JBUON 2021; 26(5): 2116-2121
ISSN: 1107-0625, online ISSN: 2241-6293 • www.jbuon.com
Email: editorial_office@jbuon.com

ORIGINAL ARTICLE

BTBD7 accelerates the epithelial-mesenchymal transition, proliferation and invasion of prostate cancer cells

Bin Chen[1,2,3], Chang Liu[4], Guohui Bai[3], Yuhang Zhu[5], Houqiang Xu[1,2]

[1]College of Life Science, Guizhou University, Guiyang, China. [2]Key Laboratory of Animal Genetics, Breeding and Production in the Plateau Mountains Region, Ministry of Education, Guizhou University, Guiyang, China. [3]Life Sciences Institute of Zunyi Medical University, Special Key Laboratory of Oral Diseases Research, Higher Education Institution in Guizhou Province, Zunyi, China. [4]Guizhou University of Traditional Chinese Medicine, Guiyang, China. [5]Affiliated Hospital of Zunyi Medical University, Zunyi, China.

Summary

Purpose: *To investigate the potential function of BTBD7 in prostate cancer (PCa) development and the underlying molecular mechanism.*

Methods: *Serum levels of BTBD7 in PCa patients were examined by qRT-PCR. Regulatory effects of BTBD7 on viability and invasiveness were detected by CCK-8 and Transwell assay, respectively. Moreover, Western blot analysis was conducted to examine protein levels of epithelial-mesenchymal transition (EMT) markers (E-cadherin and N-cadherin) in PCa cells intervened by BTBD7.*

Results: *Serum level of BTBD7 was increased in PCa patients, especially those with Gleason score ≥8 or TNM staging III+IV. Knockdown of BTBD7 attenuated the viability and invasiveness of PCa cells, which upregulated E-cadherin and downregulated N-cadherin.*

Conclusion: *Serum level of BTBD7 increases in PCa patients. It accelerates PCa development by triggering proliferative and invasive potentials, as well as EMT.*

Key words: *prostate cancer, EMT, BTBD7, proliferation, invasion*

Introduction

Prostate cancer (PCa) is the most-common reproductive system malignant tumor in men. Its incidence differs in several regions, and is higher in Europe, America and Australia, but lower in Asia and North Africa. The incidence of PCa ranks top in malignant tumors of American males, following lung cancer [1,2]. It is estimated that the 5-year survival of PCa is lower than 30% [3]. Invasiveness and metastasis are the major causes influencing the long-term prognosis of PCa.

Epithelial-mesenchymal transition (EMT) is the basic process involved in the formation of cell structure, and it also participates in the regulation of tissue repair and reconstruction [4]. However, EMT is out of control in cancers, causing a detachment of cancer cells from primary foci to metastasize to distant organs. EMT is therefore believed as the biological basis of cancer infiltration and invasion [5]. Activated EMT exerts the biological functions by mediating downstream genes. Generally speaking, upregulation of N-cadherin and downregulation or loss of E-cadherin are typical EMT markers [6].

BTBD7 locates on human chromosome 14q32.12, which encodes a 126,368-Da protein with 1,132 amino acids. BTBD7 is of great significance in epithelial dynamics and organ branching [7]. In addition, it is also involved in cancer development [8,9]. BTBD7 accelerates invasiveness and metastasis of liver cancer cells by mediating EMT [8].

Corresponding author: Houqiang Xu, MD. College of Life Science, Guizhou University, No. 2708, South Section of Huaxi Avenue, Huaxi District, Guiyang, Guizhou, China.
Tel: +86 013765056884; Email: gzdxxhq@163.com
Received: 02/06/2021; Accepted: 27/07/2021

This study aimed to explore the potential influence of BTBD7 on malignant phenotypes of PCa cells, including EMT, which guides the clinical treatment of PCa.

Methods

Baseline information of subjects

Baseline information of PCa patients (n=85) and healthy volunteers (n=85) was collected for analysis. PCa was pathologically confirmed by postoperative samples of transurethral resection of prostate or laparoscopic radical prostatectomy, or transrectal prostate biopsy. Gleason score was assessed based on the *ISUP Consensus Conference on Gleason Grading of Prostatic Carcinoma, 2014* [10]. In addition, TNM staging was standardly evaluated [11]. Venous blood sample (3 mL) was collected from each participant for isolating serum. This study was approved by the Ethic Committee of Affiliated Hospital of Zunyi Medical University and written informed consent was obtained from each patient.

