© IJHG 2021
PRINT: ISSN 0972-3757 ONLINE: ISSN 2456-6330
Int J Hum Genet, 21(4): 185-192 (2021)
DOI: 10.31901/24566330.2021/21.04.794

LncRNA DANCR Restrains Sensitivity to 5-fluorouracil in Prostate Cancer through Sponging MiR-577

Minghua Zhang[1], Xin Xiao[2], Minqiang Shen[3], Xiaohui Huang[4], Jinbiao Zhou[5] and Yinghua Jiang[6,*]

[1]Department of Urology Huzhou Cent Hosp, Affiliated Cent Hosp HuZho University, 1558 Sanhuan Rd, Huzhou, Zhejiang, Peoples R China, 313000
[2]Operation Room, Huzhou Cent Hosp, Affiliated Cent Hosp HuZhou University, 1558 Sanhuan Rd, Huzhou, Zhejiang, Peoples R China, 313000
E-mail: [1]<vxahfgiatrti@163.com>, [2]<drhpnrwqjrnr@163.com>, [3]<rynczfouxqzh@163.com>, [4]<kcitijrmiefi@163.com>, [5]<awuovjiiomzb@163.com>, [6,]<cza56885@126.com>*

KEYWORDS B Cell Leukemia/Lymphoma 2. BCL2 Associated X. Fluorouracil. Long Noncoding RNA Differentiation Antagonising Non-protein Coding RNA. MicroRNA-577. Proliferation

ABSTRACT This present study explored the functions of lncRNA DANCR on regulating sensitivity to 5-fluorouracil (5-FU) in prostate cancer in vitro. The RT-qPCR examined RNA expressions of LNCRNA DANCR in RWPE-1, VCaP, PC3 and LNCaP cells, which also measured RNA levels of miR-577 in PC3 cells. DANCR was highly expressed in prostate cancer cell lines. 5-FU (0, 1, 5 and 10¼M) treatment induced the decrease of PC3 cell viability and low RNA expressions of DANCR but increased miR-577 in PC3 cells. The luciferase reporter test detected the binding between DNACR and miR-577 . Interactions between DANCR and miR-577 were examined. Knockdown of DANCR downregulated DANCR and Bcl-2 RNA expressions but accelerated cell viability and upregulated Bax, which were enhanced by the overexpression of miR-577. Hence, DANCR might restrain sensitivity of prostate cancer cells to 5-FU by downregulating miR-577.

INTRODUCTION

Prostate cancer (PCa), the most commonly diagnosed non-cutaneous cancer in developed countries, is the second most common cancer that occurs in males and the fifth leading cause of cancer deaths (Bray et al. 2018; Torre et al. 2015). Therefore, screening and managing PCa in an early stage is one of the most challenging and controversial issues in all of medicine. As for risk factors of PCa, race, advanced ages and family history of the patient have been already verified (Goggins and Wong 2009; Grozescu and Popa 2017). Among races, Africans have the highest mortality, followed by Caucasians and the yellow race ranks last (Goggins and Wong 2009; Winterich et al. 2009). Though Asian males have the lowest risk of PCa, morbidity and mortality of PCa have been gradually growing, which mainly occurs in people over 65 years of age (Nath et al. 2012). Prostate-specific antigen has been widely used for distinguishing PCa, but it still has problems in differentiating inert carcinoma and aggressive cancer, which causes plenty of unnecessary inspections and treatments (Etzioni et al. 1998; Prensner et al. 2012). Apart from surgery, androgen-deprivation therapy, radiotherapy, ablation therapy, chemotherapy and immunotherapy are used as treatments of PCa (Evans 2018). In chemotherapy, 5-fluorouracil has already been widely used. Besides that, 5-FU alone or in combination with other drugs were adapted in clinical treatments for a variety of cancers including PCa (Satari et al. 2019; Wei et al. 2018). Unfortunately, cell toxicity and resistance limit the use of 5-FU at the clinical stage (Blondy et al. 2020; Chang et al. 2020; Longley et al. 2003). Considering this condition, it is urgent to discover new biomarkers to improve efficacy of 5-FU and to reduce its toxicity to PCa cells.

LncRNAs are abnormally expressed in many kinds of human diseases, which can help morbidity or maintain disease status (Prensner and Chinnaiyan 2011). Furthermore, lncRNAs are considered to be an essential part in progressions of cancers with participating biological processes

*Address for correspondence:
Yinghua Jiang,
198 Hongqi Road, Huzhou, Zhejiang 313000,
Peoples R China
E-mail: cza56885@126.com

such as gene expression regulation, cell cycle regulation, differentiation, transcription, etc. (Li et al. 2014; Ponting et al. 2009; Zhang et al. 2013). Because of carcinogenic or tumour suppressive function, lncRNAs have played an important role at almost every stage of PCa (Bolton et al. 2014). DANCR enhanced the invasion of PCa by suppressing TIMP2/3 (Jia et al. 2016). Besides that, LncRNA DANCR facilitated drug resistance of PCa to docetaxel with targeting miR-34a-5p and JAG1 axis (Ma et al. 2019). Based on previous studies, DANCR might be a promising therapeutical target that regulates 5-FU sensitivity in PCa cells.

