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Original Article

Large conductance voltage and Ca^{2+}-activated K^+ channels affect the physiological characteristics of human urine-derived stem cells

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Abstract: We investigated the current characteristics of large conductance voltage and Ca^{2+}-activated K^+ (BK) channels in human urine-derived stem cells (hUSCs) and the effect of BK channels on proliferation and differentiation of hUSCs. Fresh human urine (n=6) was collected from healthy donors to isolate hUSCs. Human KCNMA1 gene silencing U6 shRNA was used to down regulate the expression of BK in hUSCs. IBTX (BK channel antagonist) and NS1619 (BK channel agonist) were used to examine the effect of BK channels on hUSCs. Whole cell patch-clamping was employed to detect the current of BK channels. Flow cytometry, immunofluorescence, and western blotting were used to analyze the cell cycle and related protein levels. The results showed that the activities of BK channels were significantly decreased in P5, P7 and induced hUSCs (endothelial, urothelial and smooth muscle cells) compared with P3 hUSCs when normalized to the cell capacitance. In addition, the average BK channel current density of hUSCs was significantly decreased upon silencing BK channel expression by hnRNA. Apoptosis rates of hUSCs in iberiotoxin (IBTX) and hnRNA treatment groups were significantly increased compared with the control group, whereas treatment with BK agonist NS1619 decreased apoptosis rates. Compared with the control group, hUSCs in S phase were significantly decreased in IBTX and hnRNA treatment groups. In conclusion, BK channels play an important role in maintaining the proliferation and differentiation of hUSCs. Overexpression of BK channels in hUSCs be provide a basis for future clinical application to an overactive bladder.

Keywords: BK channel, human urine-derived stem cells, overactive bladder, proliferation, differentiation

Introduction

Overactive bladder (OAB) is a prevalent condition in both men and women. The EPIC study showed that the prevalences of OAB are 10.8% in men and 12.8% in women [1]. According to the International Continence Society (ICS) definition, OAB consists of urinary urgency with or without urge incontinence, often accompanied by frequency and nocturia. OAB seriously affects the quality of life and social interactions of patients [2]. The mechanism of OAB is still unclear. Most researchers believe that the potential mechanism of OAB is thought to be neurogenic and/or myogenic, resulting from changes in detrusor innervation, cell-to-cell communication, and myocyte excitability [3-5]. The myogenic basis is associated with an increase in the excitability and spontaneous contractile activity of the detrusor overactivity myocytes [3, 6]. In recent years, large conductance voltage and Ca^{2+}-activated K^+ (BK) channels have been considered as a novel therapeutic target for treatment of OAB because BK channels control excitability and contractility of bladder smooth muscle in normal and pathophysiological conditions [6-9].

BK channels consist of four pore-forming α subunits, which are encoded by a single *KCNMA1* gene, and four optional accessory β subunits [10, 11]. The BK channel is the only K^+ channel activated by increases in both intracellular Ca^{2+} and membrane depolarization. Therefore, it is uniquely suited to serve as a Ca^{2+}/voltage signal integrator in the modulation of detrusor smooth muscle (DSM) cell membrane excitability [9-13]. BK channels inhibit Ca^{2+} influx through

voltage-dependent calcium channels in smooth muscle cells to reduce their excitability and contractile activity [6, 9, 13]. The overall physiological function of the BK channel is to reduce membrane excitability and oppose both myogenic and nerve-evoked DSM contractions. Recent data suggest that decreases in BK channel expression, function, or regulation appear to be involved in some forms of OAB etiology and thought to be critical for the development of OAB [6-9, 13]. The high level of BK channel expression in urinary bladder smooth muscle cells (SMCs) and the lack of BK channel expression in the plasma membrane of cardiac cells, along with their unique properties that reduce membrane excitability and oppose both myogenic and nerve-evoked DSM contractions, have made BK channels very attractive intervention targets for OAB [6-9, 12, 13].

Human urine-derived stem cells (hUSCs) were first found and described by Zhang et al [14]. Similar to other types of stem cells, they have two general properties: capable of dividing and renewing themselves for long periods [15]; unspecialized and give rise to specialized cell types [16-18]. Application of hUSCs in clinical treatment of urinary system diseases has advantages. First, they are collected by noninvasive methods. Second, hUSCs can be used for autologous therapy, avoiding immune rejection after transplantation. Third, there are less ethical concerns because urine is self-provided [19-23]. Recent research including our own has shown that, compared with hUSC therapy alone, genetically modified hUSCs may offer more effective treatments for urinary system diseases [14-18].

Considering the advantages of hUSCs in clinical treatment of urinary system diseases, and that BK channels are considered as a novel therapeutic target for the treatment of OAB, we overexpressed BK channels in hUSCs as a treatment for OAB. First, we investigated the characteristics BK channel in hUSCs and the effect of BK channels on hUSCs. We also analyzed the current characteristics of BK channels in hUSCs and the effect of BK channels on the proliferation and differentiation of hUSCs.

Methods

Culture of hUSCs

As we reported previously, fresh human urine (n=6) was collected from healthy donors. After mid and last stream urine was collected, urine samples were immediately transferred to the laboratory for cell isolation and culture [24]. Briefly, urine samples were centrifuged at 500 *g* for 5 min to collect cells. The cell pellet was gently resuspended in mixed medium, including embryo fibroblast medium (EFM) and keratinocyte serum-free medium (KSFM) (1:1 ratio), and seeded in 24-well plates [passage (P) 0]. Individual hUSCs appeared at 3-5 days after initial seeding and reached 70-80% confluence after another 3-4 days. The cells were trypsinized and transferred to 6-well plates (P1). Finally, the cells were transferred to a 100-mm culture dish (P2) for expansion. P3 hUSCs were used for most experiments.

