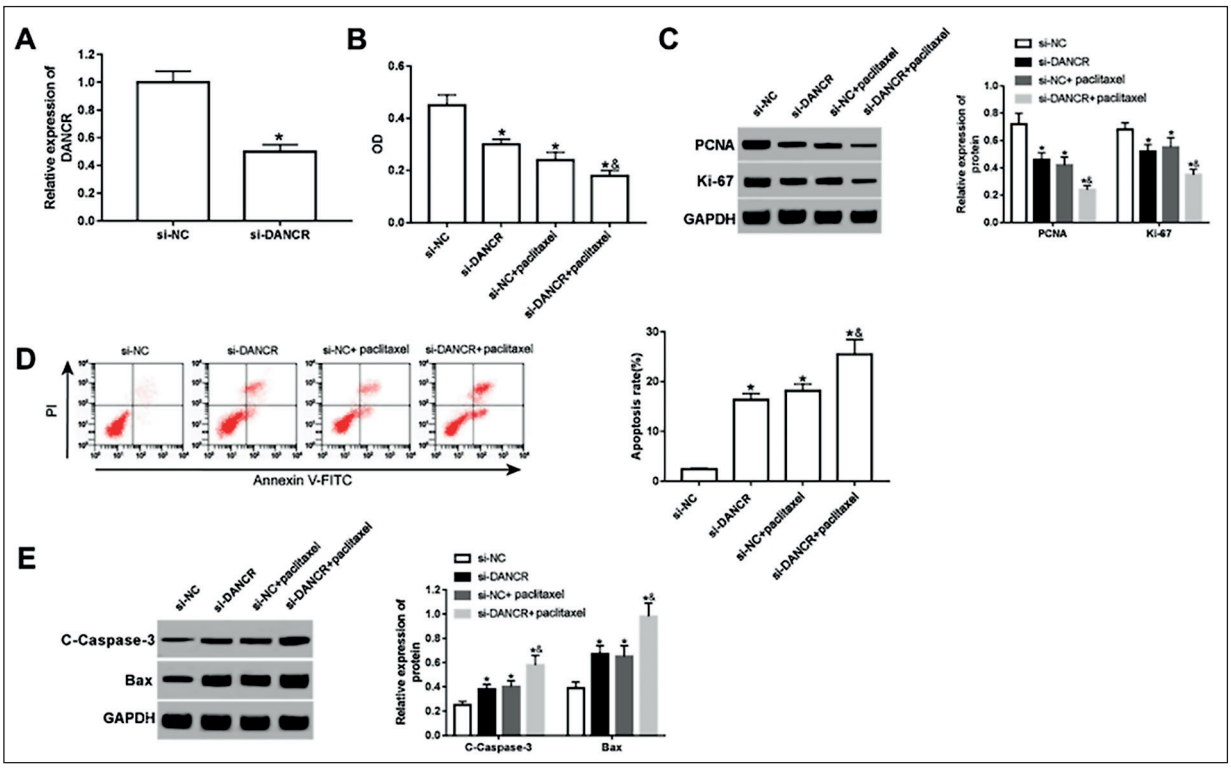

Figure 2. The effect of DANCR on paclitaxel sensitivity in prostate cancer cells. DANCR siRNA and siRNA control (si-NC) were transfected into DU145 cells and named si-DANCR and si-NC respectively. Transfected DU145 cells were treated with or without paclitaxel at 30 nM for 48 hours. ***A,*** The expression of DANCR was measured in DU145 cells transfected with si-DANCR or si-NC by qRT-PCR. ***B,*** MTT assay was used to detect the OD value at 570 nm. ***C,*** Western blot was used to detect the expression of proliferation-related protein Ki-67 and PCNA in cells. ***D,*** Flow cytometry was used to detect apoptotic changes. ***E,*** Western blot was used to detect the expression of apoptosis-related proteins C-Caspase-3 and Bax. *Compared with si-NC, $p < 0.05$; &: Compared with si-NC+Paclitaxel, $p < 0.05$.

