483

Nomiya A[1], Tagaya M[2], Oyama T[2], Kawai Y[2], Takagaki K[2], Nisimatsu H[1], Aizawa N[3], Fujimura T[1], Igawa Y[3], Homma Y[1]

1. *University of Tokyo, Department of Urology,* ***2.*** *Nippon Shinyaku Co., LTD,* ***3.*** *University of Tokyo, Department of Continence Medicine*

INCREASED EXPRESSION OF TRPA1, TRPV2, ASIC1 AND CXCL9 MRNA IN BLADDER TISSUE FROM PATIENTS WITH ULCER-TYPE INTERSTITIAL CYSTITIS

Hypothesis / aims of study

Interstitial cystitis (IC) is a chronic inflammatory disease of the bladder with unknown etiology (1). The quantitative real-time polymerase chain reaction (qRT-PCR) approach offers the opportunity to discover differential gene expression independently of any prior hypothesis of etiology, providing ideas for new therapeutic strategies. In the present study, we assessed the differential expression of the human transient receptor potential (TRP) channel gene transcripts, acid sensing ion channel (ASIC), nerve growth factor (NGF), uroplakin 3A (UPK3A) and chemokine (C-X-C motif) ligand 9 (CXCL9) in bladder tissue in IC patients and controls.

Study design, materials and methods

Patients with IC scheduled for hydrodistension or with non-invasive bladder cancer (as controls) undergoing transurethral resection were enrolled under informed consent. Diagnosis of IC was based on the clinical guidelines for interstitial cystitis and hypersensitive bladder syndrome (2). Cystoscopy was performed with a rigid 9-mm cystoscope under spinal anaesthesia and bladder specimens were obtained from 1) retrotrigonal portions in non-ulcer- type IC patients, 2) non-ulcerative retrotrigonal portions in ulcer-type IC patients, 3) ulcerative portions in ulcer-type IC patients, and 4) non-cancerous retrotrigonal portions in bladder cancer patients (non-IC bladder, BT) with cold-cup biopsy forceps, and placed immediately in ice-cold RNA later and stored at -80°C. Total RNA was extracted from bladder samples, and reverse transcribed into cDNA with reverse transcriptase. The mRNA expression levels of several TRP channels (TRPA1, TRPV1, TRPV2, TRPV4, TRPM2, TRPM7 and TRPM8), ASIC1, NGF, UPK3A and CXCL9 were compared among the three groups by qRT-PCR. The mRNA levels were expressed as the fold change in the average value for non-IC bladder tissue.

Results

After optimization of the protocol, a total of 43 specimens (non-ulcerative lesions and ulcerative lesions from ulcer type, 14; non-ulcer type, 9; bladder cancer, 6) were analyzed between October 2009 and January 2011. The subjects' background is shown in Table 1 and the qRT-PCR results are shown in Table 2.

Among the TRP channels, TRPA1 and TRPV2 showed significantly increased mRNA expression in non-ulcerative portions of ulcer-type IC compared with controls. In the same portions, a significant increase in the mRNA expression of ASIC1 and CXCL9 and a decrease in the mRNA expression of UPK3A were observed. Also, in ulcerative portions of ulcer-type IC, a significant increase in TRPV2 and CXCL9 and a significant decrease in UPK3A were found as compared with controls. However, no significant difference was observed in non-ulcer-type IC tissue, suggesting a distinct pathophysiology or disease entity between these two types of IC (Figure 1).

Interpretation of results

This study demonstrates increased expression of TRPA1, TRPV2, ASIC1 and CXCL9 mRNA and decreased expression of UPK3A mRNA in ulcer-type IC but no significant difference in non-ulcer-type IC. These findings suggest a distinct difference in pathophysiology and disease entity between these two types of IC and potential targets to novel therapy for ulcer type IC.

Concluding message

TRPA1, TRPV2, ASIC1, CXCL9 and UPK3A in the bladder may play a role in the pathophysiology of ulcer-type IC, and as such they are potential targets for novel therapy.

