Down-Regulation of the Large-Conductance Ca\textsuperscript{2+}-Activated K\textsuperscript{+} Channel, KCa1.1 in the Prostatic Stromal Cells of Benign Prostate Hyperplasia

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Large-conductance Ca\textsuperscript{2+}-activated K\textsuperscript{+} (BK\textsubscript{Ca}) channel plays an important role in the control of smooth muscle tone by modulating membrane potential and intracellular Ca\textsuperscript{2+} mobilization. BK\textsubscript{Ca} channel is functionally expressed in prostatic smooth muscle cells, and is activated by a\textsubscript{1}-adrenoceptor agonists. The main objective of this study was to elucidate the pathophysiological significance of changes in prostatic KCa1.1 expressions in benign prostatic hyperplasia (BPH). Our previous study has shown that KCa3.1 encoding intermediate-conductance KCa (IKCa) channel is up-regulated in stromal cells of implanted urogenital sinuses (UGSs) of stromal hyperplasia BPH model rats and in those of prostatic tissues from BPH patients. In the present study, the results from real-time polymerase chain reaction (PCR), Western blot, and immunohistochemical analyses showed significant down-regulation of KCa1.1 transcripts and proteins and negative correlation between KCa1.1 and KCa3.1 transcript expressions in prostatic stromal cells of both BPH model rats and BPH patients. Corresponding to down-regulation of KCa1.1 expression in stromal cells of implanted UGSs, membrane depolarization by application of the BK\textsubscript{Ca} channel blocker was disappeared. Down-regulation of KCa1.1 may be involved in the phenotype switch from contractile profile to proliferative one in prostatic stromal cells of BPH patients.

Key words Ca\textsuperscript{2+}-activated K\textsuperscript{+} channel; KCa1.1; down-regulation; benign prostatic hyperplasia; stromal cell; urogenital sinus

Large-conductance Ca\textsuperscript{2+}-activated K\textsuperscript{+} (BK\textsubscript{Ca}) channel plays a crucial role in the control of contractile tone in numerous smooth muscle cells: arteries,\textsuperscript{1-2} and gastrointestinal,\textsuperscript{3,4} and lower urinary tracts.\textsuperscript{5-7} Activation of BK\textsubscript{Ca} channels causes the smooth muscle relaxation via hyperpolarization of the membrane potential.\textsuperscript{8,9} Therefore, BK\textsubscript{Ca} channel is a potential therapeutic target for diverse urological disorders such as prostate diseases, overactive bladder, and erectile dysfunction.\textsuperscript{10-12} Several compounds have been developed as BK\textsubscript{Ca} channel activators: NS-1619, maxidil, BMS-204352, pimcaric acid, tamoxifen, 12,14-dichlorodehydroabietic acid, and bis-(1,3-dibutylbarbituric acid) trimethine oxonol [Di-BAC\textsubscript{4}](3)].\textsuperscript{11,13-16} BK\textsubscript{Ca} channel is composed of a and b subunits. BK\textsubscript{Ca} channel a subunit encoded by KCNMA1 is the pore forming protein,\textsuperscript{11,12} and BK\textsubscript{Ca} channel b subunits encoded by four different genes, KCNMB1-4 are the auxiliary proteins, which modify the biophysical and pharmacological properties such as voltage- and Ca\textsuperscript{2+}-sensitivities and gating kinetics.\textsuperscript{11,12,17} Several studies have characterized the functional roles of BK\textsubscript{Ca} channels in smooth muscle cells\textsuperscript{18,19} and interstitial cells\textsuperscript{20} isolated from human and/or rodent prostate stromal compartments by patch clamp techniques.

