

## **CXCR3 AND RELATED CHEMOKINES AS POSSIBLE BIOMARKERS FOR ULCERATIVE INTERSTITIAL CYSTITIS**

### Hypothesis / aims of study

Interstitial cystitis or painful bladder syndrome (IC/PBS) is an intractable disease affecting QOL, and its pathophysiology remains to be clarified. IC/PBS is divided into two types by cystoscopic findings, ulcerative and non-ulcerative interstitial cystitis. In the present study we investigated the genes responsible for ulcerative interstitial cystitis (IC), which could be the clue to the etiology of IC/PBS or potential biomarkers for the diagnosis of IC/PBS.

### Study design, materials and methods

Eighteen patients were enrolled in this study (9 patients with ulcerative IC and 9 patients with bladder cancer or benign prostate hyperplasia (BPH) as controls). Bladder urothelial and suburothelial tissues were taken using a cold cup biopsy forceps from a site surrounding ulcerative lesion in 9 patients with ulcerative IC and from a normal looking area in 9 patients with bladder cancer or BPH as a control group. Total RNA was extracted from the bladder samples and the expression levels of genes were compared between the IC and control groups using Whole Human Genome DNA microarray 44K (Agilent technologies). The microarray data were analyzed by using GeneSpring GX software (Agilent technologies) and Ingenuity Pathway Analysis (IPA, Ingenuity systems). In the IPA, scores of 2 or higher indicates at least a 99% confidence that a focused gene network was not generated by random chance alone. The chosen genes for further analysis were confirmed by quantitative RT-PCR. Symptoms were evaluated using the O'Leary-Sant symptom index and problem index.

### Results

Both symptom index and problem index were significantly higher in patients with ulcerative IC (14.8±0.8, 13.8±0.5) compared to the control group (5.0±1.6, 3.6±1.7), respectively. mRNA expression of known markers for bladder umbrella cells such as KRT20 and UPK 1a, 1b and 1c were decreased in the IC samples, indicating the brittle bladder urothelial barrier in ulcerative IC. Moreover we have identified 564 probes that were significantly ( $p < 0.001$ ) expressed in mRNA by more than 4 fold compared to the control group by using the volcano plot analysis. Of them 175 genes were up-regulated and 389 genes were down-regulated. Further IPA network analysis of these genes depicted the top 5 functions such as "Cell-To-Cell Communication and Signaling", "Inflammatory Disease" and "Cellular Development" (Table 1 and Fig. 1). Among these genes depicted, by using data from microarray and quantitative RT-PCR, we also confirmed the increased mRNA expression of several genes including TNF $\alpha$ , CXCR3 and CXCR3-binding chemokines in the bladder samples from IC patients (Fig. 2).

### Interpretation of results

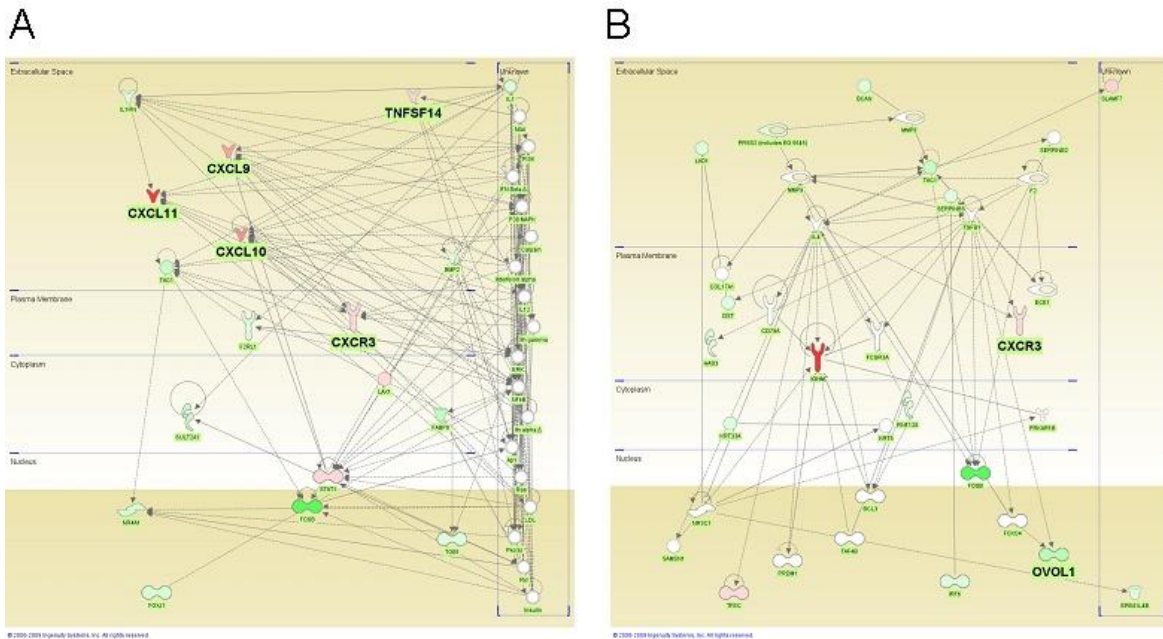
The current study using DNA microarrays analysis demonstrates the significant differences in mRNA expression in the bladder sample between ulcerative IC and control group. Moreover, over-expression of these genes depicted the top 3 functions such as "Cell-To-Cell Communication and Signaling", "Inflammatory Disease" and "Cellular Development" including TNF $\alpha$ , CXCR3 and CXCR3-binding chemokines by pathway analysis.

