

## FUNCTIONAL ROLE OF THE TRPV4 CATION CHANNEL IN STRETCH-EVOKED $Ca^{2+}$ INFLUX AND ATP RELEASE IN MOUSE UROTHELIAL PRIMARY CULTURES

### Hypothesis / aims of study

In the urinary bladder, it has been suggested that non-neuronal cells within the bladder wall which is notably the transitional epithelial cells (urothelial cells) not only function as a barrier against ions, solutes, and infection, but also participate in detection of physical and chemical stimuli.

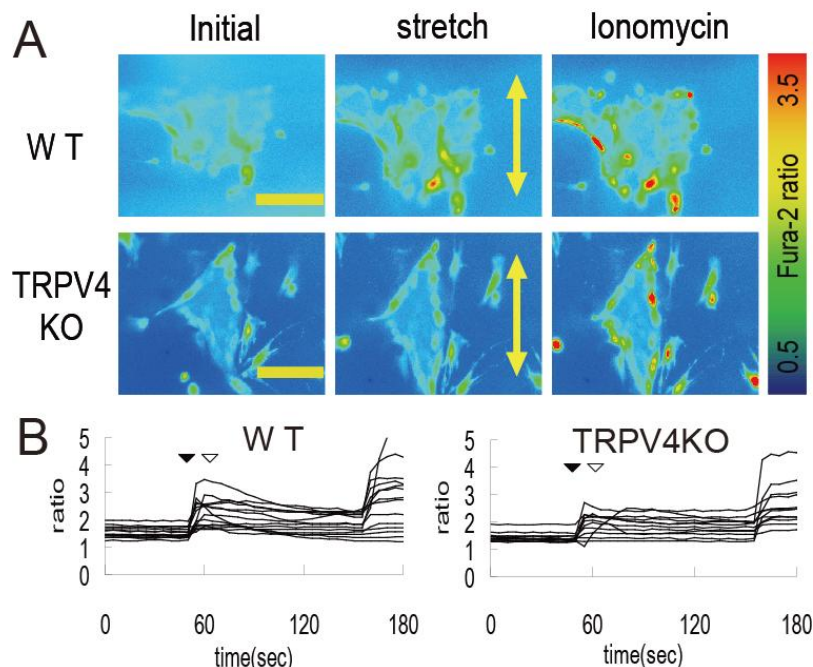
Some recent reports showed that the transient receptor potential vanilloid 4 (TRPV4) which has been proposed to be a mechanoreceptor is expressed in the urothelium and urinary bladder smooth muscle cells, and activation of the channel by specific ligands leads to augmentation of bladder contraction amplitude in cystometry and induction of bladder over activity *in vivo*. TRPV4 activation induced ATP release, which has been thought to be important for signal transmission to primary afferent nerves that control voiding reflex. Furthermore, another cystometry analysis and behavioral experiments revealed that intermicturitional interval was elongated and storage urine volume was increased in TRPV4-deficient (TRPV4KO) mice compared with wild-type (WT) mice. Thus, TRPV4 may contribute to bladder function, especially in mediating bladder distention signal to primary afferent nerves during urine storage phase. However, whether urothelial TRPV4 is required for sensing mechanical stretch, or to what extent urothelial TRPV4 contributes to stretch-evoked ATP release, has not been precisely determined.

In the present study, we examined the functional contribution of TRPV4 to stretch-dependent urothelial cell responses and to stretch-evoked adenosine triphosphate (ATP) release *in vitro*. We first established primary cell culture from mouse urothelium and expression of TRPV4 was confirmed. Because urothelial cells are physically extended on urine storage phase *in vivo*, we reproduced this phenomenon in an *in vitro* experiment using a uni-axial cell stretch system.

### Study design, materials and methods

WT (C57BL/6Cr) mice and TRPV4KO mice backcrossed on a C57BL/6Cr background were used. All experiments were performed using 8-12 week-old male mice. All the experiments were performed by comparing WT and TRPV4KO urothelial cells in order to evaluate the correlation between TRPV4 expression and stretch responses.