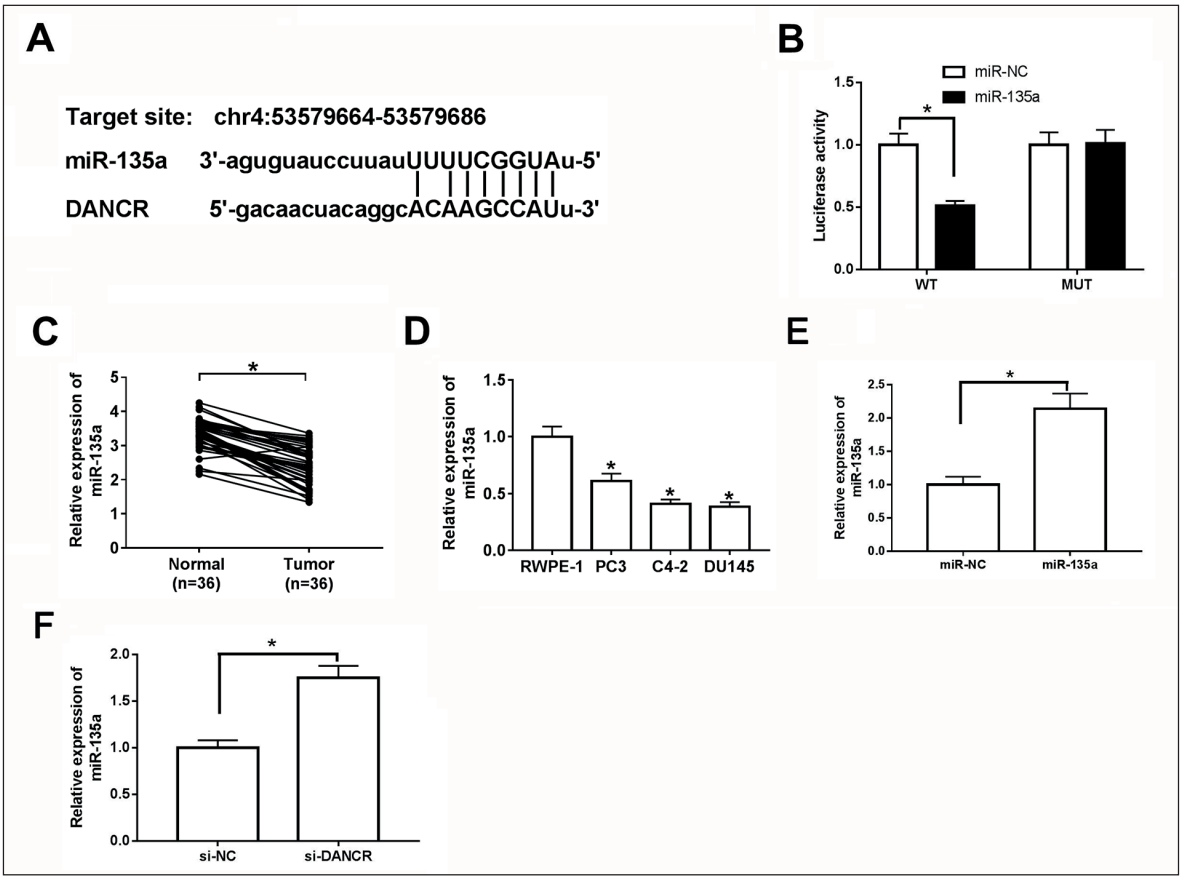

Figure 3. The association between DANCR and miR-135a. ***A,*** Using bioinformatics software starBase, we found that DANCR and miR-135a had complementary binding sites. ***B,*** Luciferase activity was measured in DU145 cells co-transfected with wild-type (WT) or mutant (MUT) luciferase reporter vector and miR-135a mimics or mimics control (miR-NC). ***C, D,*** The expression level of miR-135a was detected in prostate cancer tissues and cells by qRT-PCR. ***E,*** The expression of miR-135a was measured in DU145 cells transfected with miR-135a mimics or miR-NC by qRT-PCR. ***F,*** The abundance of miR-135a was detected in DU145 cells transfected with DANCR siRNA or siRNA control (si-NC) by qRT-PCR. *$p < 0.05$.

scribed in Figure 3B, overexpression of miR-135a led to a 50% reduction of luciferase activity in WT group, while it showed little effect in MUT group. Moreover, the expression level of miR-135a was markedly decreased in prostate cancer tissues and cells compared with that in corresponding controls (Figure 3C and 3D). Additionally, the data of qRT-PCR showed that miR-135a mimics or DANCR siRNA caused greatly increase of miR-135a abundance in DU145 cells (Figure 3E and 3F). These results suggested that DANCR can target and negatively regulate the expression of miR-135a.