Cell culture and transfection

PCa cell line PC3 and prostate cell line RWPE-1 (Cell Bank of the Chinese Academy of Sciences) were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Gibco, Rockville, MD, USA), supplemented with 10% fetal bovine serum (FBS) (Gibco, Rockville, MD, USA) in an incubator containing 5% CO_2 at 37°C.

Cells were seeded in 6-well plates for 24-h adherence. They were transfected with si-BTBD7 (sense: 5'-CCACATTTAAAGGACTGTAT-3'; anti-sense: 5'- AAAAAGCCACATTTAAAGGAC-3'), BTBD7 vector or negative control using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). Fresh medium was replaced at 6 h.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Cells were lysed with TRIzol (Invitrogen, Carlsbad, CA, USA) and the isolated RNA was reversely transcribed to cDNA using the PrimeScript RT reagent Kit (TaKaRa, Otsu, Japan). After preparing a PCR system using the SYBR Primix Ex Taq II Kit (TaKaRa, Otsu, Japan), PCR was conducted by the 7500 Real Time PCR System (Applied Biosystems, Foster City, CA, USA).

BTBD7, forward primer: 5'-AGACGCCTTCGACCATCAC-3', and reverse primer: 5'-CTCCCCTAGCTGGGTTGTAGA-3'; E-cadherin, forward primer: 5'-CGAGAGCTACACGTTCACGG-3', and reverse primer: 5'-GGGTGTCGAGGGAAAAATAGG-3'; N-cadherin, forward primer: 5'-TTTGATGGAGGTCTCCTAACACC-3', and reverse primer: 5'-ACGTTTAACACGTTGGAAATGTG-3'; β-actin, forward primer: 5'-TGAGCGCGGCTACAGCTT-3', and reverse primer: 5'-TCCTTAATGTCACGCACGATTT-3'.

Cell counting kit-8 (CCK-8) assay

2×10^3 cells suspended in 200 μL of medium per well were seeded in 96-well plates. They were induced with 10 μL of CCK-8 solution (Dojindo, Kumamoto, Japan) per well at the indicated time points. After cell culture in the dark for 2 h, absorbance (A) at 450 nm was detected.

Transwell assay

Matrigel was slowly melt on ice and diluted in phosphate buffered saline (PBS) at 1:4. Twenty μL of diluted Matrigel were coated on a Transwell insert, and dried for use. 5×10^4 cells suspended in 200 μL of serum-free medium and medium containing 10% FBS were respectively applied at the top and bottom of the prepared insert. After 24-h cell culture, cells invaded from the top to the bottom were fixed in 70% ethanol for 30 min and dyed in 0.2% crystal violet for 10 min. Invasive cells in 8 random fields per sample were captured for counting (×100).

Table 1. Comparison of baseline information between prostate cancer patients and healthy volunteers

Variables	*Control group (n=85)*	*Case group (n=85)*	t/x^2	*p*
Age (years)	54.75±4.11	55.13±3.09	0.681	0.497
Hypertension (n)				
Yes	21	28	1.405	0.310
No	64	57		
Diabetes (n)				
Yes	18	14	0.616	0.557
No	67	71		
Dyslipidemia (n)				
Yes	26	23	0.258	0.611
No	59	62		
Drinking (n)				
Yes	25	32	1.293	0.255
No	60	53		
Smoking (n)				
Yes	17	24	1.575	0.209
No	68	61		

Western blot

Cells were lysed after 15-min centrifugation at 4°C, 12,000 rpm, and 50 μg protein sample per lane was prepared for 12% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and transfer on polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, MA, USA). After blocking non-specific antigens on membranes in 5% skim milk for 1.5 h, they were induced with primary and secondary antibodies under indicated conditions. Protein signals were detected using Luminol substrate solution.

Statistics

SPSS 20.0 (IBM, Armonk, NY, USA) was used for statistical processing. Data were expressed as mean±standard deviation, and differences between groups were compared using the independent t-test. Significant difference was set at $p<0.05$.

