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TRPV4 AS STRETCH-RECEPTOR; CO-LOCALIZATION BETWEEN TRPV4 AND ADHERENCE JUNCTIONS IN UROTHELIUM OF HUMAN KIDNEY, URETER & URINARY BLADDER

Hypothesis / aims of study

We investigated the location of Transient Receptor Potential Vanilloids subtype 4 (TRPV4) in the urothelium of the kidney, ureter and bladder. TRPV4 is a calcium-specific ion-channel that is located on the cell-membranes in different circumventricular organs including the urinary bladder. TRPV4-channels are activated by different stimuli: mechanical stretch, osmosis, temperature-change and specific proteins. The activation of TRPV4-channels by mechanical stretch, has led to the hypothesis that TRPV4 functions as a mechanoreceptor in the urinary bladder. It could therefore also be involved in diseases that cause voiding dysfunction (OAB / PBS). This theory is strengthened by the dysfunctional voiding patterns and enlarged bladder capacities seen in TRPV4 knockout mice [1]. In recent immunohistochemistry & immunoprecipitation experiments we have proven a co-localization between TRPV4 and adherence junctions (AJ's) in the urothelium of the human bladder and in urothelial cell culture. AJ's have an intracellular and an extracellular domain. The first is connected to the actin cytoskeleton. The second anchors neighbouring urothelial cells to each other. Together, this forms a rigid structural network that is able to transmit the mechanical forces that originate during bladder filling and is therefore a good location to measure stretch. These findings further supports the theory that TRPV4 is a stretch-receptor. **The aim of our study** was to further explore the co-localization of TRPV4 and adherence junctions in the urothelium of the human urogenital tract (kidney, ureter, bladder). Secondarily, we wanted to explore this co-localization in another species (mouse).

Study design, materials and methods

Human tissue obtained from healthy sections of: kidney (n=1), ureter (n=1) and bladder (cancerous; n=3; non-cancerous n=2) were snap frozen and prepared for immunohistochemistry. Bladders obtained from normal mouse (n=3) & TRPV4-knockout mice (n=6) were used as positive and negative controls. Also, a well-differentiated urothelial cancer cell-culture (RT4) was used to confirm data. Tissue was cut and prepared for immunohistochemistry. Sections were fixed with 3% paraformaldehyde for 10 min, blocked with 20% goat serum in TBS-t (0,05% Tween), and stained overnight (4°C) with antibodies for TRPV4 & E-cadherine (adherence junctions), following staining with Alexa's (488 & 594) and DAPI. Specimens were analysed with binocular epifluorescent and confocal microscope.

Results

A co-localization between TRPV4 & E-cadherin (AJ's) was found throughout the urogenital tract. Strong immunofluorescence of TRPV4 was detected in the urothelium of the distal tubuli (DT), but not in the proximal tubuli (PT) of the kidney (fig 1). TRPV4 and E-cadherin in the bladder and ureter had a similar appearance. Here, TRPV4 was seen as dot-like structures (also called puncta) between adjacent umbrellacells (fig 1). These structures also stained positive for E-cadherin. In the bladder, we visualized this co-localization with confocal microscopy (fig 1; bladder). TRPV4 & E-cadherin in the normal mouse bladder urothelium was comparable to the human bladder (data not shown), but TRPV4-knockout mice showed no immunoreactivity for TRPV4 (data not shown). In urothelial cell culture, the co-localization between TRPV4 & E-cadherin was confirmed (data not shown).