Overexpression of miR-135a Improves Paclitaxel Sensitivity in Prostate Cancer Cells

To investigate the biological function of miR-135a on paclitaxel sensitivity to prostate cancer, DU145 cells were transfected with miR-135a mimics or miR-NC and then suffered from paclitaxel exposure. As displayed in Figure 4A, overexpression of miR-135a significantly decreased cell proliferation in DU145 cells and contributed to paclitaxel-induced proliferation suppression. Furthermore, addition of miR-135a reduced the expression levels of PCNA and Ki-67 protein in the presence or absence of paclitaxel (Figure 4B and 4C). In addition, the data of flow cytometry demonstrated that miR-135a overexpression increased cell apoptosis and promoted paclitaxel-induced apoptosis in DU145 cells (Figure 4D and 4E). Besides, the protein levels of C-Caspase-3 and Bax were markedly elevated by miR-135a overexpression or paclitaxel, which was aggravated by their combination (Figure 4F and 4G). These results suggested that up-regulation of miR-135a promoted paclitaxel sensitivity to prostate cancer cells.

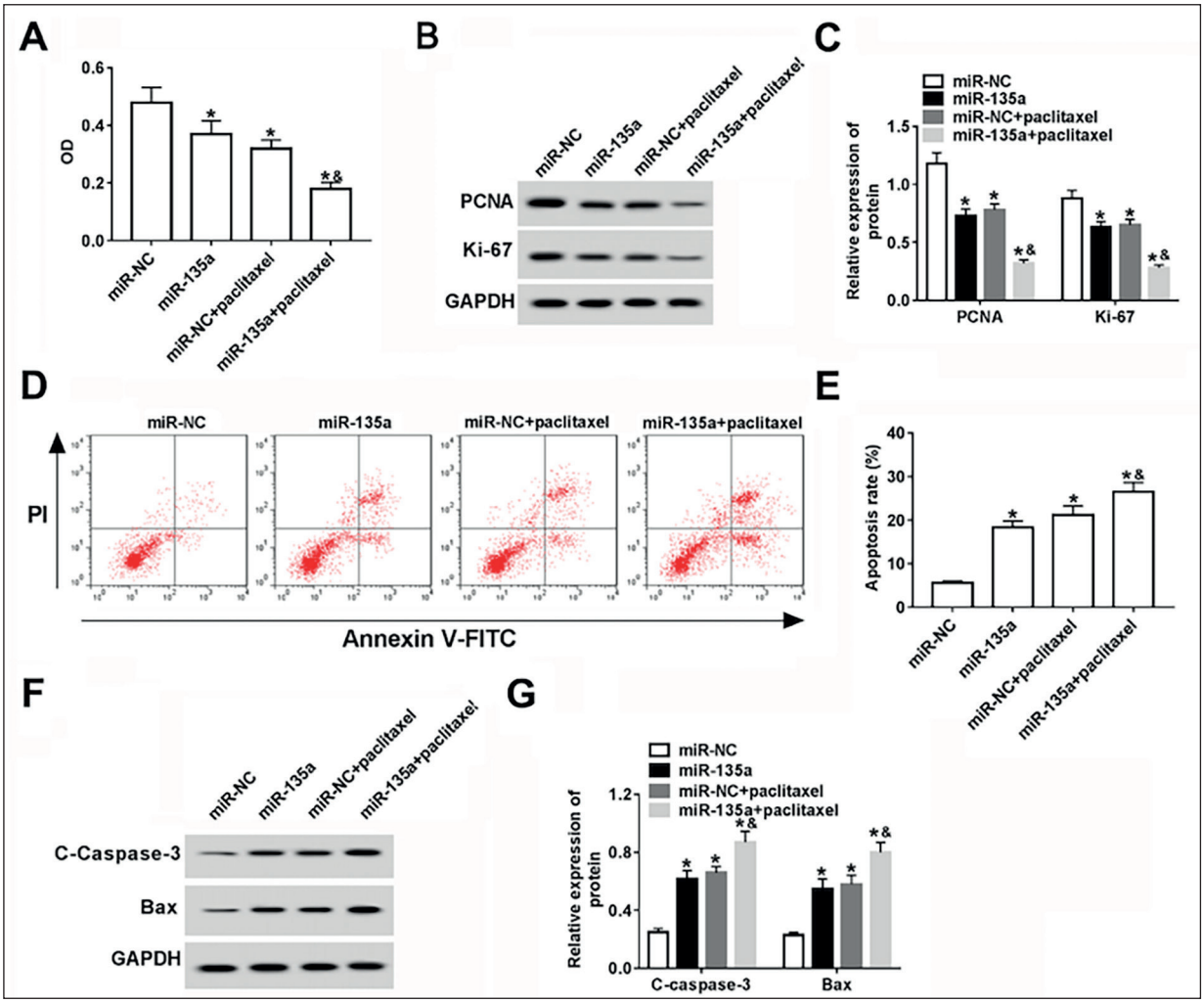

Figure 4. The effect of miR-135a on paclitaxel sensitivity in prostate cancer cells. miR-135a mimics and mimics control (miR-NC) were transfected into DU145 cells, respectively, and then treated with paclitaxel at 30 nM for 48 hours or not. ***A,*** MTT assay was performed to detect cell proliferation. ***B, C,*** The expression levels of proliferation-related protein Ki-67 and PCNA were measured in treated cells by