European Review for Medical and Pharmacological Sciences 2019; 23: 3699-3708

Long non-coding RNA DUXAP10 promotes the proliferation, migration, and inhibits apoptosis of prostate cancer cells

X.-F. WANG[1], J. CHEN[1], Y.-B. GONG[2], Y.-C. QIN[3], L. WANG[1], N.-C. LI[1]

[1]Department of Urology, Peking University Shougang Hospital, Beijing, People's Republic of China
[2]Department of Science Research, Peking University Shougang Hospital, Beijing, People's Republic of China
[3]Department of General Surgery, Chaoyang District Shuangqiao Hospital, Beijing, People's Republic of China

Lei Wang and Ningchen Li are co-corresponding authors for this paper

Abstract. – OBJECTIVE: Long non-coding RNA DUXAP10 plays a significant role in the tumorigenesis and development of human cancer. The present study was performed to investigate the role of DUXAP10 in biological functions and underlying molecular mechanisms of prostate cancer cells.

MATERIALS AND METHODS: First, the quantitative reverse transcription-polymerase chain reaction (qRT-PCR) was used to detect the expression of DUXAP10 in human prostate cancer cell lines 22RV1, PC3, and DU145. Subsequently, small interfering RNAs (siRNAs) targeting at DUXAP10 mRNA were used to downregulate DUXAP10 expression. Then, the biological functions of DUXAP10 in prostate cancer cells, proliferation, migration, and apoptosis were studied by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, colony-formation assay, cell cycle analysis, transwell migration assay, wound healing assay, and cell apoptosis assay, respectively. Finally, qRT-PCR analysis and Western blot assay were used to investigate the molecular mechanisms of DUXAP10 underlying the progression of prostate cancer.

RESULTS: Results showed that the expression of DUXAP10 was higher in PC3 and DU145 cell lines than that in the 22RV1 cell line. Additionally, the knockdown of DUXAP10 could remarkably inhibit the proliferation, migration, and induce apoptosis of prostate cancer cells, and significantly increase the number of G0/G1 cells in PC3 and DU145 cell lines. Moreover, DUXAP10 could promote the development of prostate cancer by regulating the process of epithelial-mesenchymal transition (EMT).

CONCLUSIONS: The findings of this study suggested that the down-regulation of DUXAP10 expression suppressed the progression of prostate cancer by inhibiting cell proliferation, migration and promote cell apoptosis.

Key Words
Long non-coding RNA, DUXAP10, Prostate cancer, Progression.

Introduction

Prostate cancer (PCa) has been considered as one of the most common malignancies affecting males in developed countries[1,2]. The latest data from the American Cancer Society showed that the incidence and mortality of PCa rank first and second, respectively, among all the malignancies of male patients in the United States[3]. Furthermore, the morbidity and mortality of PCa are rising rapidly among Asian men in recent years[4]. The China Cancer Statistics indicated that due to population aging and changes in diet and lifestyle, the annual new patients and deaths of PCa in China are up to 60,300 and 26,600, respectively, in 2015[5]. Although the diagnosis rate of PCa and the methods of its treatment, including surgery and radiotherapy, have been greatly improved, the recurrence, invasion and distant metastasis of PCs remain major obstacles in its treatment, resulting in a reduced rate of long-term survival of PCa patients[6-9]. Therefore, the mechanisms underlying the carcinogenesis and development of PCa shall be clarified to develop novel and more effective therapies.

With the rapid development of high-throughput sequencing technology, an increasing number of non-coding RNAs (ncRNAs) have been found. These ncRNAs can be classified into two major categories according to their size: short non-cod-

Corresponding Author: Ningchen Li, MD; e-mail: ningchenli@126.com

ing RNAs (sncRNAs) and long non-coding RNAs (lncRNAs)[10]. LncRNAs are a type of ncRNAs more than 200 nucleotides in length, which lack an open reading frame and do not encode proteins[11,12]. In recent years, several studies have implicated lncRNAs in the regulation of key cellular and physiological activities, including gene transcription, gene translation, cell cycle regulation, apoptosis, cell migration, cell proliferation, cell differentiation, and nuclear-cytoplasmic trafficking[13-17]. LncRNAs have been reported to participate in the progression of PCa; however, the studies on their biological functions and underlying molecular mechanisms in prostate cancer are still in a preliminary stage. DUXAP10 is a novel lncRNA and is located in the human chromosome 14p11.2. In this study, it was shown that DUXAP10 could markedly promote the proliferation, migration, and metastasis of PCa cells. In addition, DUXAP10 participated in the development of PCa by regulating the process of EMT.

Materials and Methods

Cell Culture

Human PCa cell lines (PC3, 22RV1, and DU145) were purchased from the Institute of Biochemistry and Cell Biology of the Chinese Academy of Sciences (Shanghai, China). These cells were cultured in Roswell Park Memorial Institute-1640 (RPMI-1640) medium (Gibco®, Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS; Thermo Fisher Scientific, Waltham, MA, USA), 100 U/mL penicillin sodium, and 100 μg/mL streptomycin (Gibco®, Thermo Fisher Scientific, Waltham, MA, USA). The culture conditions were 37°C in a humidified air atmosphere containing 5% of CO_2.

