European Review for Medical and Pharmacological Sciences 2018; 22: 8151-8160

High-glucose promotes proliferation of human bladder cancer T24 cells by activating Wnt/β-catenin signaling pathway

L. GAO[1], F.-M. XU[1], W.-J. SHI[1], S. ZHANG[1], Y.-L. LU[1], D.-K. ZHAO[1], Y.-F. LONG[2], R.-B. TENG[1], B. GE[2]

[1]Department of Urology, Affiliated Hospital of Guilin Medical University, Guilin, China
[2]Department of Urology, The Second Affiliated Hospital of Guilin Medical University, Guilin, China

L. Gao and F.-M. Xu contributed equally to this study

Abstract. – OBJECTIVE: Bladder cancer is the most prevalent genitourinary malignant disorder worldwide. We aimed to observe effects of high-glucose on bladder cancer proliferation and explore the associated mechanisms.

MATERIALS AND METHODS: Human bladder cancer cell line, T24, was divided into Blank, Control (Ctrl), 10 mmol/l, 20 mmol/l and 30 mmol/l group. T24 cell proliferation was evaluated by using multiple table tournament (MTT) assay and colony formation analysis, respectively. Quantitative Real-time PCR (qRT-PCR) assay was employed to examine mRNA expression of Wnt-5a and β-catenin. Meanwhile, Western blot assay was used to evaluate expression of Wnt-5a and β-catenin protein. The linear regression analysis was utilized to analyze correlation between Wnt-5a/β-catenin expression and T24 cell proliferation.

RESULTS: High-glucose significantly enhanced proliferation of T24 cells compared to that of Blank and Ctrl group ($p < 0.05$). High-glucose significantly promoted colony formation of T24 cells compared to that of Blank and Ctrl group ($p < 0.05$). High-glucose significantly up-regulated Wnt-5a mRNA and protein expression compared to that of Blank and Ctrl group ($p < 0.01$). High-glucose significantly increased β-catenin mRNA and protein expression compared to that of Blank and Ctrl group ($p < 0.01$). Effects of high-glucose on T24 cell proliferation were increased following with the enhanced glucose concentration. Wnt/β-catenin signaling pathway molecules were correlated with colony formation of T24 cells ($p < 0.05$).

CONCLUSIONS: High-glucose promoted the proliferation of T24 cells by activating the Wnt/β-catenin signaling pathway. This study would provide the novel targets for bladder cancer therapy.

Key Words:
Bladder cancer, High-glucose, Wnt/β-catenin pathway, Proliferation.

Introduction

Bladder cancer is the most prevalent genitourinary malignant disorder and the 4th frequently occurred cancer for men in the whole world[1,2]. Bladder is mainly characterized by the higher mortality rate and higher recurrence rate, and is unpredictable for the progression[3,4]. Every year, approximately 140430 cases were diagnosed as bladder cancer and about 29790 cases were dead[5]. The previous study reported that there were about 33% to 75% patients which were not sensitive to the chemo- or radio-therapy because of tumor metastasis and relapse[6]. Although plenty of clinical therapy for bladder cancer has been discovered, the 5-year survival rate is only about 50%[7]. Therefore, we believed that it's urgent to further investigate the development and carcinogenesis of bladder cancer, and to search the specific targets and therapeutic approaches[8,9]. Dysregulation of metabolic signaling pathway plays critical roles in cancers[10]. Meanwhile, the high-glucose is considered to act as a pathogenic and risk factor for the progression and development of the bladder cancer[11]. Researches[12,13] also reported that diabetes patients exhibited higher incidences of bladder cancer compared to the non-diabetes patients. Therefore, high glucose in diabetes is associated with the progression of bladder cancer patients. Although the tumor cells need higher-energy demand, the growth of tumor cells also requires plenty of bio-molecules and glucose acting as critical precursor for the bio-synthesis the molecules[14]. The cancer cells consume the glucose to produce the lactate and to support the oxidative phosphorylation of the mitochondria, even in the conditions with

Corresponding Author: Ruobing Teng, MD; e-mail: 18377338121@163.com

rich oxygen[15]. However, the mechanism for the high-glucose associated pathogenesis of bladder cancer has not been investigated till now. Varol et al[16] reported that the dysfunctional Wnt/β-catenin signaling pathway is considered as a common characteristic for plenty of tumors. Meanwhile, the Wnt/β-catenin signaling pathway also induces the impaired tumor cell differentiation and the un-controlled tumor cell proliferation. It has been observed[17,18] that Wnt/β-catenin signaling pathway participates in the bladder cancer cells invasion, metastasis, proliferation and differentiation. Therefore, we found that the Wnt/β-catenin signaling pathway might participate in the processes of high-glucose induced bladder cancer cell proliferation and metastasis. Therefore, we aimed to observe the effects of high-glucose and explore the associated mechanisms that increased the metastasis and proliferation of bladder cancer cells by evaluating Wnt/β-catenin signaling pathway related molecules.

Materials and Methods

Cell Culture and Trial Grouping

The human bladder cancer cell line, T24, was purchased from Shanghai Cell Bank of China Academia Sinica (Shanghai, China) and cultured in low-glucose Dulbecco's Modified Eagle Medium (DMEM, Gibco BRL. Co. Ltd, Rockville, MD, USA) supplementing with 10% fetal bovine serum (FBS, Gemini Bio-Products, Woodland, CA, USA) and 1% penicillin-streptomycin (Invitrogen/Life Technologies, Carlsbad, CA, USA). The T24 cells were cultured at 37°C in 5% CO_2 and humidified incubator (Thermo Electron Corp, Waltham, MA, USA). The cells were randomly divided into 5 groups, including Blank (cells treated with normal 5.5 mmol/l glucose), Control group (Ctrl, adding glucose at final concentration of 5.5 mmol/l and mannitol at final concentration of 25 mmol/l), 10 mmol/l group (at final concentration of 10 mmol/l), 20 mmol/l group (at final concentration of 20 mmol/l)and 30 mmol/l group (at final concentration of 30 mmol/l). The present study was approved by the Ethics Committee of Affiliated Hospital of Guilin Medical University (Guilin, China).