Western blot. ***D, E,*** Flow cytometry was performed to detect apoptotic rate in treated cells. ***F, G,*** Western blot was conducted to detect the expression of apoptosis-related proteins C-Caspase-3 and Bax. *Compared with miR-NC, $p < 0.05$; & Compared with miR-NC+Paclitaxel, $p < 0.05$.

Knockdown of miR-135a Reverses the Effect of DANCR Silence on Paclitaxel Sensitivity in Prostate Cancer Cells

In order to verify whether miR-135a was involved in knockdown of DANCR-mediated paclitaxel sensitivity to prostate cancer cells, we co-transfected DANCR siRNA and inhibitor control, DANCR siRNA and miR-135a inhibitor in DU145 cells, respectively, and cultured them with 30 nM paclitaxel. Firstly, qRT-PCR assay showed that the expression level of miR-135a in cells was significantly decreased by the introduction of inhibitor of miR-135a (Figure 5A). Moreover, miRNA-135a inhibitor could reverse the down-regulation of DANCR-induced anti-proliferation role in prostate cancer cells stimulated with paclitaxel (Figure 5B). In addition, the levels of Ki-67 and PCNA protein inhibited by DANCR knockdown were restored by miR-135a deficiency in paclitaxel-treated cells (Figure 5C and 5D). Furthermore, inhibition of miR-135a weakened silencing DANCR-induced apoptosis production in paclitaxel-treated cells (Figure 5E). Meanwhile, Western blot results showed that the expression levels of C-Caspase-3 and Bax protein in cells transfected with DANCR siRNA and miR-135a inhibitor were decreased (Figure 5F). These results suggested that down-regulation of