Extraction of Total RNA and Quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR)

Total RNA was isolated from cultured cells using a TRIzol reagent (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's protocols. The isolated RNA was then reversely transcribed into cDNA using a Rever Tra Ace qPCR RT Kit (Toyobo Co. Ltd, Osaka, Japan). The reaction was performed in a 10 μL system containing 1 μg of total RNA, 7 μL of nuclease-free water, 2 μL of 5× RT Buffer, 0.5 μL of RT Enzyme Mix, and 0.5 μL of Random Primer Mix. qRT-PCR was carried out on an ABI 7500 (Thermo Fisher Scientific, Waltham, MA, USA) instrument using a KAPA SYBR Green FAST qPCR Kit (KAPA, Wilmington, MA, US) according to the instruction of the kit. The reaction was carried out as follows: a total of 40 cycles of 95°C for 3 min, 95°C for 15 s, 58°C for 30 s and 72°C for 1 min. The reaction system was 20 μL containing 10 μL of KAPA SYBR® FAST qPCR Master Mix (2X) Universal, 0.4 μL of PCR forward primer (10 μM), 0.4 μL of PCR reverse primer (10 μM), 0.4 μL of ROX low, 8.8 μL of double-distilled water, and less than 2 μL of template DNA. The Ct values of target genes were compared with those of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) using the $2^{-\Delta\Delta CT}$ method. Each investigation was repeated three times. The specific primer sequences used for qRT-PCR were as follows:

GAPDH, forward, 5′-GAACGGGAAGCTCACTGG-3′, Reverse, 5′-GCCTGCTTCACCACCTTCT-3′. DUXAP10, forward, 5′-GGTTCAACAGTATGGCTCCAAAG-3′, Reverse, 5′-GACTGCCCATCCACAGATGAAG-3′. E-cadherin, forward, 5′-AATAGTGCCTAAAGTGCTGC-3′, Reverse, 5′-AGACCCACCTCAATCATCCT-3′. N-cadherin, forward, 5′-ATCCTACTGGACGGTTCG-3′, Reverse, 5′-TTGGCTAATGGCACTTGA-3′.

Cell Transfection

Small interfering RNAs (siRNAs) targeting at DUXAP10 mRNA and unspecific negative control oligonucleotides were synthesized by GenePharma (Shanghai, China). Then, the PCa cells were cultured in six-well plates overnight to reach 60-70% confluence before they were transfected with siRNAs using Lipofectamine 2000 (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. Finally, the cells were harvested after 48 h of transfection and the transfection efficiency was assessed by qRT-PCR analysis. The results showed that S1 and S2 were the most efficient siRNAs and thus were used for subsequent experiments.

The sequences for DUXAP10 siRNAs were as follows: si-DUXAP10 1 #: 5′-CAGCAUACUUCAAAUUCACAGCAAA-3′; si-DUXAP10 2 #: 5′-AGUUGUUUGUUAGAAUACUGGUGCU-3′; si-DUXAP10 3 #: 5′-GGAACUUCCCAAACCUCCAUGAUUU-3′

Cell Proliferation Assay

The cell proliferation was measured using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Roche Applied Science, Mannheim, Germany). The PCa cells

(PC3 and DU145 cells, 2,000 cells per well) transfected with DUXAP10 siRNAs were seeded into 96-well plates, with each well containing 200 μL of a complete culture medium. Cell viability was measured after 24, 48, 72, and 96 h of cell culture according to the manufacturer's protocol. The assay was repeated in triplicate.

Colony Formation Assay

PCa cells (PC3 and DU145 cells, 1,000 cells per well) transfected with DUXAP10 siRNAs and si-NC (negative control) were seeded into six-well plates, with each well containing 2 ml of culture medium. The cells were then cultured at 37°C in 5% CO_2 for 2 weeks, with the culture medium replaced once every 4 days. Subsequently, the medium was removed and the cells were washed twice with phosphate-buffered saline (PBS), and then, the colonies were fixed with methanol for 15 min and stained with 0.1% crystal violet for 30 min. Finally, visible colonies were counted under a low power microscope. The assay of each treatment group was repeated three times.

Cell Cycle Analysis

PC3 and DU145 cells transfected with DUXAP10 siRNAs and si-NC were harvested after 48 h of transfection, washed with PBS and fixed at 4°C overnight in pre-cooled 75% ethanol. Subsequently, the cells were stained with propidium oxide using a Cycletest™ plus DNA Reagent Kit (BD Biosciences, San Jose, CA, USA) following the recommendations provided by the manufacturer, and then analyzed by FACScan (Thermo Fisher Scientific, Waltham, MA, USA). Finally, the percentages of cells in phase G0-G1, S, and G2-M were calculated and compared for the different groups.

Cell Apoptosis Analysis

PC3 and DU145 cells transfected with DUXAP10 siRNAs and si-NC were harvested after 48 h of transfection. Subsequently, these cells were stained with fluorescein isothiocyanate (FITC) Annexin V and propidium iodide (PI) before they were incubated in the dark at room temperature according to the manufacturer's protocol. In the end, the viable, dead, early apoptotic, and late apoptotic cells were identified by FACScan (Thermo Fisher Scientific, Waltham, MA, USA) analysis.

Transwell Migration Assay

Transwell chambers (8 μm pore size; EMD Millipore, Billerica, MA, USA) were used to assess the migration ability of PC3 and DU145 cells transfected for 48 h with DUXAP10 siRNAs and si-NC. The cells (4×10^4 cells/chamber) were plated in the upper chamber of the transwell containing 200 μL of serum-free medium. Meanwhile, 900 μL of complete culture medium containing 10% of fetal bovine serum (FBS) was added to the lower chamber. After 24 h of incubation, the cells on the upper membrane of the transwell chambers were removed with a piece of cotton wool, while the cells on the lower membrane were fixed with polyoxymethylene for 10 min and then stained with 0.1% crystal violet for 30 min. Subsequently, five randomly selected fields were counted in each well. The experiments were independently repeated in triplicate.