Cell Proliferation Analysis

The T24 cell proliferation was evaluated by using the multiple table tournament (MTT, Amresco Inc., Solon, OH, USA) assay according to the previous study reported[19]. Briefly, T24 cells were digested with 0.25% trypsin (Tiangen Biotech Co. Ltd., Beijing, China) and washed with Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS), and seeded onto the 96-well plates (Corning, Corning, NY, USA) at final density of 2000 cells/well (supplementing with DMEM to volume of 200 µl, and tests were conducted for 4 repeats). The cells were cultured for 24 h and then incubated with glucose according to the grouping described for 24 h, 48 h and 72 h, respectively. After that, the cells were washed with DMEM and the MTT (Amresco Inc., Solon, OH, USA) was added to each well of 96-well plates at final concentration of 5 mg/ml at 37°C for 4 h. The culture medium was removed and the dimethylsulfoxide (DMSO, Sigma-Aldrich, St. Louis, MO, USA) was added to each well (150 µl per well) to dissolve the formazan. Finally, the absorbance of each well was measured by using iMark microplate reader (Bio-Rad Laboratories, Hercules, CA, USA) at wavelength of 490 nm. The cell proliferation was represented by calculating the optical density (OD) values.

Colony Formation Analysis

The colony formation assay was conducted due to the previously published research[20]. In brief, the T24 cells were adjusted to the density of 2000 cells/well. The cells were washed with phosphate-buffered saline (PBS, Beyotime Biotech. Shanghai, China) containing Mg^{2+} twice and fixed with 4% paraformaldehyde (Tiangen Biotech Co. Ltd., Beijing, China) for 15 min. Next, the T24 cells were cultured in CO_2 condition at 37°C for 48 h and stained with the 0.1% crystal violet (Amresco Inc., Solon, OH, USA) for 15 min. Finally, the absorbance of each well was measured with iMark microplate reader (Bio-Rad Laboratories, Hercules, CA, USA) at wavelength of 560 nm. The cell proliferation rate was illustrated by calculating the OD values. Meanwhile, the formed colonies were captured and observed under a microscope (Mode: BX51, Olympus, Tokyo, Japan).

Quantitative Real-Time PCR (qRT-PCR) Assay

The total RNAs of T24 cells were extracted with TRIzol regents (Tiangen Biotech Co. Ltd., Beijing, China) according to the manufacturer's instructions. The complementary DNAs (cDNAs) were synthesized with the QuantScript

RT Kit (Cat. No. KR103, Tiangen Biotech Co. Ltd., Beijing, China) due to instruction of manufacturer. The PCR MasterMix (Cat. No. KT201, Tiangen Biotech Co. Ltd., Beijing, China) was employed to amplify the Wnt-5a, β-catenin, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) genes by using the listed primers (Table I). Thermal cycling (CT) parameters were processed according to the followings: pre-denaturation step at 94°C for 3 min, following with 30 cycles of amplification of 94°C for 30 s, 59.3°C (Wnt-5a)/56°C (β-catenin)/59°C (GAPDH) for 30 s and 72°C for 1 min. Relative levels of Wnt-5a and β-catenin were calculated with the full-automatic gel scanning system (Mode: JS-780, Shanghai Peiqing Tech. Ltd., Shanghai, China) using the formula of $2^{-\Delta\Delta ct}$ method[21].

Western Blot Assay

T24 cells were digested and lysed by using radioimmunoprecipitation assay (RIPA, Tiangen Biotech Co. Ltd., Beijing, China) and the products were centrifuged at 5000 r/min for 10 min. The protein concentration of T24 cell supernatants was evaluated with bicinchoninic acid (BCA) Protein Assay Kit (Cat. No. PA115, Tiangen Biotech Co. Ltd., Beijing, China). Next, the proteins were separated with 15% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE, Tiangen Biotech Co. Ltd., Beijing, China) and electro-transferred onto the polyvinylidene difluoride membrane (PVDF, ZSJQ BioTech. Co. Ltd., Beijing, China). The PVDF membranes were treated with 5% skim milk (ZSJQ BioTech. Co. Ltd., Beijing, China) at room temperature for 2 h, and then incubated with rabbit anti-human Wnt-5a monoclonal antibody (1:500; Cat. No. ab174963, Abcam Biotech., Cambridge, MA, USA), rabbit anti-human β-catenin monoclonal antibody (1:2500, Cat. No. ab32572, Abcam Biotech., Cambridge, MA, USA) and mouse anti-human GAPDH monoclonal antibody (1:5000; Cat. No.TA309157, ZSJQ BioTech. Co. Ltd., Beijing, China) at 4°C overnight. Polyvinylidene difluoride (PVDF) membranes were then incubated with horseradish peroxidase (HRP)-labeled goat anti-rabbit IgG (1:1000, Cat. No. ZB-2301, ZSJQ BioTech. Co. Ltd., Beijing, China) and HRP-labeled goat anti-mouse IgG (1:1000, Cat. No. ZB-2305, ZSJQ BioTech. Co. Ltd., Beijing, China) at 37°C for 2 h. Enhanced chemiluminescence (ECL, Amresco Inc., Solon, OH, USA) was used to incubate PVDF membrane in dark for 2 min. The relative grey density of Western blot bands was scanned and analyzed with Quantity One software (version: 4.66, Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Statistical Analysis

Data in this study were described as mean ± standard deviation (SD) and analyzed with SPSS software 19.0 (IBM Corp., IBM SPSS Statistics for Windows, Armonk, NY, USA). The Student's *t*-test was used to analyze the differences between two groups. The Tukey's post-hoc test was used to validate analysis of variance (ANOVA) for comparing the differences among the multiple groups. Linear regression analysis was utilized to analyze correlation between Wnt-5a/β-catenin expression and T24 cell proliferation. The experiments or tests were conducted at least for six repeats. A statistical significance was defined as $p < 0.05$.