Benign prostatic hyperplasia (BPH) is a proliferative process of both the stromal and epithelial elements of the prostate arising in the periurethral and transition zones of the gland.\textsuperscript{21} The major component of clinical BPH specimens is stromal extracellular matrix, which is organized by smooth muscle, fibrous tissue element, and collagen.\textsuperscript{22} a\textsubscript{1}-Adrenoceptor antagonists are clinically used to decrease the periurethral and transition zones of the gland.\textsuperscript{21} Several studies have characterized the functional roles of BK\textsubscript{Ca} channels in smooth muscle cells\textsuperscript{18,19} and interstitial cells\textsuperscript{20} isolated from human and/or rodent prostate stromal compartments by patch clamp techniques.

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This study is focused on the changes in KCa1.1 expression and BK\textsubscript{Ca} channel activity in prostatic stromal cells of stromal hyperplasia BPH. Expressions of KCa1.1 transcripts and proteins in implanted UGSs of stromal hyperplasia BPH model rats were compared with the normal host prostate by real-time polymerase chain reaction (PCR), Western blot, and immunohistochemical analyses. Similar examinations were performed using human prostate needle-biopsies and surgical prostate specimens of BPH patients. Additionally, we measured the...
depolarization responses induced by pharmacological blockade of BK$_{Ca}$ channel in both isolated stromal cells from implanted UGSs and normal prostates.

MATERIALS AND METHODS

RNA Extraction, Reverse Transcription (RT)-PCR, and Real-Time PCR Experimental model rats for BPH with stromal hyperplasia were prepared as previously reported.25) Implanted urogenital sinuses (UGSs) and normal host prostates in stromal hyperplasia BPH model rats were dissected 4 weeks after implantation. All experiments were carried out in accordance with the guiding principles for the care and use of laboratory animals (the Science and International Affairs Bureau of the Ministry of Education, Culture, Sports, Science and Technology of Japan) and also with the approval of the ethics committees of Nagoya City University and Taiho Pharmaceutical Co., Ltd. Total RNAs from human normal prostates were purchased from BD Biosciences (San Jose, CA, U.S.A.) and BioChain (Hayward, CA, U.S.A.) (21–50 years old, three distinct Lot No. samples). Total RNA extraction and reverse-transcription were performed as previously reported.25) We enrolled 7 patients with BPH aged 54–86 (mean age: 70.4±4.5 year-old). Prostate needle-biopsy samples and surgical prostate specimens from BPH patients were obtained with informed consent from all patients before the study by explaining the purpose and methods. Normal human prostatic tissues were obtained from patients who had undergone radical cystoprostatectomy for bladder cancer. We confirmed that no malignancies were pathologically found in these prostatic tissues. The study was also approved by the ethics committee of Nagoya City University. BPH was diagnosed on the basis of International Prostatic Symptom Score (IPSS), the Quality of Life (QOL) index, digital rectal examination, ultrasonography, uroflowmetry, and prostate needle-biopsy. The resulting cDNA products were amplified with gene-specific primers, designed using Primer Express$^\text{TM}$ software (Ver 1.5) (Applied Biosystems, Foster City, CA, U.S.A.).

Quantitative, real-time PCR was performed with the use of SYBR Green chemistry on an ABI 7700 sequence detector system (Applied Biosystems) as previously reported.25) Following PCR primers for rat clones were used for real-time PCR: KCa1.1 (GenBank accession number: NM_031828, 408–509), 102 bp; KCa3.1 (NM_023021, 837–937), 101 bp; β-actin (ACTB) (NM_031144, 419–519), 101 bp; β-actin (ACTB) (NM_023021, 837–937), 101 bp; β-actin (ACTB) (NM_023021, 837–937), 101 bp. The following PCR primers were calculated, yielding transcriptional quantitation results of KCa1.1 transcript and protein expressions, real-time PCR, Western blotting, and immunohistochemical analyses were performed in normal host prostates and implanted UGSs of Stromal Hyperplasia BPH Model Rats. In real-time PCR examination, the expression of KCa1.1 transcripts in implanted UGSs was much lower than in normal host prostate (Fig. 1A). Expressions relative to β-actin (ACTB) (arbitrary unit) were 0.034±0.003 and 0.016±0.002 (n=8 for each, p<0.01) in normal host prostates and implanted UGSs, respectively. No significant differences in C$_i$ values at threshold 0.2 among two

antibodies (Alomone Labs, Jerusalem, Israel), and then incubated with anti-rabbit horseradish peroxidase-conjugated immunoglobulin G (IgG) (Millipore, Billerica, MA, U.S.A.). An enhanced chemiluminescence detection system (GE Healthcare Japan, Tokyo, Japan) was used for detection of the bound antibody. The resulting images were analyzed by a LAS-1000 device (FUJIFILM, Tokyo, Japan).