MicroRNAs (miRNA) are endogenous noncoding RNAs, which are small (21-22 nucleotides). MiRNAs mainly regulate gene expressions through the degradation or translation suppression of messenger RNA (Correia de Sousa et al. 2019). As biomarkers, miRNAs were also used to identify types of PCa (Abramovic et al. 2020; Fabris et al. 2016). MiRNA-29b-3p not only suppressed proliferation of PCa cells and induced apoptosis in vitro, but also reduced xenograft tumour growth in mice (Sur et al. 2019). MiR-577 targeted SMURF1, causing decreased SNHG3 expression, proliferation, invasiveness and migration but facilitated apoptosis of PCa cells (Li et al. 2020). Moreover, the negative correlation between DANCR and miR-577 has been reported in colorectal cancer detection and osteoarthritis (Fan et al. 2018; Wang et al. 2018). Nevertheless, their interaction was never mentioned in PCa. In order to enrich detections of DANCR in PCa, miR-577 was applied for checking the chemosensitivity of PCa to 5-FU with DANCR expression.

Objectives

The present study aimed to examine RNA expressions of lncRNA DANCR and miR-577 in prostate cancer cells with 5-FU treatment and changes of cell viability and apoptotic factors.

Experimental Data

Main Reagents

The main reagents used for the study included RWPE-1 (ATCC, USA), VCaP (ATCC, USA), PC3 (ATCC, USA), LNCaP (ATCC, USA), RPMI-1640 medium (Gibco™, USA), ten percent foetal bovine serum (Gibco™, USA), pcDNA3.1 vector (Invitrogen, USA), Lipofectamine 3000 (Invitrogen™, USA), TRIzol™ Reagent (Invitrogen™, USA), NGS Reverse Transcription Kit (Ion Torrent™, USA), 5-FU (Sigma-Aldrich, USA), CCK-8 (Sigma-Aldrich, USA), and psiCHECK™-2 vector (Promega, USA).

METHODOLOGY

Cell Culture

Human normal prostate epithelial cell line RWPE-1 and prostate cancer cell lines (VCaP, PC3 and LNCaP) were all acquired form ATCC (USA). Later, the cells were all cultivated in RPMI-1640 medium (Gibco™, USA) containing ten percent foetal bovine serum (FBS) at 37^0C and five percent CO_2. Cells in log phase were selected for further experiments.

Cell Transfection

Small interfering RNAs of DANCR were produced by GenePharma (Shanghai, China) called siNC and siDANCR. MirVana™ miRNA inhibitor and mimics (Invitrogen™, USA) were performed to change levels of miR-577. Overexpression of DANCR was created by pcDNA3.1 vector from Invitrogen™ (USA). Then, PC3 cells were applied for transfection with Lipofectamine 3000 (Invitrogen™, USA). Cells were first incubated in a 12-well plate with 1×10^5 cells per well and transfection was conducted after cell confluences reached fifty percent, cells were grouped and transfected with siNC, siDANCR, NC inhibitor, miR-577 inhibitor, siDANCR with miR-577 mimics, oeNC and oeDANCR at 37^0C for 4 hours. Later, cells were cultivated at RPMI-1640 medium with ten percent FBS for 48 hours at 37^0C, with five percent CO_2. Expressions of DANCR and miR-577 were quantified through RT-qPCR.

RT-qPCR

Total RNA was extracted from RWPE-1, VCaP, PC3 and LNCaP cell lines following instructions of the TRIzol™ Reagent (Invitrogen™, USA). Later, reverse transcription was applied

for obtaining cDNA using the NGS Reverse Transcription Kit (Ion Torrent™, USA). Thereafter, PCR was processed for amplification. Conditions were listed as following, that is, pre-denaturation, 95^0C, 5 minutes, denaturation, 95^0C, 30 seconds, annealing and extension, 60^0C, 30 seconds, 35 cycles. Sequences of primers were displayed as, DANCR, F (forward), 5'- GCCACTATGTAGAGGGTTTC-3', and R (reverse), 5'- ACCTGCGCTAAGAA-3' (Jia et al. 2016), miR-577, F, 5í- TGCGGTAGATAAAATATTGG-3', and R, 5'- GTGCAGGGTCCGAGGT-3' (Wang et al. 2018), Bcl-2, F, 5'- GAGTACCTGAACCGGCATCT-3', and R, 5'- GGTATGCACCCAGAGTGATG-3' (Li et al. 2017), Bax, F, 5'- CAGGATGCGTCCACCAAGAA-3', and R, 5'- AGTAGAAGAGGGCAACCACG-3' (Li et al. 2017), GAPDH, F, 5'- ATGGGGAAGGTGAAGGTCGG-3', and R, 5'- GACGGTGCCATGGAATTTGC-3' (Jia et al. 2016), and U6, F, 5'- GCTTCGGCAGCACATATACTAAAAT-3', and R, 5'- CGCTTCACGAATTTGCGTGTCAT-3' (Wang et al. 2018). Relative expressions were calculated using $2^{-\Delta\Delta Ct}$ methods, and GAPDH and U6 were internal references for DANCR and miR-577, respectively. The experiment was repeated three times.

