European Review for Medical and Pharmacological Sciences 2018; 22: 3370-3377

# Mechanism of lncRNA DUXAP8 in promoting proliferation of bladder cancer cells by regulating PTEN

M.-G. LIN, Y.-K. HONG, Y. ZHANG, B.-B. LIN, X.-J. HE

Department of Urinary Surgery, The First Affiliated Hospital of Medical College Shantou University, Shantou, China

**Abstract. – OBJECTIVE: To evaluate the lncRNA DUXAP8 expression in bladder cancer and its mechanism.**

**PATIENTS AND METHODS: Clinical specimens were analyzed. The expression of lncRNA in bladder cancer and adjacent tissues was detected using qRT-PCR. The $\chi^2$-test analysis was used to analyze the relationship between lncRNA DUXAP8 expression and clinicopathological information in patients with bladder cancer. The tumor cell activity and cell proliferation were measured by cell counting kit-8 (CCK8) and colony formation assay. We utilized polymerase chain reaction (PCR) to access PTEN expression in bladder cancer and adjacent tissues. Pearson correlation analysis was utilized for evaluating the relationship between PTEN and lncRNA DUXAP8. Western blot was used for detecting protein expression.**

**RESULTS: LncRNA DUXAP8 expression was higher in bladder cancer tissues; it was in a positive correlation with the TNM stage and tumor size, but negatively correlated with the total survival time. Knockdown of DUXAP8 decreased cell viability and cellular proliferation. Lower expression of PTEN gene was found in bladder cancer compared with that in adjacent tissues. Pearson correlation analysis showed that PTEN was negatively correlated with DUXAP8; knockdown of DUAP8 increased the expression of PTEN. Overexpressing DUAP8 increased protein level of PTEN, but decreased cell viability.**

**CONCLUSIONS: Our results pointed out that lncRNA DUXAP8 was overexpressed in bladder cancer tissues, which can promote the progression of bladder cancer through inhibiting PTEN.**

Key Words
Bladder cancer, lncRNA, DUXAP8, PTEN.

## Introduction

Bladder cancer is the most common malignancy of the urinary system. Its incidence is high and with a clear upward trend, seriously endangers human health[1]. Globally, bladder cancer is the seventh in men's tumors and seventeenth in women[2]. Current treatment of bladder cancer is not satisfied, as it cannot effectively decrease the tumor progression and recurrence. The survival rate of patients with metastatic bladder cancer was low after comprehensive treatment, and the 5-year survival rate reported in the literature was only 62%[3]. Therefore, inhibiting the progress of bladder cancer has become a key issue in the current treatment. It is a hot topic to find new molecular targets to explore the molecular mechanism of bladder cancer progression.

LncRNAs are a kind of non-coding RNAs in the nucleus or cytoplasm that are over 200 nt in length. They have a relatively long nucleotide chain, as well as specific and complex secondary spatial structures[4]. It is a class of important epigenetic regulators, and regulates DNA methylation, histone modification and chromatin remodeling through the epigenetic, transcriptional regulation and post-transcriptional levels as in the formation of RNA, thereby silencing or activating genes[5].

With the in-depth research, evidence indicated that lncRNAs participated in the process of tumorigenesis through various biological regulatory mechanisms, affecting the biological characteristics of tumor proliferation, infiltration and metastasis, and even the prognosis and survival of tumor patients. Khaitan et al[6] analyzed lncRNA expression profiles in the melanoma cell line WM1552C and melanocytes. They found that 77 lncRNAs were significantly expressed in the WM1552C cell line, suggesting that lncRNAs were closely related to tumorigenesis. Yuan et al[7] observed that lncRNA-activated TGF-β (LncRNA-ATB) was abnormally expressed in hepatocellular carcinoma (HCC) and metastasis, which was related to poor prognosis.

LncRNA DUXAP8 was located on chromosome 20q11 with 2307 bp in length[8]. Gene sequence of lncRNA DUXAP8 was detected from



*Corresponding Author:* Mingen Lin, MD, Ph.D; e-mail: Me_lin20@163.com

tumor cell lines and proved to have a regulatory role in cell cycle and reproductive development[9]. So far, no studies have been carried out on investigating the effect of lncRNA DUXAP8 on bladder cancer and its underlying mechanism.