Lentivirus transduction

For lentivirus transduction, P3 hUSCs were seeded at 2000 cells/cm^2 in a 6-well plate and then infected by a human KCNMA1 gene-silencing U6 shRNA construct in a retroviral green fluorescent protein vector (Obio Technology Co, Ltd, Shanghai, China) at a multiplicity of infection of 40 with 5 mg/ml polybrene (Sigma-Aldrich, USA). The medium was changed after 12 h. The viruses were collected for further experiments after 3 days by ultracentrifugation.

Flow cytometry

Cells seeded in 6-well plates (1000 cells/cm^2) were treated with 3×10^{-6} mol/L NS1619 (Sigma-Aldrich), an agonist of BK, or 2×10^{-7} mol/L iberiotoxin (IBTX; Sigma-Aldrich) an antagonist of BK, for 48 h. Cells transduced with the human KCNMA1 gene-silencing lentivirus (KCNMA1$^{-/-}$) were incubated for 72 h. The cells were harvested by trypsin-EDTA treatment and washed with phosphate-buffered saline (PBS) twice. Then, the cells were incubated at room temperature in binding buffer (500 μL) containing Annexin V-FITC (2 μL) and propidium iodide for 15 min. Cells were analyzed for apoptosis in a MoFlo XDP flow cytometer (Beckman Coulter, USA). To analyze the cell cycle, cells were collected by the same method and fixed in precooled 70% ethanol at 4°C overnight, centrifuged at 1000 rpm for 10 min, and then treated with propidium iodide and RNase. A total of 1×10^4 gated cells were acquired in each sample and analyzed using Flowmax software (Beckman Coulter).

Endothelial, urothelial, and smooth muscle differentiation of hUSCs in vitro

A single clone of P3 hUSCs was plated at a density of 1000 cells/cm^2 was cultured in medium for 14 days to differentiate into three lineages: endothelial cells (ECs), SMCs, and urothelial cells (UCs). Endothelial Growth Medium-2 containing 30 ng/mL vascular endothelial growth factor (VEGF) was used for EC differentiation. Equal volumes of Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) and EFM containing 2.5 ng/mL transforming growth factor-β1, 5 ng/mL platelet-derived growth factor-BB, and 10% FBS were used for SMC differentiation. DMEM containing 10% FBS was mixed with KSFM at a 1:4 ratio and supplemented with 30 ng/mL epidermal growth factor for UC differentiation. The differentiation medium was replaced every third day. All growth factors were purchased from R&D Systems (Minneapolis, MN).

Confocal microscopy

As we reported previously [25], hUSCs grown on glass coverslips were fixed with 4% paraformaldehyde for 30 min and then washed. After permeabilization with cold acetone for 3 min, the fixed hUSCs were blocked with 1% bovine serum albumin at room temperature and then incubated with the primary antibodies including mouse anti-CD31, mouse anti-desmin, and mouse anti-AE1/AE3 at 4°C overnight. The next day, the hUSCs were washed with PBS for 10 min three times and then incubated with anti-mouse Alexa Fluor 488 (1:200; Beyotime, Shanghai, China) for 2 h at room temperature. After washing with PBS for 10 min three times, the hUSCs were counterstained with 4,6-diamidino-2-phenylindole (Sigma-Aldrich, St. Louis, MO) for 10 min at room temperature. Then, the hUSCs were observed under a laser scanning confocal microscope (Leica, Solms, Germany).

Western blot analysis

Proteins of each sample were extracted according to a standard protocol as reported previously [26]. Equal amounts of proteins (40 μg) from each sample were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and then transferred to polyvinylidene fluoride membranes (Immobilon-PSQ; Millipore, Billerica, MA, USA). The membranes were incubated with blocking solution (5% bovine serum albumin) for 2 h at room temperature. Then, the membranes were incubated with primary antibodies including rabbit anti-cyclin-dependent kinase 2, rabbit anti-cyclin D1, rabbit anti-BK-α, rabbit anti-BK-β, mouse anti-glyceraldehyde-3-phosphate dehydrogenase, and mouse anti-tubulin at 4°C overnight. The following day, the cells were washed with Tris-buffered saline containing 0.1% Tween 20 for 10 min three times and then incubated with the horseradish peroxidase-conjugated secondary antibody (1:5000, Beyotime) for 2 h at room temperature. Detection of immunoreactive bands was performed by enhanced chemiluminescence (Millipore) with the ChemiDoc XRS+Image System (Bio-Rad Laboratories, Bay Street, CA).

Electrophysiology

The electrophysiological properties of BK currents in cultured hUSCs were investigated in glass coverslip-adhered single cells by the whole cell patch-clamp technique. For whole cell recordings, the pipette solution consisted of 140 mM KCL, 1 mM $MgCl_2$, 0.05 mM EGTA, and 10 mM Hepes with the pH adjusted to 7.2 by sodium hydroxide. The bath solution consisted of 134 mM NaCl, 6 mM KCL, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 10 mM glucose, and 10 mM Hepes with the pH adjusted to 7.4 by sodium hydroxide. The pipette resistance varied between 4 and 6 MΩ. BK currents were recorded using a HEKA EPC10 USB amplifier (HEKA Elektronik, Germany) and filtered at a threshold frequency of 2.9 KHz. The BK current was recorded in voltage-clamp mode by applying voltage steps in 10 mV increments from -40 to 80 mV for 200 ms with a holding potential of -70 mV. Data were analyzed using FitMaster software (HEKA Elektronik). The density of BK currents was normalized to the cell capacitance.

Statistical analysis

All statistical analyses were conducted using R (http://www.R-project.org/). Data are presented as the mean ± standard deviation. Chi square, analysis of variance, and a t-test with Bonferroni correction were used for analysis of contingency tables depending on the sample sizes. A value of $P<0.05$ was considered as significantly different.