Immunohistochemical Analysis The implanted UGSs from stromal hyperplasia BPH model rats (4 weeks after implantation) and surgical prostate specimens from BPH patients were fixed in 10% neutral-buffered formalin, embedded in paraffin, and thin-sectioned at 2 μm.25,26) Tissue sections were deparaffinized in xylene followed by rehydration in graded alcoholic solutions and Ca$^{2+}$, Mg$^{2+}$-free phosphate buffered saline (PBS) (−). Endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide in methanol for 30 min at 4°C followed by washing with PBS (−). Anti-KCa1.1 antibody was used for immunostaining. All sections were developed by the avidin-biotin-complex method. The sections were also stained with hematoxylin and eosin staining and Masson's trichrome. Microscopic images of each section were captured on a computer and analyzed with an image analysis system (Win ROOF; Tech-Jam, Osaka, Japan).

Measurement of Membrane Potential Membrane potential was monitored using the voltage-sensitive dye, DiBAC$_4$(3) (Dojindo, Kumamoto, Japan) by the ARUGUS-HiSCA system (Hamamatsu Photonics, Hamamatsu, Japan).28) Cell isolation from normal rat prostate and implanted UGS was performed as previously reported.25) Prior to the fluorescence measurements with DiBAC$_4$(3), isolated cells were preincubated in normal N-(2-hydroxyethyl)piperazine-N’2-ethanesulfonic acid (HEPES) buffer (in mM, 137 NaCl, 5.9 KCl, 2.2 CaCl$_2$, 1.2 MgCl$_2$, 10 glucose, and 10 HEPES, pH 7.4) containing 100 nm DiBAC$_4$(3) for 30 min at room temperature. The staining cells were continuously incubated with 100 nm DiBAC$_4$(3) through experiments. For membrane potential imaging, the fluorescence emission was collected using a 505 nm dichroic mirror and a BA filter (>520 nm). Data collection and analysis were performed using the ARUGUS-HiSCA system. The sampling interval was 10 s.

Statistical Analysis Statistical significance between two groups was evaluated using Welch’s or Student’s t-test after the F-test. Data are presented as the means±S.E.M.

RESULTS

Expressions of KCa1.1 Transcripts and Proteins in Implanted UGSs of Stromal Hyperplasia BPH Model Rats We have recently shown the down-regulation of KCa1.1 in implanted UGSs of stromal hyperplasia BPH model rats by DNA microarray analysis.26) In order to obtain more definite results of KCa1.1 transcript and protein expressions, real-time PCR, Western blotting, and immunohistochemical analyses were performed in normal host prostates and implanted UGSs of stromal hyperplasia BPH model rats. In real-time PCR examination, the expression of KCa1.1 transcripts in implanted UGSs was much lower than in normal host prostate (Fig. 1A). Expressions relative to β-actin (ACTB) (arbitrary unit) were 0.034±0.003 and 0.016±0.002 (n=8 for each, p<0.01) in normal host prostates and implanted UGSs, respectively. No significant differences in C$_i$ values at threshold 0.2 among two
1.0, the expressions of KCa1.1 proteins were 0.16 (Fig. 2C). When KCa1.1 transcripts and proteins in prostate needle-biopsies were higher in implanted UGSs than in normal host prostates. When KCa1.1-positive signals were rarely detected in stromal compartments of normal host prostates (72.8 ± 6.5%, n = 8) than in those of normal prostates (89.0 ± 3.7%, n = 3) (Fig. 2Ba). When primary antibody was preincubated with the excess antigen peptides, KCa1.1-positive cells in the stromal compartments of normal host prostates disappeared (Fig. 2Ab). When primary antibody was preincubated with the excess antigen peptides, KCa1.1-positive cells in the stromal compartments of normal host prostates disappeared (Fig. 3Bb). These results were almost consistent with those obtained from implanted UGSs of stromal hyperplasia BPH model rats.

