225

Janssen D A W[1], Hoenderop J[2], Heesakkers J[1], Schalken J A[1]
1. *dept. of Urology; Radboud University Nijmegen Medical Centre,* ***2.*** *dept. of Physiology; Radboud University Nijmegen Medical Centre*

TRPV4 IS INVOLVED IN CELL JUNCTION FORMATION IN THE UROGENITAL TRACT EPITHELIA. AN ULTRASTRUCTURAL STUDY

Hypothesis / aims of study

Transient receptor potential vanilloid subtype 4 (TRPV4) is a non specific cation channel that is located in the epithelium of the urinary bladder, the ureter and the distal section of the kidney tubuli. Transgenic TRPV4 deficient mouse have a phenotype that displays bladder dysfunction. Therefore TRPV4 channels are being investigated as a mechanoreceptor in the bladder and as a potential pharmacological target for OAB. Besides this, numerous TRP-channels are associated with carcinogenesis. Previous research has demonstrated a molecular connection between TRPV4 and barrier forming adherence junctions in all above mentioned tissues [1]. Recent reports showed an abnormal cell junction formation in the skin epithelium of TRPV4 deficient mice. The aim of this study is to investigate cell junction formation in the urogenital tract of humans and in TRPV4 knockout mice.

Study design, materials and methods

The location of TRPV4, Adherence Junctions (AJ's) and Tight junctions (TJ) was investigated with immunofluorescence assays, Western blotting, Immunoprecipitation (IP) and qPCR techniques on non cancerous tissue sections from human cystectomies (n=4) , ureterectomies (n=1) and nefrectomies (n=2) and kidney and bladder tissues from wild type and TRPV4 -/- mice. Subsequent evaluation of urothelial cell junctions was investigated in bladder tissues from wild type and TRPV4 -/- mice with Transmission Electron Microscopy (TEM).

Results

Results from our qPCR and immunofluorescence experiments demonstrate that TRPV4 channels are located in the urothelium of the bladder, ureter and the epithelial cells of the distal collecting ducts of the kidney (fig 1). TRPV4 co localizes with adherence junctions throughout the urogenital tract. Immunofluorescence assays demonstrated a qualitative and quantitative reduction of cell junction formation (predominantly AJ's) in TRPV4 -/- kidney and bladder epithelium. TEM evaluation confirmed this and showed a remarkable increase in intercellular space between adjacent urothelial cells in bladders from TRPV4 -/- mouse (fig 2).

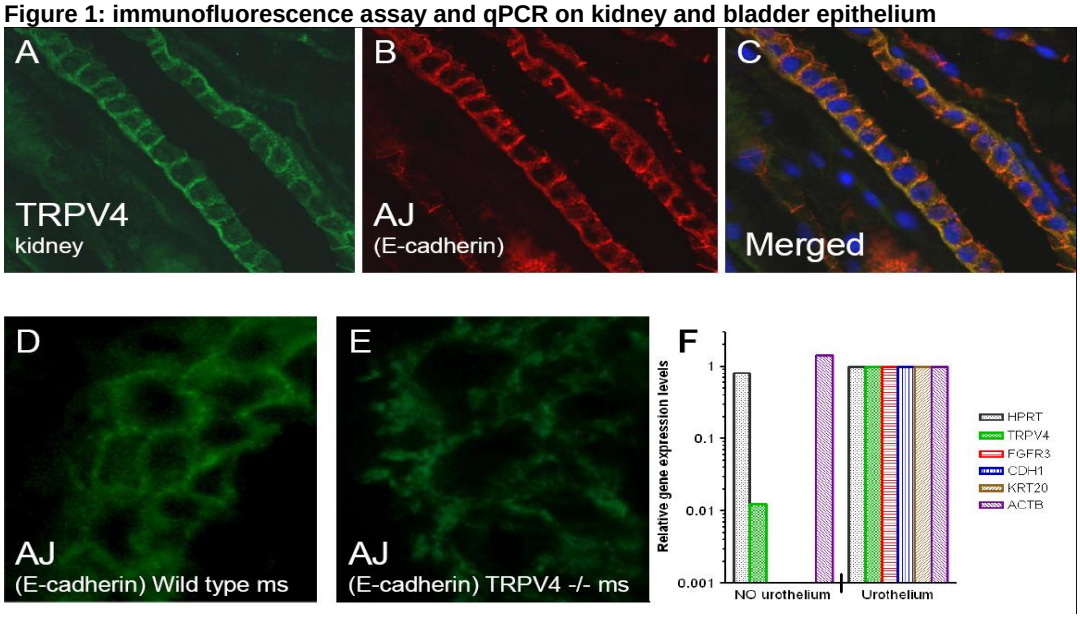
Interpretation of results

TRPV4 channels are connected to epithelial adherence junctions throughout the urogenital tract and play a role in cell junction formation. An absence of TRPV4 channels causes reduced urothelial cell adhesion and most likely leading to a leaky urothelium.