Results

High-Glucose Enhanced Proliferation of T24 Cells

The MTT results showed that there were no significant differences for T24 cell proliferation between Blank group and Ctrl group, at 24 h, 48 h and 72 h, respectively (Table II, $p > 0.05$). Comparing with the Blank or Ctrl group, the high-glucose (10 mmol/l, 20 mmol/l and 30 mmol/l) treated T24 cells exhibited signifi-

Table I. Primers for the qRT-PCR assay.

Genes		Sequences	Length (bp)
Wnt-5a	Forwards	5'-TGCGTGTTGGGTTGAAGATA-3'	311
	Reverse	5'-TTTGATGGCACTGTTTGGAA-3'	
β-catenin	Forwards	5'-CGACAGACTGCCTTCAGATCTT-3'	162
	Reverse	5'-CCTTCAGCACTCTGCTTGTG-3	
GAPDH	Forwards	5'-TGCACCACCAACTGCTTAGC-3'	112
	Reverse	5'-GGCATGGACTGTGGTCATGAG-3'	

Table II. Proliferation of T24 cells undergoing high-glucose environment.

Groups	OD values (A490)		
	24 h	48 h	72 h
Blank	0.4210 ± 0.0164	0.6277 ± 0.1567	1.0457 ± 0.5403
Ctrl	0.4123 ± 0.0101	0.6510 ± 0.2726	1.0565 ± 0.6575
10 mmol/l	0.4347 ± 0.0158*,#	07130 ± 0.1270*,#	1.2053 ± 0.7129*,#
20 mmol/l	0.475 ± 0.0157*,#	0.7653 ± 0.1772*,#	1.3837 ± 0.5861*,#
30 mmol/l	0.5024 ± 0.0136*,#	0.8259 ± 0.2584*,#	1.5391 ± 0.4499*,#

*$p < 0.05$ *vs*. Blank group, #$p < 0.05$ *vs*. Ctrl group.

cantly higher proliferative activities, at 24 h, 48 h and 72 h, respectively (Table II, $p < 0.05$). Meanwhile, the proliferation of T24 cells was enhanced following with the increased concentration of glucose (ranging from 10 mmol/l to 30 mmol/l) (Table II), showing significant dose-effect relationship.

High-Glucose Promoted the Colony Formation of T24 Cells

The colony formation assay indicated that there were no remarkable differences for the formed colonies between Blank group and Ctrl group (Figure 1, $p > 0.05$). The colonies in High-glucose treatment T24 cells were significantly increased compared to that of Blank or Ctrl group (Figure 1, $p < 0.01$). Moreover, the colony proliferative rate in 10 mmol/l, 20 mmol/l and 30 mmol/l group was (47.73% ± 5.72%), (67.32% ± 5.2%) and (145.43% ± 10.36%), respectively (Figure 1). The statistical analysis showed that the colony proliferative rate was increased significantly following with increased concentration of glucose (Figure 1, $p < 0.01$).

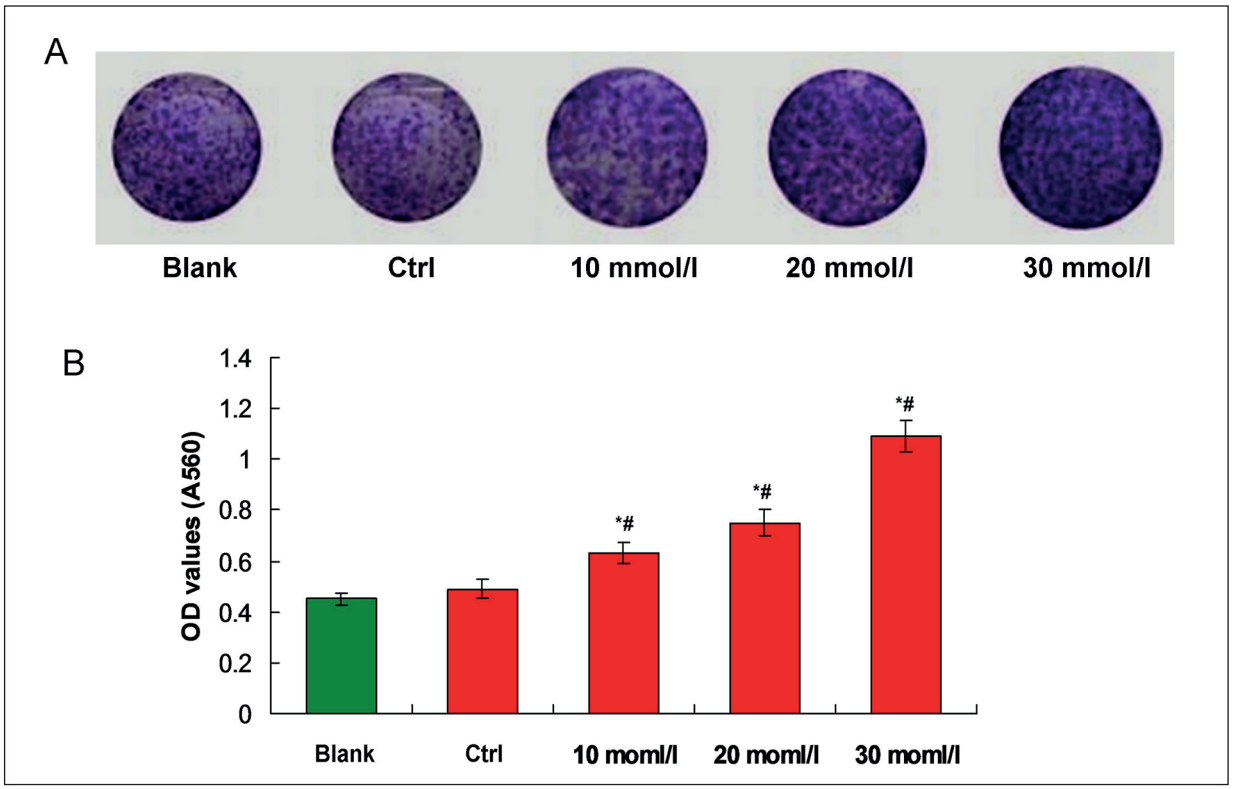

Figure 1. Proliferation of T24 cells undergoing high-glucose treatment. ***A.*** Colony formation in different groups. ***B.*** Statistical analysis for colony formation. *$p < 0.01$ *vs.* Blank group, #$p < 0.01$ *vs.* Ctrl group.