Wound Healing Assay

First, PC3 and DU145 cells were transfected with specific DUXAP10 siRNAs and si-NC for 48 h and then seeded into six-well plates (1×10^5 cells per well). At 90-95% of confluence, the culture medium was removed and the monolayer of the cells was wounded by manual scraping with a sterile 200 μL pipette tip. Then, the cell monolayer was washed twice with PBS and cultured at 37°C in a humidified air atmosphere containing 5% CO_2. Next, at 0 h, 18 h, and 24 h, respectively, after the wound was created, the images of the cell monolayer were taken using a TH4-200 inverted microscope (Olympus Corporation, Tokyo, Japan). Finally, the migration rate was assessed by measuring the distance among the cells.

Western Blot

Total protein was isolated from PC3 and DU145 cells transfected with DUXAP10 siRNAs and si-NC for 48 h using a radioimmunoprecipitation assay (RIPA) buffer according to its instructions. The protein concentration was measured using a BCA protein assay kit (Thermo Fisher Scientific, Waltham, MA, USA). Sixty micrograms of protein were separated using 8% of sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto 0.4 μm nitrocellulose membranes (Sigma-Aldrich, St. Louis, MO, USA). The membranes were then incubated with specific primary antibodies at room temperature for 1 h. Subsequently, the membranes were washed three times using phosphate-buffered saline and Tween 20 (PBST) and incubated with the corresponding secondary antibody at room temperature for 1 h. The β-actin antibody was used as the control. Primary an-

ti-β-actin, anti-E-cadherin, and anti-N-cadherin antibodies were purchased from Santa Cruz Biotechnology Inc. (Dallas, TX, USA).

Statistical Analysis

SPSS 20.0 (IBM Corporation, Armonk, NY, USA) and GraphPad Prism 5.0 software (GraphPad Software Inc., La Jolla, CA, USA) were used for all statistical analysis and image processing. The difference between the two groups was evaluated using Student's *t*-test. $p < 0.05$ indicated a significant difference.

Results

DUXAP10 Expression in PCa Cells

The expression of DUXAP10 in a panel of PCa cell lines, including PC3, DU145, and 22RV1 cells, was detected by the qRT-PCR analysis. Results showed that DUXAP10 expression was significantly higher in PC3 and DU145 cells than that in 22RV1 cells (Figure 1A). Therefore, PC3 and DU145 cells were selected for subsequent experiments. Next, to investigate the biological role of DUXAP10 dysregulation in PCa cells and to identify the most effective siRNA for DUXAP10 knockdown, three short interference RNAs targeting at DUXAP10 mRNA and one unspecific negative control siRNA were used to down-regulate the DUXAP10 expression. The results of qRT-PCR analysis suggested that si-DUXAP10 1 # and si-DUXAP10 2 # demonstrated a higher inhibition efficiency than si-DUXAP10 3 # in PC3 and DU145 cells (Figure 1B). Thus, si-DUXAP10 1 # and si-DUXAP10 2 # were selected for subsequent biological function assays.

Knockdown of lncRNA DUXAP10 Inhibited the Proliferation and Colony Formation of PCa Cells

To clarify the effect of lncRNA DUXAP10 on the proliferation of PCa cells, an MTT assay was performed in PC3 and DU145 cells transfected with siRNA-DUXAP10 and si-NC. Results indicated that the knockdown of DUXAP10 significantly inhibited the proliferation of PC3 and DU145 cells (Figure 1C, D). Meanwhile, the colony formation assays were carried out to further investigate the role of DUXAP10 in the proliferation of PC3 and DU145 cells. Results showed that the colony formation numbers were significantly decreased after PC3 and DU145 cells were transfected with siRNA-DUXAP10 (Figure 1E, F, G, H). All the above data suggested that the down-regulation of lncRNA DUXAP10 significantly inhibited the proliferation of PCa cells.

Knockdown of lncRNA DUXAP10 Promoted Cell Cycle Arrest and Induced the Apoptosis of PCa Cells

To investigate the effects of DUXAP10 on the cell cycle progression and apoptosis of PCa cells, a cell cycle analysis, and a cell apoptosis assay were performed. The results of cell cycle analysis showed that the percentage of G0/G1 cells significantly increased after PCa cells were transfected with siRNA-DUXAP10, along with significantly reduced proportions of cells in phase S and G2/M (Figure 2A, B). In addition, the results of cell apoptosis assay indicated that the numbers of early apoptotic and late apoptotic PC3 and DU145 cells were significantly increased after the transfection with siRNA-DUXAP10 (Figure 2C, D).

Knockdown of lncRNA DUXAP10 Inhibited the Migration of PCa Cells

The progression of human cancer is mainly caused by the migration and metastasis of cancer cells. Therefore, in this study, a transwell migration assay and a wound healing assay were conducted to assess the role of DUXAP10 in the metastasis of PCa cells. The transwell assay revealed that the down-regulation of DUXAP10 expression significantly suppressed the migration of PCa cells (Figure 3A, B). Moreover, the results of the wound healing assay showed that the decreased DUXAP10 expression inhibited the migration of PC3 and DU145 cells, consistent with the results of the transwell migration assay (Figure 3C, D, E, F).

LncRNA DUXAP10 Promoted the Metastasis of PCa Cells via Regulating the Process of EMT

Considering that the EMT process has been implicated in the migration and metastasis of human cancer, the effect of lncRNA DUXAP10 on the EMT process was studied in PCa cells by analyzing the expression of EMT markers at mRNA and protein levels after the expression of DUXAP10 was down-regulated. Results from the qRT-PCR analysis showed that DUXAP10 knockdown could increase the mRNA level of E-cadherin, while decreasing the mRNA level of N-cadherin in PC3 and DU145 cells (Figure 4A, B). Meanwhile, the Western blot assays indicated that the protein level of E-cadherin was increased while the protein level of N-cadherin was reduced in PCa cells transfected with siRNA-DUXAP10 (Figure 4C-F).

