224

Miyamoto T[1], Nakagomi H[1], Kira S[1], Mochizuki T[2], Koizumi S[3], Tominaga M[4], Takeda M[1]

1. *Department of Urology, Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi,* ***2.*** *Yamanashi Kosei Hospital,* ***3.*** *Department of Pharmacology, Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi,* ***4.*** *Division of Cell Signaling, Okazaki Institute for Integrative Bioscience (National Institute for Physiological Sciences), National Institutes of Natural Sciences*

PIEZO1, A NOVEL MECHANOSENSOR IN THE BLADDER UROTHELIUM

Hypothesis / aims of study

In recent years, Piezo1 and Piezo2 have been newly identified as mechanically activated cation channels, and Piezo1 has been reported to be expressed in the mouse bladder [1]. In this study, we examined the localization of Piezo1 in the mouse bladder and its effect upon stretch stimulation in mouse urothelial primary culture cells.

Study design, materials and methods

(1) Animals: Wild-type (C57BL/6Cr) mice were used. All experiments were performed using 8- 12-week-old male mice.
(2) Preparation of primary urothelial cell cultures: Whole bladders were taken from anesthetized mice, and urothelial cells were prepared as previously described [2].
All the experiments with primary urothelial cell cultures were performed after the cells had formed clusters after 72-h of cultivation.
(3) Quantitative RT-PCR: mRNA was extracted from mouse urothelial primary culture cells, and expression of the *Piezo1* gene was confirmed using by the Smart Cycler System.
(4) For RNA interference, Lipofectamine RNAimax was used for transfecting 10 nM of siRNA.
Primary culture cells were transfected with Piezo1 siRNA and control siRNA.
(5) Immunostaining: Expression of the Piezo1 protein in mouse whole bladder specimens and urothelial primary culture cells was confirmed using an anti-Piezo1 antibody.
(6) Mechanical stretch experiment: Primary urothelial cells were seeded in an elastic silicone chamber, and the silicone chamber was extended using a cell-stretch system (modified version of STB150, STREX). Stretch stimulation was applied at a preset stretch speed (100 μm/s) and distance (200 μm).
(7) Measurement of Ca^{2+} concentrations: Cells were loaded with a fluorescent Ca^{2+} indicator (10 μM fura-2-AM) at 37 °C for 40 min. By Using a Ca^{2+} imaging system, responses to mechanical stretch stimulation were examined in urothelial primary culture cells. Ionomycin (5 μM) was applied at the final step in each experiment for normalization and to assess cell viability. We compared the stretch-induced changes in intracellular Ca^{2+} concentrations between the control group, the Piezo1-knockdown group, and the Piezo1 inhibitor GsMTX4-treated group. GsMTX4 is a selective stretch-activated ion channels inhibitor [3].
(8)Photon imaging of ATP release: ATP release from urothelial primary culture cells was detected using a luciferin-luciferase bioluminescence assay. Data were imaged using Aquacosmos software and analyzed using ImageJ 1.41 software. We compared the stretch-induced ATP release from urothelial primary culture cells between the control group, the Piezo1-knockdown group, and the GsMTX4-treated group.

Results

Piezo1 was highly expressed in the mouse urothelium (Figure 1).
The stretch-induced Ca^{2+} influx was significantly lower in the Piezo1-knockdown and GsMTX4 -treated groups than in the control group (Figure 2 and 3).
Furthermore, stretch-induced ATP release from urothelial cells was significantly lower in the Piezo1- knockdown and GsMTX4 -treated groups than in the control group.

Interpretation of results

First, Piezo1 is expressed in bladder urothelial cells especially in the basal and intermediate layers of mouse bladders. Second, Piezo1 mediates stretch-induced Ca^{2+} influx and ATP release in primary urothelial cell cultures. Therefore, it is thought that Piezo1 act as a mechanosensor in the bladder urothelium. Third, GsMTX4 attenuates the stretch-induced Ca^{2+} influx in bladder urothelial cells and reduces stretch-induced ATP release from urothelial cells. GsMTX4 may be used to treat bladder disorders such as overactive bladder.

Concluding message

Piezo1 functions as a mechanosensor in the bladder urothelium and is thought to be a key molecule for afferent nerve signal transduction.

Figure 1 Immunostaining of mouse bladder tissue and primary urothelial cell culture.
(A) Anti-Piezo1 antibody signals are highly detected in the urothelial layer. (B) Piezo1 is also expressed in urothelial primary culture cells.

