available at www.sciencedirect.com
journal homepage: www.europeanurology.com

European Association of Urology

Bladder Cancer

Transient Receptor Potential Vanilloid Type 2 (TRPV2) Expression in Normal Urothelium and in Urothelial Carcinoma of Human Bladder: Correlation with the Pathologic Stage

Sara Caprodossi [a], *Roberta Lucciarini* [a], *Consuelo Amantini* [a], *Massimo Nabissi* [a], *Giacomo Canesin* [a], *Patrizia Ballarini* [b], *Adriana Di Spilimbergo* [c], *Marco Andrea Cardarelli* [c], *Lucilla Servi* [d], *Gabriele Mammana* [d], *Giorgio Santoni* [a,*]

[a] *Department of Experimental Medicine and Public Health, University of Camerino, Camerino (MC), Italy*
[b] *Department of Molecular, Cellular and Animal Biology, University of Camerino, Camerino (MC), Italy*
[c] *Pathology and Cytodiagnostic Unit, ASUR 9, Macerata, Italy*
[d] *Urology Operative Unit, ASUR 9, Macerata, Italy*

Article info

Article history:
Accepted October 5, 2007
Published online ahead of print on October 16, 2007

Keywords:
Bladder
Transient receptor potential vanilloid type 2 (TRPV2)
Urothelial carcinoma
Urothelium

Abstract

Objective: To evaluate the expression of transient receptor potential vanilloid type 2 (TRPV2) in normal human bladder and urothelial carcinoma (UC) tissues.
Methods: Bladder specimens were obtained by transurethral resection or radical cystectomy. TRPV2 mRNA expression in normal human urothelial cells (NHUCs), UC cell lines, and formalin-fixed paraffin-embedded normal ($n = 6$) and cancer bladder tissues ($n = 58$) was evaluated by polymerase chain reaction (PCR) and quantitative real-time PCR (RT-PCR). TRPV2 protein expression was assessed by cytofluorimetric and confocal microscopy analyses in NHUCs and UC cells and by Western blotting and immunohistochemistry in normal and UC tissues.
Results: Enhanced TRPV2 mRNA and protein expression was found in high-grade and -stage UC specimens and UC cell lines. Both the full-length TRPV2 (hTRPV2) and a short splice-variant (s-TRPV2) were detected in NHUC and normal bladder specimens, whereas a progressive decline of s-TRPV2 in pTa, pT1, and pT2 stages was observed, up to a complete loss in pT3 and pT4 UC specimens.
Conclusions: Normal human urothelial cells and bladder tissue specimens express TRPV2 at both the mRNA and protein levels. A progressive loss of s-TRPV2 accompanied by a marked increase of hTRPV2 expression was found in high-grade and -stage UC tissues.

* Corresponding author. Department of Experimental Medicine and Public Health, University of Camerino, Via Madonna delle Carceri 9, 62032, Camerino (MC), Italy.
Tel. +39 0737 403312/403319.
E-mail address: giorgio.santoni@unicam.it (G. Santoni).

1. Introduction

Bladder carcinoma is the second most common neoplasm of the urinary tract. More than 90% of primary bladder carcinomas are urothelial carcinomas (UCs) derived from the uroepithelium, 6–8% are squamous cell carcinomas, and 2% are adenocarcinomas [1]. Bladder UC can be present as superficial tumours (Ta and T1 stages) with high rates of recurrence and progression and muscle-invasive UC (T2–T4); they have a high risk of progression despite radical cystectomy and other treatments [2].

Recent findings indicate that urothelium represents not only a barrier protecting the underlying tissue, but also a responsive structure capable of detecting physiologic and chemical stimuli and releasing several signalling molecules [3,4]. Among the sensory receptors belonging to the TRP family, transient receptor potential vanilloid type 2 (TRPV2; vanilloid receptor-like 1 or VRL-1) [4,5] is highly expressed in sensory dorsal root ganglion neurons, although transcripts were found in non-neuronal tissues [6]. In rat urinary bladder TRPV2 mRNA is expressed in urothelial and smooth muscle cells [7].