PTEN gene is a tumor suppressor gene with bispecific protein phosphatase activity, it can affect the modulation of cell nuclear cycle and cell-cell adhesion[10]. PTEN is located on the short arm of chromosome 10 (10q23.3), with a total length of about 200 000. The encoded protein is more complex and has a variety of physiological functions. It cannot only act on the nucleus and regulate the cell cycle, but also functions on the cell membrane to participate in the intercellular interaction and adhesion[11].

## Patients and Methods

### Expression Analysis of LncRNA DUXAP8

Cancer tissues and adjacent normal tissues in 31 patients with bladder cancer who were pathologically confirmed by our hospital, were harvested. This study was approved by the Ethics Committee of The First Affiliated Hospital of Medical College Shantou University. Signed written informed consents were obtained from all participants before the study. LncRNAs differentially expressed in bladder cancer and adjacent tissues were accessed by qRT-PCR and normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

### Cell Culture and Transfection

The bladder cancer cell lines SV-HUC-1, T24, RT4, and J82 were purchased from the ATCC Cell Bank (Manassas, VA, USA) and cultured in Roswell Park Memorial Institute 1640 (RPMI 1640) medium (Gibco, Grand Island, NY, USA) containing 10% fetal bovine serum (FBS) (HyClone, South Logan, UT, USA). The above cells were cultured in accordance with the conventional cell culture method in the aseptic table and maintained in a 37°C, 5% $CO_2$ incubator. Bladder cancer cells were transfected with three DUXAP8 siRNAs (si-DUXAP8 1 #, 2 #, and 3 #) and a scrambled negative control siRNA (si-NC) sequence purchased from Invitrogen (Carlsbad, CA, USA) using Lipofectamine 2000. Medium was replaced after transfection for 6 h. Interference sequences were si-DUXAP8 1# F: UUUAGACCCAUUCUCGUAUGGAGGU; R; ACCUCCAUACGAGAAUGGGUCUAAA; si-DUXAP8 2# F; CAGCAUACUUCAAAUUCACAGCAAA; R: UUUGCUGUGAAUUUGAAGUAUGCUG; si-DUXAP8 3# F; UUUAGACCCAUUCUCGUAUGGAGGU; R: ACCUCCAUACGAGAAUGGGUCUAAA.

### RNA Extraction and qRT-PCR

We used TRIzol reagent (Invitrogen, Carlsbad, CA, USA) to extract total RNA from tissues or cultured cells. In short, reverse transcription kit (TaKaRa, Dalian, China) was utilized to reversely transcribe the RNA into cDNA. Real-time PCR was carried out using SYBR Premix Ex Taq (TaKaRa, Dalian, China). GAPDH expression normalized the results.

### CCK8 Assay for Cell Viability

Cell counting kit-8 (CCK8, Beyotime Institute of Biotechnology, Shanghai, China) was used to test the viability of bladder cancer cells. RT4 and T24 cells transfected with si-DUXAP8 (3000 cells per well) were seeded in 96-well plates. After cell adherence, 10 μL of CCK-8 solution were added in each well and cells were maintained in a 5% $CO_2$ incubator at 37°C. After 1 h, OD values at 450 nm were recorded. All groups were replicated for 5 wells.

### Dual Luciferase Reporter Gene Assay

Transfected cells were seeded in the 24-well plates. After incubation for 48 h, relative luciferase activity was detected based on the recommendations of Dual-Glo®Luciferase Assay System (Promega, Madison, WI, USA).

### Colony Formation Assay

After transfecting cells, they were seeded in 6-well plates and the culture medium was changed every 4 days. 14 days later, the cells were fixed with methanol and stain with 0.1% crystal violet (Sigma-Aldrich, St. Louis, MO, USA). Finally, the visible colonies were counted. The experiment was repeated for three times.