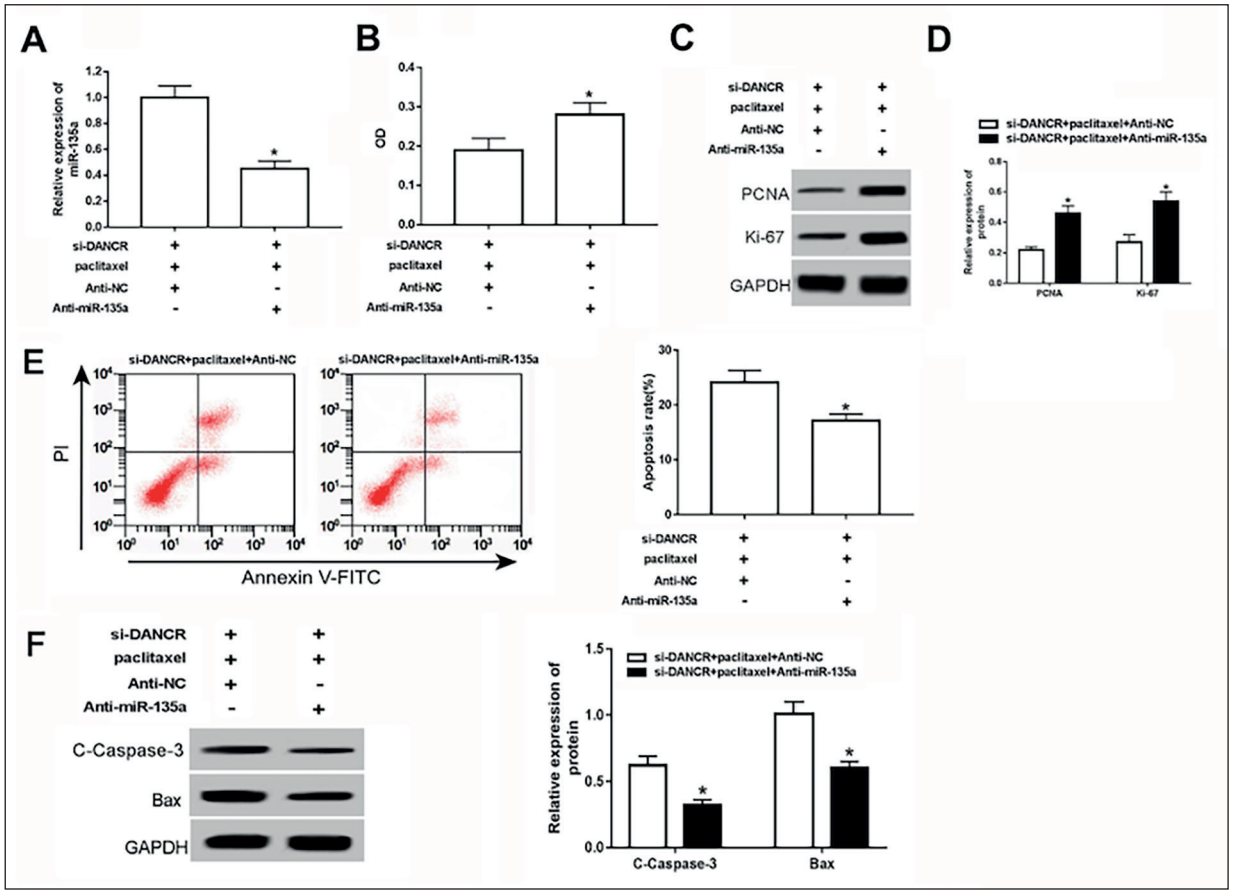

Figure 5. The effect of miR-135a knockdown on silencing DANCR-mediated paclitaxel sensitivity in prostate cancer cells. DU145 cells were co-transfected with DANCR siRNA and miR-135a inhibitor (anti-miR-135a) or inhibitor control (anti-NC) and then treated with 30 nM paclitaxel for 48 hours. ***A,*** qRT-PCR was used to detect the expression of miR-135a in cells. ***B,*** Cell proliferation was measured in treated DU145 cells by MTT. ***C, D,*** The protein levels of Ki-67 and PCNA were measured in treated cells by Western blot. ***E***, Cell apoptosis was detected in treated cells by flow cytometry. ***F,*** The protein levels of C-Caspase-3 and Bax were detected in treated DU145 cells by Western blot. $*p < 0.05$.

miR-135a reversed silence of DANCR-mediated promoting effect on paclitaxel sensitivity to prostate cancer cells.

Discussion

In our study, we found that the expression of DANCR was enhanced in prostate cancer tissues and cells, indicating that high expression of DANCR may be related to the occurrence of prostate cancer. We also observed that down-regulation of DANCR expression in prostate cancer cells by siRNA technology inhibited cell proliferation and increased cell apoptosis, suggesting that down-regulation of DANCR can play a role in the anti-malignant phenotype of prostate cancer cells. Moreover, DANCR knockdown aggravated paclitaxel-induced proliferation inhibition and apoptosis production, which fully demonstrated that down-regulation of DANCR could increase the sensitivity of prostate cancer cells to paclitaxel. In our experiments, we found that DANCR can target the expression of miR-135a, and overexpression of miRNA-135a increased the paclitaxel sensitivity of prostate cancer cells, while deficiency of miR-135a reversed the down-regulation of DANCR-mediated paclitaxel sensitivity in prostate cancer. These findings suggested that down-regulation of DANCR could enhance the paclitaxel sensitivity of prostate cancer cells by sponging miR-135a.

LncRNAs play important roles in the progression and chemoresistance of prostate cancer[9,10]. DANCR is a newly discovered lncRNA molecule related to tumors in recent years[11]. High expres-

sion of DANCR in rectal cancer patients was associated with poor tumor progression and prognosis[12]. Furthermore, DANCR could also promote osteosarcoma and gliomas progression by acting as miRNA sponge or competitive endogenous RNA (ceRNA)[13-15]. In our research, we found that DANCR is highly expressed in prostate cancer, suggesting the carcinogenic role of DANCR in prostate cancer, which was revealed by which DANCR knockdown decreased proliferation and induced apoptosis. Moreover, silencing DANCR improved the sensitivity of prostate cancer cells to paclitaxel, which is also in agreement with that promoting chemoresistance other cancers[16,17]. These findings detected that down-regulation of DANCR may be one of the promising ways to increase chemotherapy outcomes of patients with prostate cancer.