High-Glucose Up-Regulated Wnt-5a Expression

In order to clarify the mechanism triggering the high-glucose associated T24 cell proliferation, the Wnt/β-catenin signaling pathway molecule, Wnt-5a[22], was evaluated. The qRT-PCR results indicated that there were no significant differences for the Wnt-5a mRNA levels between Blank group and Ctrl group (Figure 2, $p > 0.05$). However, the high-glucose treatment (10 mmol/l, 20 mmol/l and 30 mmol/l glucose) significantly increased the mRNA levels of Wnt-5a compared to that of Blank or Ctrl group (Figure 2, $p < 0.01$). Moreover, the Western blot assay (Figure 3A) was also conducted to verify the Wnt-5a expression. Similar to the qRT-PCR findings, the Western blot results also showed that the high-glucose treatment significantly enhanced the Wnt-5a expressions compared to that of Blank or Ctrl group (Figure 3B, $p < 0.01$).

High-Glucose Increased β-Catenin Expression

In this study, the other Wnt/β-catenin signaling pathway molecule, β-catenin[23], was also examined by using qRT-PCR and Western blots assay. The qRT-PCR results showed that there were no significant differences for the β-catenin mRNA between Blank group and Ctrl group (Figure 4, $p > 0.05$). However, the β-catenin mRNA levels in High-glucose groups were significantly increased compared to that of Blank or Ctrl group (Figure 4, $p < 0.01$). Furthermore, the Western blot results also indicated that the high-glucose treatment significantly increased the Wnt-5a expression compared to that of Blank or Ctrl group (Figure 5, $p < 0.01$).

Wnt/β-Catenin Signaling Pathway Molecules Were Correlated With Colony Formation of T24 Cells

In order to clarify the Wnt/β-catenin signaling pathway associated colony formation, the correlations between Wnt-5a/β-catenin and colony formation were evaluated. The results showed that the Wnt-5a was positively correlated with the colony formation in T24 cells (Figure 6A, $p < 0.05$). Meanwhile, the β-catenin was also positively correlated with the colony formation in the T24 cells (Figure 6B, $p < 0.05$).

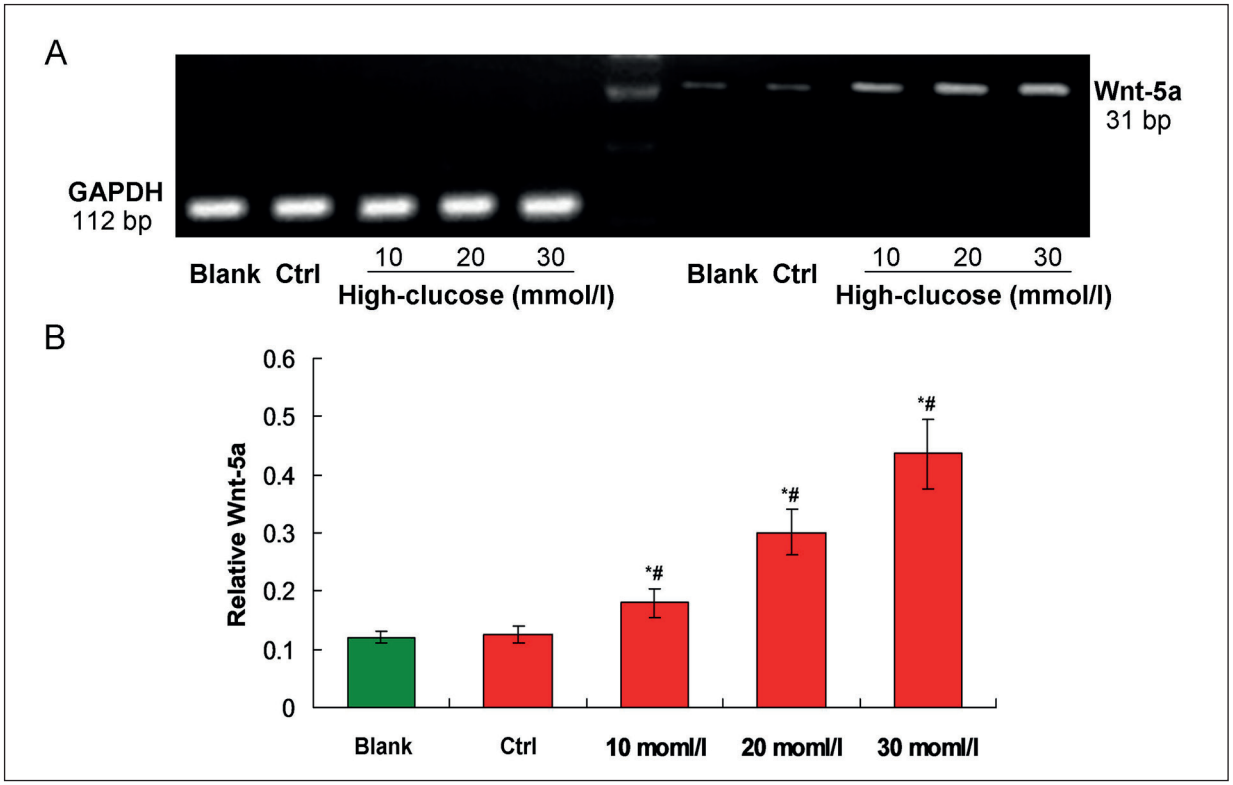

Figure 2. qRT-PCR analysis for the Wnt-5a mRNA expression in each group. A. Image for qRT-PCR amplified genes. ***B.*** Statistical analysis for Wnt-5a mRNA expression. *$p < 0.01$ *vs.* Blank group, #$p < 0.01$ *vs.* Ctrl group.