### Western Blot

Transfected cells were harvested and lysed by lysate solution, total protein was obtained, add appropriate amount of sodium dodecylsulphate (SDS) loading buffer was added to dissolve the cross-linking of proteins. Protein samples were subjected to polyacrylamide gel electrophoresis (PAGE). Then, proteins were transferred onto a polyvinylidene fluoride (PVDF) (Merck, Millipore, Billerica, MA, USA) membrane. Corresponding primary anti-

**Table I.** Correlation between DUXAP8 expression and clinicopathological information in patients with bladder cancer (n=31).

| Clinicopathologic features | Number of cases | DUXAP8 expression | | *p*-value |
|---|---|---|---|---|
| | | Low (n=15) | High (n=16) | |
| ***Gender*** | | | | 0.8528 |
| Male | 16 | 8 | 8 | |
| Female | 15 | 7 | 8 | |
| ***Age (years)*** | | | | 0.5761 |
| <60 | 14 | 6 | 8 | |
| ≥60 | 17 | 9 | 8 | |
| ***Tumor size*** | | | | 0.0198* |
| <3CM | 14 | 10 | 4 | |
| ≥3CM | 17 | 5 | 12 | |
| ***TNM stage*** | | | | 0.0064** |
| I-II | 17 | 12 | 5 | |
| III-IV | 14 | 3 | 11 | |
| ***Histological grade*** | | | | 0.2001 |
| Low | 17 | 10 | 7 | |
| High | 14 | 5 | 9 | |
| ***Lymph node metastasis*** | | | | 0.1244 |
| No | 23 | 13 | 10 | |
| Yes | 8 | 2 | 6 | |

*$p<0.05$

body was used for incubation at 4°C overnight, the secondary antibody was used at room temperature for 1 h. Membranes were exposed to enhanced chemiluminescence (ECL).

### Statistical Analysis

We used statistical product and service solutions (SPSS) 22.0 statistical software (IBM, Armonk, NY, USA) for data analysis, and GraphPad Prism 5.0 (Version X; La Jolla, CA, USA) for image editing. Kaplan-Meier survival curves were used for survival analysis, and clinicopathological data were estimated by $\chi^2$-test. $p<0.05$ indicated a statistical significant difference. *$p<0.05$, **$p<0.01$, and ***$p<0.001$.

## Results

### LncRNA DUXAP8 Is Highly Expressed in Bladder Cancer

We collected 31 cases of bladder cancer and 31 cases of adjacent tissues. The qRT-PCR results revealed that DUXAP8 was highly expressed in bladder cancer cells ($p<0.001$) (Figure 1A). Next, we used Kaplan-Meier survival analysis to investigate the correlation between DUXAP8 expression and prognosis in patients with bladder cancer. Lower survival of patients with bladder cancer overexpressing DUXAP8 was observed in comparison with those presented low expression of DUXAP8 (Figure 1B, $p=0.0498$, R=2.550). In addition, DUXAP8 expression in III + IV bladder cancer tissues was found higher than those in I + II bladder cancer tissues ($p<0.05$), and it was overexpressed in bladder cancer tissues larger than 3 cm ($p<0.05$) (Figure 1C and 1D).

### Screening of Cell Lines and Interference Sequencing

We extracted the total RNA in the cell lines (SV-HUC-1, T24, RT4, and J82) and the relative expression of DUXAP8 in these cells was detected by qRT-PCR. DUXAP8 expression in T24 and RT4 cells was significantly increased (Figure 2A); therefore, we selected T24 and RT4 cell lines for the knockdown experiments. Next, three different DUXAP8 siRNAs were conducted and transfected into T24 and RT4 cells, which were further verified to be effective by qRT-PCR. Moreover, si-DUXAP8 1# showed a more efficient interference than si-DUXAP8 2# and 3# (Figure 2B). As a result, we chose si-DUXAP8 1 # for subsequent experiments.