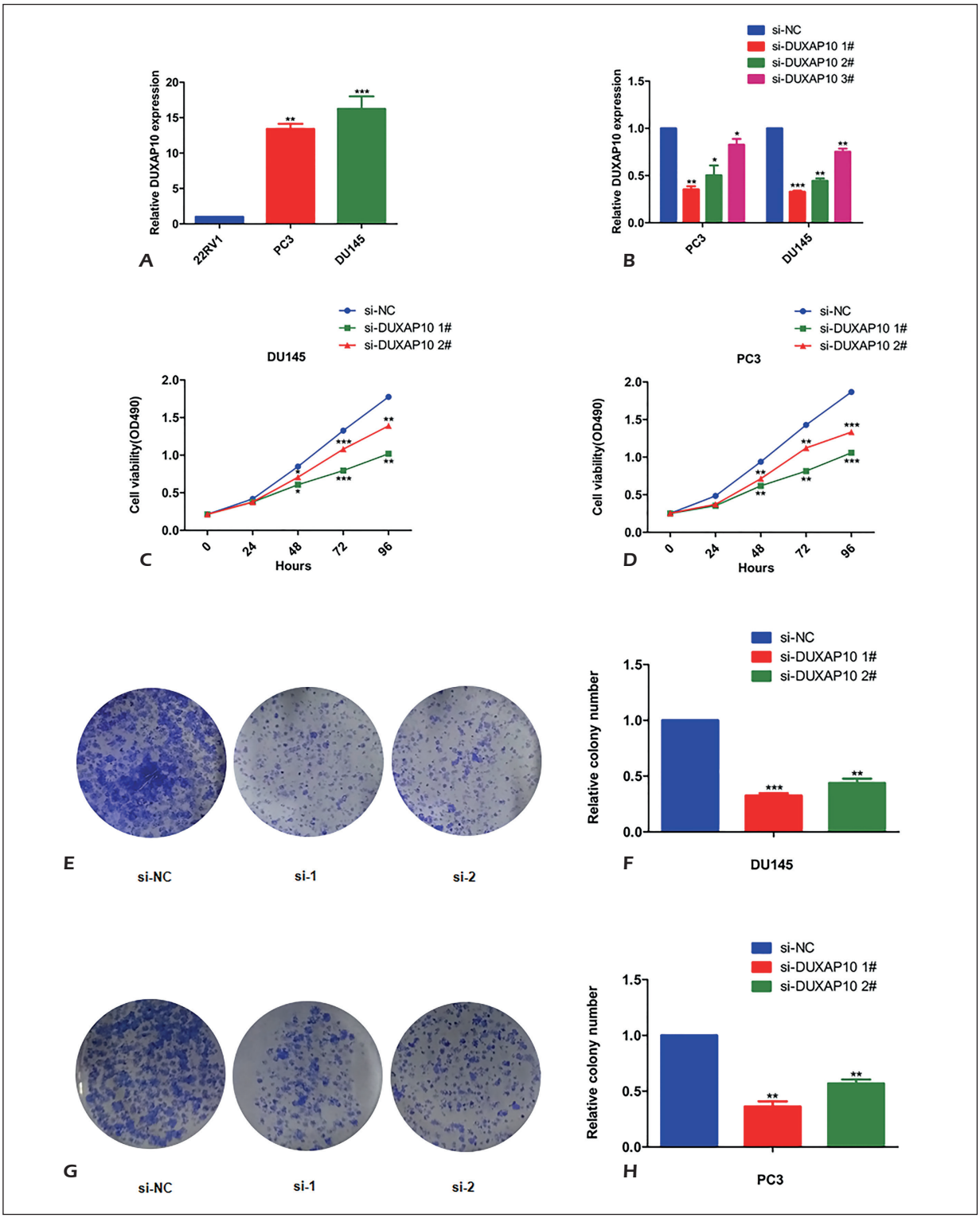

Figure 1. Down-regulation of lncRNA DUXAP10 inhibited the proliferation of prostate cancer cells. ***A,*** DUXAP10 expression in a panel of prostate cancer cell lines (PC3, DU145 and 22RV1). ***B,*** DUXAP10 expression in PC3 and DU145 cells after transfection with siRNA-DUXAP10 and si-NC. ***C-D***, Proliferation of PC3 and DU145 cells transfected with siRNA-DUXAP10 and si-NC was measured by MTT assays. ***E-H***, Colony formation assays were performed to measure the clone formation ability of prostate cancer cells transfected with siRNA-DUXAP10 and si-NC. *p<0.05, **p<0.01, ***p<0.001.