Results

Baseline information of PCa patients

A total of 85 PCa patients and 85 healthy volunteers were recruited in case group and control group, respectively. No significant differences in age, incidence of hypertension, diabetes and dyslipidemia, and history of drinking and smoking were detected between groups ($p>0.05$) (Table 1). Therefore, baseline information was comparable between PCa patients and healthy volunteers.

Serum level of BTBD7 increased in PCa patients

Compared with controls, serum level of BTBD7 was higher in PCa patients (Figure 1A). Subsequently, PCa patients were categorized by Gleason

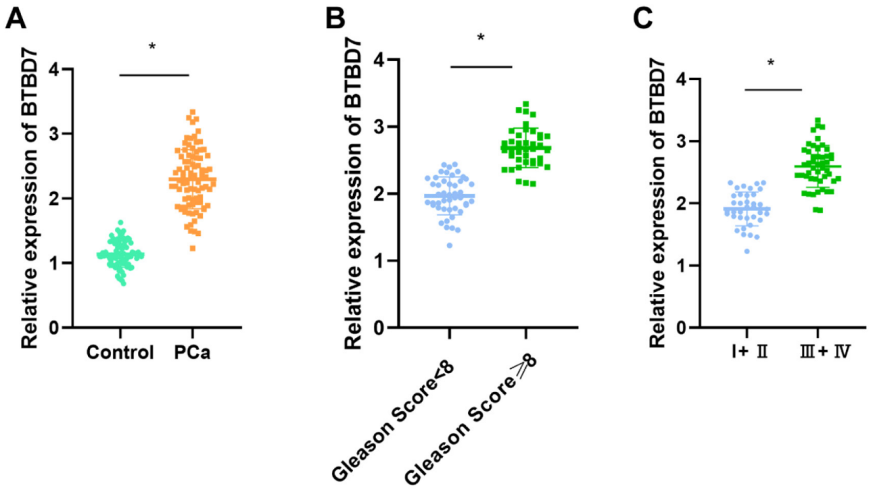

Figure 1. Serum level of BTBD7 increased in PCa patients. **A:** Serum level of BTBD7 in PCa patients (n=85) and healthy volunteers (n=85). **B:** Serum level of BTBD7 in PCa patients categorized by Gleason score. **C:** Serum level of BTBD7 in PCa patients categorized by TNM staging (*$p<0.05$).

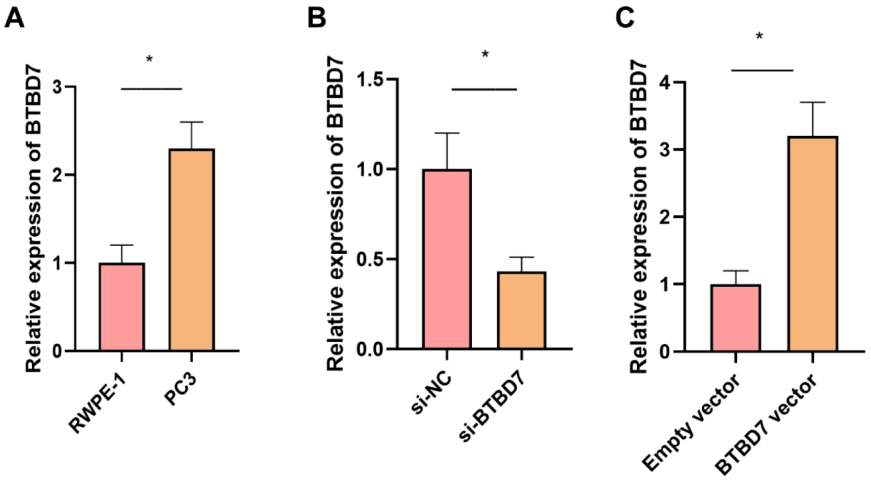

Figure 2. level of BTBD7 in PCa cells. **A:** Relative level of BTBD7 in RWPE-1 and PC3 cells. **B:** Transfection efficacy of si-BTBD7 in PC3 cells. **C:** Transfection efficacy of BTBD7 vector in PC3 cells. (*$p<0.05$).

score and TNM staging. In comparison to PCa patients with Gleason score < 8, serum level of BTBD7 was higher in those with Gleason score ≥ 8 (Figure 1B). A higher serum level of BTBD7 was detected in stage III+V PCa patients than that of stage I+II patients (Figure 1C) indicating that BTBD7 may affect the progression of PCa.