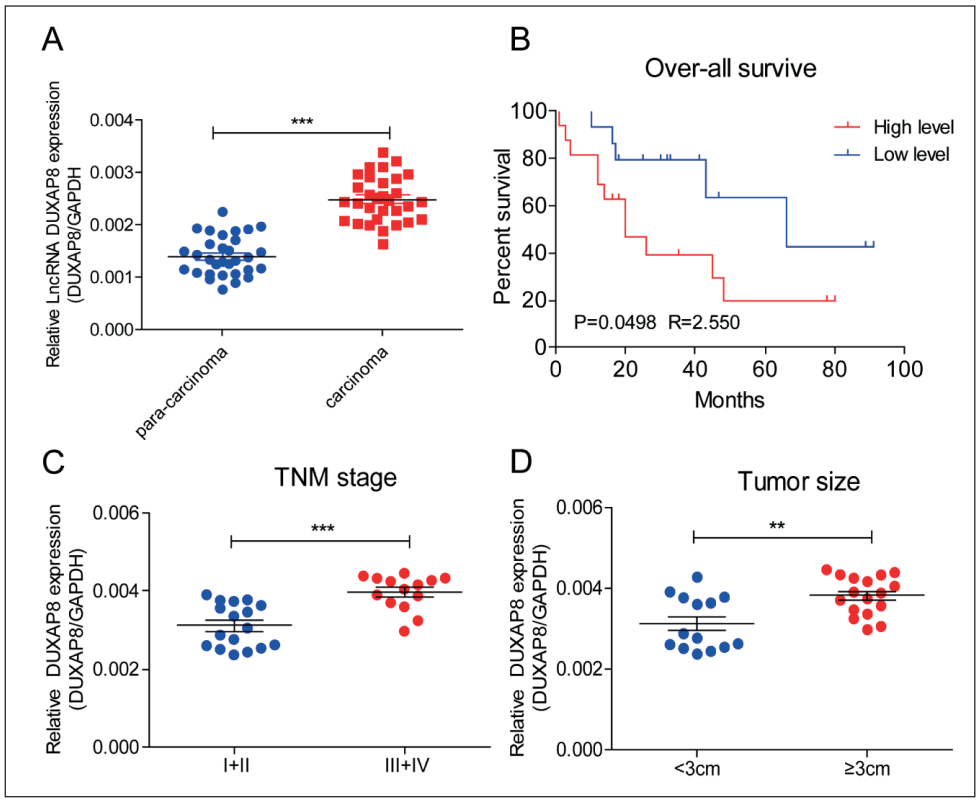


**Figure 1.** DUXAP8 is highly expressed in bladder cancer. **A**, Expression of DUXAP8 in 31 bladder cancer tissues was higher than that in 31 adjacent tissues. **B**, The overall survival rate in patients with bladder cancer overexpressing DUXAP8 was significantly lower than that of low expression patients. **C**, Expression of DUXAP8 in III + IV bladder cancer was higher than that in I + II bladder cancer. **D**, Expression of DUXAP8 in tumors over 3 cm was higher than less than 3 cm.

### *Knockdown of DUXAP8 Decreased Cell Viability and Inhibited Proliferation of Bladder Cancer Cells*

CCK8 assay suggested that the OD450 value of RT4 and T24 cells transfected with si-DUXAP8 1# decreased compared with those transfected with si-NC negative control, indicating that knockdown of DUXAP8 reduced the viability of RT4 and T24 cells (Figure 2D, E). Colony formation assay showed that transfection of si-DUXAP8 resulted in a significantly lower ability of colony capacity after that DUXAP8 was more down-regulated in RT4 and T24 cells than in the negative control (Figure 2F). These findings indicated that DUXAP8 may serve as an oncogene involved in promoting proliferation of bladder cancer cells.

### *PTEN is the Target Gene of DUXAP8 in Bladder Cancer*

By clinical sample analysis, PTEN was found lowly expressed in cancer tissues than in adjacent cancerous tissues (Figure 3A). Further analysis of the relationship between PTEN gene and DUXAP8 showed that DUXAP8 was negatively correlated with PTEN in clinical samples (Figure 3B). Western blot pointed out that overexpressed PTEN resulted in increased protein levels in RT4 and T24 cell lines, indicating that PTEN suppressed the proliferative ability of bladder cancer cells (Figure 3C). In RT4 and T24 cell lines with knockdown of DUXAP8, protein level of PTEN was elevated (Figure 3D), suggesting that DUXAP8 regulated bladder cancer progression through PTEN. CCK8 assay showed that overexpression of PTEN decreased cell viability (Figure 3E, F), which illustrated that PTEN had the capacity in inhibiting cell growth of bladder cancer.

## Discussion

Bladder cancer is one of the most common malignant tumors in the urinary system. Its incidence rate ranks fifth in the world and it is the second most serious malignant tumor in the urinary system. Due to its high morbidity and mortality, bladder cancer has gradually become a research hotspot in the urinary system[12]. Great advances have been made from exploring the biological features of bladder cancer and its potential mechanism[13]. However, the genetic regulation of bladder cancer remains unclear.