Expression of KCa1.1 in Prostate Needle-Biopsy Samples from BPH Patients We determined the expressions of KCa1.1 transcripts and proteins in prostate needle-biopsies from BPH patients (human BPH samples) by real-time PCR analysis. Normal prostates showed a relatively high expression of KCa1.1 transcripts, whereas human BPH samples showed a low expression of them (Fig. 3A). Expressions (arbitrary unit) were 0.034 ± 0.004 (n = 3) and 0.006 ± 0.002 (n = 7, p < 0.01) in normal human prostates and human BPH samples, respectively. We next performed immunohistochemical staining of KCa1.1 proteins in surgical prostate specimens from BPH patients. We enrolled patients with BPH aged 61–81 (mean age: 73.1 ± 4.4 year-old), and the prostate volume was 166.3 ± 63.0 mL (n = 10). The mean serum prostate-specific antigen (PSA) level was 9.0 ± 1.9 ng/mL (n = 10) in human BPH samples. In the normal human prostates, KCa1.1-positive signals were highly detected in stromal compartments (Fig. 3Ba), whereas, in human BPH samples, KCa1.1-positive signals were rarely detected in stromal compartments (Fig. 3Bc). KCa1.1-positive cells were quantified by counting brown-stained cells within the total number of cells in randomly selected fields at ×400 magnification. The fraction of KCa1.1-positive cells was much smaller in the stromal compartments of implanted UGSs (15.1 ± 3.0%, n = 4) than in those of normal host prostates (72.8 ± 6.5%, n = 4, p < 0.01) (Fig. 2C). When primary antibody was preincubated with the excess antigen peptides, KCa1.1-positive cells in the stromal compartments of normal host prostates disappeared (Fig. 2Ab). These results suggest that phenotypic changes from ‘contractile’ prostatic stromal cells in normal host prostates to ‘proliferative’ ones in implanted UGSs may associate with the down-regulation of KCa1.1.

Expression of KCa1.1 in Prostate Needle-Biopsy Samples from BPH Patients We examined the expression of KCa1.1 transcripts and proteins in normal host prostates and implanted UGSs of stromal hyperplasia BPH model rats. We determined the expressions of KCa1.1 transcripts and proteins in prostate needle-biopsies from BPH patients (human BPH samples) by real-time PCR analysis. Normal prostates showed a relatively high expression of KCa1.1 transcripts, whereas human BPH samples showed a low expression of them (Fig. 3A). Expressions (arbitrary unit) were 0.034 ± 0.004 (n = 3) and 0.006 ± 0.002 (n = 7, p < 0.01) in normal human prostates and human BPH samples, respectively. We next performed immunohistochemical staining of KCa1.1 proteins in surgical prostate specimens from BPH patients. We enrolled patients with BPH aged 61–81 (mean age: 73.1 ± 4.4 year-old), and the prostate volume was 166.3 ± 63.0 mL (n = 10). The mean serum prostate-specific antigen (PSA) level was 9.0 ± 1.9 ng/mL (n = 10) in human BPH samples. In the normal human prostates, KCa1.1-positive signals were highly detected in stromal compartments (Fig. 3Ba), whereas, in human BPH samples, KCa1.1-positive signals were rarely detected in stromal compartments (Fig. 3Bc). KCa1.1-positive cells were quantified by counting brown-stained cells within the total number of cells in randomly selected fields at ×400 magnification. The fraction of KCa1.1-positive cells was much smaller in the stromal compartments of BPH samples (33.4 ± 6.2%, n = 11, p < 0.01) than in those of normal prostates (89.0 ± 3.7%, n = 3) (Fig. 3Bd). When primary antibody was preincubated with the excess antigen peptides, KCa1.1-positive cells in the stromal compartments of normal human prostates disappeared (Fig. 3Bb). These results were almost consistent with those obtained from implanted UGSs of stromal hyperplasia BPH model rats.