Expression level of BTBD7 in PCa cells

qRT-PCR data showed a higher level of BTBD7 in the PCa cell line PC3 than in the prostate cell line RWPE-1 (Figure 2A). Later, transfection efficacy of si-BTBD7 and BTBD7 vector was respectively tested in PC3 cells, and both of them presented a great transfection efficacy (Figure 2B,2C).

Knockdown of BTBD7 inhibited the proliferative and invasive abilities of PCa

CCK-8 assay identified that knockdown of BTBD7 markedly reduced the viability of PC3 cells from 48 h to 96 h (Figure 3A). Besides, the invasive ability was attenuated by transfection of si-BTBD7 in PC3 cells (Figure 3B).

Overexpression of BTBD7 promoted the proliferative and invasive abilities of PCa

We further examined the phenotype changes of PC3 cells overexpressing BTBD7. As expected, overexpression of BTBD7 markedly promoted the proliferative and invasive abilities of PCa cells (Figure 4A,4B).

BTBD7 triggered EMT of PCa

To illustrate the correlation between BTBD7 and EMT in PCa cells, relative levels of E-cadherin and N-cadherin were examined. Knockdown of BTBD7 upregulated E-cadherin, and downregulated N-cadherin in PC3 cells (Figure 5A,5B). Opposite expression changes were observed following overexpression of BTBD7 (Figure 5C,5D) suggesting that BTBD7 accelerated PCa progression by triggering EMT.

Discussion

PCa is a commonly diagnosed malignant tumor in men. The 5-year survival of early stage PCa is up to 90% [12]. Nevertheless, about 15-20% PCa pa-

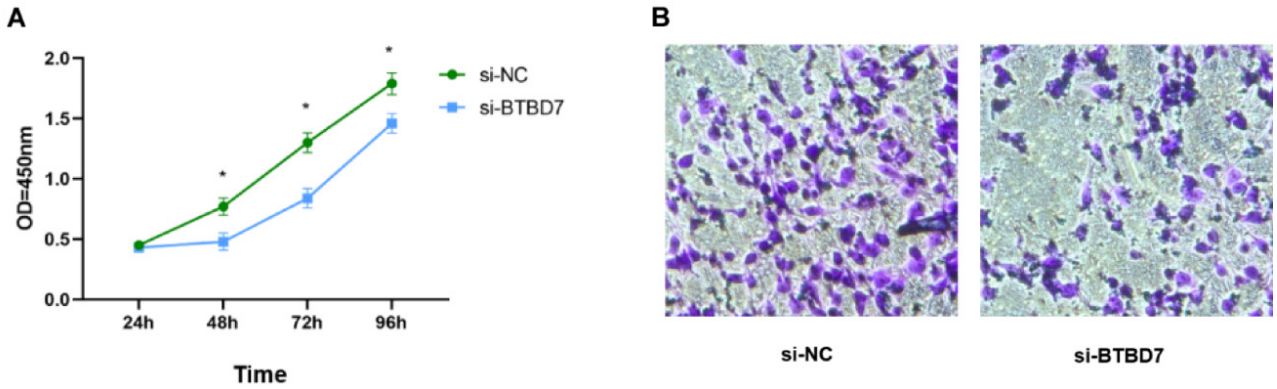

Figure 3. Knockdown of BTBD7 inhibited proliferative and invasive abilities of PCa. **A:** Cell viability in PC3 cells with BTBD7 knockdown. **B:** Cell invasion in PC3 cells with BTBD7 knockdown (*p<0.05).