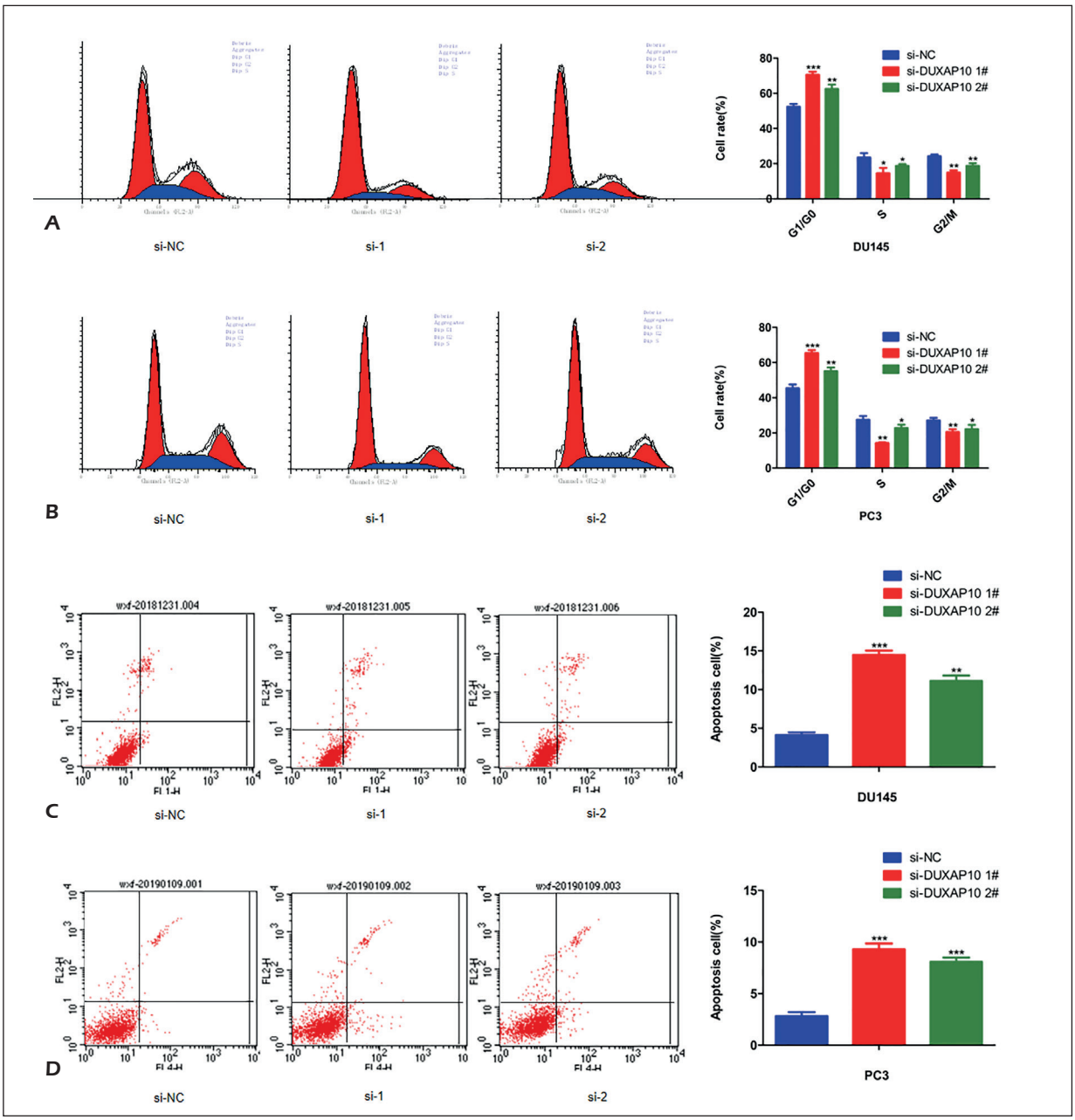

Figure 2. Knockdown of lncRNA DUXAP10 suppressed the cell cycle progression and induced the apoptosis of prostate cancer cells. ***A-B***, A cell cycle analysis was carried out to investigate the cell cycle progression of PC3 and DU145 cells after they were transfected with siRNA-DUXAP10 and si-NC. ***C-D***, Number of apoptotic PC3 and DU145 cells was measured by a cell apoptosis assay after they were transfected with siRNA-DUXAP10 and si-NC.

Discussion

PCa is one of the most common malignancies in the genitourinary system of males. Considering the high risk of PCa, an increasing number of studies have focused on the etiology and pathogenesis of PCa. However, the tumorigenesis and progression of PCa are a complex process involving the inactivation of tumor suppressor genes and the activation of oncogenes. Therefore, the specific molecular mechanisms of PCa remain unclear[18,19]. In recent years, several studies[20] have revealed that lncRNAs play an increasingly vital role in the tumorigenesis and development of human cancer, including PCa, by regulating gene expression at transcrip-

