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 Trpm7 knockout (KO) mice were generated by using Cre-Lox system. Trpm7 full mice were crossed with Uppda-CreERT mice. After tamoxifen injections, urothelium-specific targeted deletion, desensitized 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 Trpm7 KO mice compared to the cells of control mice. Study on voiding behavior showed a significantly smaller voided volume in Trpm7 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 immune cell infiltration in the submucosal layer of Trpm7 KO mice, most likely due to an inflammation. In transmission electron microscopy, immature intercellular junctions were observed in the urothelium of Trpm7 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 knockout induced by tamoxifen was confirmed by using patch-clamp recordings with the primary urothelial cells, as a reduction of the Mg²⁺-inhibitable current, which is a major component of the intercellular junction 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 Trpm7 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 Trpm7 KO mice exhibit immature intercellular junctions in vivo. Since the increased permeability of this study suggests to cause hypersensitive bladder afferent nerves and a significant decrease in voided volume is a common pathogenesis of interstitial cystitis, our Trpm7 KO mice may serve as a good animal model for interstitial cystitis.
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 Trpm7 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

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