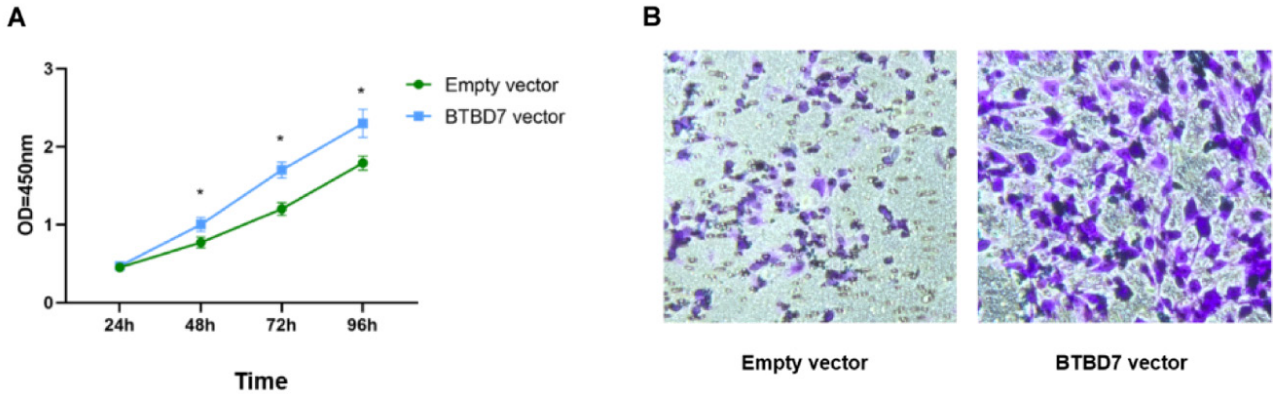

Figure 4. Overexpression of BTBD7 promoted proliferative and invasive abilities of PCa. **A:** Cell viability in PC3 cells with BTBD7 overexpression. **B:** Cell invasion in PC3 cells with BTBD7 overexpression (*p<0.05).

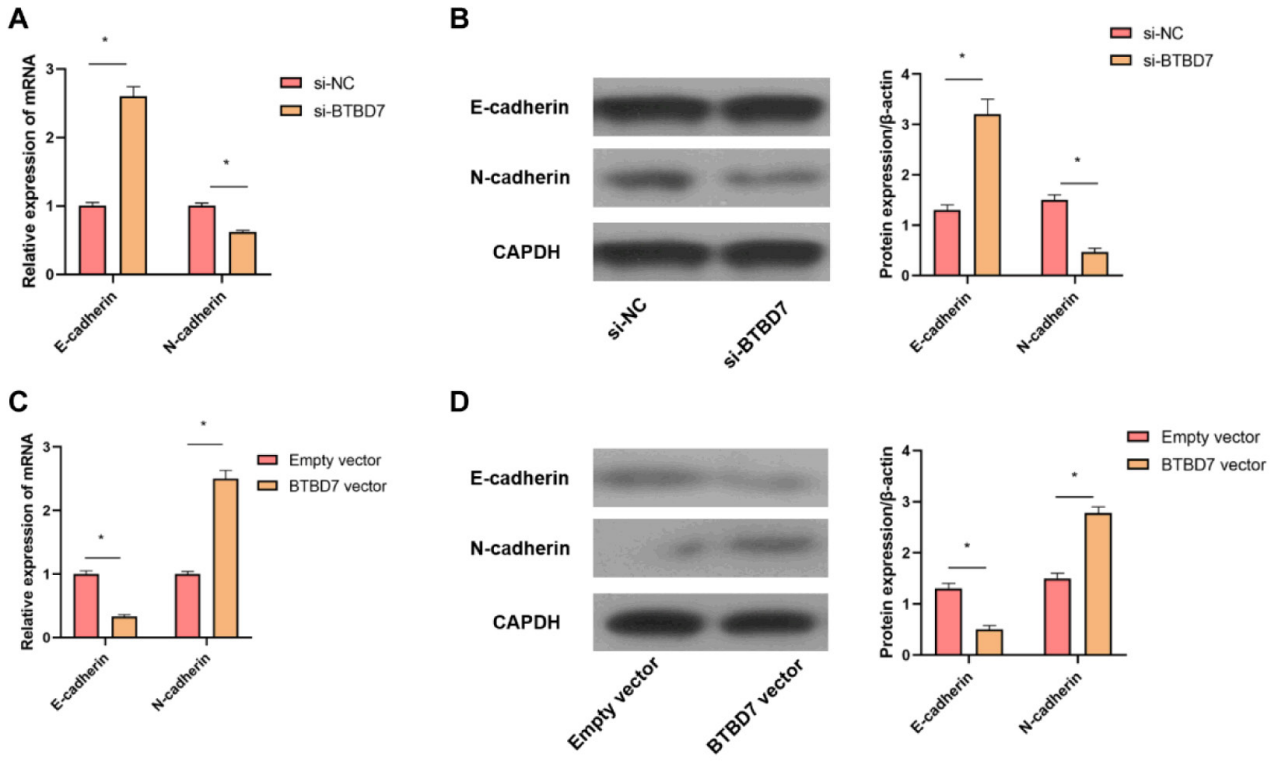

Figure 5. BTBD7 triggered EMT of PCa. **A,B:** The mRNA and protein levels of E-cadherin and N-cadherin in PC3 cells with BTBD7 knockdown. **C,D:** The mRNA and protein levels of E-cadherin and N-cadherin in PC3 cells with BTBD7 overexpression (*p<0.05).