Cell Toxicity Detection

After transfection, PC3 cells containing siNC, siDANCR, NC inhibitor, miR-577 inhibitor, siDANCR with miR-577 mimics, oeNC and oeDANCR in log phase were gathered and treated by 5-FU (0, 1μM, 5μM and 10μM, Sigma-Aldrich, USA) (Satari et al. 2019), and then seeded onto a 96-well plate with 1ṁ10^4 cells per well and kept for culturing for 48 hours. Then, 10μl CCK-8 (Sigma-Aldrich) was added into each well and incubated with cells for another 2 hours at 37^0C. Finally, optical density (OD) values were analysed at 450nm wavelength using the Multiskan™ FC Microplate Photometer (Thermo Scientific™, USA).

Dual Luciferase Reporter Assay

Putative binding sites of DANCR with miR-577 were provided by LncBase Predicted v.2 (http://carolina.imis.athena-innovation.gr). Afterwards, wild type sequences of DANCR with putative binding sites of miR-577 and its mutant type were inserted into psiCHECKμ-2 vector (Promega, USA) for creating DANCR-wt and DANCR-mut. Then, miR-577 inhibitor with DANCR-wt/mut and NC inhibitor with DANCR-wt/mut were co-transfected into PC3 cells using Lipofectamine 3000. Relative luciferase activities were quantified with GloMaxÆ Discover Microplate Reader (Promega, USA) for 48 hours after transfection.

Statistical Analysis

All experiments were run in a triplicate and data were displayed as mean ± SD. GraphPad Prism 7 (USA) was applied to analyse data. Comparisons among groups were examined with Student's t-test and one-way ANOVA. $P<0.05$ was considered to have statistical significance.

RESULTS

5-FU Downregulated Viability of Prostate Cancer Cell and Inhibited Expression of lncRNA DANCR

In order to confirm the role of DANCR, expressions of DANCR were first checked in RWEP-1, VCaP, PC3 and LNCaP cell lines, which showed that RNA levels of DANCR were increased in PCa cell lines compared to REPE-1 cell line, especially in PC3 cell line (Fig.1A, $^{**}P<0.05$). Then, 5-FU was applied to treat PC3 cells for measuring suppression in cell viability. With concentrations of 5-FU growing, cell viabilities of PC3 cells were reduced in a dose dependent manner (Fig. 1B, $^{**}P<0.05$). Meanwhile, DANCR was declined in a dose dependent manner with 5-FU densities growing (Fig. 1C, $^{**}P<0.05$). Considering DANCR was inhibited with 5-FU application, overexpression of DANCR was performed to check functions of DANCR. After overexpression, the expression of DANCR was upregulated compared to the control group after 5-FU (10ĕM) treatment (Fig. 1D, $^{**}P<0.05$). Then, toxicity of 5-FU to PC3 cells with overexpressed DANCR were evaluated, indicating that upregulation of DANCR reduced 5-FU toxicity (Fig. 1E, $^{**}P<0.05$).