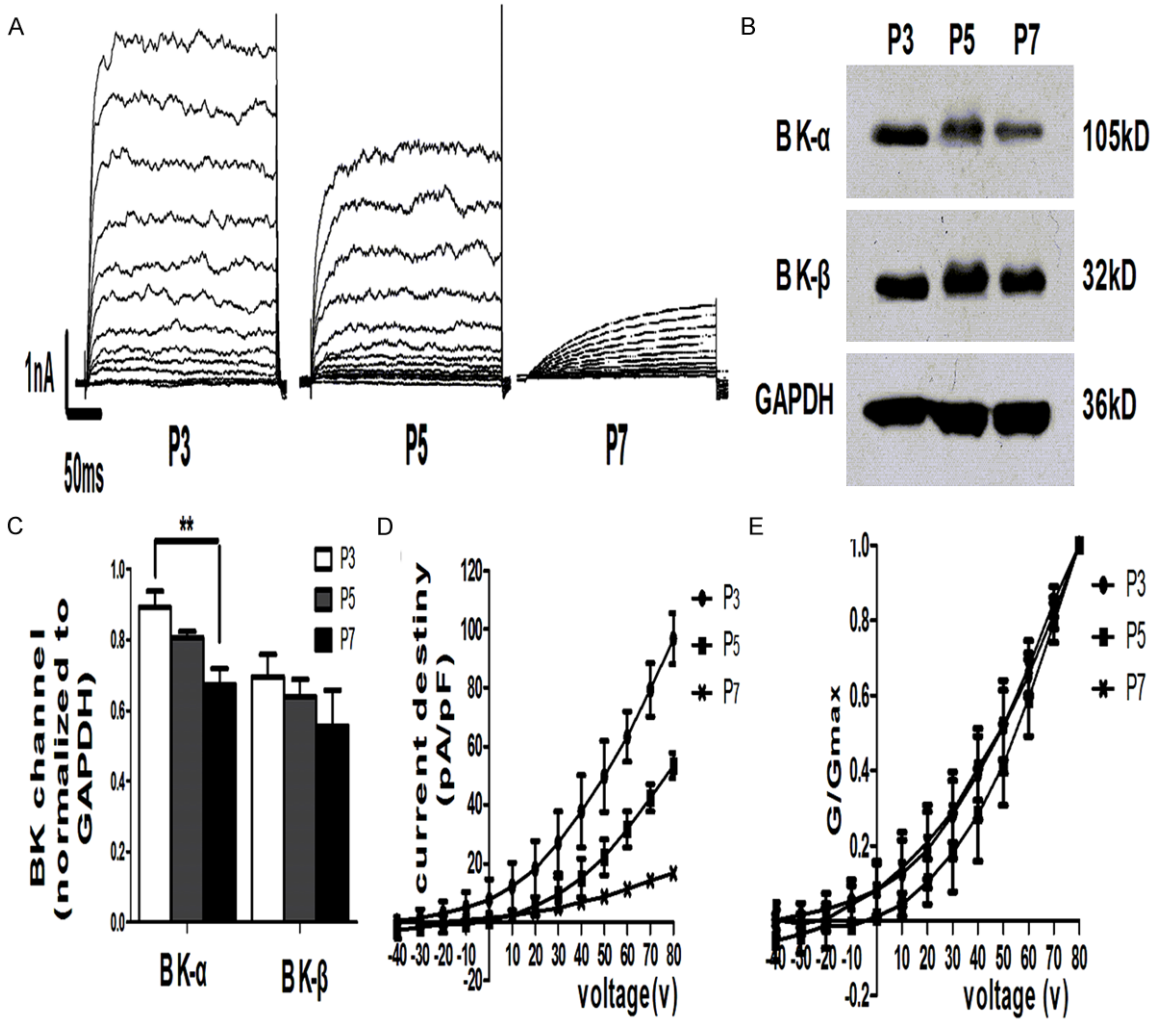

Figure 1. Altered BK channel currents in different passages of hUSCs. Patch clamping showed that the current of BK channels gradually decreased from P3 to P7 in hUSCs (A, D and E). Western blotting showed that BK α subunits gradually decreased from P3 to P7 in hUSCs (B and C). P, passage; hUSCs, human urine-derived stem cells.

Results

Changes of BK channel currents in different passages of hUSCs

BK channel currents in different passages of cultured hUSCs were recorded using the voltage-clamp protocol. Average hUSC capacitances were 33.45, 38.76, and 42.16 pF in P3, P5, and P7 cultured hUSCs, respectively (**Figure 1A**). hUSCs responded with a gradual increase of current when evoked using -40-80 mV in 10 mV increments with a 100 ms duration. Western blotting showed that the level of BK-α protein decreased gradually ($P<0.01$, n=4) from P3 to P7, but no change was observed in BK β-subunits ($P>0.05$, n=6, **Figure 1B** and **1C**). The average current density of BK channels in hUSCs was significantly decreased at P5 and P7 compared with P3 cultured cells when normalized to the cell capacitance ($P<0.001$, **Figure 1D** and **1E**).

Silencing of BK channels in hUSCs induces apoptosis

We examined the activities of hUSCs under different conditions of the BK channel. The average current density of BK channels in hUSCs was significantly decreased upon silencing of BK channels by hnRNA ($P<0.01$, **Figure 2A**). The apoptosis rates of hUSCs in IBTX and hnRNA treatment groups were significantly increased compared with control groups ($P<0.01$, n=6, **Figure 2B** and **2C**), whereas the BK agonist NS1619 decreased the apoptosis rate ($P<0.05$, n=6, **Figure 2B** and **2C**).