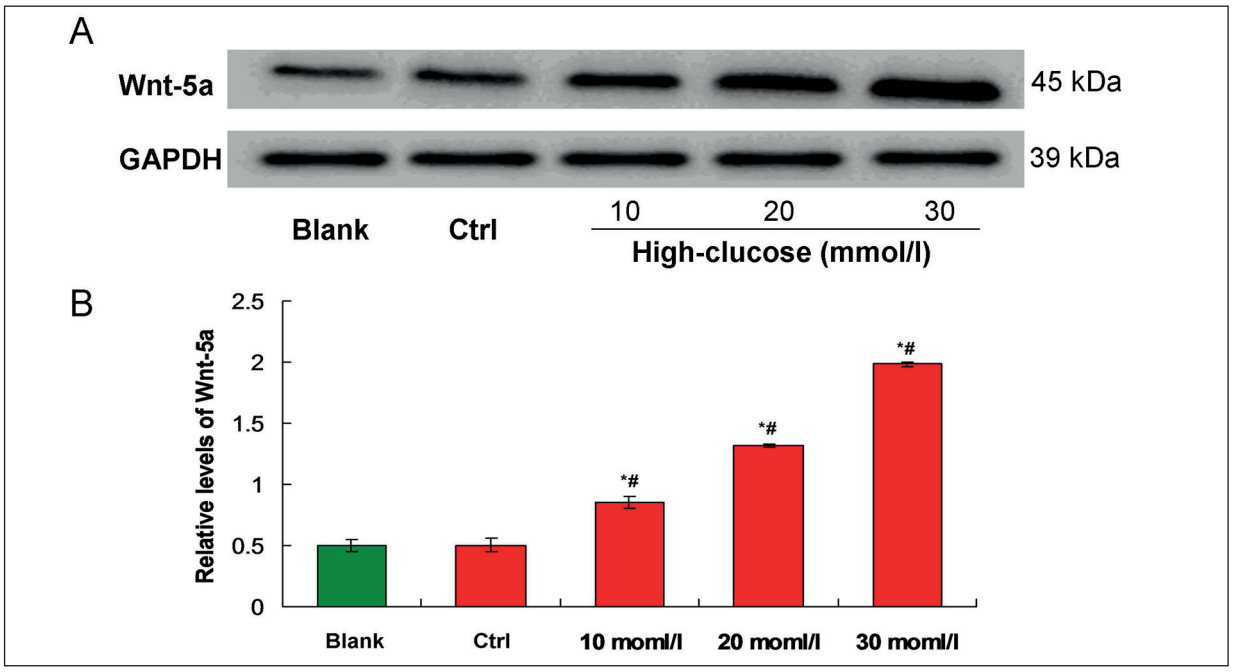

Figure 3. Western blot assay for Wnt-5a protein expression in different groups. ***A.*** Western blot image for Wnt-5a in each group. ***B.*** Statistical analysis for Wnt-5a protein expression. *$p < 0.01$ *vs.* Blank group, $^{\#}p < 0.01$ *vs.* Ctrl group.

Discussion

Following with the quick development of society, enhancement of quality of life and achievement of population aging, the diabetes mellitus has been become a global disease threatening human health[24,25]. Previous investigations[26-29] reported that the hyperglycemia

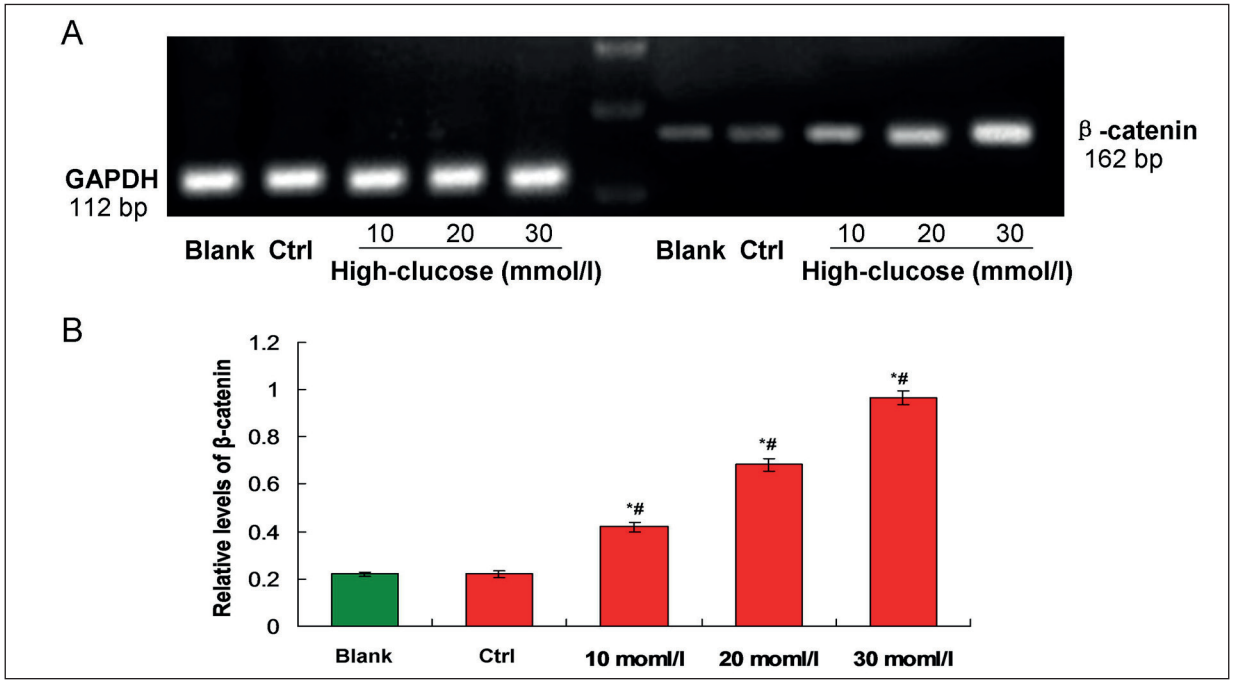

Figure 4. qRT-PCR analysis for β-catenin mRNA expression in different groups. ***A.*** qRT-PCR image for the amplified β-catenin gene. ***B.*** Statistical analysis for β-catenin mRNA expression. *$p < 0.01$ *vs.* Blank group, $^{\#}p < 0.01$ *vs.* Ctrl group.