Abnormal expression of genes plays a crucial role in the development and progression of blad-

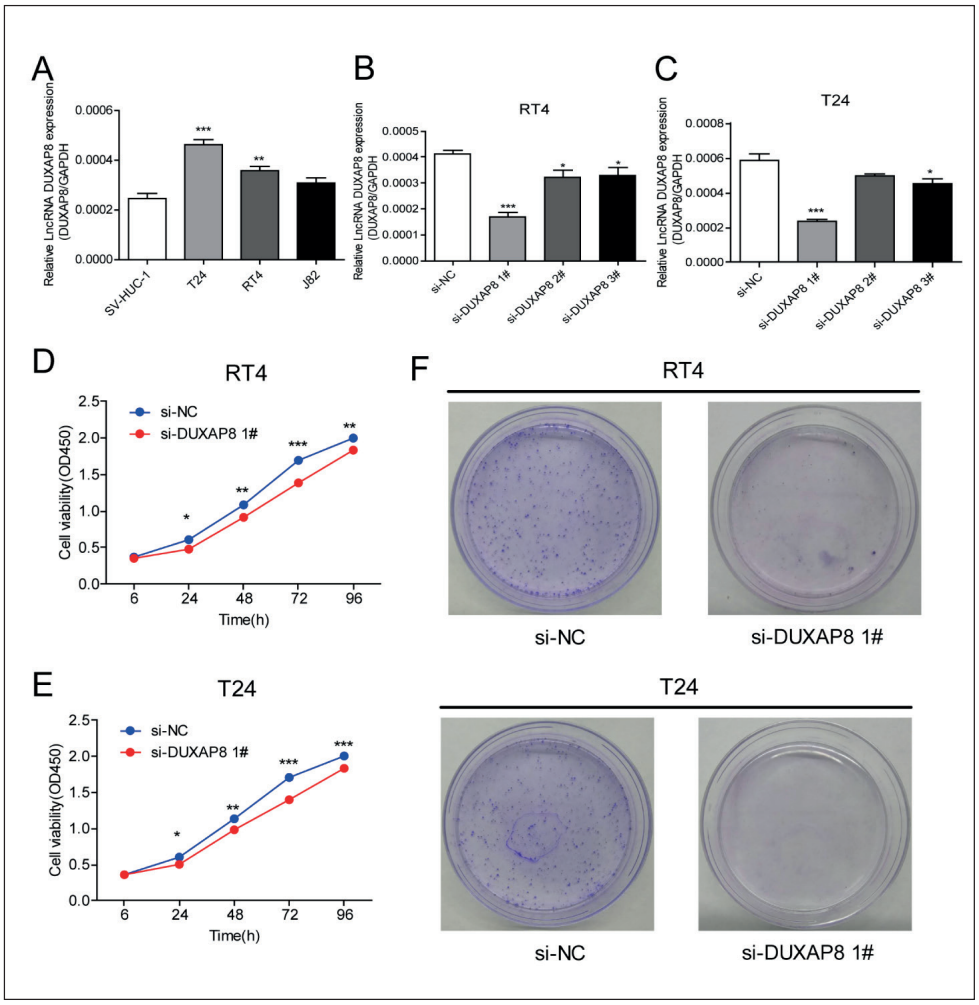


**Figure 2.** Low expression of DUXAP8 inhibits proliferation of bladder cancer cells. **A**, DUXAP8 expression in bladder normal and cancer cell lines (SV-HUC-1, T24, RT4, J28). **B**, Transfection of si-DUXAP8 in RT4 cell lines. **C**, Transfection of si-DUXAP8 in RT4 cell lines. **D**, CCK8 assay showed that cell viability in the RT4 cell line was decreased after DUXAP8 knockdown. **E**, CCK8 assay showed that cell viability in the T24 cell line was decreased after DUXAP8 knockdown. **F**, Colony formation assay showed that proliferation bladder cancer cells was inhibited after DUXAP8 knockdown.