LncRNA usually functions as ceRNA or sponge of miRNA to exhibit its role. This study first demonstrated that miR-135a was a target of DANCR. We found that DANCR and miR-135a also have complementary binding sites and down-regulation of DANCR can increase the expression of miR-135a in prostate cancer cells. Previous studies have shown that miR-135a plays an important role in the progression of tumors, miR-135a is low-expressed in tumors and plays a protective role in the progression of tumors[18-20]. Our results showed that miR-135a expression was decreased in prostate cancer tissues and cells and its overexpression played anti-proliferation and pro-apoptotic roles *in vitro*, suggesting the tumor-suppressive role of miR-135a, which is also consistent with former works[21-24]. Moreover, previous study indicated that miR-135a could enhance drug sensitivity to cisplatin and paclitaxel in ovarian cancer[25]. Similarly, this research also showed that the sensitivity of prostate cancer cells to paclitaxel was also enhanced by miR-135a. In addition, downregulation of DANCR increased-sensitivity of paclitaxel in prostate cancer was reversed by decreasing the expression of miR-135a, uncovering that DANCR regulated paclitaxel sensitivity by sponging miR-135a.

Conclusions

DANCR expression was elevated in prostate cancer. This study was the first to demonstrate DANCR knockdown as a sensitizer of paclitaxel in prostate cancer cells. Moreover, we explored a novel mechanism underlying that the function of DANCR was associated with miR-135a, which provides a new idea for improving paclitaxel-based chemotherapy efficacy for prostate cancer patients.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Fizazi K, Tran N, Fein L, Matsubara N, Rodriguez-Antolin A, Alekseev B, Ozguroglu M, Ye D, Feyerabend S, Protheroe A, De Porre P, Kheoh T, Park Y, Todd MB, Chi K; LATITUDE Investigators. Abiraterone plus prednisone in metastatic, castration-sensitive prostate cancer. N Engl J Med 2017; 377: 352-360.
2) Kopczyńska E. Role of microRNAs in the resistance of prostate cancer to docetaxel and paclitaxel. Contemp Oncol (Pozn) 2015; 19: 423-427.
3) Nie W, Ge HJ, Yang XQ, Sun X, Huang H, Tao X, Chen WS, Li B. LncRNA- UCA1 exerts oncogenic functions in non-small cell lung cancer by targeting miR-193a-3p. Cancer Lett 2016; 371: 99-106.
4) Peng W, Wang Z, Fan H. LncRNA NEAT1 impacts cell proliferation and apoptosis of colorectal cancer via regulation of akt signaling. Pathol Oncol Res 2017; 23: 651-656.
5) Zhang L, Yang C, Chen S, Wang G, Shi B, Tao X, Zhou L, Zhao J. Long noncoding RNA DANCR is a positive regulator of proliferation and chondrogenic differentiation in human synovium-derived stem cells. DNA Cell Biol 2017; 36: 136-142.
6) Liu Y, Zhang M, Liang L, Li J, Chen YX. Over-expression of lncRNA DANCR is associated with advanced tumor progression and poor prognosis in patients with colorectal cancer. Int J Clin Exp Pathol 2015; 8: 11480-11484.
7) Yuan S, Wang J, Yang F, Tao Q, Zhang J, Wang L, Yang Y, Liu H, Wang Z, Xu Q, Fan J, Liu L, Sun S, Zhou W. Long noncoding RNA DANCR increases stemness features of hepatocellular carcinoma by derepression of CTNNB1. Hepatology 2016; 63: 499-511.
8) Jia J, Li F, Tang XS, Xu S, Gao Y, Shi Q, Guo W, Wang X, He D, Guo P. Long noncoding RNA DANCR promotes invasion of prostate cancer through epigenetically silencing expression of TIMP2/3. Oncotarget 2016; 7: 37868-37881.
9) Xue D, Lu H, Xu HY, Zhou CX, He XZ. Long noncoding RNA MALAT1 enhances the docetaxel resistance of prostate cancer cells via miR-145-5p-mediated regulation of AKAP12. J Cell Mol Med 2018; 22: 3223-3237.
10) Jiang H, Xiong W, Chen L, Lv Z, Yang C, Li Y. Knockdown of the long noncoding RNA HOTTIP inhibits cell proliferation and enhances cell sensitivity to

cisplatin by suppressing the Wnt/β-catenin pathway in prostate cancer. J Cell Biochem 2019; 120: 8965-8974.