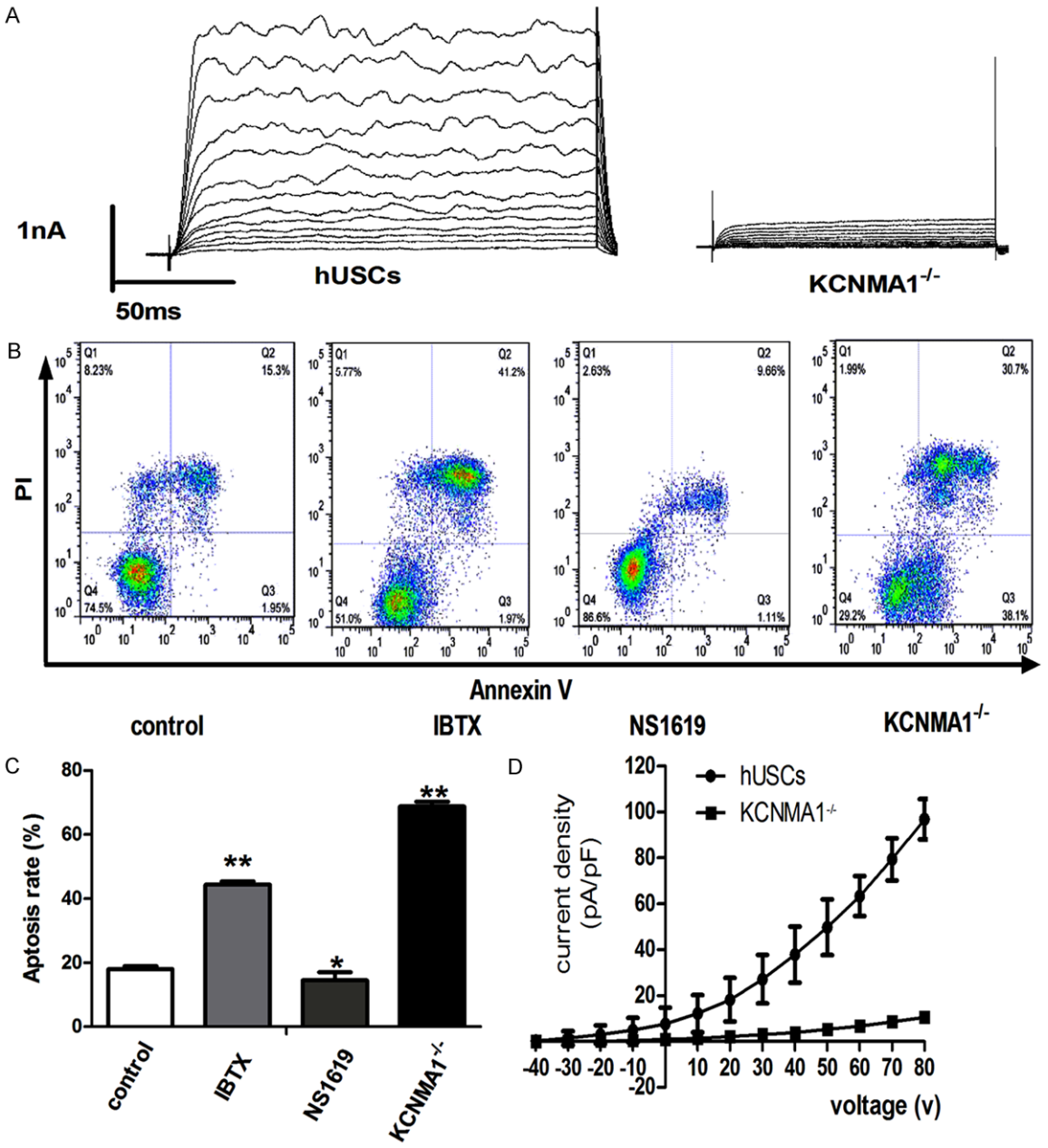

Figure 2. Silencing BK channels in hUSCs and altered apoptosis under different treatments. BK channel currents in control and BK knockdown hUSCs (A and D). Flow cytometric analysis showing decreased apoptosis rates of hUSCs in IBTX and KCNMA1 knockdown groups compared with the control group. However, it was increased in the IBTX group (B and C). *P<0.05; **P<0.01.

Changes of the cell cycle in the four treatments of BK channels

Flow cytometry was employed to detect changes in the cell cycle of hUSCs in IBTX, NS1619, KCNMA1 knockdown and control groups (**Figure 3A**). Compared with the control group, hUSCs in S phase were significantly decreased by IBTX and hnRNA treatments (P<0.01, **Figure 3B**). Therefore, loss-of-function of the BK channel may act on G1/S phase and inhibit cell growth.

Differentiation of hUSCs into ECs, UCs, and SMCs

hUSCs were successfully induced into ECs, UCs, and SMCs. The three types of induced

