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Invited review

TRPV6: From identification to function

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Contents

1. Identification, genomic organisation and properties of the TRPV6 channel

The TRPV6 channel belong to the TRPV (vanilloid) subfamily of transient receptor (TRP) channels which is named after the first member TRPV1 that can be activated by vanilloid like compounds [1,2]. TRPV6 was first isolated from rat small intestine [3] followed by the identification of the human and mouse orthologues [4,5]. Among the members of the mammalian *Trp* gene family, the TRPV6 channel (formerly called ECAC2, CaT1, CaT-like) exhibits specific features: a) TRPV6 and its closest relative TRPV5 represent the most Ca^{2+} selective *Trp* ion channels. Both are mainly expressed in Ca^{2+}-transporting epithelia where they are assumed to play an important role in Ca^{2+} (re)absorption. b) The translation of the *Trpv6* gene is initiated 120 bp upstream of the annotated AUG at a non-AUG codon. c) A coupled polymorphism of *Trpv6* exists in humans causing a three amino acid (aa) exchange resulting in a so called derived *Trpv6* haplotype. d) Several human malignancies show an upregulation of the *Trpv6* gene expression (e.g. prostate and breast cancer). e) Homozygous *Trpv6*-deficient male mice are hypofertile due to a disordered Ca^{2+} homeostasis in the epididymal fluid resulting in a defective sperm maturation.

The *Trpv6* gene is located on chromosome 7q33-q34 (human), chromosome 6 (mouse) and chromosome 4 (rat) in adjacent to its closest relative, *Trpv5* (7q35 in human). It is likely that *Trpv5* and *Trpv6* arose by gene duplication from an ancestral gene. The chromosomal organization of *Trpv6* is highly conserved among several species; e.g. in mouse, the *Trpv6* gene spans 15 exons and extends over a region of ~15.7 kb. *Trpv6* has a broader expression pattern than the *Trpv5* gene which is mainly expressed in the kidney of mammals. TRPV5 plays a role in Ca^{2+} re-uptake in the kidney and thereby prevents loosing Ca^{2+} ions via the urine [6] whereas, depending on the species, TRPV6 is supposed to be responsible for Ca^{2+} uptake in the intestine [4,7]. The intact TRPV6 channel is also an essential component for the Ca^{2+} uptake by the epididymal epithelium [8]. This uptake mechanism is responsible for the

Abbreviations: aa, amio acid; ARE, AU-rich elements; bp, base pair; CaT1, calcium transport protein 1; CaT-like, calcium transport protein-like; CMV, Cytomegalovirus; DNA, deoxyribonucleic acid; ECAC, epithelial Ca^{2+} channel; EphB6, ephrin receptor B6; EVH1, enabled vasodilator stimulated phophoprotein (VASP) homology domain 1; ES, embryonic stem cells; HEK, (human embryonic kidney) cells; kb, kilo base; NCC, NaCl cotransporter; NFAT, nuclear factor of activated T-cell; N-terminal, amino-terminal; OCRL, oculocerebrorenal syndrome of Lowe; PHAII, pseudo-hypoaldosteronism type II; PIP2, phosphatidylinositol 4,5-bisphosphate; PMCA, plasma membrane Ca^{2+} ATPase; PRM, proline rich motifs; PTP1B, tyrosine-protein phosphatase non-receptor type 1B; RT-PCR, reverse transcriptase polymerase chain reaction; SCCD, semicircular canal duct; TNF, tumor necrosis factor; TM, transmembrane segment; TRP, transient receptor potential; SH3, (SRC homology 3) domain; SRC, proto-oncogene tyrosine-protein kinase; WNK, with no lysine (K) kinase; WW, (tryptophan-tryptophan) domain; 1,25(OH)2D3, (*1,25*-dihydroxy-Vitamin D3).

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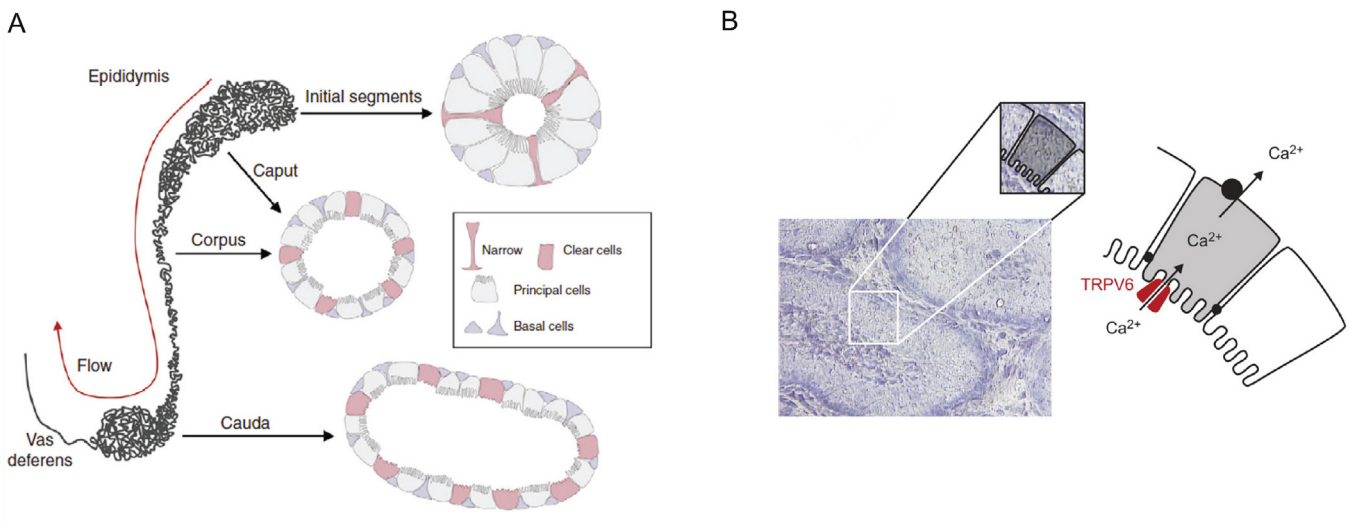