- (1) Extracted *m*-RNA from mouse urothelial primary culture cells and confirmed the expression of several TRP channel genes using by RT-PCR method.
- (2) Expression of TRPV4 protein was confirmed using an anti-TRPV4 antibody in mouse whole bladder specimen and urothelial primary culture cells.
- (3) Using a  $Ca^{2+}$ -imaging system, responses to application of 4 $\alpha$ -phorbol 12, 13 didecanoate (4 $\alpha$ -PDD), TRPV4-selective agonist, and mechanical stretch stimulation were examined in the urothelial primary culture cells.
- (4) Using a photon imaging system, ATP release from the urothelial primary cultures upon mechanical stretch stimulation was examined.



### Results

RT-PCR showed that TRPV4 was highly expressed in mouse urothelial cells and an immunofluorescent analysis revealed TRPV4 expression in urothelium, typically in the basal and intermediate layers, but much lesser extent in other cell types of the bladder. TRPV4-like immunoreactivity was observed in the urothelial primary culture cells from WT mouse bladder, whereas it was completely absent in ones from the bladder in TRPV4KO mice.

Intracellular  $Ca^{2+}$  measurements in urothelial primary culture cells from WT mice revealed a response to 4 $\alpha$ -PDD, which was absent in the cells from TRPV4KO mice.

We established a cell-stretch system which can be attached to a fluorescent microscope for  $Ca^{2+}$ - and photon-imaging. Stretch stimulation evoked robust intracellular  $Ca^{2+}$  increase in WT urothelial cells, however, such intracellular  $Ca^{2+}$  increase response was significantly attenuated in TRPV4KO cells (Fig. A and B). Stretch-evoked  $Ca^{2+}$  increase in WT urothelial cells was partially reduced in the presence of ruthenium red (RR), a broad TRP channel blocker, whereas that in TRPV4KO cells did not show such reduction.

Potent ATP release occurred following stretch stimulation or 4 $\alpha$ -PDD administration in WT urothelial cells, which was dramatically suppressed in TRPV4KO cells. Stretch-dependent ATP release was almost completely eliminated in the presence of RR or in the absence of extracellular Ca<sup>2+</sup> in WT cells.

#### Interpretation of results

Firstly, we demonstrated that TRPV4 is highly expressed in urothelial cells especially in basal and intermediate layers of mice bladders. It is physiologically very interesting because afferent nerves and detrusor smooth muscles are presented under urothelium, to which transmitter signals such as ATP from urothelium can be directly conveyed.

Secondly, we confirmed that 4 $\alpha$ -PDD, a selective TRPV4 agonist, induced robust intracellular Ca<sup>2+</sup> increase only in WT urothelial cells in the Ca<sup>2+</sup>-imaging system, indicating that functional TRPV4 is retained in urothelial primary cultures.

Thirdly, using a cell-stretch system, we found that urothelial cells responded to mechanical stretch stimuli, and released ATP via TRPV4 activation, suggesting that TRPV4 is mechanically activated upon stretch stimulation, leading to release of ATP as a signal transmitter.

These results raise a possibility that TRPV4 senses distension of the bladder urothelium, which is converted to ATP signal to regulate voiding reflex pathway on urine storage phase, and could contribute to abnormal over activity of the bladder (OAB) in pathological conditions.

#### Concluding message

In the present study, we provided the evidence that TRPV4 channel is the candidate molecule involved in mechanosensory transduction in urinary bladder. Recently, distension during urine storage phase has been speculated to activate urothelial cells and release various chemical mediators (e.g. ATP, prostaglandin, NO, acetylcholine, tachykinin), which then transfer signals to afferent nerves or muscle cells. Our results claim that TRPV4 is a key player at least in stretch-induced ATP release.

Given that TRPV4 is critically involved in the sensing mechanism in bladder, development of chemicals modulating TRPV4 activity would hopefully lead to the treatment of bladder disorders such as OAB.

#### References

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<b><i>Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?</i></b>	<b>Yes</b>
<b><i>Name of ethics committee</i></b>	<b>Yamanashi CCTM</b>