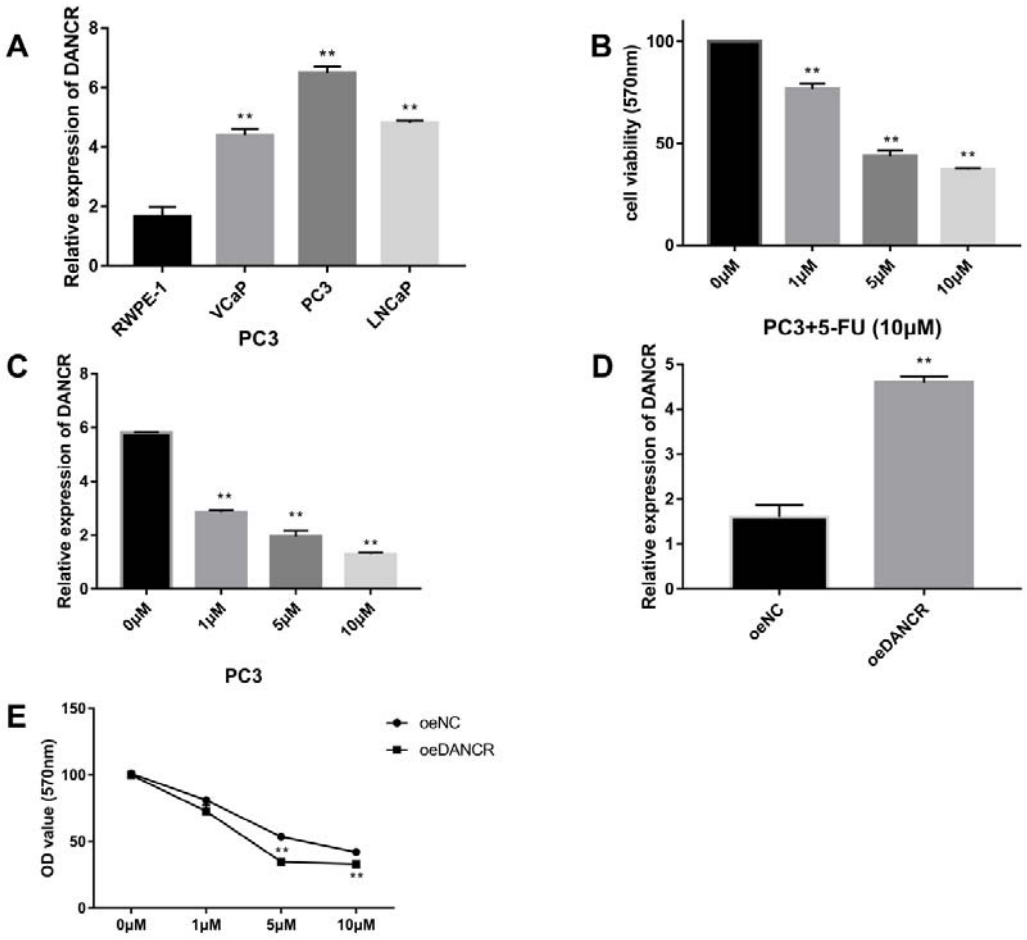

Fig. 1. 5-FU downregulated viability of prostate cancer cell and inhibited expression of lncRNA DANCR
A: Relative RNA expression of lncRNA DANCR were measured using RT-qPCR in RWEP-1, VCaP, PC3 and LNCaP cell lines, **P<0.05
B: Cell viabilities of PC3 cells with upregulated concentrations of 5-FU (0, 1μM, 5μM and 10μM) were evaluated by CCK-8, **P<0.05
C: Expressions of DANCR in PC3 cells with 5-FU treatment (0, 1μM, 5μM and 10μM) were examined using RT-qPCR, **P<0.05
D: RNA levels of DANCR after overexpression were assessed using RT-qPCR, **P<0.05
E: Toxicity of PC3 cells with the overexpression of DANCR were evaluated through CCK-8, **P<0.05. All results were from three independent experiments
Source: Authors

MiR-577, the Target of lncRNA DANCR, Expressed Higher in 5-FU-Treated PC3 Cells and Promoted Cell Toxicity

Through LncBase Predicted v.2, putative binding sites of miR-577 with DANCR were provided (Fig. 2A). Furthermore, the binding between them was evaluated using luciferase reporter test, showing that miR-577 inhibitor and DANCR-wt group showed the highest fluorescence compared with other three groups (Fig. 2B, **P<0.05). According to this, miR-577 ex-

pression in PC3 cells with 5-FU (0, 1μM, 5μM and 10μM) were detected, reminding that the RNA level of miR-577 was upregulated dose dependently (Fig. 2C, **P<0.05). Hence, suppressed miR-577 was employed and miR-577 was reduced (Fig. 2D, **P<0.05). Later, cell toxicity of 5-FU in PC3 cells with miR-577 inhibitor was measured, revealing that the toxicity was reduced by the transfection of miR-577 inhibitor (Fig. 2E, **P<0.05). Each experiment was repeated three times.

MiR-577 Hindered Roles of lncRNA DANCR and Upregulated 5-FU Sensitivity in Prostate Cancer Cells

As functions of DANCR and miR-577 and their binding were measured, the interaction be-