tients may suffer from cancer invasion and metastasis, and the 5-year survival of metastatic PCa is lower than 30%. It is necessary to clarify the molecular mechanism of PCa metastasis, thus guiding the prevention and health management of metastatic cases.

BTBD7 contains 1,130 amino acid residues and two BTB/POZ domains located in the nucleus and cytoplasm, respectively. The BTB/POZ domain contains about 100 amino acid residues that are highly variable to form a spatial conformation with a unique three-dimensional fold and a larger contact plane [13,14]. The BTB/POZ domain-containing proteins are functional in eukaryotic cells. They are able to mediate tissue and organ development, protein degradation, cell movement and apoptosis, and tumor formation [15,16]. Our results showed that serum level of BTBD7 increased in PCa patients, and its level was correlated to Gleason score and TNM staging. *In vitro* experiments concluded that BTBD7 could promote the proliferative and invasive abilities of PCa cells.

For epithelium-originated malignant tumors, EMT strengthens the invasive and metastatic abilities of tumor cells. Cadherins are Ca^{2+}-dependent glycoproteins that are responsible for maintaining tissue morphology and coordinating cell movement [17]. N-cadherin and E-cadherin belong to the cadherin family. Through stabilizing cell adhesion, E-cadherin inhibits invasiveness and metastasis. It is reported that E-cadherin is widely expressed in epithelial malignant tumors, and its level is correlated to tumor stage. E-cadherin is downregulated or deficient in lowly differentiated breast cancer, gastric cancer and liver cancer cells [18,19]. N-cadherin is only expressed in neuroectoderm or mesoderm [20]. It is abundantly expressed in nerve cells and hematopoietic cells, but barely expressed in normal epithelial cells. Previous evidence has shown that N-cadherin is upregulated in PCa cells, which is correlated to a higher Gleason grade and advanced stage of PCa [21]. Nalla et al [22] reported the presence of cadherin conversion in PCa cells, that is, the conversion of E-cadherin to N-cadherin. Cadherin conversion is a critically important mechanism of initiating EMT. An *in vitro* experiment demonstrated that the intervention of BTBD7 in MDCK cells downregulates E-cadherin, which loosens cell adhesion and strengthens migratory ability [7]. Fan et al [9] indicated that lowly expressed E-cadherin in NSCLC and BTBD7 are closely related to poor prognosis. Silence of BTBD7 in lung cancer cells can upregulate E-cadherin and attenuates their migratory ability [14]. Consistently, our data showed upregulation of E-cadherin and downregulation of N-cadherin in PCa cells with BTBD7 knockdown. We believe that BTBD7 is a key molecule triggering EMT of PCa.

Conclusions

Collectively, serum level of BTBD7 was increased in PCa patients, which triggered the proliferative and invasive abilities, as well as EMT of PCa cells. Our findings provide novel ideas for clinical diagnosis and treatment of PCa. However, the conclusion needs to be further validated in *in vivo* experiments and the potential therapeutic function of BTBD7 in PCa should be supported by more clinical trials.

Funding acknowledgements

This study was supported by the National Natural Science Foundation of China (31860242); Construction Projects of Medical Biomaterial Rsearch & Development Talent Base in Guizhou Province (No.[2018]3).

Conflict of interests

The authors declare no conflict of interests.