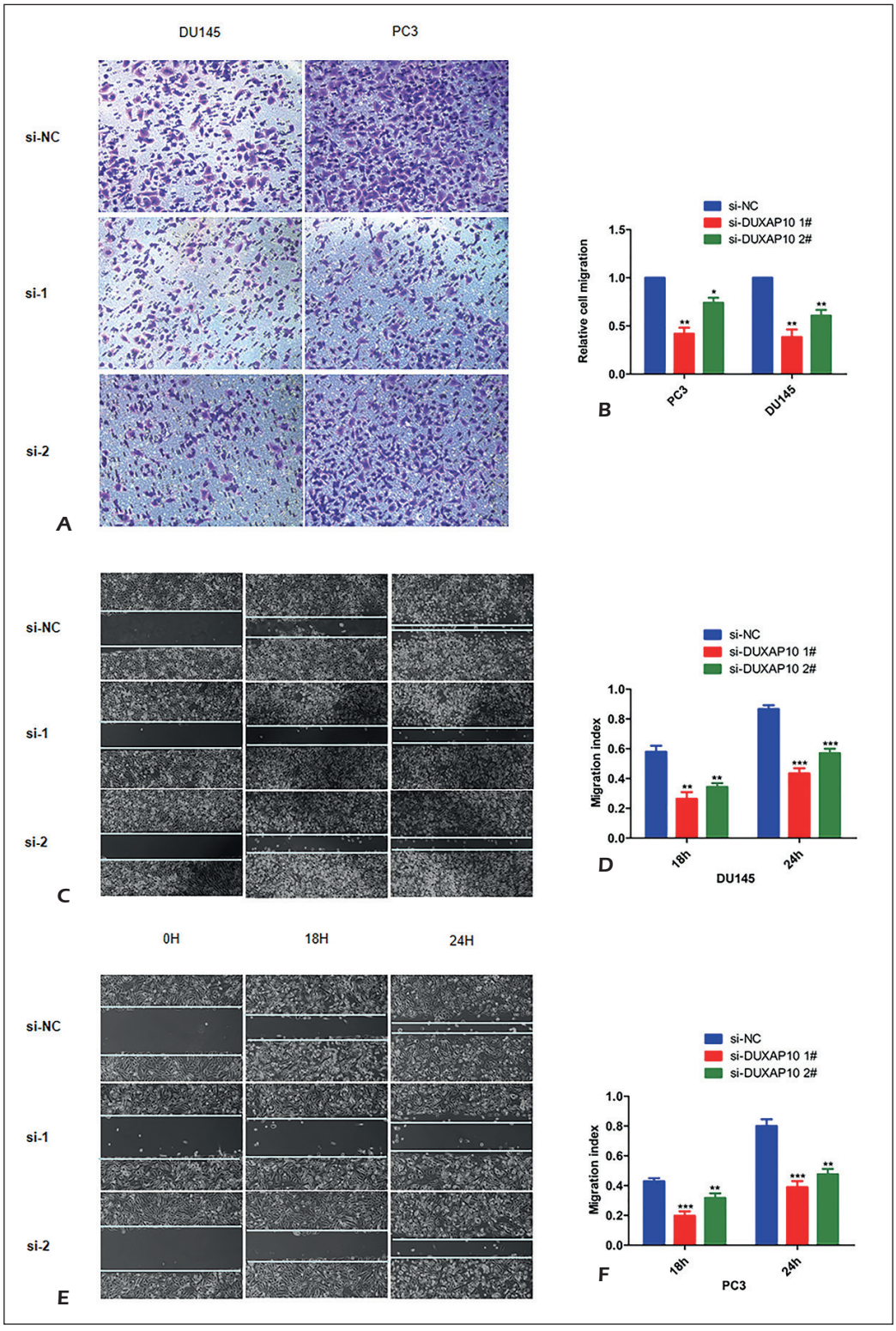

Figure 3. Knockdown of lncRNA DUXAP10 inhibited the migration of prostate cancer cells. ***A-B,*** Transwell migration assays were conducted to detect the migration ability of PC3 and DU145 cells transfected with siRNA-DUXAP10 and si-NC. ***C-F,*** Wound healing assays were used to investigate the migration ability of PC3 and DU145 cells transfected with siRNA-DUXAP10 and si-NC.

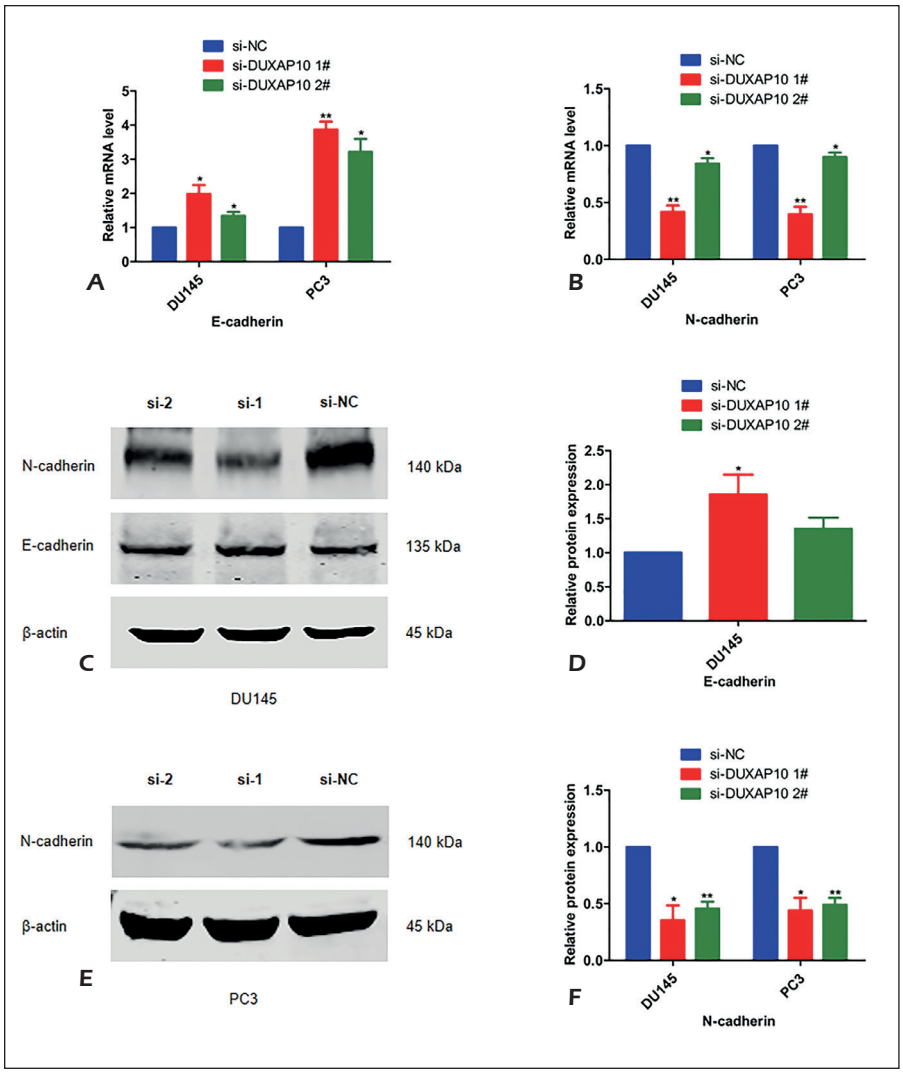

Figure 4. LncRNA DUXAP10 accelerated the metastasis of prostate cancer cells via regulating the process of EMT. ***A-B,*** MRNA levels of E-cadherin and N-cadherin were measured by qRT-PCR analysis following the transfection with anti-DUXAP10 siRNAs. ***C-F,*** Protein levels of E-cadherin and N-cadherin were measured by Western blot assays in PC3 and DU145 cells transfected with anti-DUXAP10 siRNAs.

