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TRPM7 CONTRIBUTES TO INTERCELLULAR JUNCTION FORMATION IN THE UROTHELIUM – POSSIBLE LINK TO THE PATHOPHYSIOLOGY OF INTERSTITIAL CYSTITIS

Hypothesis / aims of study
Transient receptor potential melastatin 7 (TRPM7) is a calcium-permeable non-selective cation channel with a unique kinase domain in its C-terminal region (1). Previous studies suggest its involvement in cellular and total body magnesium homeostasis as well as cancer cell adhesion and migration *in vitro* (2). In the urinary bladder, functional expression of TRPM7 has been reported in the urothelium (3). However, its physiological significance *in vivo* still remains unknown. The purpose of this study was to reveal the physiological role of TRPM7 in mouse urothelium *in vivo*.

Study design, materials and methods
The urothelium-specific inducible *Trpm*7 knockout (KO) mice were generated by using Cre-Lox system. *Trpm*7 fl/fl mice were crossed with Upk3a-CreERT mice. After tamoxifen injections, urothelium-specific targeted deletion, decreased TRPM7 proteins and magnesium-inhibitable currents were confirmed using genomic PCR, immunohistochemistry, immunocytochemistry and patch-clamp recordings in primary urothelial cells, respectively. Voiding behavior was observed with free-moving and awake mice by using modified metabolic cages. To clarify the morphological features of the bladder in *Trpm7* KO mice, histological analysis by using hematoxylin-eosin staining, confocal microscopy and transmission electron microscopy were performed.

Results
The magnesium-inhibitable cation currents as well as the acid-inducible currents were significantly smaller in the urothelial cells of *Trpm*7 KO mice compared to the cells of control mice. Study on voiding behavior showed a significantly smaller voided volume in *Trpm*7 KO mice (mean voided volume 0.28 ± 0.08 g in KO mice, 0.36 ± 0.04 g in control mice, maximum voided volume 0.51 ± 0.06 g in KO mice, 0.65 ± 0.05 g in control mice, $p < 0.05$, n = 6-8). Histological analysis showed partial but substantial edema (Fig.1B, two-way arrow) with and immune cell infiltration in the submucosal layer of *Trpm*7 KO mice, most likely due to an inflammation. In transmission electron microscopy, immature intercellular junctions were observed in the urothelium of *Trpm*7 KO mice (Fig.2 C and D) but not in control mice (Fig.2 A and B). Moreover, in the urothelium of control mice, TRPM7 protein was observed in intercellular junctions co-localizing with E-cadherin ~~to reveal the function of TRPM7 in the urothelium. A functional TRPM7 knockdown induced by tamoxifen was confirmed by using patch-clamp recordings with the primary urothelial cells, as a reduction of the Mg^{2+}-inhibitable curre~~, ~~which is~~ a major component of the intercellular junction~~s. complex, was observed in confocal microscopy.~~

Interpretation of results
These results suggest that TRPM7 is involved in the formation of intercellular junctions in mouse urothelium. The immature intercellular junctions in the urothelium of *Trpm*7 KO mice might lead to a disruption of barrier function resulting in an inflammation which may affect voiding behavior *in vivo*.

Concluding message
To our knowledge, this is the first study that demonstrates *Trpm*7 KO mice exhibit immature intercellular junctions *in vivo*. Since the increased permeability of the urothelium that causes hypersensitive bladder afferent nerves and a significant decrease in voided volume is a common pathogenesis of interstitial- cystitis, our *Trpm*7 KO mice may serve as a good animal model for interstitial cystitis.

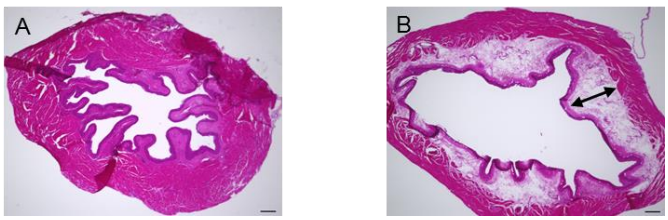

Figure1. Representative images of the bladder from control (A) and *Trpm7* knockout (B) mice with hematoxylin-eosin staining. Scale bar, 200μm.

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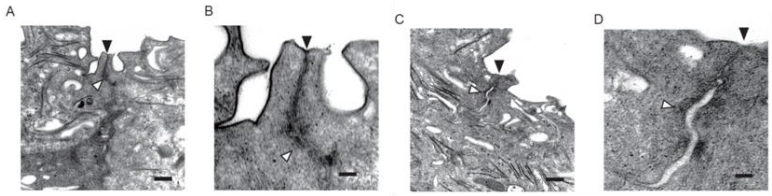

Figure 2. Representative images of transmission electron microscopy of the intercellular junctions of the superficial layer of the urothelial cells of control mice (A and B), or *Trpm*7 knockout mice (C and D). Black arrowheads indicate tight junctions and white arrowheads indicate the end of junction complexes containing adherence junctions. Scale bar, 200nm (A and C), 50nm (B and D).

References
1. Handb Exp Pharmacol, 2014. 223; 521-546.
2. Cancer Res, 2012. 72(16); 4250-4261.
3. Am J Physiol Renal Physiol, 2010. 298(3); F682-701.

Disclosures
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