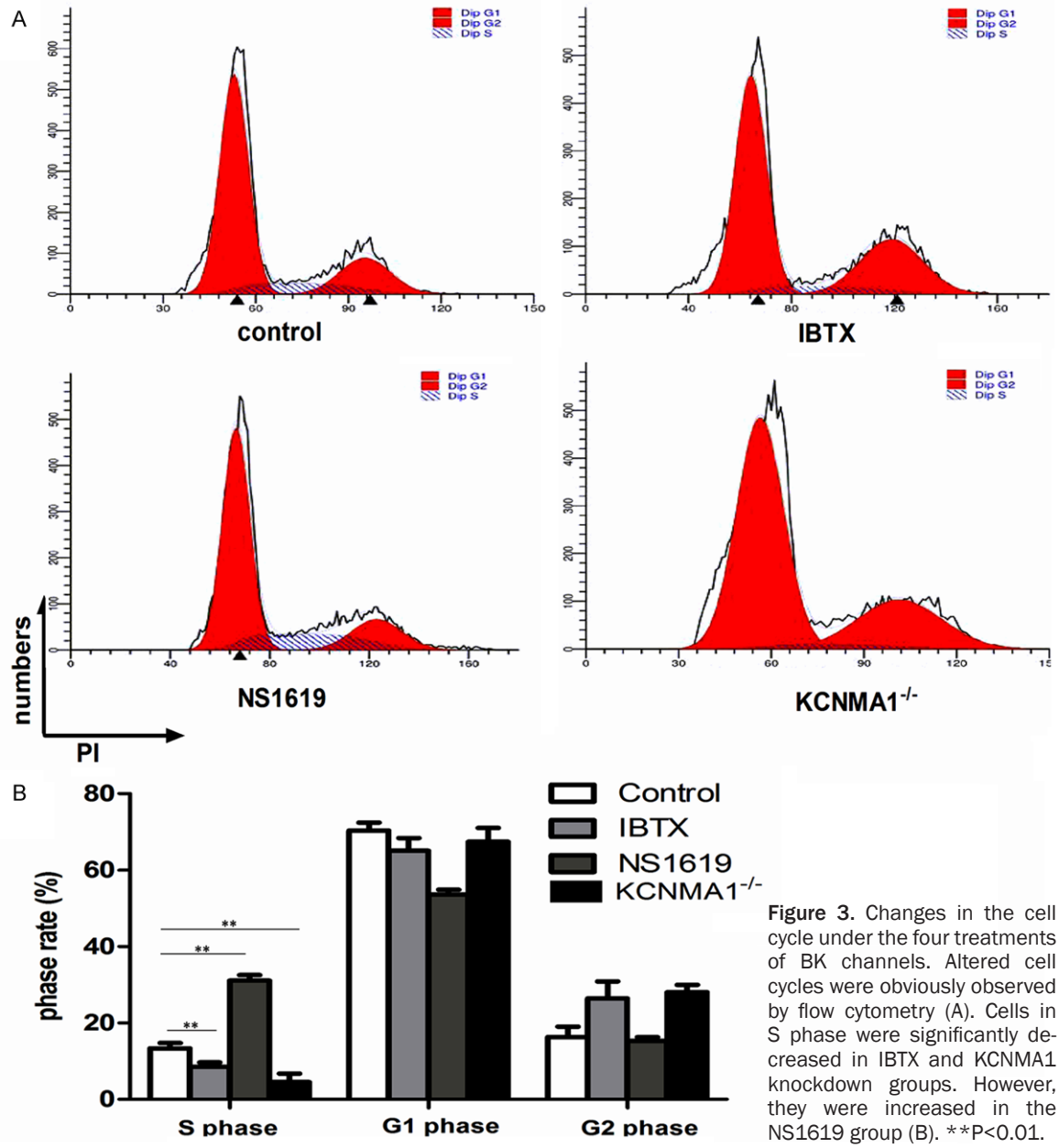

Figure 3. Changes in the cell cycle under the four treatments of BK channels. Altered cell cycles were obviously observed by flow cytometry (A). Cells in S phase were significantly decreased in IBTX and KCNMA1 knockdown groups. However, they were increased in the NS1619 group (B). **P<0.01.

cells were identified by morphology (**Figure 4A**), immunofluorescence staining of markers (**Figure 4B**), and western blot analysis (P<0.05, n=6, **Figure 4C**). The average current density of BK channels was significantly decreased in the three types of induced cells compared with P3 cultured hUSCs when normalized to the cell capacitance (P<0.001, **Figure 5**).

Discussion

This study provides evidence regarding the importance of BK channels for the proliferation and differentiation of hUSCs. BK channels are necessary for maintenance and differentiation of hUSCs and the lack of BK channels can induce apoptosis of hUSCs. In addition, the current density of BK channels was significantly decreased at P5 and P7 compared with P3 cultured cells and significantly decreased after directed differentiation of hUSCs to ECs, UCs, or SMCs.

It has been 8 years since the discovery of hUSCs. hUSCs have already been applied to directed differentiation [17-19, 24], tissue engi-