der cancer, which is currently a research hot spot. As a consequence, future study of the pathogenesis of bladder cancer and molecular mechanism is urgent, as well as investigation of the appropriate new treatment site, thereby guiding a potential direction of diagnosing and treating bladder cancer. Long non-coding RNA (lncRNA) is a kind of non-coding RNA molecules whose length is 200nt-100kb. They are widely found in the nucleus and cytoplasm, and do not participate or are rarely involved in protein preparation due to the lack of open reading frame[14]. LncRNAs participate in the biological behavior of tumors, but the specific mechanism needs to be explored. LncRNAs modulate gene transcription *via* the modification of chromatin; the regulation is at the epigenetic and transcriptional level[15]. LncRNA is closely related to tumor. Abnormal expression of lncRNA was greatly involved in the development and progression of bladder cancer[16]. Our study suggested that great efforts should be made on the biological function and clinical significance of lncRNAs derived from pseudogenes. Many investigations[17-19] have reported that there were many lncRNAs, such as UCA1, PVT1, MEG3, etc., closely related to bladder cancer. UCA1 was overexpressed in embryonic and bladder carcinomas, whilst rarely expressed in normal adult tissues and adjacent tissues. UCA1 was thought to be involved in embryogenesis and carcinogenesis and may be used as a diagnostic molecular marker[18,20]. A study[21] found that PVT1 can promote cell differentiation and inhibit apoptosis, which was lowly expressed in bladder cancer tissues and cell lines. MEG3 served as a tumor suppressor and it has been highlighted a lower expression in bladder cancer respect to normal tissues. It has been confirmed that overexpression of MEG3 could suppress the proliferation and colony capacity of bladder cancer cell lines via promoting apoptosis[22,23].

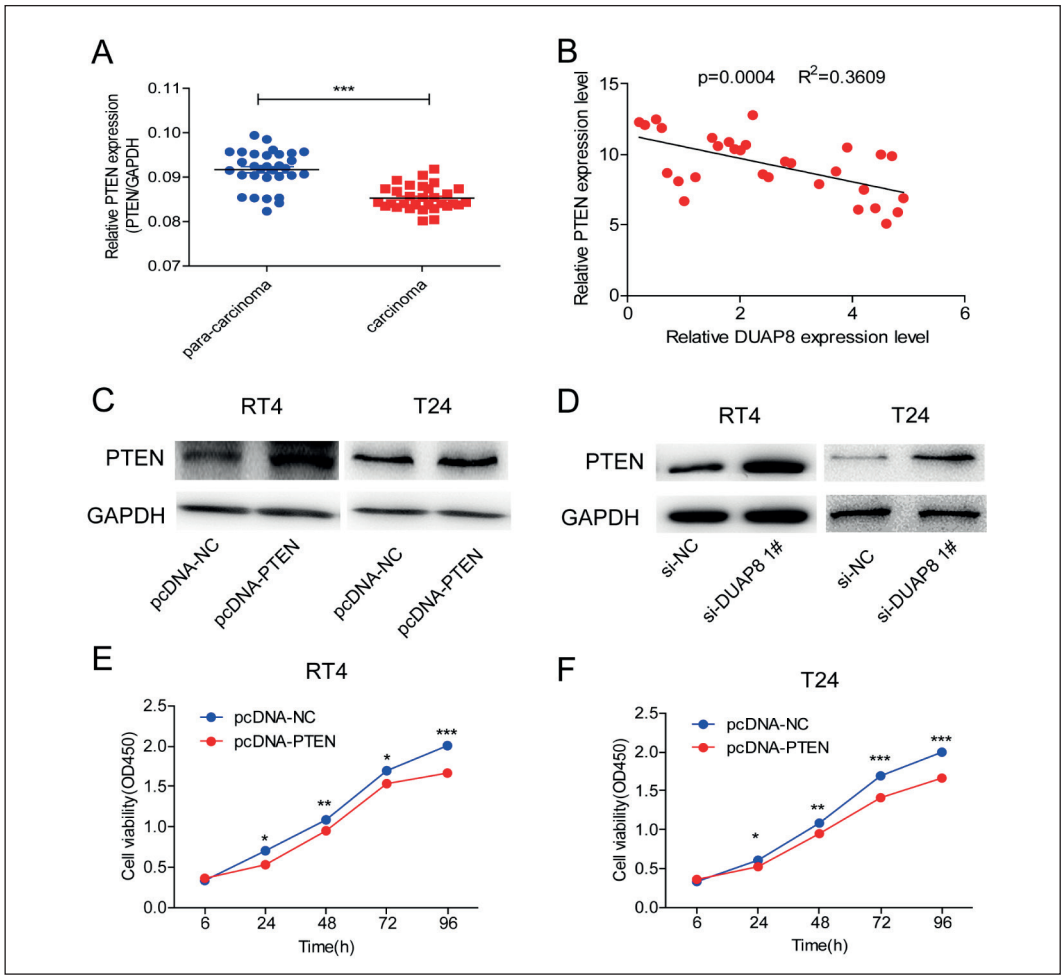


**Figure 3.** PTEN is lowly expressed in bladder cancer tissues, and is regulated by DUXAP8. **A**, PTEN expression in bladder cancer tissues was lower than that in adjacent tissues. **B**, Pearson correlation analysis showed that DUXAP8 and PTEN were negatively correlated in clinical samples. **C**, Protein level of DUXAP8 in RT4 and T24 cell lines was increased after overexpressing PTEN. **D**, Protein level of PTEN in RT4 and T24 cell lines were increased after DUXAP8 knockdown. **E-F**, In PT4 *(left)*, T24 *(right)* cell lines, cell viability was decreased after PTEN was overexpressed.