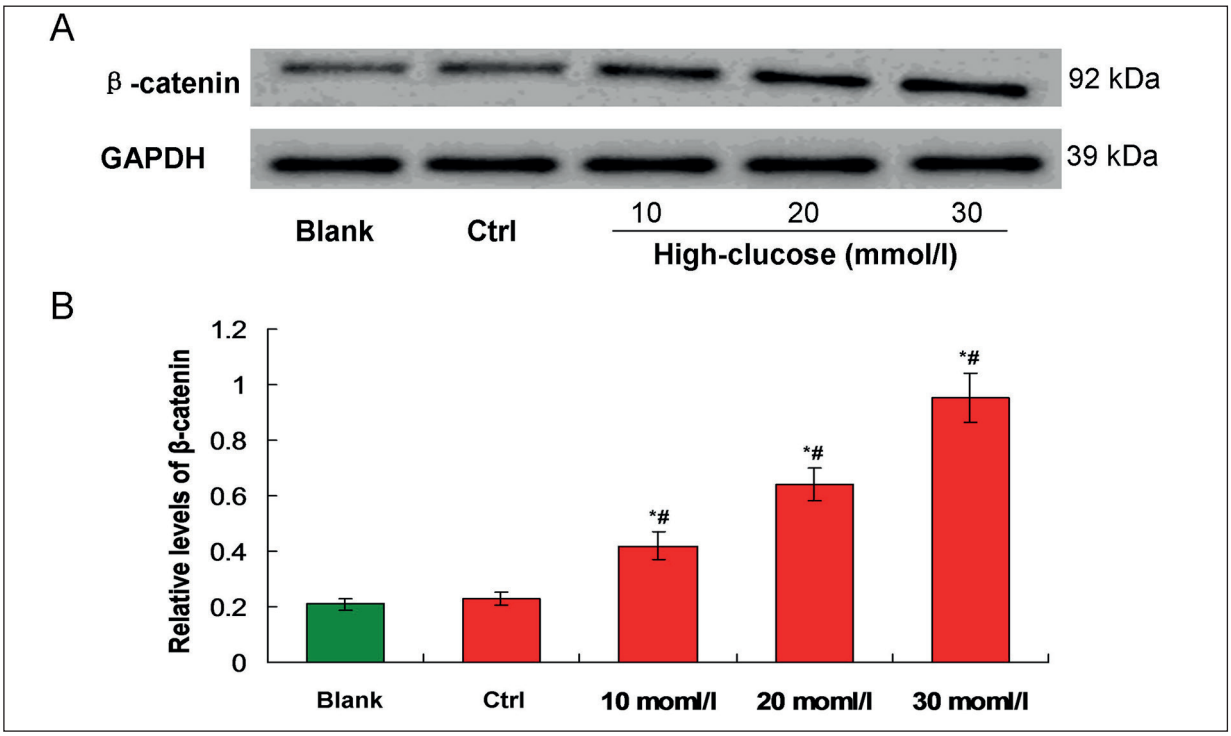

Figure 5. Evaluation of the β-catenin expression by using Western blot assay in different groups. A. Western blot image for the β-catenin in each group. B. Statistical analysis for β-catenin protein expression. *$p < 0.01$ *vs.* Blank group, #$p < 0.01$ *vs.* Ctrl group.

is closely associated with the occurrence of cancers, such as liver cancer, prostate cancer, breast cancer and bladder cancer. The occurrence rates of hyperglycemia in cancer patients are significantly higher compared to that of normal individuals. The long-term high-glucose could damage the enzyme system participating in aerobic metabolism and increase anaerobic glycolysis by activating oncogenes and silencing anti-oncogenes, finally promoting the tumor proliferation[30]. The conclusion of oxidative stress playing critical roles in the pathogenesis of metabolic diseases has been proven to be reasonable by some researches[31-33]. These reports showed that reducing reactive oxygen species (ROS) could improve the hyperlipidemia, insulin-resistant state and hepatic steatosis. Therefore, higher concentration of glucose could provide the continuous, exclusive, direct energy and create appropriate circumstance for tumor growth, and eventually promote the proliferation of cancer cells. In the present study, MTT assay and colony formation assay were used to evaluate the proliferation of T24 cells. The results indicated that the high-glucose could significantly promote the proliferation of T24 cells compared to that of normal glucose circumstance. The classical Wnt/β-catenin signaling pathway participates in multiple physiologic func-

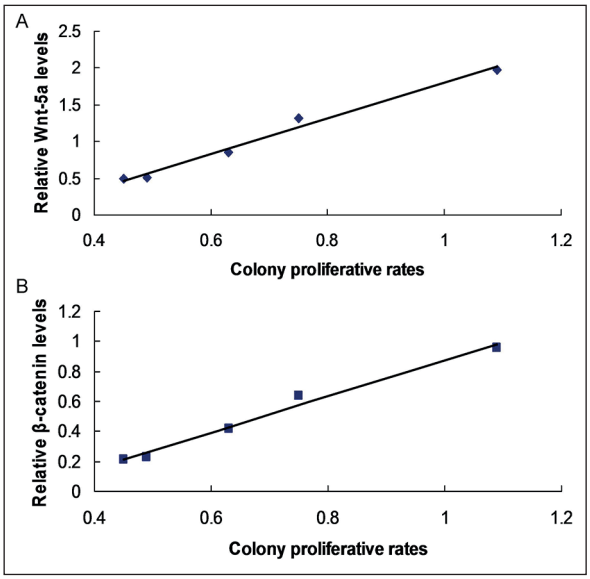

Figure 6. Correlation analysis between Wnt/β-catenin signaling molecules and T24 cell proliferation by using linear regression method. ***A.*** Correlation between Wnt-5a and T24 cell proliferation. ***B.*** Correlation between β-catenin and T24 cell proliferation.