tional, post-transcriptional, and epigenetic levels. Wu et al[21] recently found that, as an independent prognostic risk factor in PCa, the expression of lncRNA LINC01296 was significantly higher in PCa tissues than that in adjacent normal tissues. In addition, LINC01296 could promote the proliferation, migration, and invasion of PCa cells via participating in the modulation of the PI3K–Akt–mTOR signaling pathway and epithelial-mesenchymal transition. LncRNA LOXL1-AS1 is encoded on the opposite strand of the lysyl oxidase-like 1 (LOXL1) gene. Long et al[22] has found that LOXL1-AS1 could interact with miR-541-3p *in vivo*, while the down-regulation of LOXL1-AS1 expression could suppress the expression of CCND1 and block the cell cycle progression of PCa cells. Furthermore, the down-regulation of lncRNA PVT1 could markedly suppress the proliferation and migration of PCa cells by reducing the phosphorylation of p38, a mitosis-associated molecule involved in the proliferation and migration of human cancer cells[23-25]. The previous research has also shown that the expression of pseudogene derived from lncRNA DUXAP10 was significantly higher in colorectal cancer tissues than that in adjacent normal tissues. Meanwhile, the knockdown of DUXAP10 could inhibit the proliferation of cancer cells while inducing cell apoptosis and cell cycle arrest of colorectal cancer cells. Furthermore, DUXAP10 could significantly suppress the expression of p21 and PTEN by binding to lysine-specific demethylase 1 (LSD1), thereby promoting the occurrence and development of colorectal cancer[26]. Lv et al[27] found that the expression of DUXAP10 was markedly upregulated in bladder cancer cells

compared with that in normal bladder cells, and the knockdown of DUXAP10 inhibited cancer cell proliferation and promoted their apoptosis by mediating the PI3K/Akt/mTOR signaling. In addition, lncRNA DUXAP10 was overexpressed in human pancreatic cancer tissues compared with that in adjacent normal tissues, and the level of DUXAP10 was positively correlated with the clinical stages of pancreatic cancer and lymph node metastasis. Moreover, DUXAP10 could promote the proliferation of pancreatic cancer cells by interacting with RNA-binding proteins EZH2 and LSD1, and accelerate the migration, invasion, and inhibit the apoptosis of pancreatic cancer cells[28]. In addition, lncRNA DUXAP10 was similarly upregulated in ovarian cancer and chronic myeloid leukemia and could promote the tumorigenesis and progression of these malignancies[29,30]. The present study has focused on the biological functions of DUXAP10 in PCa cells. It was found that the down-regulation of DUXAP10 could remarkably inhibit the proliferation and migration while inducing the apoptosis of PCa cells. Meanwhile, the knockdown of DUXAP10 could significantly suppress the cell cycle progression of PC3 and DU145 cells. The results of this study also indicated that lncRNA DUXAP10 may function as an oncogenic lncRNA and hence may be considered as a new target for the diagnosis and treatment of PCa. Scholars[31,32] have also found that numerous lncRNAs could promote the migration and metastasis of various types of tumors by regulating the EMT process. To confirm whether DUXAP10 could participate in the EMT process of PC3 and DU145 cells, the mRNA and protein levels of EMT markers were analyzed in this study after the expression of DUXAP10 was knocked down. Results from the qRT-PCR analysis and Western blot assays suggested that the down-regulation of DUXAP10 could decrease the expression of N-cadherin while increasing the expression of E-cadherin, thus confirming that DUXAP10 acts as a significant regulator of the EMT process in PCa cells. We think this is the first study carried out to clarify the biological functions and potential molecular mechanisms of lncRNA DUXAP10 in PCa cell lines. Nevertheless, more studies are required to measure the expression levels of lncRNA DUXAP10 in PCa tissues and adjacent normal tissues. Meanwhile, the correlation between DUXAP10 expression and clinicopathological characteristics of PCa shall be further investigated.

Conclusions

In summary, the results of this study showed that the decreased expression of lncRNA DUXAP10 could significantly inhibit the proliferation, migration and cell cycle progression of PCa cells, while inducing their apoptosis. In addition, DUXAP10 could promote the migration and metastasis of PCa cells via regulating the process of EMT.

Acknowledgements

This work was supported by the Shijingshan District-Supported Key Medical Specialities Project (No. 2018004) and the Peking University Shougang Hospital Key Clinical Construction Project (No. 2017-05).

Conflict of Interests

The Authors declared that they have no conflicts of interests.

References

1) Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman, D, Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 2015; 136: E359-E386.
2) Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015; 65: 87-108.
3) Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin 2016; 66: 7-30.
4) Ito K. Prostate cancer in Asian men. Nat Rev Urol 2014; 11: 197-212.
5) Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ, He J. Cancer statistics in China, 2015. CA Cancer J Clin 2016; 66: 115-132.
6) Cancer Genome Atlas Research Network. The molecular taxonomy of primary prostate cancer. Cell 2015; 163: 1011-1025.
7) Cucchiara V, Cooperberg MR, Dall'Era M, Lin DW, Montorsi F, Schalken JA, Evans CP. Genomic markers in prostate cancer decision making. Eur Urol 2018; 73: 572-582.
8) Wade CA, Kyprianou N. Profiling prostate cancer therapeutic resistance. Int J Mol Sci 2018; 19. pii: E904.
9) Aird J, Baird AM, Lim MCJ, McDermott R, Finn SP, Gray SG. Carcinogenesis in prostate cancer: the role of long non-coding RNAs. Noncoding RNA Res 2018; 3: 29-38.
10) Khvorova A, Wolfson A. New competition in RNA regulation. Nat Biotechnol 2012; 30: 58-59.
11) ENCODE Project Consortium. An integrated encyclopedia of DNA elements in the human genome. Nature 2012; 489: 57-74.

