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TRPV4 AS A MECHANORECEPTOR IN THE HUMAN BLADDER; A CO-LOCALIZATION BETWEEN TRPV4 AND ADHERENCE JUNCTIONS THE UROTHELIUM.

Hypothesis / aims of study

Transient Receptor Potential Vanilloid receptor 4 (TRPV4) is a calcium specific ion-channel that is activated by physical stimuli like 'stretch'. It is found in different circumventricular organs in the body. Much of the localization of TRPV4 in the human bladder is unknown, but expression of TRPV4 in the urothelium of the mouse urinary bladder has already been reported. This may imply that in the urinary bladder, TRPV4 has a sensory role in detecting changes in 'stretch' in the bladder and therefore might also be involved in the sensory disregulation that is seen in diseases like OAB and PBS. To measure stretch, a receptor should be linked to rigid structures that are able to transmit mechanical forces that originate during bladder filling. Anchoring cell-junctions like adherence & tight junctions, are good candidates for this because they are intracellulary connected to the actin cytoskeleton and extracellulary anchor neighboring urotheliumcells to each other, thus forming a rigid structural network. We hypothesized that if TRPV4 plays a role in detecting 'stretch' in the urothelium, one of these two junctions would be a logical site. **The aim of our study** was to investigate the localization of TRPV4 in the human bladder urothelium and to see if there was a co-localization with tight junctions or adherence junctions.

Study design, materials and methods

Snap frozen bladder tissue obtained from healthy sections of cancerous (n=3) and non-cancerous (n=2) cystectomy patients. Bladders obtained from normal mouse (n=3) and 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 (PFA) & stained overnight (4°C) with antibodies for TRPV4, ZO-1 (tight junctions) & E-cadherin (adherence junctions), following staining with Alexa's (488 & 594) and DAPI. Specimens were analyzed with binocular epifluorescent and confocal microscope.

Results

Adherence junctions in the human bladder were clearly visualized with E-cadherin. These were seen as evenly distributed bright dot-like structures between the connecting cell membranes of neighboring urothelium cells. The adherence junctions between umbrella cells had a profound expression of TRPV4. The expression of TRPV4 in bladders of normal and TRPV4 knockout mice showed staining of the urothelium in the normal, but not in the knockout mice. The same co-localization between TRPV4 & E-cadherin was also seen in the mouse bladder. Comparative results were seen in the urothelial cell culture. No correlation was seen with TRPV4 and zona occludens.

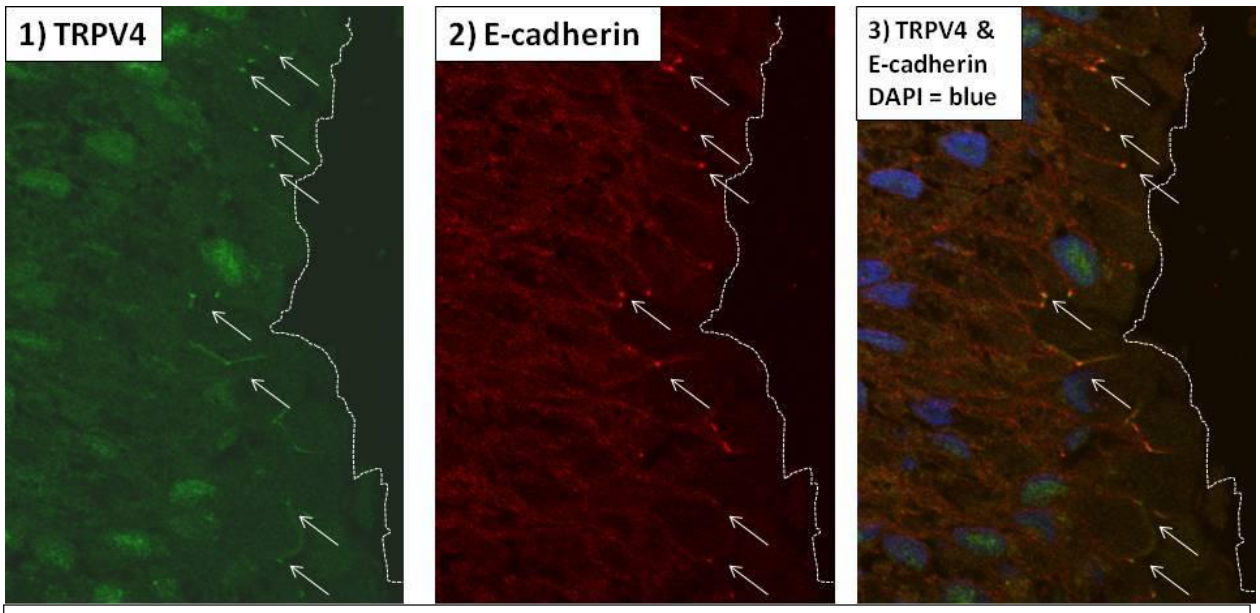

Fig1: epifluorescence confocal images of bladder showing TRPV4 (green) and E-cadherin (adherence junctions) and merged images. The dotted white line marks the urothelium-lumen-border. Note the evenly ditributed dot-like structures between the umbrellacells (white arrows). These represent adherence junctions 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.

Fig 2 shows immunohistochemical stainings on a well differentiated urothelial cancer cell culture (RT4) with TRPV4 & E-cadherin (adherence junctions). A high density of TRPV4 is seen at adherence junctions. Nuclei staining with DAPI. Pictures taken at 60x with binocular fluorescence microscope.

Interpretation of results

Our results confirm that TRPV4 is connected to the rigid structural network that includes adherence junctions and the actin cytoskeleton. To take into consideration the enlarged bladder capacities and the dysfunctional voiding seen in TRPV4-knockout mice, our findings provide new evidence on a molecular base, that TRPV4 is involved in measuring bladder stretch. TRPV4 could therefore also be involved in dysfunctional voiding pathology and be a potential therapeutic target for OAB.

Concluding message

TRPV4 is located near specific adherence junctions between umbrella cells, implying a link between motoric 'stretch' and urothelial sensation. These results provide new evidence that TRPV4 in the urothelium is involved in the sensation of stretch in the urinary bladder.

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