tions, such as cell proliferation, differentiation and migration[34]. The abnormal activation of Wnt/β-catenin signaling pathway involves in the pathologic processes of bladder cancer by inhibiting glycogen synthase kinase 3β (GSK-3β) and triggering accumulation of β-catenin[35]. β-catenin is the classical and central molecule for down-regulating the down-stream factors in Wnt/β-catenin signaling pathway, and modulates tumor cell proliferation, invasion and tissue repair[35]. Hirata et al[36] also discovered that the expression of endo-nuclear β-catenin in bladder cancer cells was significantly increased. However, when the β-catenin gene was knocked out, the proliferation and invasion of bladder cancer were significantly decreased. The Wnt-5a, a critical molecule for the Wnt/β-catenin signaling pathway, mainly codes growth factor, participates in the tumor growth and differentiation and promotes the intercellular signal transduction[37,38]. Malgor et al[39] reported that Wnt-5a highly expressed in the bladder cancer tissues and was positively correlated with pathological grading, tumor-node-metastasis (TNM) classification and prognosis of bladder cancer. In our study, we found that the high-glucose induced the activation of Wnt/β-catenin signaling pathway, which may be the potential pathway that interlinks the diabetes mellitus and tumors[40]. Our findings showed that the high-glucose significantly enhanced the expression of Wnt-5a and β-catenin in T24 cells, consistently with the previous report[40]. To date, there were many hypotheses for the high-glucose associated Wnt/β-catenin signaling pathway mediated bladder cancer cell proliferation and invasion[40-42]; however, there were even no one well recognized mechanisms. Therefore, the mechanisms for the high-glucose and Wnt/β-catenin signaling pathway associated bladder cancer also need to be further investigated. Although some significant findings were obtained in this study, there were also a few limitations. On one hand, there might be plenty genes or molecules involved in the pathogenesis of bladder cancer; however, we only evaluated a few genes in this study. We would clarify, in following studies, the effects of the other genes or molecules on the bladder cancer. On the other hand, Wnt/β-catenin signaling pathway has not been evaluated in the bladder cancer patients with diabetes, which would be significant for the clinical therapy of bladder cancer.

Conclusions

We revealed the effects of high-glucose circumstance on the proliferation of T24 cells and evaluated the regulative functions of Wnt/β-catenin signaling pathway. High-glucose promoted the proliferation of T24 cells by activating the Wnt/β-catenin signaling pathway. We clarified the pathogenetic mechanism providing a novel target for bladder cancer therapy.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Acknowledgements

This study was granted by National Natural Science Foundation of China (Grant No. 81660425) and Project of Scientific Research and Technology Development of Guilin City (Grant No. 20170226).

References

1) Sigel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin 2016; 66: 7-30.
2) Lin MG, Hong YK, Zhang Y, Lin BB, He XJ. Mechanism of lncRNA DUXAP8 in promoting proliferation of bladder cancer cells by regulating PTEN. Eur Rev Med Pharmacol Sci 2018; 22: 3370-3377.
3) Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA Cancer J Clin 2015; 65: 5-29.
4) Bansal N, Gupta AK, Gupta A, Sankhwar SN, Mahdi AA. Serum-based protein biomarkers of bladder cancer: a pre- and post-operative evaluation. J Pharm Biomed Anal 2016; 124: 22-25.
5) Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011; 61: 69-90.
6) Chamie K, Litwin MS, Bassett JC, Daskivich TJ, Lai J, Hanley JM, Konety BR, Saigal CS; Urologic Diseases in American Project. Recurrence of high-risk bladder cancer: a population-based analysis. Cancer 2013; 119: 3219-3227.
7) Chen J, Wang L, Tang Y, Gong G, Liu L, Chen M, Chen Z, Cui Y, Li C, Cheng X, Qi L, Zu X. Maspin enhances cisplatin chemosensitivity in bladder cancer T24 and 5637 cells and correlates with prognosis of muscle-invasive bladder cancer patients receiving cisplatin based neoadjuvant chemotherapy. J Exp Clin Cancer Res 2016; 35: 2.
8) Liu Q, Liu H, Cheng H, Li Y, Li X, Zhu C. Downregulation of long noncoding RNA TUG1 inhibits proliferation and induces apoptosis through the TUG/miR-142/ZEB2 axis in bladder cancer cells. Onco Targets Ther 2017; 10: 2461-2471.

9) Yuan H, Yu S, Cui Y, Men C, Yang D, Gao Z, Zhu Z, Wu J. Knockdown of mediator subunit med19 suppresses bladder cancer cell proliferation and migration by downregulating Wnt/beta-catenin signaling pathway. J Cell Mol Med 2017; 21: 3254-3263.

10) Hanhan D, Weingerg RA. Hallmarks of cancer: the Warburg effect: the metabolic requirements of cell proliferation. Science 2009; 324: 1029-1033.

11) Lee YH, Yeh CH. Laminar shear stress inhibits high glucose-induced migration and invasion in human bladder cancer cells. In Vitro Cell Dev Biol Anim 2018; 54: 120-128.

12) Oh JJ, Kang MY, Jo JK, Lee HM, Byun SS, Lee SE, Lee S, Hong SK. Association between diabetes mellitus and oncological outcomes in bladder cancer patients undergoing radical cystectomy. Int J Urol 2015; 22: 1112-1117.

13) Turati F, Polesel J, Di Maso M, Montella M, Libra M, Grimaldi M, Tavani A, Serraino D, La Vecchia C, Bosetti C. Diabetes mellitus and the risk of bladder cancer: an Italian case-control study. Br J Cancer 2015; 113: 127-130.

14) Zhao W, Chang C, Cui Y, Zhao X, Yang J, Shen L, Hou Z, Zhang Z, Ye C, Hasenmayer D, Perkins R, Huang X, Yao X, Yu L, Huang R, Zhang D, Guo H, Yan J. Steroid receptor coactivator-3 regulates glucose metabolism in bladder cancer cell through coactivation of hypoxia inducible factor 1 alpha. J Biol Chem 2014; 289: 11219-11229.

15) Zhang X, Yang X, Yang C, Li P, Yuan W, Deng X, Cheng Y, Li P, Yang H, Tao J, Lu Q. Targeting protein kinase CK2 suppresses bladder cancer cell survival via the glucose metabolic pathway. Oncotarget 2016; 7: 87361-87372.