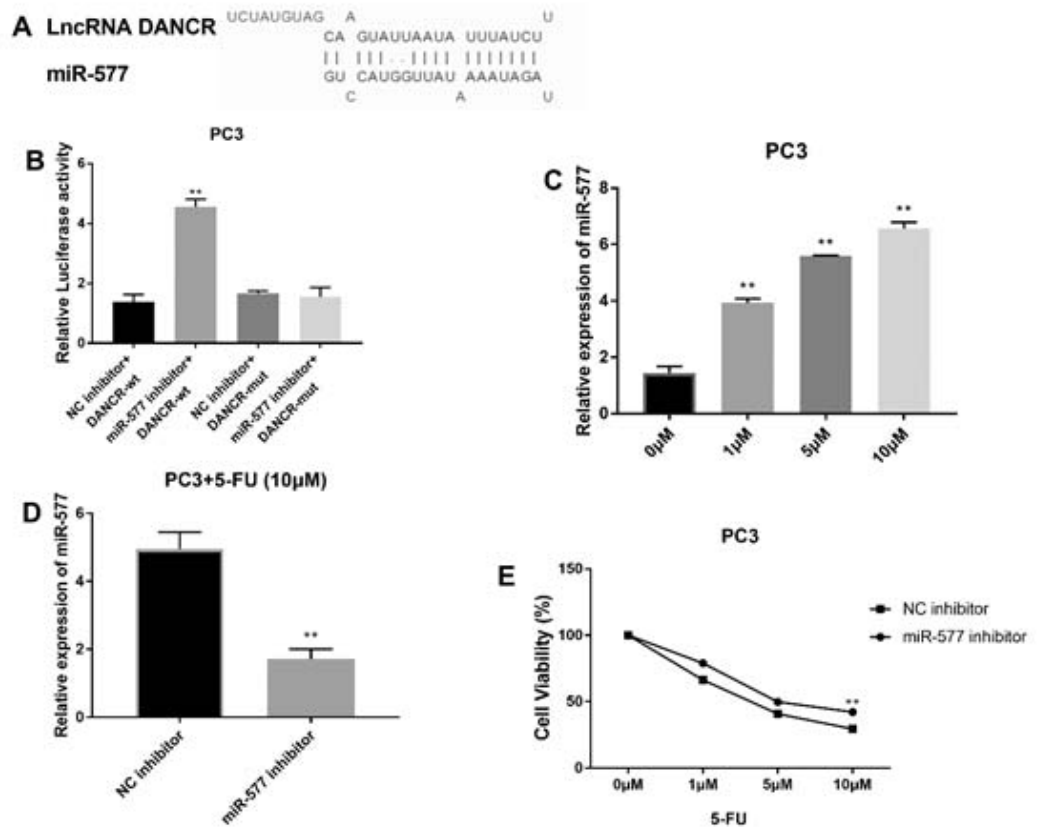

Fig. 2. MiR-577 was the target of lncRNA DANCR and expressed higher in 5-FU treated prostate cancer cell line
A: Putative binding sites between miR-577 and DANCR were predicted by LncBase Predicted v.2 (http://carolina.imis.athena-innovation.gr)
B: The binding between NC inhibitor+DANCR-wt/mut and miR-577+DANCR-wt/mut were determined using dual luciferase reporter assay, **P<0.05
C: Relative RNA expression of miR-577 in PC3 cells after 5-FU treatment (0, 1μM, 5μM and 10μM) were validated by RT-qPCR, **P<0.05
D: Expressions of miR-577 with NC inhibitor and miR-577 inhibitor were detected in PC3 cells with 5-FU (10μM) using RT-qPCR, **P<0.05
E: Toxicity of PC3 cells with 5-FU (10μM) and inhibitor of NC or miR-577 were evaluated using CCK-8, **P<0.05
Source: Authors

tween those two biomarkers was analysed. As DANCR was upregulated and miR-577 was repressed, knockdown of DANCR and overexpression of miR-577 were created to make a full-scale cognition. After the transfection of inhibited DANCR and overexpressed miR-577, expression of miR-577 was upregulated while DANCR level was obviously decreased (Fig. 3A, $^{**}P<0.05$). Meanwhile, toxicity was checked, showing that toxicity was promoted by suppressed transfection of DANCR, which was enhanced by miR-577 mimics (Fig. 3B, $^{**}P<0.05$). Moreover, mRNA expression of Bcl-2 was reduced while Bax was upregulated with the transfection of siDANCR and miR-577 mimics (Fig. 3C, $^{**}P<0.05$). All experiments were run in a triplicate.

DISCUSSION

Although extensive research of prostate cancer has been conducted, the mechanism of PCa and its treatments are still mysterious (Grozescu and Popa 2017). Besides that, over-diagnosis and overtreatment increase risk in PCa treatment. Therefore, it is necessary to find new biomarkers to improve ways for dealing with this disease. 5-fluorouracil (5-FU) is a kind of chemotherapeutant for preventing cellular metabolism of cancer cells (Wigmore et al. 2010). Moreover, effects of 5-FU on PCa have been measured as well, which showed that 5-FU could accelerate apoptosis and suppress proliferation of prostate cancer cells (Manogue et al. 2018). Therefore, 5-FU was chosen for measuring its antiproliferative capacity. According to the previous study of, lncRNA NEAT1 facilitated 5-FU resistance in colorectal cancer cells through promoting expressions of ALDH1 and c-Myc and chromatin remodelling (Zhu et al. 2020). LncRNA DANCR suppressed sensitivity to cisplatin in glioma cells, in which DANCR upregulated AXL, causing the activation of PI3K/Akt/NF-κB signaling path-

