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TRANSIENT RECEPTOR POTENTIAL C1 AND C4 IN INTERSTITIAL CYSTITIS - IMMUNOHISTOCHEMICAL ANALYSIS OF THE BLADDER TISSUE

Hypothesis / aims of study

Interstitial cystitis (IC) is still a disease of mystery. A previous study demonstrated increased expression of genes involved in pronociceptive inflammatory reactions in IC including TRPM2, TRPV1, TRPV2, TRPV4, ASIC1, and CXCL9 in Hunner type IC (HIC) but not in non-Hunner type IC (NHIC) (1). In addition, increased mRNA expression of TRPC1 and TRPC4 in IC bladder was also shown (2); however, localization and function of TRPC1 and TRPC4 were not analyzed. In this study, immunohistochemical expression of TRPC1 and TRPC4 was investigated for its location and correlation with clinical parameters of IC.

Study design, materials and methods

Patients with IC scheduled for hydrodistension or those with non-invasive bladder cancer undergoing transurethral resection (as controls) were enrolled. Diagnosis of IC was based on the clinical guidelines for IC and hypersensitive bladder (3).

Bladder biopsy specimens were obtained from 1) NHIC, 2) background mucosa (or out of Hunner lesion) of HIC, and 3) non-cancerous portions of bladder cancer as control. Bladder samples were fixed in 10% buffered formalin, embedded in paraffin, and then cut into 5-μm sections. For immunohistochemical assessment, bladder sections were dewaxed in xylene and rehydrated through decreasing concentrations of ethanol. Slides were incubated with anti-TRPC1 antibody (ab74819, Abcam) or anti-TRPC4 antibody (ab111841, Abcam) overnight in a humid chamber at 4°C. Immunohistochemistry was detected using Dako envision system following manufacturer's guidance. For each section, expression of TRPC1 or TRPC4 in epithelium and interstitial layer were assessed using a five point scoring system (0, no remarkable dying; 1, weak dying; 2, normal dying; 3, strong dying; 4, very strong dying). Severity of bladder inflammation was scored 0 to 3 according to degree of inflammatory cell infiltration, edema, ulceration and hemorrhage.

Patients' symptom at enrolment was assessed by O'Leary and Sant's symptom index (OSSI), problem index (OSPI), visual analogue scale for pain (VAS), frequency volume chart data (daily urinary frequency, average voided volume), and the maximum distended bladder capacity at hydrodistension.

Level of TRPC1 or TRPC4 expression was analyzed by the Wilcoxon rank sum test, and its relation with clinical parameters was evaluated by Pearson product-moment correlation coefficient, with $p < 0.05$ considered statistically significant.

Results

Bladder samples from 37 subjects (NHIC, 8; HIC, 26; control, 3) were evaluated. All the IC patients were compatible with NIDDK criteria (Table 1).

Table 1: Patients' background

Variables	Control	NHIC	HIC
Patients (male /female)	3 (2/1)	8 (2/6)	26 (2/24)
Age at diagnosis	69.7±12.3	51.4±21.6	69.7±9.43
O'Leary & Sant's symptom index	2.73±1.01	11.6±3.78*	15.0±3.58*
O'Leary & Sant's problem index	2.73±1.27	9.88±2.59*	13.3±3.23*
Visual analogue scale for pain (0-10)	0.55±0.69	5.25±2.77*	7.55±1.85*
Daily urinary frequency	7.67±1.82	14.9±5.54*	18.5±6.80*
Average voided volume (ml)	186±29.7	118±41.0*	104±43.1*
Distended bladder capacity (ml)	920±110	612±226*	504±199*

*: $p < 0.05$ versus control

Staining intensity of TRPC1 and C4 appears to be stronger in IC tissue than control (Figure 1). In particular, histochemical score of TRPC1 was significantly larger in epithelium and interstitial tissue in both NHIC and HIC, while the score of TRPC4 was significantly greater in epithelium of HIC and interstitial tissue of NHIC alone (Table 2). TRPC1 expression significantly correlated with daily urinary frequency, average voided volume, maximum bladder capacity at hydrodistension, and severity of histological inflammation in HIC (Table 3). Correlation with clinical parameters was ambiguous for TRPC1 and TRIPC4 expressions in NHIC and for TRPC4 expression in HIC.

Figure 1. Representative sections of immunohistochemical staining of TRPC1 and TRPC4 in bladder mucosa (magnification: x100) - scale bar: 200μm.