Table 1. Subjects' background

Variables	BT (control)	Non-ulcer-type IC	Ulcer-type IC
Number of patients	6	9	14
(male/ female)	(4/2)	(4/5)	(2/12)
Mean age (range)	74.0±9.63 (61–88)	52.3±20.5 (20–73)	70.4 ±12.2 (36–83)
BMI	22.4±2.89	23.5±4.43	22.0±2.26

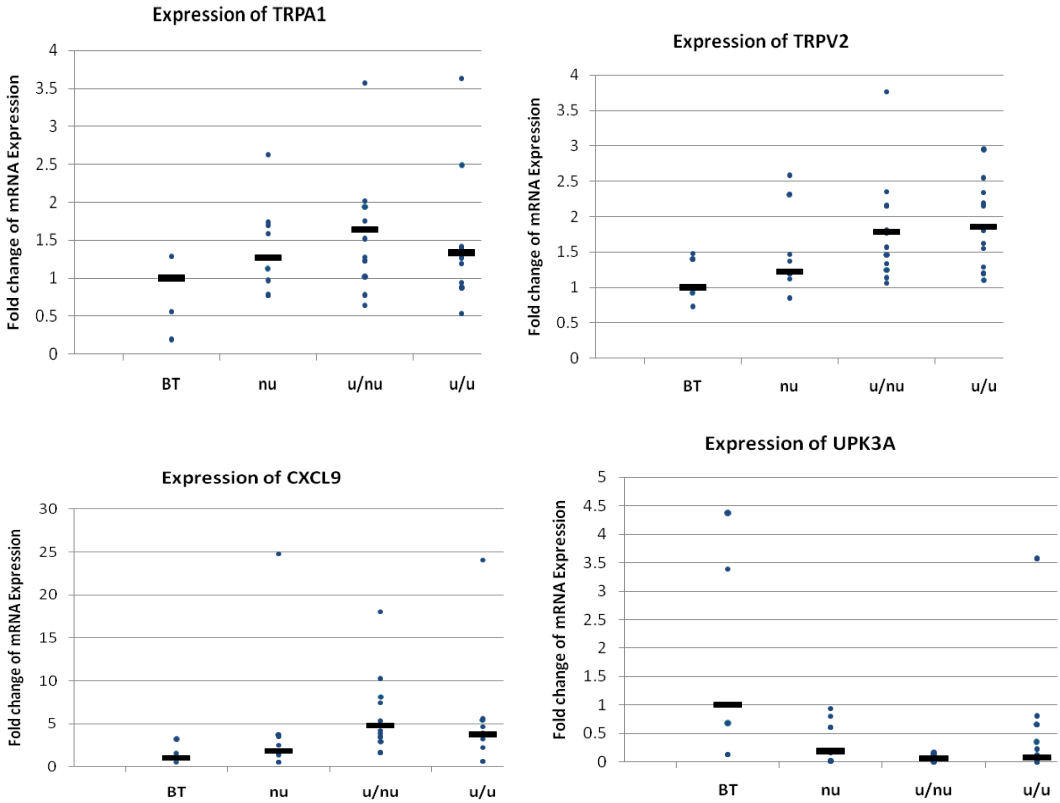
Table 2. mRNA expression in the bladder tissue with interstitial cystitis (fold change of control)

	Non-ulcer type IC		Ulcer type IC			
Gene Symbol			Non-ulcerative lesion		Ulcerative lesion	
	Fold change	p-value (n=9)	Fold change	p-value (n=14)	Fold change	p-value (n=14)
ACTB	0.87	0.272	1.39	0.179	1.38	0.179
TRPA1	1.27	0.388	1.64	0.041*	1.34	0.353
TRPV1	1.32	0.145	1.47	0.076	0.61	0.779
TRPV2	1.23	0.456	1.79	0.005**	1.86	0.009**
TRPV4	1.08	0.776	0.83	0.274	0.79	0.153
TRPM2	0.40	0.328	1.17	0.968	1.02	0.841
TRPM7	0.95	0.955	1.02	0.779	0.96	0.779
TRPM8	2.39	0.224	1.45	0.091	1.51	0.109
NGF	2.45	0.181	1.57	0.207	0.61	0.659

ASIC1	1.22	0.272	1.57	0.009**	0.91	0.779
UPK3A	0.19	0.224	0.07	0.009**	0.08	0.033**
CXCL9	1.82	0.224	4.78	0.000**	3.78	0.006**

GAPDH mRNA levels were used as an internal normalization control.
The p-values were determined by the Wilcoxon rank-sum test. (*p<0.05, **p<0.01)

Figure 1. mRNA expression of TRPA1, TRPV2, CXCL9 and UPK3A in the bladder tissue



BT: bladder tumor (control), nu: non-ulcer type IC, u/nu: non-ulcerative lesion of ulcer type IC, u/u: ulcerative lesion of ulcer type IC

References

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Is this a clinical trial?	**No**
What were the subjects in the study?	**HUMAN**
Was this study approved by an ethics committee?	**Yes**
Specify Name of Ethics Committee	**University of Tokyo Institutional Review Board**
Was the Declaration of Helsinki followed?	**Yes**
Was informed consent obtained from the patients?	**Yes**