Expressions of BKCa Channel β1 Subunit (KCNNMBI) Transcripts and Proteins in Implanted UGSs of Stromal Hyperplasia BPH Model Rats and Prostate Needle-Biopsy Samples from BPH Patients We examined the expression of KCNNMB1 transcripts in normal host prostates and implanted UGSs of stromal hyperplasia BPH model rats.
UGSs of stromal hyperplasia BPH model rats using DNA microarray and real-time PCR analyses. DNA microarray analysis was performed as previously reported and showed no significant differences of KCNMB1 transcript expression between both groups (probe ID 1369767_a_at, 1.28 ± 0.11 in implanted UGSs, p > 0.05) (Fig. 4A). Similarly, real-time PCR analysis showed no significant differences of KCNMB1 transcripts between both groups (n=8 for each, p>0.05) (Fig. 4B). Expression levels of KCNMB2-4 transcripts were very low (less than 0.001 as arbitrary unit) in both groups, and no significant differences of their expressions were found (not shown). The expression levels of KCNMB1 proteins in implanted UGSs were verified by Western blot analysis. As shown in Fig. 4C (upper panel), the anti-KCNMB1 antibody recognized a single band at approximately 30 kDa, similar to the predicted molecular weight of rat KCNMB1 proteins. Densitometric analysis revealed that no significant differences of KCNMB1 protein expressions were detected between normal host prostates and implanted UGSs (n=4 for each, p>0.05) (Fig. 4C, lower panel). When anti-KCNMB1 antibody was preincubated with the excess antigen peptide, a band signal was disappeared (not shown). No significant differences of KCNMB1 transcript expressions were also observed between normal human prostates and human BPH samples (Fig. 4D).

Correlation between $K_{Ca}1.1$ and $K_{Ca}3.1$ Transcript Expressions in Implanted UGSs of Stromal Hyperplasia BPH Model Rats and Prostate Needle-Biopsy Samples from BPH Patients

Our recent report has shown the up-regulation of $K_{Ca}3.1$ in implanted UGSs of stromal hyperplasia BPH model rats and prostate needle-biopsies from BPH patients, and the wet weight was positively correlated with the expression of $K_{Ca}3.1$ transcripts in implanted UGSs. In proliferating, non-contractile VSMCs of experimental restenosis models, a major molecular component of Ca$^{2+}$-activated $K^+$ channel has transferred from $K_{Ca}1.1$ to $K_{Ca}3.1$. Present study showed that the wet weights were negatively correlated with $K_{Ca}3.1$ transcript expression in implanted UGSs.
with the expression of $K_{Ca1.1}$ transcripts relative to ACTB (arbitrary unit) in implanted UGSs [$n=8$, $R$ (correlation coefficient for linear fitting) $= -0.87$, $p=0.005$] (Fig. 5A). We further evaluated the correlation between $K_{Ca1.1}$ and $K_{Ca3.1}$ transcript expressions in implanted UGSs of stromal hyperplasia BPH model rats and prostate needle-biopsies from BPH patients. Data on implanted UGS weights and $K_{Ca3.1}$ expressions were obtained from the same data sources in our previous study.26 Expressions of $K_{Ca1.1}$ and $K_{Ca3.1}$ transcripts in implanted UGSs were plotted as $K_{Ca1.1}$ vs. $K_{Ca3.1}$ (Fig. 5B). Similarly, negative correlation between $K_{Ca1.1}$ and $K_{Ca3.1}$ expression ($n=7$, $R=-0.83$, $p=0.016$) (Fig. 5C). In surgical prostate specimens from BPH patients, negative correlation between prostate volumes and percentages of $K_{Ca1.1}$-positive cells (not shown) was not detected, however, negative correlation between PSA levels and percentages of $K_{Ca1.1}$-positive cells was detected ($R=-0.85$, $p=0.002$) (Fig. 5D). $K_{Ca1.1}$ and $K_{Ca3.1}$ transcript expressions in normal prostates from rats and humans were also shown in Figs. 5B and C (closed triangles), respectively. These findings support our hypothesis that a major molecular component of Ca$^{2+}$-activated K$^+$ channel in prostatic stromal cells with proliferative profile is transferred from $K_{Ca1.1}$ to $K_{Ca3.1}$.