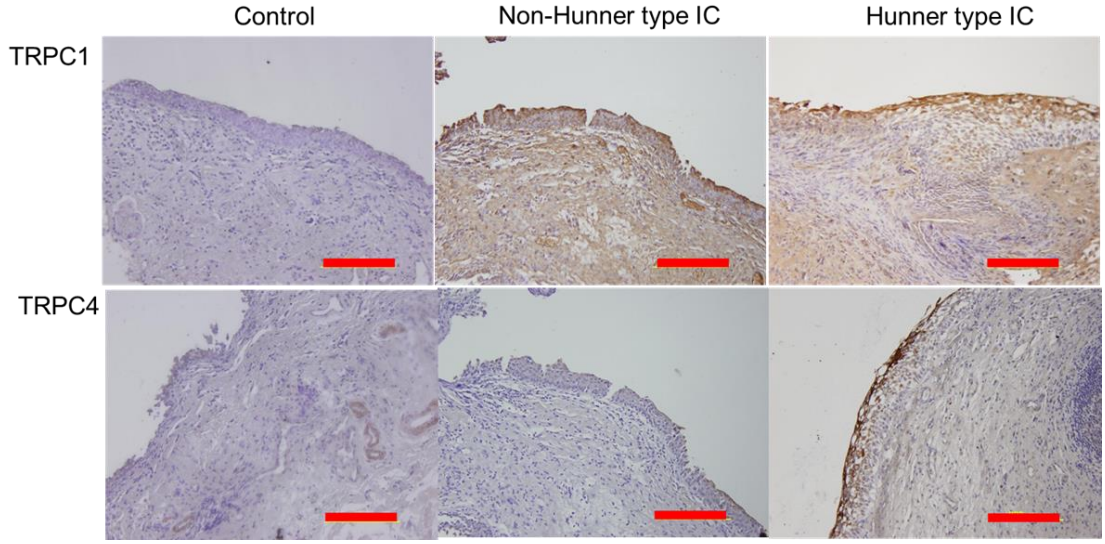

Table 2. The mean score of TRPC1 and TRPC4 expression in the bladder tissue

	Control	NHIC	p-value	HIC	p-value
TRPC1 (epithelium)	0.67	3.13	0.000	2.96	0.000
TRPC1 (interstitial layer)	0.67	1.75	0.015	1.35	0.038
TRPC4 (epithelium)	1.67	2.12	0.086	2.88	0.001
TRPC4 (interstitial layer)	0.33	1.38	0.035	0.92	0.103

P-value: vs. control.

Table 3. Correlation between TRPC expression scores and clinical parameters

	NHIC				HIC			
	TRPC1e	TRPC1i	TRPC4e	TRPC4i	TRPC1e	TRPC1i	TRPC4e	TRPC4i
OSSI	-0.634	**-0.757***	0.153	-0.243	0.082	-0.072	0.174	0.322
	0.091	**0.030**	0.718	0.563	0.691	0.725	0.394	0.109
OSPI	-0.383	-0.638	0.207	-0.459	0.159	0.091	0.103	0.189
	0.349	0.088	0.623	0.252	0.438	0.657	0.617	0.354
VAS	-0.234	-0.522	-0.277	-0.084	0.284	0.382	-0.141	-0.184
	0.578	0.184	0.506	0.843	0.160	0.054	0.494	0.368
Frequency	-0.075	0.109	0.406	0.151	**0.555****	**0.526****	0.372	**0.453***
	0.860	0.797	0.319	0.721	**0.004**	**0.007**	0.067	**0.023**
AVV	-0.325	0.020	-0.282	-0.580	**-0.470***	-0.288	-0.164	-0.271
	0.431	0.963	0.499	0.131	**0.018**	0.163	0.433	0.191
Distended volume	0.215	-0.125	-0.085	-0.405	**-0.461***	**-0.480***	0.116	-0.110
	0.609	0.769	0.841	0.320	**0.018**	**0.013**	0.572	0.594
Severity of inflammation	NA	NA	NA	NA	**0.494***	**0.413***	-0.007	0.064
					0.010	**0.036**	0.973	0.758

*: p<0.05, **: p<0.01 e: epithelium, i: interstitial layer, AVV: average voided volume.
The upper and lower panels represent Pearson's correlation coefficient and p-value, respectively.

Interpretation of results

The present study demonstrated increased expression of TRPC1 and TRPC4 in IC bladder. Expression of TRPC1 in HIC was correlated with urinary frequency, smaller bladder capacity, and inflammation severity.

Concluding message

The results of this study support the hypothesis that TRPC1 and TRPC4 have some role in the pathogenesis of IC. Further researches are required to elucidate the function of TRPC1 and TRPC4 in the bladder mucosa in connection with pathogenesis of IC.

References

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Disclosures

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