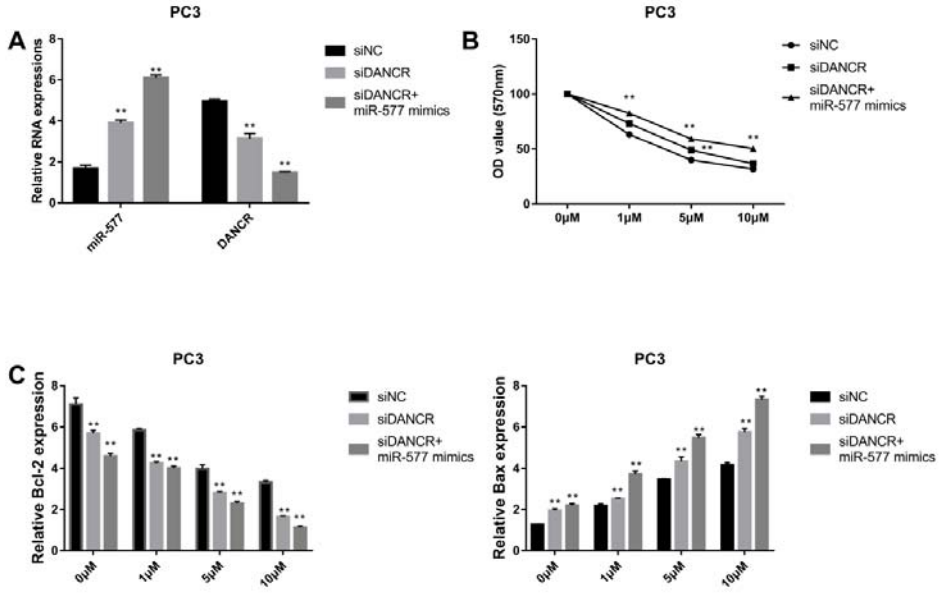

Fig. 3. MiR-577 upregulated 5-FU sensitivity in prostate cancer cells by restraining lncRNA DANCR
A: Relative RNA expressions of DANCR and miR-577 were assessed with RT-qPCR after DANCR inhibition and miR-577 overexpression, $^{**}P<0.05$
B: Toxicities of PC3 cells with DANCR inhibition and miR-577 overexpression were analysed by CCK-8, $^{**}P<0.05$
C: Bcl-2 and Bax expressions in 5-FU-treated PC3 cells were examined by RT-qPCR after DANCR inhibition and miR-577 overexpression, $^{**}P<0.05$
Source: Authors

way (Ma et al. 2018). As mentioned, lncRNA DANCR could sponge miR-135a to regulate sensitivity to paclitaxel in prostate cancer (Zhao et al. 2019). Moreover, LncRNA DANCR promoted JAG1 by competitively sponging miR-34a-5p, resulting in increased resistance to docetaxel in PCa cells (Ma et al. 2019). However, whether lncRNA DANCR can regulate 5-FU sensitivity has not been mentioned in prostate cancer. Hence, this study would examine functions of DANCR and its roles on regulating chemosensitivity of 5-FU. First, expressions of DANCR were higher in prostate cancer cell lines, especially in PC3 cell line. Besides that, 5-FU treatment reduced cell viabilities and expressions of DANCR in PC3 cells. Moreover, overexpressed LncRNA DANCR upregulated expression of DANCR and reduced toxicity of 5-FU in PC3 cells. Therefore, DANCR might be the biomarker that alleviated chemosensitivity of 5-FU to prostate cancer cells.

According to the research of Jiang et al. (2017) miR-577 suppressed proliferation and induced cell cycle arrest of colorectal cancer cells and increased sensitivity to 5-FU (Jiang et al. 2017), whereas no studies have mentioned its function in modulating 5-FU sensitivity in PCa. In this study, through LncBase v.2, miR-577 was verified to have putative binding sites with lncRNA DANCR and following luciferase reporter assay verified that the wild type of DANCR could directly bind miR-577 in PC3 cells. Furthermore, miR-577 expressions were significantly upregulated with growing concentrations of 5-FU and suppressed miR-577 downregulated its expression and so was the cell toxicity of PC3 cells. Therefore, miR-577 was the target of DANCR, which could upregulate sensitivity to 5-FU in PC3 cells. Thereafter, interactions between miR-577 and DANCR were detected, which showed that miR-577 overexpression could enlarge the downregulation DANCR after DANCR suppression. Moreover, the toxicity of 5-FU to PC3 cells was facilitated with inhibition of DANCR, which was magnified after miR-577 overexpression. Besides that, Bcl-2 expression was downregulated but Bax was promoted. Therefore, miR-577 might be the promising biomarker that restrained functions of DANCR in modulating 5-FU sensitivity in prostate cancer cells.