Concluding message

These result suggest that TRPV4 channels, besides being important for sensory functions, is also involved in epithelial barrier formation.

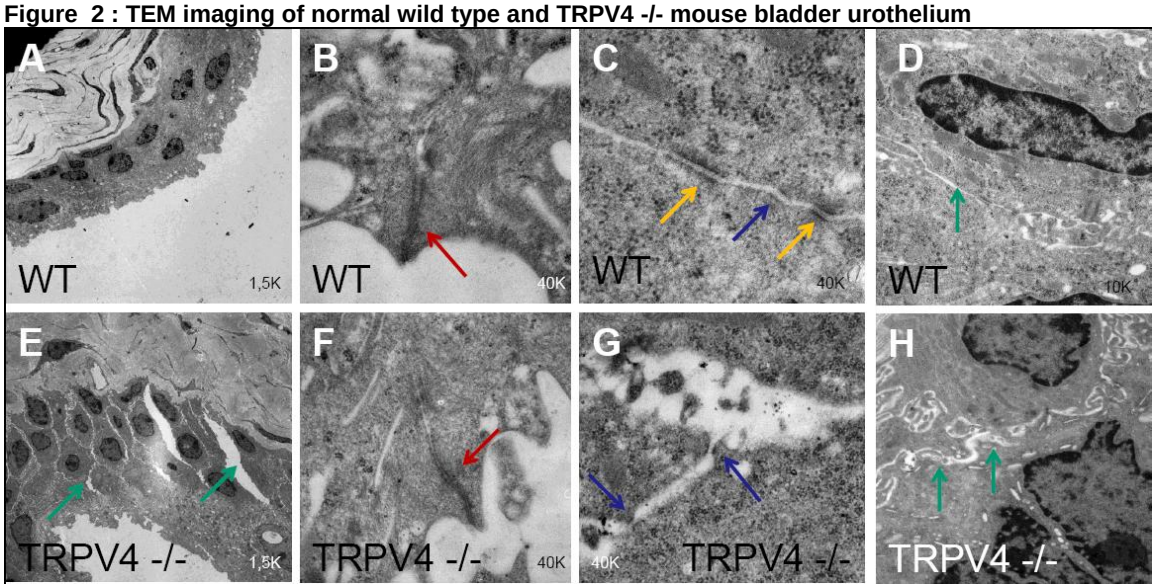
Figure 1: immunofluorescence assay and qPCR on kidney and bladder epithelium



A,B,C: TRPV4 and E-cadherin (AJ's) colocalize of human kidney epithelium (collecting ducts)
D, E: normal cell junction formation in wild type mouse bladder urothelium **(D)** versus abnormal cell junction formation in TRPV4 -/- mouse bladder **(E)**.

F: qPCR for TRPV4 gene expression in human bladder biopsies with and without urothelium (HE controled). Urothelial markers FGF-R (Fibroblast Growth Factor Receptor), CDH1 (E-cadherin) and KRT20 (Kerantin 20) were used to identify the quantitiy of urothelial cells in the biopsy. HRPT and ACTB were used as control genes to quantify total amount of cells in biopsy. TRPV4 gene expression was detected in samples with urothelium and almost no TRPV4 was expressed found in biopsy without urothelium.

Figure 2 : TEM imaging of normal wild type and TRPV4 -/- mouse bladder urothelium



A, B, C, D: TEM imaging of wild type bladder with normal urothelium. **A)** overview, B) normal tight junction (red arrow), **C)** normal adherence junctions (orange arrow) and desmosomes (blue arrow), **D)** narrow intercellular space between adjacent urothelial cells (green arrow).
E, F, G, H: TEM imaging of TRPV4 -/- mouse bladder urothelium. **E)** overview with enlarged intercellular spaces between cells (green arrow), **F)** tight junction (red arrow), **G)** desmosomes (blue arrow), but no adherence junctions present, **H)** enlarged intercellular spaces between adjacent urothelial cells (green arrow).

References

1. The mechanoreceptor TRPV4 is localized in adherence junctions of the human bladder urothelium: a morphological study. J Urol. 2011 Sep;186(3):1121-7. Epub 2011 Jul 23.

Disclosures

Funding: Pfizer OAB-LUTS compatitive grant **Clinical Trial:** No **Subjects:** HUMAN **Ethics Committee:** Tissue was obtained with approval of CMO regio Arnhem-Nijmegen (human ethical committee). Mouse tissue was obtained with approval of the Radboud Univerity Animal Ethical Committee (RUDEC) **Helsinki:** Yes **Informed Consent:** Yes