11) Zhang J, Tao Z, Wang Y. Long non-coding RNA DANCR regulates the proliferation and osteogenic differentiation of human bone-derived marrow mesenchymal stem cells via the p38 MAPK pathway. Int J Mol Med 2018; 41: 213-219.

12) Ma X, Wang X, Yang C, Wang Z, Han B, Wu, Zhuang L. DANCR acts as a diagnostic biomarker and promotes tumor growth and metastasis in hepatocellular carcinoma. Anticancer Res 2016; 36: 6389-6398.

13) Jiang N, Wang X, Xie X, Liao Y, Liu N, Liu J, Miao N, Shen, Peng J. lncRNA DANCR promotes tumor progression and cancer stemness features in osteosarcoma by upregulating AXL via miR-33a-5p inhibition. Cancer Lett 2017; 405: 46-55.

14) Wang Y, Zeng X, Wang N, Zhao W, Zhang X, Teng S, Zhang Y, Lu Z. Long noncoding RNA DANCR, working as a competitive endogenous RNA, promotes ROCK1-mediated proliferation and metastasis via decoying of miR-335-5p and miR-1972 in osteosarcoma. Mol Cancer 2018; 17: 89.

15) Yang JX, Sun Y, Gao L, Meng Q, Yang BY. Long non-coding RNA DANCR facilitates glioma malignancy by sponging miR-33a-5p. Neoplasma 2018; 65: 790-798.

16) Ma Y, Zhou G, Li M, Hu D, Zhang L, Liu P, Lin K. Long noncoding RNA DANCR mediates cisplatin resistance in glioma cells via activating AXL/PI3K/Akt/NF-κB signaling pathway. Neurochem Int 2018; 118: 233-241.

17) Xu YD, Shang J, Li M, Zhang YY. LncRNA DANCR accelerates the development of multidrug resistance of gastric cancer. Eur Rev Med Pharmacol Sci 2019; 23: 2794-2802.

18) Zhao X, Sun Z, Li H, Jiang F, Zhou J, Zhang L. MiR-135a-5p modulates biological functions of thyroid carcinoma cells via targeting VCAN 3'-UTR. Cancer Biomark 2017; 20: 207-216.

19) Zhang T, Wang N. MiR-135a confers resistance to gefitinib in non-small cell lung cancer cells by up-regulation of RAC1. Oncol Res 2018; 26: 1191-1200.

20) Xie Y, Li F, Li Z, Shi Z. MiR-135a suppresses migration of gastric cancer cells by targeting TRAF5-mediated NF-κB activation. Onco Targets Ther 2019; 12: 975-984.

21) Wan X, Pu H, Huang W, Yang S, Zhang Y, Kong Z, Yang Z, Zhao P, Li A, Li T, Li Y. Androgen-induced miR-135a acts as a tumor suppressor through downregulating RBAK and MMP11, and mediates resistance to androgen deprivation therapy. Oncotarget 2016; 7: 51284-51300.

22) Tribollet Y, Barenton B, Kroiss A, Vincent S, Zhang L, Forcet C, Cerutti C, Périan S, Allioli N, Samarut J, Vanacker JM. MiR-135a inhibits the invasion of cancer cells via suppression of ERRα. PLoS One 2016; 11: e0156445.

23) Xu B, Tao T, Wang Y, Fang F, Huang Y, Chen S, Zhu W, Chen M. hsa-miR-135a-1 inhibits prostate cancer cell growth and migration by targeting EGFR. Tumour Biol 2016; 37: 14141-14151.

24) Xu B, Lu X, Zhao Y, Liu C, Zhang X, Chen S, Zhu W, Zhang L, Chen M. MicroRNA-135a induces prostate cancer cell apoptosis via inhibition of STAT6. Oncol Lett 2019; 17: 1889-1895.

25) Fukagawa S, Miyata K, Yotsumoto F, Kiyoshima C, Nam SO, Anan H, Katsuda T, Miyahara D, Murata M, Yagi H, Shirota K, Yasunaga S, Kato K, Miyamoto S. MicroRNA-135a-3p as a promising biomarker and nucleic acid therapeutic agent for ovarian cancer. Cancer Sci 2017; 108: 886-896.