CONCLUSION

LncRNA DANCR reduced chemosensitivity of 5-FU in prostate cancer cells through directly suppressing miR-577, suggesting that those two biomarkers might be potential factors to regulate5-FU treatment in prostate cancer in vitro. Nevertheless, in vivo and clinical detections are needed for getting further knowledge about functions of LncRNA DANCR and miR-577.

RECOMMENDATIONS

In prostate cancer cells, lncRNA DANCR was upregulated, and its overexpression restrained sensitivity to 5-FU. In contrast, miR-577, the target of DANCR, was upregulated by 5-FU treatment, which enhanced sensitivity to 5-FU in prostate cancer cells after knockdown of DANCR. Hence, DANCR might be a target for 5-FU treatment. This study provided a new biomarker for increasing efficacy of 5-FU in PCa but further detections were requested.

ABBREVIATION LIST

- LncRNA DANCR: Long noncoding RNA Differentiation Antagonising Non-protein Coding RNA
- 5-FU: 5-Fluorouracil
- MiR-577: MicroRNA-577
- PCa: Prostate Cancer
- ATCC: American Type Culture Collection
- CCK-8: Cell Counting Kit-8
- SD: Standard Deviation

REFERENCES

Abramovic I, Ulamec M, Katusic Bojanac A, Bulic-Jakus F, Jezek D, Sincic N 2020. Mirna in prostate cancer: Challenges toward translation. *Epigenomics*, 12(6): 543-558.

Blondy S, David V, Verdier M, Mathonnet M, Perraud A, Christou N 2020. 5-fluorouracil resistance mechanisms in colorectal cancer: From classical pathways to promising processes. *Cancer Sci*, 111(9): 3142-3154.

Bolton EM, Tuzova AV, Walsh AL, Lynch T, Perry AS 2014. Noncoding rnas in prostate cancer: The long and the short of it. *Clin Cancer Res*, 20(1): 35-43.

Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A 2018. Global cancer statistics 2018: Globocan estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*, 68(6): 394-424.

Chang CW, Lee HC, Li LH, Chiang Chiau JS, Wang TE, Chuang WH, Chen MJ, Wang HY, Shih SC, Liu CY, Tsai TH, Chen YJ 2020. Fecal microbiota transplantation prevents intestinal injury, upregulation of toll-like receptors, and 5-fluorouracil/oxaliplatin-induced toxicity in colorectal cancer. *Int J Mol Sci*, 21(2): 1-23.

Correia De Sousa M, Gjorgjieva M, Dolicka D, Sobolewski C, Foti M 2019. Deciphering mirnasí action through mirna editing. *Int J Mol Sci*, 20(24): 1-22.

Etzioni R, Cha R, Feuer EJ, Davidov O 1998. Asymptomatic incidence and duration of prostate cancer. *Am J Epidemiol*, 148(8): 775-785.

Evans AJ 2018. Treatment effects in prostate cancer. *Mod Pathol*, 31(S1): S110-121.

Fabris L, Ceder Y, Chinnaiyan AM, Jenster GW, Sorensen KD, Tomlins S, Visakorpi T, Calin GA 2016. The potential of micrornas as prostate cancer biomarkers. *Eur Urol*, 70(2): 312-322.

Fan X, Yuan J, Xie J, Pan Z, Yao X, Sun X, Zhang P, Zhang L 2018. Long non-protein coding rna dancr functions as a competing endogenous rna to regulate osteoarthritis progression via mir-577/sphk2 axis. *Biochem Biophys Res Commun*, 500(3): 658-664.

Goggins WB, Wong G 2009. Cancer among asian indians/pakistanis living in the united states: Low incidence and generally above average survival. *Cancer Causes Control*, 20(5): 635-643.

Grozescu T, Popa F 2017. Prostate cancer between prognosis and adequate/proper therapy. *J Med Life*, 10(1): 5-12.

Jia J, Li F, Tang XS, Xu S, Gao Y, Shi Q, Guo W, Wang X, He D, Guo P 2016. Long noncoding rna dancr promotes invasion of prostate cancer through epigenetically silencing expression of timp2/3. *Oncotarget*, 7(25): 37868-37881.

Jiang H, Ju H, Zhang L, Lu H, Jie K 2017. Microrna-577 suppresses tumor growth and enhances chemosensitivity in colorectal cancer. *J Biochem Mol Toxicol*, 31(6): 1-7.

Li G, Zhang H, Wan X, Yang X, Zhu C, Wang A, He L, Miao R, Chen S, Zhao H 2014. Long noncoding rna plays a key role in metastasis and prognosis of hepatocellular carcinoma. *Biomed Res Int*, 2014: 1-8.