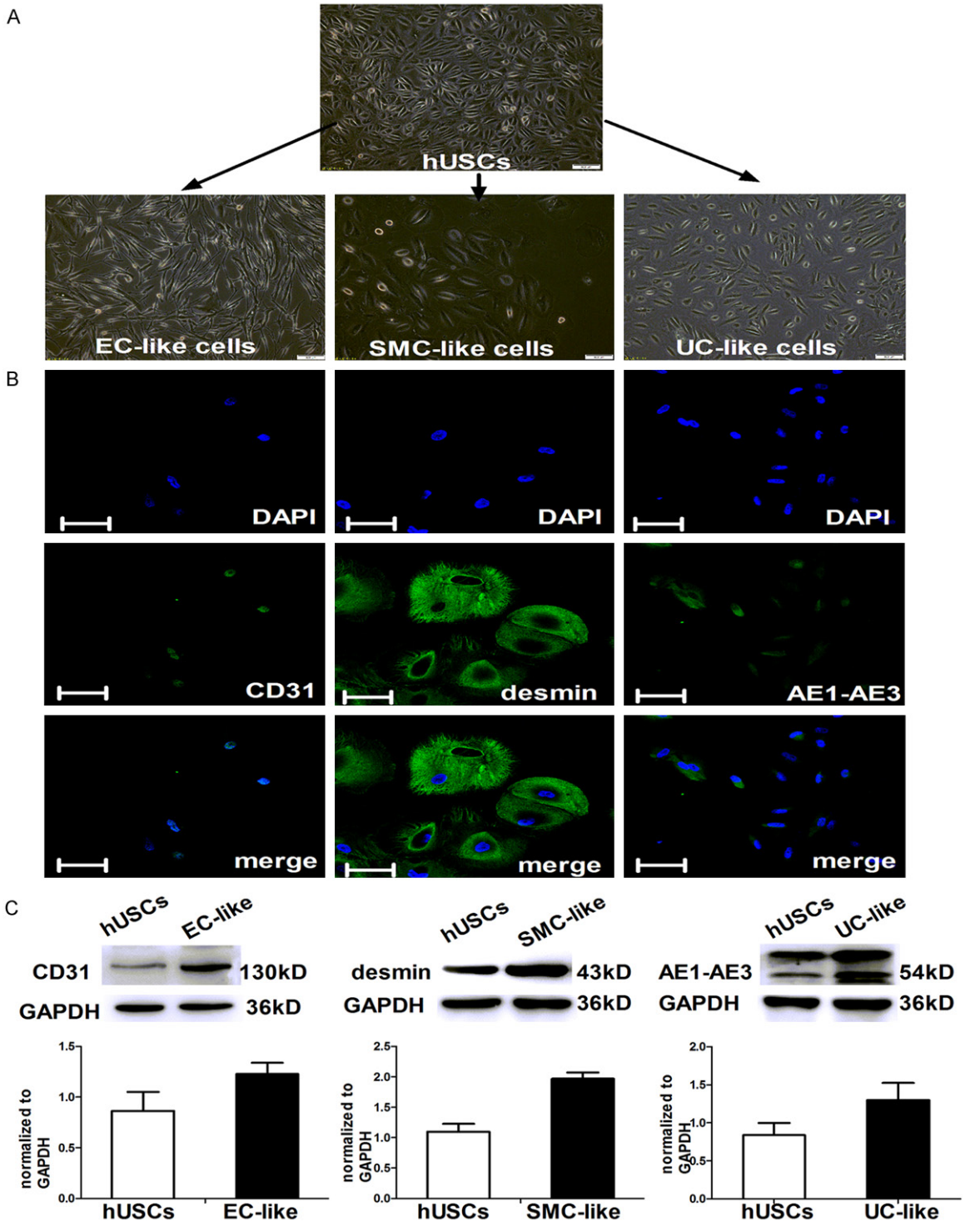

Figure 4. Differentiation of hUSCs into ECs, UCs, and SMCs. Morphology of non-induced hUSCs and induced ECs, UCs, and SMCs (A). Immunofluorescence staining of EC marker CD31, UC markers AE1/AE3, and SMC marker desmin (B). Western blotting of the cell markers (C).

neering [19, 27, 28] and genetic modification [29]. For the treatment of urinary system diseases, hUSCs have some advantages. First, collection methods are simple, safe, low cost, and non-invasive. Second, hUSCs can be used for autologous therapy avoiding immune rejec-

