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EXPRESSION PROFILE OF UROTHELIAL TRANSCRIPTION FACTORS IN BLADDER BIOPSIES WITH INTERSTITIAL CYSTITIS

Hypothesis / aims of study
Interstitial cystitis (IC) is considered to manifest Hunner's lesion or glomerulation in the bladder based on NIDDK criteria, but the pathology remains elusive. While Hunner's lesion displays an inflammatory gene signature, intrinsic abnormalities of the urothelium *per se* are largely unknown. Tissue specific master transcription factors (TFs) play pivotal roles in human development and disease, and we sought to characterize IC pathology based on the expression profile of urothelial master TFs.

Study design, materials and methods
To identify candidate urothelial master TFs, we used bladder carcinoma cell lines which are derived from the urothelial stem cells but display two contrasting characteristics: epithelial or mesenchymal. Gene expression was measured with quantitative RT-PCR. From the initial screening of 170 TFs (human homologs of Drosophila segmentation genes and known master TFs from a database), 28 TFs were selected. Subsequently we purified mRNA from bladder biopsies of IC patients and measured gene expression levels of known urothelial marker genes and candidate master TFs. Multivariate expression data were analyzed with SPSS software.

Results
mRNA expression of the 28 candidate TFs as well as known urothelial marker genes were measured in IC specimens sampled endoscopically. IC biopsies included various clinical status, including ulcerative type biopsies (Hunner's lesion or 'apparently normal' urothelium counterpart from the same patient) and non-ulcerative type biopsies (urothelium with or without glomerulation during hydrodistension). Initially, we noted that E-cadherin expression (encoded by CDH1 gene) had a huge variation among different IC specimens (Figure 1A, Log2 expression range: from 5.29 to 14.96, 815-fold expression difference). Endoscopic handling could have resulted in significant variation of epithelial/stromal ratio and we presumed that the contaminated stroma (i.e. submucosal tissue and inflammatory cells) significantly affected data interpretation. Therefore, we changed the internal control of RT-PCR from 18s ribosomal RNA to CDH1, thereby exclusively focusing on urothelial status. UPK1A expression (uroplakin 1A, a known marker of urothelial cells) was lower in IC biopsies from Hunner's lesion (ulcer positive) compared to urothelium from both ulcerative and non-ulcerative patients (collectively defined as ulcer negative) (Figure 1). Moreover, expression of other urothelial marker genes, TP63, SHH or FOXA1 was lower in Hunner's lesion. Conversely, retinoic acid receptor RARA expression was higher in ulcer positive compared to ulcer negative urothelium (Figure 2). Among 28 candidate TFs, expression of EVX1, OVOL1, EHF, ELF3 or GRHL2 was lower in Hunner's lesion. Conversely, expression of vitamin D receptor VDR, ELF4, IRF1 (Figure 3), ETS2, NR4A2 or ZBTB7B was higher in ulcer positive compared to ulcer negative urothelium. We noted that several factors are associated with each other and the multivariate expression data could be decomposed to fewer number of dimensions. Factor analysis reduced the expression complexity of known urothelial markers and the candidate TFs and we chose four axes for convenience of classification (Figure 4). From the known markers, retinoic acid receptors RARA and RXRA were classified into the first axis (Figure 4A, Principal Axis: PA1), and the second axis (PA2) was related to the urothelial basal cell marker, KRT5 (Figure 4A). Other known markers, KRT7, UPK1B, and UPK3A were classified into PA2 but these three were inversely correlated with KRT5 (Figure 4B). The third axis (PA3) included four markers, TP63, KRT20, UPK1A, and UPK3B (Figure 4A), which are closely associated with each other (Figure 4B). The fourth axis (PA4) included SHH and FOXA2 (Figure 4A). Most candidate TFs were classified according to these criteria but some were under-detected (KLF3, KLF15, NR5A2 and FOS) in IC biopsies, and EHF and CEBPG were not classified into the four PAs, due to poor correlation with other factors (Figure 4A). Finally, we sought to characterize IC biopsies according to these multivariate expression profiles (Figure 5). Principal component (PC) analysis produced a similar multivariate classification as that obtained in factor analysis. We drew a scatter plot as shown in Figure 5 according to these four principal components. Although there was no remarkable segregation between ulcer statuses (black circles: Hunner's lesion, white circles: 'apparently normal' urothelium counterparts from ulcerative type patients and urothelium from non-ulcerative type patients), biopsies from Hunner's lesion (black) tended to be located in particular PC areas (Figure 5).