TRPV2 contains six transmembrane domains, a putative pore-loop region, a cytoplasmic amino terminus with three ankyrin-repeat domains, and a cytoplasmic carboxy terminus. TRPV2 is a non-selective cation channel with high Ca^{2+} permeability; it acts as a heat sensor with a temperature threshold of 50–52 °C [5] and is activated by agonists such as 2-aminoethoxydiphenyl borate and Δ9-tetrahydrocannabinol (THC) [8]. Interestingly, the mouse ortholog of TRPV2 is activated by growth factors including insulin growth factor 1 (IGF-1), platelet-derived growth factor, and the neuropeptide head activator [9].

TRPV2 is also described as a stretch-activated channel and can mediate hypotonic swelling and stretch-induced $[Ca^{2+}]_i$ increase in vascular smooth muscle cells [10]. The TRPV cation channel family shows homeostatic and regulatory functions (calcium influx, apoptosis, and cell proliferation). In addition, recent data indicate that changes in their expression contribute to malignant growth and progression [11,12] and a role for TRPV6 and TRPV1 in the progression of prostate and bladder cancer and gliomas was reported [13–15].

At present, no data concerning the TRPV2 channel expression in normal and tumour human bladder tissues are available, and it is still unknown whether changes in TRPV2 expression could be associated with the disease stage in UC. Therefore, we investigated both the mRNA and protein expression levels of TRPV2 in normal and UC bladder tissues with different pathologic grades and stages.

2. Materials and methods

2.1. Bladder specimens and cell lines

Specimens from normal human bladder (NB, $n = 6$) were obtained by multiple cold-cup biopsy during diagnostic cystoscopy (cystoscopic control during tension- and free vaginal tape [TVT] procedures for the treatment of female stress urinary incontinence). None of the patients had urinary tract infections and the urodynamic assessment showed no functional disorders of the lower urinary tract. Macroscopic examination of the bladder mucosa did not reveal any pathology (cancer) at time of diagnosis. Specimens from bladder cancer tissues were obtained by transurethral resection of superficial UC ($n = 22$) or after radical cystectomy for muscle-invasive bladder cancer ($n = 36$). All patients gave their consent and a local ethics committee approved this study.

All the specimens were embedded in paraffin, and 5–7-μm thick sections were collected on slides ($n = 6$, for each specimen) and processed for immunohistochemistry. Bladder tumours were histologically diagnosed according to the World Health Organization classification.

Normal human urothelial cells (NHUCs) from Oligene (Berlin, Germany) were cultured in the Oligene Urothelial Cell Media System. Human RT4 (low-grade), EJ (high-grade), and J82 (high-grade) UC cell lines were from American Type Culture Collection (ATCC; Rockville, MD) and maintained in RPMI 1640 medium (Flow Laboratories, Irvine, Scotland) supplemented with 10% heat-inactivated fetal calf serum (FCS; Euroclone, Devon, United Kingdom), 2 mM N-[2-hydroxyethl]piperazine N′-[2-ethanesulfonic acid] (HEPES), 2 mM L-glutamine, 100 IU/ml penicillin, and 100 μg/ml streptomycin at 37 °C, 5% CO_2.

Full-length (hTRPV2)–plasmid-transfected HeLa cells were kindly provided by Dr. Lucia Ricci-Vitiani (Istituto Superiore di Sanità, Roma, Italy).

2.2. RNA extraction, reverse transcription, and DNA amplification

Total RNA was isolated from NHUCs, UC cell lines, and bladder tissues using the RNeasy Mini Kit (Qiagen, Valencia, CA, USA) as described [16]; total RNA from normal human bladder tissue (HBL) was purchased from Chemicon International (Temecula, CA, USA).

Total RNA from fixed paraffin-embedded tissue slices was isolated by Optimum™ FFPE RNA isolation kit (Ambion Diagnostic, Austin, TX, USA) as described [15]. Reverse transcription was performed using the High-Capacity cDNA Archive Kit (PE Applied Biosystems, Foster City, CA, USA).

The TRPV2 coding sequence was amplified using the following primers: forward 5'-tcctaggatgacctcaccct-3' and reverse 5'-gccatcagttggactggag-3', and the polymerase chain reaction (PCR) profile was 10 s at 98 °C, 20 s at 65 °C, 1 min at