### Concluding message

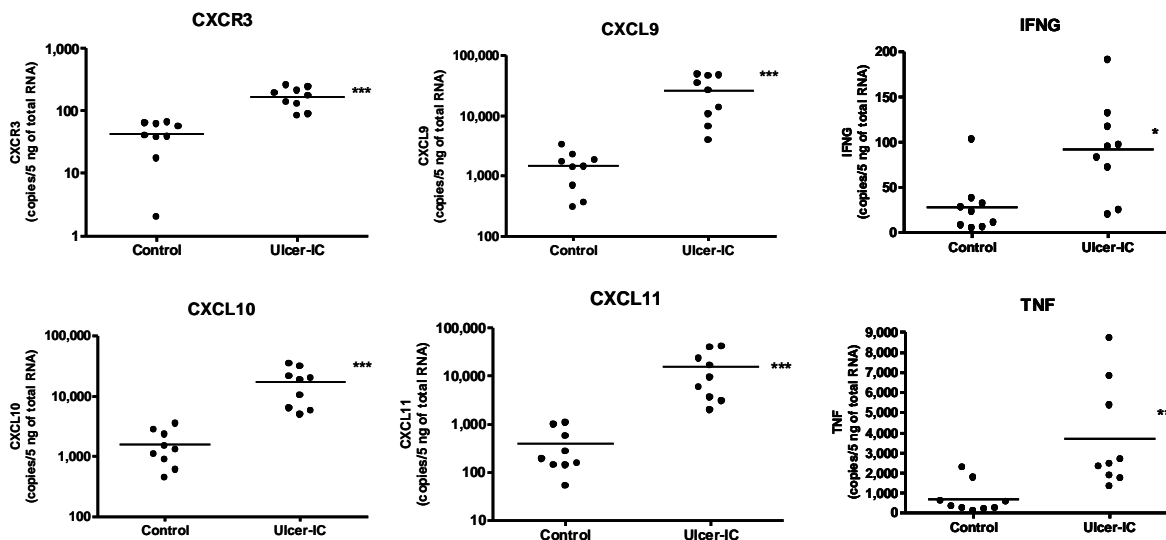
This study shows that the genes including CXCR3 and CXCR3-binding chemokines, which act on the immune system, are significantly up-regulated in the bladder tissue obtained from ulcerative interstitial cystitis patients. Therefore, it is suggested that these genes are involved in the etiology of ulcerative interstitial cystitis, and might be a potential biomarker for this disease.

**Table 1:** The Top 5 gene network functions extracted by the IPA analysis

ID	Associated Network Functions	Score
1	Cell-To-Cell Signaling and Interaction, Hematological System Development and Function, Immune and Lymphatic System Development and Function	35
2	Inflammatory Disease, Connective Tissue Disorders, Skeletal and Muscular Disorders	30
3	Cellular Development, Skeletal and Muscular System Development and Function, Cancer	30
4	Visual System Development and Function, Cancer, Neurological Disease	25
5	Tissue Morphology, Behavior, Nervous System Development and Function	9



**Fig. 1** The gene networks representing “Cell-To-Cell Communication and Signaling (ID1)” [A] and “Inflammatory Disease, etc. (ID2)” [B] extracted by the IPA analysis



**Fig. 2** Results of quantitative RT-PCR. Increased mRNA expression of several genes, including TNF $\alpha$ , CXCR3 and CXCR3-binding chemokines, demonstrated in the bladder samples from uncreative IC patients.

<i>Specify source of funding or grant</i>	none
<i>Is this a clinical trial?</i>	No
<i>What were the subjects in the study?</i>	HUMAN
<i>Was this study approved by an ethics committee?</i>	Yes
<i>Specify Name of Ethics Committee</i>	Ethics Committee of Shinshu University School of Medicine
<i>Was the Declaration of Helsinki followed?</i>	Yes
<i>Was informed consent obtained from the patients?</i>	Yes