It has been shown that DUXAP8 can promote the development of GC by binding EZH2 and SUZ12 (two key components of PRC2) to apparently inhibit the expression of PLEKHO1[24]. Sun et al[8] found that the pseudogenes DUXAP8 may play an oncogene role in non-small cell lung cancer (NSCLC) by binding to EZH2 and LSD1 for silencing transcriptions of EGR1 and RHOB, which may provide a new therapeutic target for bladder cancer.

Our study found that pseudogene-derived lncRNA DUXAP8 was more upregulated in bladder cancer tissues than that of the adjacent normal ones, indicating its potential role in the progression of bladder cancer. To date, little has been done on the role of lncRNA DUUXAP8 in tumor. Some studies have reported the mechanism of DUXAP8 in gastric cancer and non-small cell lung cancer, but its role in bladder cancer has not been studied. Our study investigated the expressions of lncRNA DUXAP8 and PTEN in bladder cancer and further analyzed the relationship between DUXAP8 expression and pathological data of bladder cancer patients. Additionally, the relationship between DUXAP8 and PTEN gene was explored. The results of the CCK8 and colony formation assay showed that decreased level of DUXAP8 can inhibit the viability and proliferation of bladder cancer cells, respectively. Western blot showed that PTEN protein expression decreased after overexpression of PTEN protein, while knockdown

of DUXAP8 significantly increased the protein level of PTEN. The results showed that DUXAP8 had the ability to regulate bladder cancer by modulating PTEN gene, thus influencing the progress of bladder cancer.

## Conclusions

Our results pointed out that lncRNA DUXAP8 was overexpressed in bladder cancer tissues, which can promote the progression of bladder cancer through inhibiting PTEN.

### Funding Acknowledgements

This work was supported by the Guangdong Medical Research foundation (A2018103), Shantou Science and Technology Fund (2017026) and Guangdong Natural Science Fund Project (No. 2015A030310078).

### Conflict of Interest

The authors declared no conflict of interest.