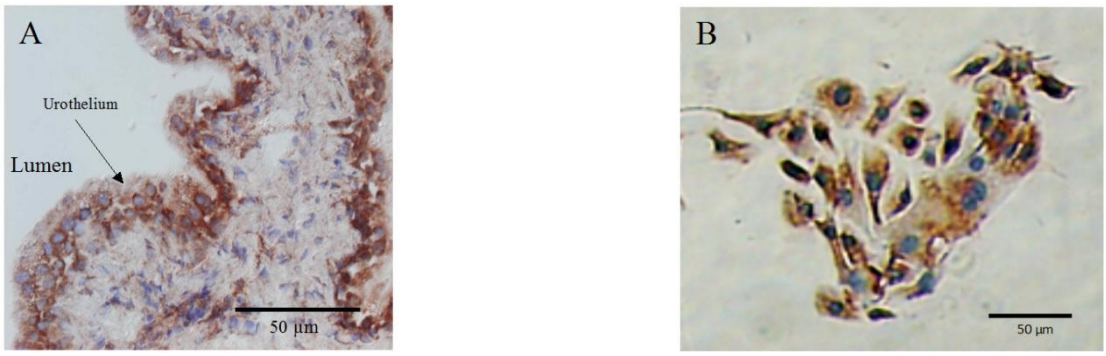


Figure 2 Ca^{2+} responses to stretch stimulation in primary urothelial cell cultures.
The upper panel (A, B, and C) shows the representative pseudo-color images of the control group. The bottom panel (D, E, and F) shows the representative pseudo-color images of the Piezo1-Knockdown group.
(A and D) Pre-stretch phase; (B and E) Post-stretch phase; (C and F) Addition of ionomycin

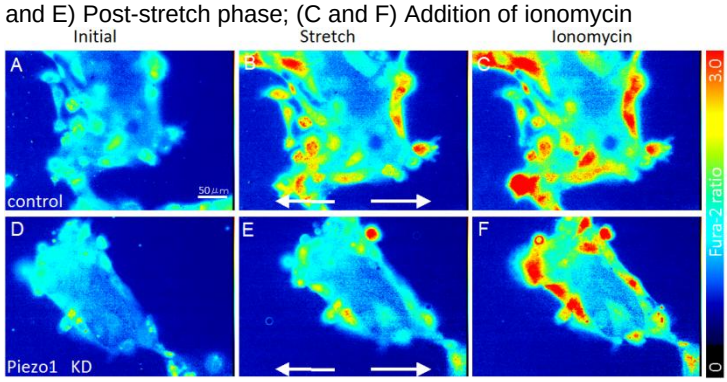
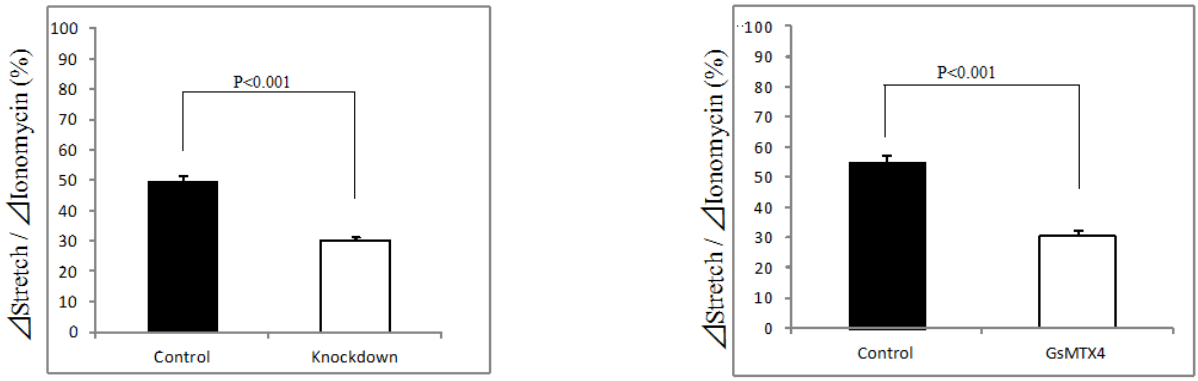


Figure 3 The average peak Ca^{2+} increases is significantly reduced in the Piezo1-knockdown group and the GsMTX4-treated group as compared to the control group upon stretch.



References
1. Bertrand, C., et al.Piezo1 and Piezo2 are essential components of distinct mechanically activated cation channels. Science 2010; 330
2. Mochzuki, T., et al. The TRPV4 cation channel mediates stretch-evoked Ca2+ influx and ATP release in primary urothelial cell cultures. J Biol. Chem., 2009;284
3. Chilman, B., et al. The mechanosensitive ion channel Piezo1 is inhibited by the peptide GsMTX4. Biochemistry, 2011; 50

Disclosures
Funding: none **Clinical Trial:** No **Subjects:** ANIMAL **Species:** mouse **Ethics Committee:** the University of Yamanashi Animal Care and Use Committee