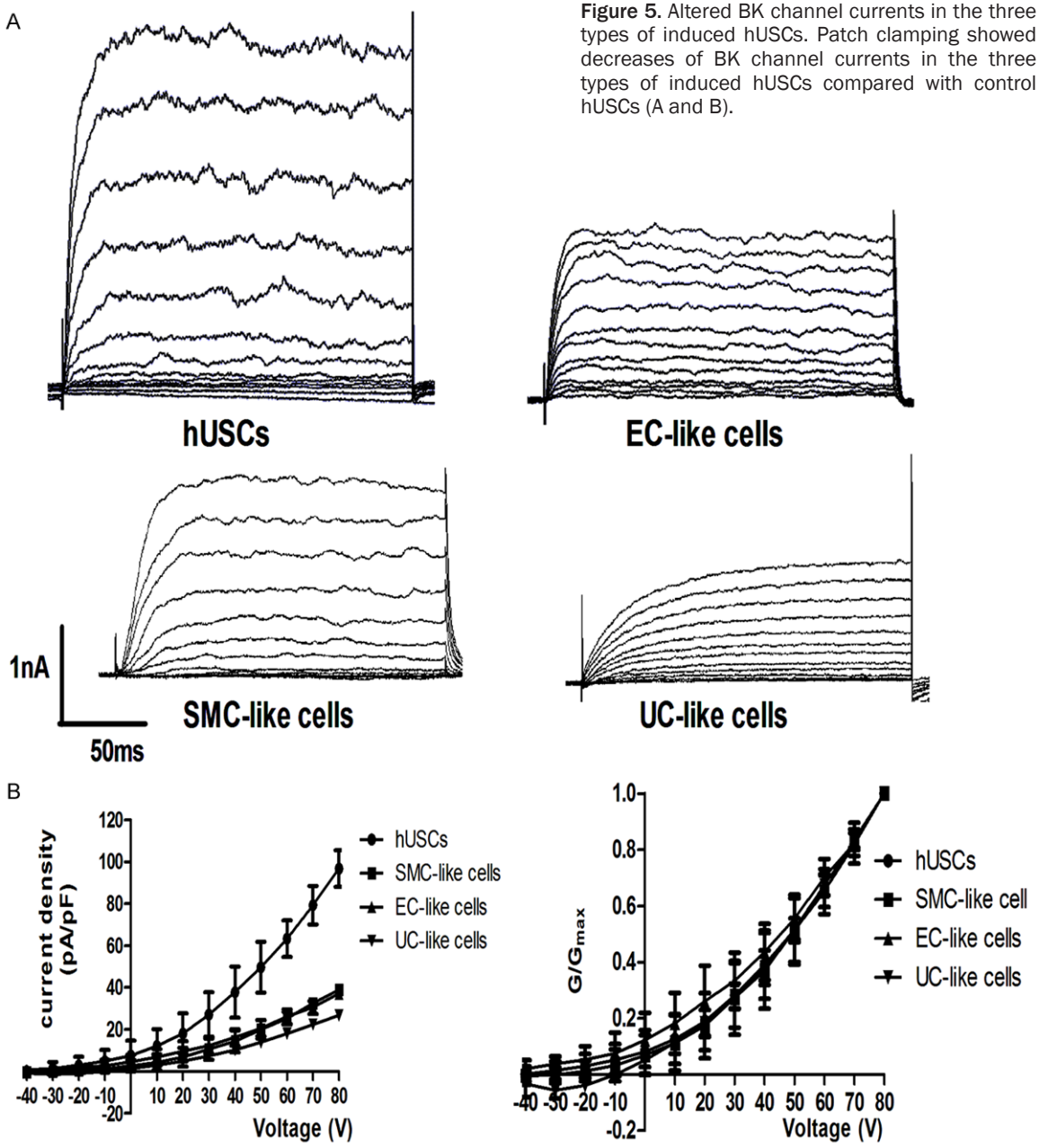

Figure 5. Altered BK channel currents in the three types of induced hUSCs. Patch clamping showed decreases of BK channel currents in the three types of induced hUSCs compared with control hUSCs (A and B).

tion after transplantation. Third, they have less ethical concerns because urine is self-provided [19-23]. Considering the advantages of hUSCs in clinical treatment of urinary system diseases, and that BK channels are considered as a novel therapeutic target for the treatment of OAB, we explored overexpression of BK channels in hUSCs as a treatment for OAB. First, we elucidated the current characteristics of BK channels in hUSCs and the effect of BK channels on proliferation and differentiation of hUSCs. We found BK channels are important for proliferation and differentiation of hUSCs based on the following observations: (1) higher passages of hUSCs had lower current densities of the BK channel; (2) Silencing the BK channel in hUSCs increased cells in S phase of the cell cycle and induced apoptosis; (3) Different kinds of directed differentiation of hUSCs led to lower activities of BK channels.

Recent studies including our own have shown that, compared with hUSCs and gene therapy alone, genetically modified hUSCs may offer

more effective treatments for urinary system diseases [14-18]. Genetically modified stem cell therapy have several advantages: 1) in vitro genetic modification of stem cells can be used to optimize the type of gene, levels of transgene delivery and expression efficacies; 2) in vitro gene modification is simpler and more efficient than in vivo experiments, and 3) it offers the opportunity to regulate the expression levels of the transgene within stem cells for tissue repair [19]. In animal studies, hUSCs genetically modified with fibroblast growth factor-2 have improved erectile dysfunction in a type 2 diabetic rat model [29]. Moreover, VEGF-expressing hUSCs can be potentially used for cell therapy in the treatment of stress urinary incontinence [30]. However, genetically modified hUSC therapy has not been reported for OAB. In the current study, we revealed the physiological effects of BK channels in hUSCs, which might provide the basis for BK genetically modified hUSC treatment of OAB. Compared with P5 and P7 cultured hUSCs, we found that P3 hUSCs had a strong advantage in maintaining the current and stability of hUSCs. This result suggests that P3 hUSCs could be used as key effector cells in future research of OAB. In fact, BK channel gene therapy has gained attention recently for the treatment of OAB. Recombinant BK channel α subunits can be directly and locally expressed in the human bladder to exert long lasting, DSM tissue-specific effects without significant side effects [6-9, 13]. Similarly, hUSC-based BK overexpression might provide insights into treatment of OAB because of their ability to self-renew without immune rejection. This novel approach can also avoid short transfer effectors such as “naked” DNA. In future studies, we want to investigate the effects BK channel overexpression in hUSCs for the treatment of OAB in animal models.

In conclusion, we examined the effect of BK channels on the proliferation and differentiation of hUSCs. The results indicate that BK channels are necessary for hUSC maintenance, which provides a basis for future clinical application.

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Disclosure of conflict of interest

None.

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References

[1] Irwin DE, Milsom I, Hunskaar S, Reilly K, Kopp Z, Herschorn S, Coyne K, Kelleher C, Hampel C, Artibani W, Abrams P. Population-based survey of urinary incontinence, overactive bladder, and other lower urinary tract symptoms in five countries: results of the EPIC study. Eur Urol 2006; 50: 1306-1314.

[2] Eapen RS, Radomski SB. Review of the epidemiology of overactive bladder. Res Rep Urol 2016; 8: 71-76.

[3] Brading AF. A myogenic basis for the overactive bladder. Urology 1997; 50: 68-73.

[4] de Groat WC. A neurologic basis for the overactive bladder. Urology 1997; 50: 53-56

[5] Bulmer P, Abrams P. The unstable detrusor. Urol Int 2004; 72: 1-12.

[6] Li L, Jiang C, Song B, Yan J, Pan J. Altered expression of calcium-activated K and Cl channels in detrusor overactivity of rats with partial bladder outlet obstruction. BJU Int 2008; 101: 1588-94.

[7] Hristov KL, Afeli SA, Parajuli SP, Cheng Q, Rovner ES, Petkov GV. Neurogenic detrusor overactivity is associated with decreased expression and function of the large conductance voltage- and Ca(2+)-activated K(+) channels. PLoS One 2013; 8: e68052.

[8] Xin W, Cheng Q, Soder RP, Petkov GV. Inhibition of phosphodiesterases relaxes detrusor smooth muscle via activation of the large-conductance voltage- and Ca^{2+}-activated K^+ channel. Am J Physiol Cell Physiol 2012; 302: C1361-1370.

[9] Petkov GV. Central role of the BK channel in urinary bladder smooth muscle physiology and pathophysiology. Am J Physiol Regul Integr Comp Physiol 2014; 307: R571-584.