## References

1) Meng FM, Meng FM, Song XL. MiR-576-3p is a novel marker correlated with poor clinical outcome in bladder cancer. Eur Rev Med Pharmacol Sci 2017; 21: 973-977.
2) Kakehi Y, Hirao Y, Kim WJ, Ozono S, Masumori N, Miyanaga N, Nasu Y, Yokomizo A. Bladder Cancer Working Group report. Jpn J Clin Oncol 2010; 40 Suppl 1: i57-i64.
3) Mayr R, Fritsche HM, Pycha A, Pycha A. Radical cystectomy and the implications of comorbidity. Expert Rev Anticancer Ther 2014; 14: 289-295.
4) Schaukowitch K, Kim TK. Emerging epigenetic mechanisms of long non-coding RNAs. Neuroscience 2014; 264: 25-38.
5) Sun M, Kraus WL. From discovery to function: The expanding roles of long noncoding RNAs in physiology and disease. Endocr Rev 2015; 36: 25-64.
6) Khaitan D, Dinger ME, Mazar J, Crawford J, Smith MA, Mattick JS, Perera RJ. The melanoma-upregulated long noncoding RNA SPRY4-IT1 modulates apoptosis and invasion. Cancer Res 2011; 71: 3852-3862.
7) Yuan JH, Yang F, Wang F, Ma JZ, Guo YJ, Tao QF, Liu F, Pan W, Wang TT, Zhou CC, Wang SB, Wang YZ, Yang Y, Yang N, Zhou WP, Yang GS, Sun SH. A long noncoding RNA activated by TGF-beta promotes the invasion-metastasis cascade in hepatocellular carcinoma. Cancer Cell 2014; 25: 666-681.
8) Sun M, Nie FQ, Zang C, Wang Y, Hou J, Wei C, Li W, He X, Lu KH. The pseudogene DUXAP8 promotes non-small-cell lung cancer cell proliferation and invasion by epigenetically silencing EGR1 and RHOB. Mol Ther 2017; 25: 739-751.
9) Nunes FD, de Almeida FC, Tucci R, de Sousa SC. Homeobox genes: a molecular link between development and cancer. Pesqui Odontol Bras 2003; 17: 94-98.
10) Lu X, Liu DP, Xu Y. The gain of function of p53 cancer mutant in promoting mammary tumorigenesis. Oncogene 2013; 32: 2900-2906.
11) Leslie NR, den Hertog J. Mutant PTEN in cancer: worse than nothing. Cell 2014; 157: 527-529.
12) Malmsträ M PU, Busch C, Bo JNN. Recurrence, progression and survival in bladder cancer. Scand J Urol Nephrol 1987; 21: 185-195.
13) Lee SR, Roh YG, Kim SK, Lee JS, Seol SY, Lee HH, Kim WT, Kim WJ, Heo J, Cha HJ, Kang TH, Chung JW, Chu IS, Leem SH. Activation of EZH2 and SUZ12 regulated by E2F1 predicts the disease progression and aggressive characteristics of bladder cancer. Clin Cancer Res 2015; 21: 5391-5403.
14) Brosnan CA, Voinnet O. The long and the short of noncoding RNAs. Curr Opin Cell Biol 2009; 21: 416-425.
15) Paul J, Duerksen JD. Chromatin-associated RNA content of heterochromatin and euchromatin. Mol Cell Biochem 1975; 9: 9-16.
16) Lalevee S, Feil R. Long noncoding RNAs in human disease: emerging mechanisms and therapeutic strategies. Epigenomics 2015; 7: 877-879.
17) Chen M, Zhuang C, Liu Y, Li J, Dai F, Xia M, Zhan Y, Lin J, Chen Z, He A, Xu W, Zhao G, Guo Y, Cai Z, Huang W. Tetracycline-inducible shRNA targeting antisense long non-coding RNA HIF1A-AS2 represses the malignant phenotypes of bladder cancer. Cancer Lett 2016; 376: 155-164.
18) Wang X, Gong Y, Jin B, Wu C, Yang J, Wang L, Zhang Z, Mao Z. Long non-coding RNA urothelial carcinoma associated 1 induces cell replication by inhibiting BRG1 in 5637 cells. Oncol Rep 2014; 32: 1281-1290.
19) Wang F, Li X, Xie X, Zhao L, Chen W. UCA1, a non-protein-coding RNA up-regulated in bladder carcinoma and embryo, influencing cell growth and promoting invasion. FEBS Lett 2008; 582: 1919-1927.
20) Wang XS, Zhang Z, Wang HC, Cai JL, Xu QW, Li MQ, Chen YC, Qian XP, Lu TJ, Yu LZ, Zhang Y, Xin DQ, Na YQ, Chen WF. Rapid identification of UCA1 as a very sensitive and specific unique marker for human bladder carcinoma. Clin Cancer Res 2006; 12: 4851-4858.
21) Zhuang C, Li J, Liu Y, Chen M, Yuan J, Fu X, Zhan Y, Liu L, Lin J, Zhou Q, Xu W, Zhao G, Cai Z, Huang W. Tetracycline-inducible shRNA targeting long non-coding RNA PVT1 inhibits cell growth and

induces apoptosis in bladder cancer cells. Oncotarget 2015; 6: 41194-41203.

22) Zhou Y, Zhang X, Klibanski A. MEG3 noncoding RNA: a tumor suppressor. J Mol Endocrinol 2012; 48: R45-R53.

23) Zhang X, Zhou Y, Mehta KR, Danila DC, Scolavino S, Johnson SR, Klibanski A. A pituitary-derived MEG3 isoform functions as a growth suppressor in tumor cells. J Clin Endocrinol Metab 2003; 88: 5119-5126.

24) Ma HW, Xie M, Sun M, Chen TY, Jin RR, Ma TS, Chen QN, Zhang EB, He XZ, De W, Zhang ZH. The pseudogene derived long noncoding RNA DUXAP8 promotes gastric cancer cell proliferation and migration via epigenetically silencing PLEKHO1 expression. Oncotarget 2017; 8: 52211-52224.