References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011;61:69-90.
2. Bargiota A, Oeconomou A, Zachos I, Samarinas M, L PL, Tzortzis V. Adverse effects of androgen deprivation therapy in patients with prostate cancer: Focus on muscle and bone health. JBUON 2020;25:1286-94.
3. Herbert C, Liu M, Tyldesley S et al. Biochemical control with radiotherapy improves overall survival in intermediate and high-risk prostate cancer patients who have an estimated 10-year overall survival of >90%. Int J Radiat Oncol Biol Phys 2012;83:22-7.
4. Thevenot PT, Saravia J, Jin N et al. Radical-containing ultrafine particulate matter initiates epithelial-to-mesenchymal transitions in airway epithelial cells. Am J Respir Cell Mol Biol 2013;48:188-97.
5. Bitting RL, Boominathan R, Rao C et al. Development of a method to isolate circulating tumor cells using mesenchymal-based capture. Methods 2013;64:129-36.
6. Davalos V, Moutinho C, Villanueva A et al. Dynamic epigenetic regulation of the microRNA-200 family mediates epithelial and mesenchymal transitions in human tumorigenesis. Oncogene 2012;31:2062-74.
7. Onodera T, Sakai T, Hsu JC, Matsumoto K, Chiorini JA, Yamada KM. Btbd7 regulates epithelial cell dynamics and branching morphogenesis. Science 2010;329:562-5.
8. Tao YM, Huang JL, Zeng S et al. BTB/POZ domain-containing protein 7: epithelial-mesenchymal transition promoter and prognostic biomarker of hepatocellular carcinoma. Hepatology 2013;57:2326-37.
9. Fan C, Miao Y, Zhang X et al. Btbd7 contributes to reduced E-cadherin expression and predicts poor prognosis in non-small cell lung cancer. BMC Cancer 2014;14:704.
10. Epstein JI, Egevad L, Amin MB, Delahunt B, Srigley JR, Humphrey PA. The 2014 International Society of Urological Pathology (ISUP) Consensus Conference on Gleason Grading of Prostatic Carcinoma: Definition of Grading Patterns and Proposal for a New Grading System. Am J Surg Pathol 2016;40:244-52.
11. Abdel-Rahman O. Assessment of the prognostic value of the 8th AJCC staging system for patients with clinically staged prostate cancer; A time to sub-classify stage IV? Plos One 2017;12:e188450.
12. Wisniewski T, Winiecki J, Makarewicz R, Zekanowska E. The effect of radiotherapy and hormone therapy on osteopontin concentrations in prostate cancer patients. JBUON 2020;25:527-30.
13. Onodera T, Sakai T, Hsu JC, Matsumoto K, Chiorini JA, Yamada KM. Btbd7 regulates epithelial cell dynamics and branching morphogenesis. Science 2010;329:562-5.
14. Shu J, Wang L, Han F, Chen Y, Wang S, Luo F. BTBD7 Downregulates E-Cadherin and Promotes Epithelial-Mesenchymal Transition in Lung Cancer. Biomed Res Int 2019;2019:5937635.
15. Daley WP, Matsumoto K, Doyle AD et al. Btbd7 is essential for region-specific epithelial cell dynamics and branching morphogenesis in vivo. Development 2017;144:2200-11.
16. Cheng D, Qian W, Meng M, Wang Y, Peng J, Xia Q. Identification and Expression Profiling of the BTB Domain-Containing Protein Gene Family in the Silkworm, Bombyx mori. Int J Genomics 2014;2014:865065.
17. Zheng J, Zhao S, He X et al. The up-regulation of long non-coding RNA CCAT2 indicates a poor prognosis for prostate cancer and promotes metastasis by affecting epithelial-mesenchymal transition. Biochem Biophys Res Commun 2016;480:508-14.
18. Brasch J, Harrison OJ, Honig B, Shapiro L. Thinking outside the cell: how cadherins drive adhesion. Trends Cell Biol 2012;22:299-310.
19. Pal M, Koul S, Koul HK. The transcription factor sterile alpha motif (SAM) pointed domain-containing ETS transcription factor (SPDEF) is required for E-cadherin expression in prostate cancer cells. J Biol Chem 2013;288:12222-31.
20. Ando K, Uemura K, Kuzuya A et al. N-cadherin regulates p38 MAPK signaling via association with JNK-associated leucine zipper protein: implications for neurodegeneration in Alzheimer disease. J Biol Chem 2011;286:7619-28.
21. Tanaka H, Kono E, Tran CP et al. Monoclonal antibody targeting of N-cadherin inhibits prostate cancer growth, metastasis and castration resistance. Nat Med 2010;16:1414-20.
22. Nalla AK, Estes N, Patel J, Rao JS. N-cadherin mediates angiogenesis by regulating monocyte chemoattractant protein-1 expression via PI3K/Akt signaling in prostate cancer cells. Exp Cell Res 2011;317:2512-21.