12) Brosnan CA, Voinnet O. The long and the short of noncoding RNAs. Curr Opin Cell Biol 2009; 21: 416-425.
13) Rinn JL, Chang HY. Genome regulation by long noncoding RNAs. Annu Rev Biochem 2012; 81: 145-166.
14) Lee JT. Epigenetic regulation by long noncoding RNAs. Science 2012; 338: 1435-1439.
15) Su Y, Wu H, Pavlosky A, Zou LL, Deng X, Zhang ZX, Jevnikar AM. Regulatory non-coding RNA: new instruments in the orchestration of cell death. Cell Death Dis 2016; 7: e2333.
16) Perry RB, Ulitsky I. The functions of long noncoding RNAs in development and stem cells. Development 2016; 143: 3882-3894.
17) Huarte M. The emerging role of lncRNAs in cancer. Nat Med 2015; 21: 1253-1261.
18) Miłek K, Kaczmarczyk-Sekuła K, Strzępek A, Dyduch G, Białas M, Szpor J, Gołąbek T, Szopiński T, Chłosta P, Okoń K. Mast cells influence neoangiogenesis in prostatic cancer independently of ERG status. Pol J Pathol 2016; 67: 244-249.
19) Zhou X, Chen Q, Wang H, Zhang C, Fu B, Wang G. Specific expression of lncRNA RP13-650J16.1 and TCONS_00023979 in prostate cancer. Biosci Rep 2018; 38. pii: BSR20171571.
20) Wang X, Wang L, Gong Y, Liu Z, Qin Y, Chen J, Li N. Long noncoding RNA LINC01296 promotes cancer-cell proliferation and metastasis in urothelial carcinoma of the bladder. Onco Targets Ther 2018; 12: 75-85.
21) Wu J, Cheng G, Zhang C, Zheng Y, Xu H, Yang H, Hua L. Long noncoding RNA LINC01296 is associated with poor prognosis in prostate cancer and promotes cancer-cell proliferation and metastasis. Onco Targets Ther 2017; 10: 1843-1852.
22) Long B, Li N, Xu XX, Li XX, Xu XJ, Liu JY, Wu ZH. Long noncoding RNA LOXL1-AS1 regulates prostate cancer cell proliferation and cell cycle progression through miR-541-3p and CCND1. Biochem Biophys Res Commun 2018; 505: 561-568.
23) Wan B, Wu HY, Lv DJ, Zhou XM, Zhong LR, Lei B, Zhang SB, Mao XM. Downregulation of lncRNA PVT1 expression inhibits proliferation and migration by regulating p38 expression in prostate cancer. Oncol Lett 2018; 16: 5160-5166.
24) Xia P, Zhang R, Ge G. C/EBPβ mediates TNF-α-induced cancer cell migration by inducing MMP expression dependent on p38 MAPK. J Cell Biochem 2015; 116: 2766-2777.
25) Cheng G, Gao F, Sun X, Bi H, Zhu Y. Paris saponin VII suppresses osteosarcoma cell migration and invasion by inhibiting MMP-2/9 production via the p38 MAPK signaling pathway. Mol Med Rep 2016; 14: 3199-3205.
26) Lian Y, Xu Y, Xiao C, Xia R, Gong H, Yang P, Chen T, Wu D, Cai Z, Zhang J, Wang K. The pseudogene derived from long non-coding RNA DUXAP10 promotes colorectal cancer cell growth through epigenetically silencing of p21 and PTEN. Sci Rep 2017; 7: 7312.
27) Lv XY, Ma L, Chen JF, Yu R, Li Y, Yan ZJ, Cheng Y, Ma Q. Knockdown of DUXAP10 inhibits proliferation and promotes apoptosis in bladder cancer cells via PI3K/Akt/mTOR signaling pathway. Int J Oncol 2018; 52: 288-294.
28) Lian Y, Xiao C, Yan C, Chen D, Huang Q, Fan Y, Li Z, Xu H. Knockdown of pseudogene derived from lncRNA DUXAP10 inhibits cell proliferation, migration, invasion, and promotes apoptosis in pancreatic cancer. J Cell Biochem 2018; 119: 3671-3682.
29) Zhang Q, Wang WW, Xu TH, Xu ZF. Highly expressed long non-coding RNA DUXAP10 promotes proliferation of ovarian cancer. Eur Rev Med Pharmacol Sci 2018; 22: 314-321.
30) Yao R, Feng WT, Xu LJ, Zhong XM, Liu H, Sun Y, Zhou LL. DUXAP10 regulates proliferation and apoptosis of chronic myeloid leukemia via PTEN pathway. Eur Rev Med Pharmacol Sci 2018; 22: 4934-4940.
31) Lv J, Fan HX, Zhao XP, Lv P, Fan JY, Zhang Y, Liu M, Tang H. Long non-coding RNA Unigene56159 promotes epithelial-mesenchymal transition by acting as a ceRNA of miR-140-5p in hepatocellular carcinoma cells. Cancer Lett 2016; 382: 166-175.
32) Xu ZY, Yu QM, Du YA, Yang LT, Dong RZ, Huang L, Yu PF, Cheng XD. Knockdown of long non-coding RNA HOTAIR suppresses tumor invasion and reverses epithelial-mesenchymal transition in gastric cancer. Int J Biol Sci 2013; 9: 587-597.