Li T, Xing Y, Yang F, Sun Y, Zhang S, Wang Q, Zhang W 2020. Lncrna snhg3 sponges mir-577 to up-regulate smurf1 expression in prostate cancer. *Cancer Med*, 9(11): 3852-3862.

Li Z, Zheng X, Li P, Itoua ES, Moukassa D, Ndinga Andely F 2017. Effects of acupuncture on mrna levels of apoptotic factors in perihematomal brain tissue during the acute phase of cerebral hemorrhage. *Med Sci Monit*, 23: 1522-1532.

Longley DB, Harkin DP, Johnston PG 2003. 5-fluorouracil: Mechanisms of action and clinical strategies. *Nat Rev Cancer*, 3(5): 330-338.

Ma Y, Zhou G, Li M, Hu D, Zhang L, Liu P, Lin K 2018. Long noncoding rna dancr mediates cisplatin resistance in glioma cells via activating axl/pi3k/akt/nf-Śb signaling pathway. *Neurochem Int*, 118: 1-38.

Ma Y, Fan B, Ren Z, Liu B, Wang Y 2019. Long noncoding rna dancr contributes to docetaxel resistance in prostate cancer through targeting the mir-34a-5p/jag1 pathway. *Onco Targets Ther*, 12: 5485-5497.

Manogue C, Ledet E, Guddati AK, Lewis B, Sartor O 2018. Extreme prostate-specific antigen response to infusional 5-flourouracil in castrate-resistant prostate cancer. *Oncologist*, 23(3): 383-385.

Nath A, Singh JK, Vendan SE, Priyanka, Sinha S 2012. Elevated level of prostate specific antigen among prostate cancer patients and high prevalence in the gangetic zone of bihar, india. *Asian Pac J Cancer Prev*, 13(1): 221-223.

Ponting CP, Oliver PL, Reik W 2009. Evolution and functions of long noncoding rnas. *Cell*, 136(4): 629-641.

Prensner JR, Chinnaiyan AM 2011. The emergence of lncrnas in cancer biology. *Cancer Discov*, 1(5): 391-407.

Prensner JR, Rubin MA, Wei JT, Chinnaiyan AM 2012. Beyond psa: The next generation of prostate cancer biomarkers. *Sci Transl Med*, 4(127): 127rv123.

Satari A, Amini SA, Raeisi E, Lemoigne Y, Heidarian E 2019. Synergetic impact of combined 5-fluorouracil and rutin on apoptosis in pc3 cancer cells through the modulation of p53 gene expression. *Adv Pharm Bull*, 9(3): 462-469.

Sur S, Steele R, Shi X, Ray RB 2019. Mirna-29b inhibits prostate tumor growth and induces apoptosis by increasing bim expression. *Cells*, 8(11): 1-11.

Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A 2015. Global cancer statistics, 2012. *CA Cancer J Clin*, 65(2): 87-108.

Wang B, Sun L, Li J, Jiang R 2018. Mir-577 suppresses cell proliferation and epithelial-mesenchymal transition by regulating the wnt2b mediated wnt/beta-catenin pathway in non-small cell lung cancer. *Mol Med Rep*, 18(3): 2753-2761.

Wang Y, Lu Z, Wang N, Feng J, Zhang J, Luan L, Zhao W, Zeng X 2018. Long noncoding rna dancr promotes colorectal cancer proliferation and metastasis via mir-577 sponging. *Exp Mol Med*, 50(5): 1-17.

Wei Y, Yang P, Cao S, Zhao L 2018. The combination of curcumin and 5-fluorouracil in cancer therapy. *Arch Pharm Res*, 41(1): 1-13.

Wigmore PM, Mustafa S, El-Beltagy M, Lyons L, Umka J, Bennett G 2010. Effects of 5-fu. *Adv Exp Med Biol*, 678: 157-64.

Winterich JA, Grzywacz JG, Quandt SA, Clark PE, Miller DP, Acuļa J, Dignan MB, Arcury TA 2009. Menís knowledge and beliefs about prostate cancer: Education, race, and screening status. *Ethn Dis*, 19(2): 199-203.

Zhang H, Chen Z, Wang X, Huang Z, He Z, Chen Y 2013. Long non-coding rna: A new player in cancer. *J Hematol Oncol*, 6: 1-7.

Zhao HF, Zhang ZC, Shi BK, Jiang XZ 2019. Dancr sponges mir-135a to regulate paclitaxel sensitivity in prostate cancer. *Eur Rev Med Pharmacol Sci*, 23(16): 6849-6857.

Zhu Y, Hu H, Yuan Z, Zhang Q, Xiong H, Hu Z, Wu H, Huang R, Wang G, Tang Q 2020. Lncrna neat1 remodels chromatin to promote the 5-fu resistance by maintaining colorectal cancer stemness. *Cell Death Dis*, 11(11): 962.

Paper received for publication in August, 2021
Paper accepted for publication in November, 2021