Fig. 1. The TRPV6 channel is an essential component for the Ca^{2+} uptake by the epididymal epithelial cells. A) Schematic representation of the murine epididymal duct and the different cell types of the epididymal epithelium. The red line shows the flow of spermatozoa during the maturation process in the epididymis. B) In the apical membrane of epididymal epithelial cells TRPV6 is responsible for Ca^{2+} uptake from the epididymal fluid.

decrease of the Ca^{2+} concentration in the epididymal fluid along the passage of spermatozoa, generating a luminal Ca^{2+} gradient with higher Ca^{2+} concentration in the caput epididymis (proximal) of the epididymal duct and lower Ca^{2+} concentration in the cauda epididymis (distal) (Fig. 1A). So one can assume that a decrease of the Ca^{2+} concentration in the epididymal fluid is a prerequisite for proper sperm maturation and viability during the passage of the epididymal duct. *Trpv6* is also expressed in the placenta, in the prostate and in the exocrine pancreas where TRPV6 might be important to retain the intracellular Ca^{2+} concentration [4,5,9].

The functional TRPV6 channel comprises four identical subunits that form a complex. A single TRPV6 subunit consists of 6 transmembrane segments (TM1 to TM6) (Fig. 2). The tetrameric TRPV6 channel is an inwardly rectifying Ca^{2+} selective ion channel and TRPV6 currents can be observed after Ca^{2+}addition from the outside to a Ca^{2+} free extracellular solution [7,10]. A high affinity calmodulin binding site located at the C-terminal region of the channel facilitates the inactivation of TRPV6 in the presence of Ca^{2+} [11]. Replacing a conserved glycine residue at position 516 within the cytosolic TM4-TM5 linker of the human TRPV6 protein by a serine leads to an open conformation of the channel [12]. Thereby, the constitutive Ca^{2+} entry is enhanced and the inactivation is prevented. A second mutation (T621A) into TRPV6_G516S partially rescues the TRPV6 function. According to the crystal structure the T621 is located at the distal end of TM6 within a short linker between TM6 and the helix formed by the TRP domain [13]. These findings demonstrate that the TM4-TM5 linker and the TM6-TRP-domain linker are important components for proper TRPV6 channel gating. Permeation of other divalent ions is also observed with $Ca^{2+} > Ba^{2+} > Sr^{2+} > Mg^{2+}$ [7,10]. A recent study of TRPV5 shows that mutations of a tryptophan (W583) at the intracellular mouth of the pore in TM6 region lead to enhanced Ca^{2+} uptake and cell death. This tryptophan residue is also highly conserved in TRPV6 [14]. Under non-physiological conditions, TRPV6 channels are also permeable for monovalent ions like Na^{+} in the absence of extracellular Ca^{2+}, but the currents are about 10× times larger than those observed for Ca^{2+} ions [7,10].

Currently, all electrophysiological experiments were performed in cells over-expressing the truncated *Trpv6* cDNA, but comparison of the biophysical properties of heterologous expressed full-length cDNA or the truncated cDNA demonstrate similar results [15]. However, the translocation to the plasma membrane is more efficient and a five times higher amount of the truncated TRPV6 protein is necessary for the production of equal current amplitudes [15]. So far, no TRPV6 currents were measured in acutely isolated primary cells that endogenously express *Trpv6* transcripts like pancreatic acinar cells although similar protocols are used. The reason for this is not clear. Therefore other methods are necessary to characterize TRPV6 channel activity in primary cells, e.g. $^{45}Ca^{2+}$ uptake measurements. Via this radioactive Ca^{2+} uptake assay, Weissgerber et al. [8,16] demonstrated that the Ca^{2+} concentration of the fluid in the cauda epididymis of *Trpv6*-deficient mice was 100 times higher than that of wild type controls.

2. Expression pattern of TRPV6 in different species

The cDNA was first cloned from rat small intestine [3] and human placenta [4], but interestingly the expression patterns are not equal across different species and depend on the method used for detection transcripts and proteins. For example, the murine *Trpv6* gene is expressed in placenta, pancreas, prostate, uterus and several parts of the small intestine, esophagus, stomach, colon and epididymis as demonstrated by RT-PCR and Northern blots [5,8,17]. Using immunostaining TRPV6 expression was demonstrated in the apical cell membrane at the tips of the villi of intestinal enterocytes in the murine duodenum [18]. Additionally, murine *Trpv6* transcripts or proteins are reported in bone and in different regions along the nephron tubules at the apical domain of the late distal convoluted tubule, the connecting tubule, and the cortical and medullary collecting ducts [19] and semicircular canal duct (SCCD) of the vestibular system [20]. Contrary to this, *Trpv6* transcripts in the rat kidney are not detectable by Northern blots [3] and could be only weakly or not detected in human kidney [4,7]. A TRPV6 expression in human small intestine and healthy prostate has never been demonstrated convincingly but are highly expressed in the healthy mouse prostate. Using TRPV6 specific antibodies which were applicable to discriminate between tissues from wild type and *Trpv6*-deficient mice, TRPV6 proteins have been identified in human placenta [15,21], mouse prostate and epididymis [8,16]. After antibody affinity purification followed by mass spectrometry TRPV6 derived peptides were also detected in placenta and the human cancer cell lines T47D [15]. It should be mentioned here, that the expression of *Trpv6* transcripts in human normal and benign hyperplastic prostate is low or even not detectable but is