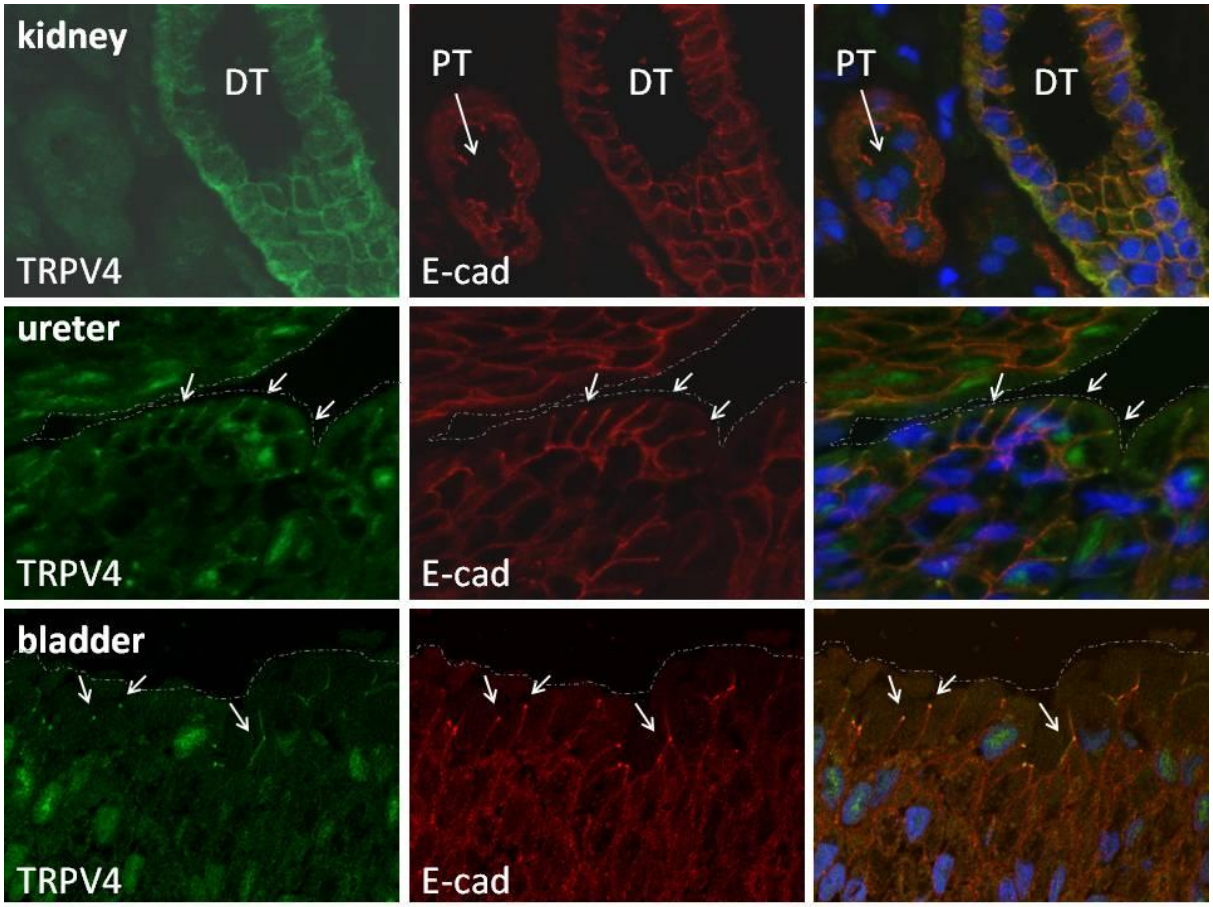

Fig1: immunofluorescence images of kidney, ureter (binocular) & bladder (confocal) showing TRPV4 (green) and E-cadherin (adherence junctions) and merged images. **Kidney** : strong immunofluorescence of TRPV4 in only the distal tubuli (DT) and not in the proximal tubuli (PT). In the DT, a strong co-localization between TRPV4 & E-cadherin is seen. **Ureter & bladder :** the dotted white line marks the urothelium-lumen-border. Note the evenly ditributed dot-like structures between the umbrellacells (white arrows). These represent adherence junctions that also stain for TRPV4. The staining of some nuclei with TRPV4 is also seen in mouse, but not in TRPV4-Knockout mice and is probably caused by transcription of TRPV4-protein and PFA fixation.

Interpretation of results

Our results confirm the co-localization of TRPV4 and AJ's in the urothelium of the kidney, ureter and bladder, meaning TRPV4 is connected to the rigid structural network that includes the intracellular actin-cytoskeleton and extracellular anchoring cell-junctions. These findings support the theory that TRPV4 is involved in the sensation of stretch in the bladder and the ureter. The existence of the co-localization between TRPV4 and AJ's in the distal tubuli of the kidney could mean TRPV4 has a two roles in the kidney. One is stretch sensation. The other a possible osmosensor.

Concluding message

TRPV4 co-localizes with adherence junctions in the urothelium of the bladder, the ureter & the distal tubuli of the kidney. These findings suggest that TRPV4 is involved in the sensation of stretch in the urothelium of the bladder, ureter and kidney.

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Is this a clinical trial?	**No**
What were the subjects in the study?	**NONE**