Effects of Pharmacological Blockade of BK$_{Ca}$ Channel Activity on Membrane Potential in Isolated Stromal Cells from Implanted UGSs We investigated whether membrane depolarization by BK$_{Ca}$ channel blockade was observed in isolated stromal cells with spindle shapes from implanted UGSs of stromal hyperplasia BPH model rats. We evaluated effects of 1 $\mu$m paxilline (PAX), a selective BK$_{Ca}$ channel blocker on membrane potential in isolated stromal cells from implanted UGSs using voltage-sensitive dye, DiBAC$_4$(3). Depolarization and hyperpolarization result in the increase and decrease in DiBAC$_4$(3) fluorescence intensities, respectively. At the end of the experiments, cells were exposed to the bathing solution, in which 140 mM Na$^+$ was substituted with K$^+$, to measure the fluorescence intensity at 0 mV ($F_{140K}$). (A) Effects of 1 $\mu$m paxilline (PAX) on membrane potential were monitored in isolated stromal cells from normal host prostates (Aa) and implanted UGSs of stromal hyperplasia BPH model rats (Ab) by DiBAC$_4$(3), a voltage-sensitive dye. Experiments were carried out in the constant presence of 100 nM DiBAC$_4$(3). At the end of the experiments, cells were exposed to the bathing solution, in which 140 mM Na$^+$ was substituted with K$^+$, to measure the fluorescence intensity at 0 mV ($F_{140K}$). (B) The ratios ($\Delta F_{140K}/\Delta F_{0}$) of PAX-induced fluorescence changes ($\Delta F_{140K}$) to 140 m$s$ K$^+$-induced fluorescence changes ($\Delta F_{0}$) in normal host prostate and implanted UGSs were summarized as means±S.E.M. **$p<0.01$ vs. normal host prostate.
BKCa channels indirectly communicate Ca2+ signal pathways to membrane potential changes required for various cellular processes. The principle finding of the present study is that a major molecular component of Ca2+-activated K+ channel in prostatic smooth cells of implanted UGSs of stromal hyperplasia BPH model rats and in those of needle-biopsy samples and surgical prostate specimens from BPH patients is transferred from KCa1.1 to KCa3.1.

BKCa channels are activated by Ca2+ sparks from intracellular Ca2+ stores and generate spontaneous transient outward currents (STOCs). Hyperpolarization by BKCa channel activation leads to the decrease in intracellular Ca2+ concentration and to the relaxation of smooth muscles. In cavernous and urinary bladder smooth muscles from knock-out mice lacking the KCa1.1 gene, hypercontractility by loss of BKCa channel activity leads to erectile dysfunction and overactive bladder, respectively. In these smooth muscles, BKCa channels play essential roles for cholinergic and purinergic receptor-mediated contractility and β-adrenergic receptor- and nitric oxide-mediated relaxation. In human and rodent prostates, adrenergic, cholinergic, and nitricergic nerve terminals form close contacts with prostatic smooth muscle cells, which have α1-adrenoceptor-, α2-adrenoceptor-, β-adrenoceptor-, muscarinic receptor-, and nitric oxide (NO)-mediated signaling pathways. It has been reported that physiological stimulation of α-adrenoceptors induces BKCa channel activation in human prostatic smooth muscle cells. Present study showed that prostatic stromal KCa1.1 expressions in implanted UGSs from stromal hyperplasia BPH model rats and in human BPH samples were lower than in ones of normal prostates (Figs. 1–3). In consistent with these expression profiles, smaller depolarizing responses by pharmacological blockade of BKCa channel were observed in isolated stromal cells from implanted UGSs (Fig. 6). The adrenergic-, cholinergic, and non-adrenergic, noncholinergic innervations and the various types of receptor-mediated tone regulation in implanted UGSs of stromal hyperplasia BPH model rats have not been determined yet. In addition, it is difficult to obtain freshly-punctured, non-fixed needle-biopsy samples from BPH patients. Therefore, it remains unclear whether hypercontractility by loss of BKCa channel activity is involved in the lower urinary tract symptoms associated with BPH.