16) Varol N, Konac E, Bilen CY. Does Wnt/β-catenin pathway contribute to the stability of DNMT1 expression in urological cancer cell lines? Exp Biol Med (Maywood) 2015; 240: 624-630.

17) Pierzynski JA, Hildebrandt MA, Kamat AM. Genetic variants in the Wnt/β-catenin signaling pathway as indicators of bladder cancer risk. J Urol 2015; 194: 1771-1776.

18) Mao XW, Xiao JQ, Xu G, Li ZY, Wu HF, Li Y, Zheng YC, Zhang N. CUL4B promotes bladder cancer metastasis and induces epithelial-to-mesenchymal transition by activating the Wnt/β-catenin signaling pathway. Oncotarget 2017; 8: 77241-77253.

19) Xie J, Chen Y, Meng F, Shu T, Liu T, Zhang L, Zhang ZX. Study on the relationship between the RASSF10 gene and the biological behavior of hepatocellular carcinoma cells. Eur Rev Med Pharmacol Sci 2017; 21: 3576-3580.

20) Zhang JF, Zhang GY, Hu XM, Luo ZP, Ma YZ. MicroRNA-384 downregulates SETD8 expression to suppress cell growth and metastasis in osteosarcoma cells. Eur Rev Med Pharmacol Sci 2018; 22: 1602-1608.

21) Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2-$^{\Delta\Delta}$ct method. Methods 2001; 25: 402-408.

22) Bi LL, Han F, Zhang XM, Li YY. LncRNA MT1JP acts as a tumor inhibitor via reciprocally regulating Wnt/β-catenin pathway in retinoblastoma. Eur Rev Med Pharmacol Sci 2018; 22: 4204-4214.

23) Wang Y, Li H, Song SP. β-Arrestin 1/2 aggravates podocyte apoptosis of diabetic nephropathy via Wnt/β-catenin pathway. Med Sci Monit 2018; 24: 1724-1732.

24) Alberti KG, Zimmet P. Global burden of disease-where does diabetes mellitus fit in? Nat Rev Endocrinol 2013; 9: 258-260.

25) Bertuzzi F, Colussi G, Lauterio A, De Carlis L. Intramuscular islet allotransplantation in type 1 diabetes mellitus. Eur Rev Med Pharmacol Sci 2018; 22: 1731-1736.

26) Yang WS, Shu XO, Gao J, Li HL, Cai H, Yang G, Ji BT, Rothman N, Gao YT, Zheng W, Xiang YB. Prospective evaluation of type 2 diabetes mullitus on the risk of primary liver cancer in Chinese men and women. Ann Oncol 2013; 24: 1679-1685.

27) Moreira DM, Anderson T, Gerber L.The association of diabetes mellitus and high-grade prostate cancer in a multi-center biopsy series. Cancer Causes Control 2011; 22: 977-983.

28) Larsson SC, Mantzoros CS, Wolk A. Diabetes mellitus and risk of breast cancer, a meta analysis of cohort studies. Int J Cancer 2007; 121: 856-862.

29) Fang H, Yao B, Yan Y, Xu H, Liu Y, Tang H, Zhou J, Cao L, Wang W, Zhang J, Zhao L, Chen X, Zhang F, Zhao Y. Diabetes mellitus increases the risk of bladder cancer, an updated meta-analysis of observational studies. Diabetes Technol Ther 2013; 15: 914-922.

30) Card P, Lincet H. The cancer tumor: a metabolic parasite? Bull Cancer 2013; 100: 427-433.

31) Houstis N, Rosen ED, Lander ES. Reactive oxygen species have a causal role in multiple forms of insulin resistance. Nature 2006; 440: 944-948.

32) Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, Nakayama O, Mskishima M, Matsuda M, Shimomura I. Increased oxidative stress in obesity and its impact on metabolic syndrome. J Clin Invest 2004; 114: 1752-1761.

33) Zhang LL, Zhang ZJ. Sestrin 2 aggravates oxidative stress of neurons by decreasing the expression of Nrf2. Eur Rev Med Pharmacol Sci 2018; 22: 3493-3501.

34) Larue L, Delmas V. The WNT/beta-catenin pathway in melanoma. Front Biosci 2006; 11: 733-742.

35) Clevers H, Nusse R. Wnt/β-catenin signaling and disease. Cell 2012; 149: 1192-1205.

36) Hirata H, Hinoda Y, Ueno K, Shahryari V, Tabatabai ZL, Dahiya R. MicroRNA-1826 targets VEGFC, beta-catenin (CTNNB1) and MEK1 (MAP2K1) in human bladder cancer. Carcinogenesis 2012; 33: 41-48.

37) Mikels AJ, Nusse R. Purified Wnt-5a protein activates or inhibits β-catenin-TCF signaling depending on receptor context. PLoS Biol 2006; 4: e115-e116.

38) Wu BQ, Cao Y, Bi ZG. Suppression of adriamycin resistance in osteosarcoma by blocking Wnt/β-catenin signal pathway. Eur Rev Med Pharmacol Sci 2017; 21: 3185-3192.

39) Malgor R, Crouser S, Greco D, Brockett C, Coschigano K, Nakazawa M, Jenkinson S. Correlation of Wnt5a expression with histopathological grade/stage in urothelial carcinoma of the bladder. Diagn Pathol 2013; 8: 139.

40) Garcia-Jimenez C, Garcia-Martinez JM, Chocarro-Calvo A, De la Vieja A. A new link between diabetes and cancer: enhanced Wnt-β-catenin signaling by high glucose. J Mol Endocrinol 2014; 52: R51-R66.

41) Chocarro-Calvo A, Garcia-Martinez JM, Ardila-Gonzalez S, De la Vieja A, Garcia-Jimenez G. Glucose-induced β-catenin acetylation enhances Wnt signaling in cancer. Mol Cell 2013; 49: 474-486.

42) Wang SY, Yang SX, Zhao XX, Chen F, Shi J. Expression of the Wnt/beta-catenin signal pathway in patients with acute renal injury. Eur Rev Med Pharmacol Sci 2018; 22: 4661-4667.