[10] Atkinson NS, Robertson GA, Ganetzky B. A component of calcium-activated potassium channels encoded by the Drosophila slo locus. Science 1991; 253: 551-555.

[11] Hoa NT, Ge L, Tajhya RB, Beeton C, Cornforth AN, Abolhoda A, Lambrecht N, DaCosta-Iyer M, Ouyang Y, Mai AP, Hong E, Shon J, Hickey MJ, Erickson KL, Kruse CA, Jadus MR. Small cell lung cancer cells express the late stage gBK tumor antigen: a possible immunotarget for

the terminal disease. Am J Transl Res 2014; 6: 188-205.
[12] Contreras GF, Castillo K, Enrique N, Carrasquel-Ursulaez W, Castillo JP, Milesi V, Neely A, Alvarez O, Ferreira G, González C, Latorre R. A BK (Slo1) channel journey from molecule to physiology. Channels (Austin) 2013; 7: 442-58.
[13] Petkov GV. Role of potassium ion channels in detrusor smooth muscle function and dysfunction. Nat Rev Urol 2011; 9: 30-40.
[14] Zhang Y, McNeill E, Tian H, Soker S, Andersson KE, Yoo JJ, Atala A. Urine derived cells are a potential source for urological tissue reconstruction. J Urol 2008; 180: 2226-2233.
[15] Kang HS, Choi SH, Kim BS, Choi JY, Park GB, Kwon TG, Chun SY. Advanced properties of urine derived stem cells compared to adipose tissue derived stem cells in terms of cell proliferation, immune modulation and multi differentiation. J Korean Med Sci 2015; 30: 1764-1776.
[16] Wang C, Hei F, Ju Z, Yu J, Yang S, Chen M. Differentiation of urine-derived human induced pluripotent stem cells to alveolar type II epithelial cells. Cell Reprogram 2016; 18: 30-36.
[17] Guan J, Zhang J, Guo S, Zhu H, Zhu Z, Li H, Wang Y, Zhang C, Chang J. Human urine-derived stem cells can be induced into osteogenic lineage by silicate bioceramics via activation of the Wnt/beta-catenin signaling pathway. Biomaterials 2015; 55: 1-11.
[18] Liu G, Pareta RA, Wu R, Shi Y, Zhou X, Liu H, Deng C, Sun X, Atala A, Opara EC, Zhang Y. Skeletal myogenic differentiation of urine-derived stem cells and angiogenesis using microbeads loaded with growth factors. Biomaterials 2013; 34: 1311-1326.
[19] Li L, Zhang D, Li P, Damaser M, Zhang Y. Virus integration and genome influence in approaches to stem cell based therapy for andro-urology. Adv Drug Deliv Rev 2015; 82-83: 12-21.
[20] Kloskowski T, Nowacki M, Pokrywczyńska M, Drewa T. Urine--a waste or the future of regenerative medicine? Med Hypotheses 2015; 84: 344-349.
[21] Qin D, Long T, Deng J, Zhang Y. Urine-derived stem cells for potential use in bladder repair. Stem Cell Res Ther 2014; 5: 69.
[22] Liu G, Wang X, Sun X, Deng C, Atala A, Zhang Y. The effect of urine-derived stem cells expressing VEGF loaded in collagen hydrogels on myogenesis and innervation following after subcutaneous implantation in nude mice. Biomaterials 2013; 34: 8617-8629.
[23] Bharadwaj S, Liu G, Shi Y, Wu R, Yang B, He T, Fan Y, Lu X, Zhou X, Liu H, Atala A, Rohozinski J, Zhang Y. Multipotential differentiation of human urine-derived stem cells: potential for therapeutic applications in urology. Stem Cells 2013; 31: 1840-1856.
[24] Chen W, Xie M, Yang B, Bharadwaj S, Song L, Liu G, Yi S, Ye G, Atala A, Zhang Y. Skeletal myogenic differentiation of human urine-derived cells as a potential source for skeletal muscle regeneration. J Tissue Eng Regen Med 2017; 11: 334-341.
[25] Zhao J, Song Q, Wang L, Dong X, Yang X, Bai X, Song B, Damaser M and Li L. Detrusor myocyte autophagy protects the bladder function via inhibiting the inflammation in cyclophosphamide-induced cystitis in rats. PLoS One 2015; 10: e0122597.
[26] Zhao J, Wang L, Dong X, Hu X, Zhou L, Liu Q, Song B, Wu Q, Li L. The c-Jun N-terminal kinase (JNK) pathway is activated in human interstitial cystitis (IC) and rat protamine sulfate induced cystitis. Sci Rep 2016; 6: 19670.
[27] Bodin A, Bharadwaj S, Wu S, Gatenholm P, Atala A, Zhang Y. Tissue-engineered conduit using urine-derived stem cells seeded bacterial cellulose polymer in urinary reconstruction and diversion. Biomaterials 2010; 31: 8889-8901.
[28] Wu S, Liu Y, Bharadwaj S, Atala A, Zhang Y. Human urine-derived stem cells seeded in a modified 3D porous small intestinal submucosa scaffold for urethral tissue engineering. Biomaterials 2011; 32: 1317-1326.
[29] Ouyang B, Sun X, Han D, Chen S, Yao B, Gao Y, Bian J, Huang Y, Zhang Y, Wan Z, Yang B, Xiao H, Songyang Z, Liu G, Zhang Y, Deng C. Human urine-derived stem cells alone or genetically-modified with FGF2 Improve type 2 diabetic erectile dysfunction in a rat model. PLoS One 2014; 9: e92825.
[30] Zhang D, Wei G, Li P, Zhou X, Zhang Y. Urine-derived stem cells: A novel and versatile progenitor source for cell-based therapy and regenerative medicine. Genes Dis 2014; 1: 8-17.