Several reports have shown down-regulation of KCa1.1 in smooth muscle cells during hypertension and overactive bladder. However, these reports have not defined the regulatory mechanisms of KCa1.1 gene expression. In vascular smooth muscles of experimental restenosis model, the drastic changes in major molecular component of Ca2+-activated K+ channel from KCa1.1 to KCa3.1 have been reported. Also, it has been reported that several repressors contributing to the switching from contractile phenotype to proliferative one has been identified in vascular smooth muscle cells. In the present study, at least 50% cells showed the switching from KCa1.1-positive to KCa1.1-negative in normal prostate (Fig. 3Bd). Similar results were obtained in implanted UGSs (Fig. 2C). In addition, our previous report has shown no KCa3.1-positive cells in stromal cells of normal prostate. These strongly suggest that the phenotypic switching from KCa1.1-positive cells to KCa1.1-negative ones may generate drastically in prostatic stromal cells. In the present study, we could not determine whether the silencing of KCa1.1 gene and pharmacological blockade of KCa1.1 induce 1) up-regulation of KCa3.1 and 2) prostatic stromal cell differentiation. Further transcriptional regulatory network analysis will be needed to clarify the mechanisms underlying the phenotypic switching from KCa1.1-positive to KCa1.1-negative in prostatic stromal cells from BPH patients.

Kundu et al. have identified KCa3.1 promoters, which contain multiple female sex hormone, estrogen-responsive sequences. In male sex hormones, androgens and their metabolites are critical in regulating prostatic growth, therefore, they may regulate the expression of KCa3.1 transcripts via genomic mechanism. In arterial and corporal smooth muscles, testosterone induces vasorelaxant effects by the activation of BKCa channels via non-genomic pathway. We have shown that KCa1.1 expression in amygdala complex of the rat is gender-dependent and that castration in male rats results in significant down-regulation of KCa1.1, however, it remains unclear the mechanisms underlying genomic regulation of testosterone-mediated KCa1.1 gene expression. PSA expression is often used to measure androgen receptor activity. Of interest, present study showed negative correlation between PSA levels and KCa1.1 expressions in human BPH samples (Fig. 5D). It can be suggested that in cells which possess androgen receptors, the activation of them may suppress the KCa1.1 expression.

A genetic polymorphism in human BKCa channel β1 subunit (hKCNMB1) (E65K) results in a gain-of-function mutation, and is linked to a reduced diastolic hypertension and to protection against infarction and stroke. In the present study, no significant differences of KCNMB expression between normal prostates and implanted UGSs/human BPH samples (Fig. 4), and a genetic polymorphism in KCNMB1 was not found in human BPH samples.

In summary, in stromal hyperplasia BPH patients, phenotypic changes from ‘contractile’ prostatic stromal cells to ‘proliferative’ ones may associate with the changes in major molecular component of Ca2+-activated K+ channel from KCa1.1 to KCa3.1. The increase in prostatic smooth muscle tone via α1-adrenoceptors leads to bladder outflow obstruction in men with BPH. The decrease in BKCa channel activity mediated by down-regulation of KCa1.1 could be associated with the mechanism underlying 1) the increase in the prostatic smooth muscle tone and 2) hypersensitivity to α1-adrenoceptor blockers in BPH patients.

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