Interpretation of results
IC might be characterized based on the urothelial master TF.

Concluding message
We developed a method to characterize IC biopsies based on the urothelial master TF signature and Hunner's lesion might be defined as 'decreased progenitors and higher regeneration'.
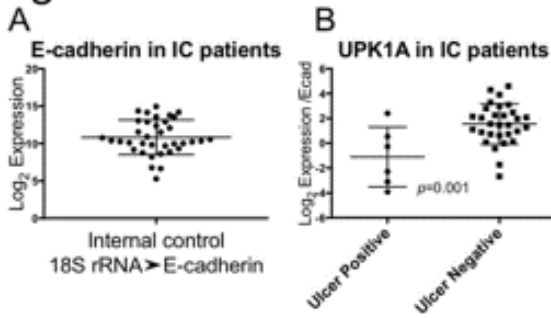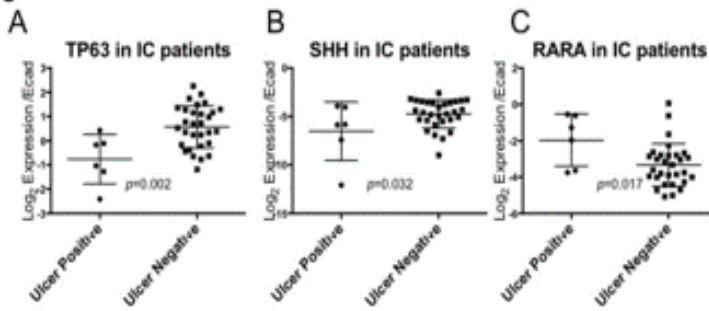
Figure 1

A
E-cadherin in IC patients

Internal control
18S rRNA ➤ E-cadherin

B
UPK1A in IC patients

p=0.001

Ulcer Positive / Ulcer Negative

Figure 2

A
TP63 in IC patients

p=0.002

Ulcer Positive / Ulcer Negative

B
SHH in IC patients

p=0.032

Ulcer Positive / Ulcer Negative

C
RARA in IC patients

p=0.017

Ulcer Positive / Ulcer Negative

Figure 3

A
Lower in Hunner's lesion

EVX1 in IC patients

p<0.001

OVOL1 in IC patients

p=0.022

EHF in IC patients

p=0.024

Ulcer Positive / Ulcer Negative

B
Higher in Hunner's lesion

VDR in IC patients

p=0.007

ELF4 in IC patients

p=0.007

IRF1 in IC patients

p=0.014

Ulcer Positive / Ulcer Negative

Figure 4

A

B

Figure 5

A

PC2(KRT5)

PC1(RA)

B

PC4(SHH)

PC3(TP63)

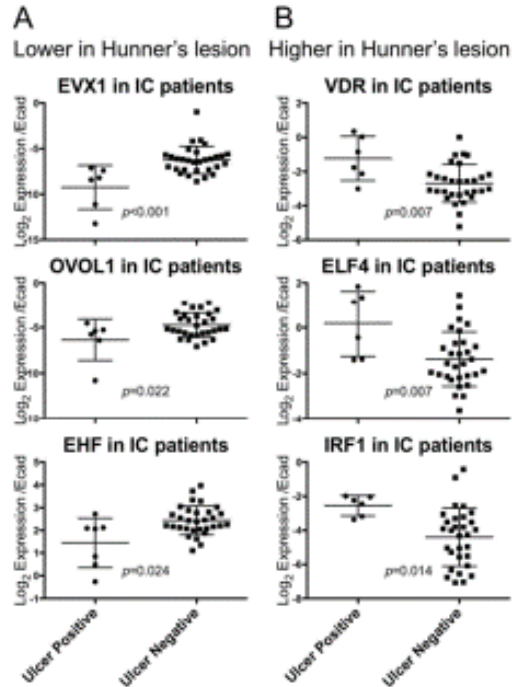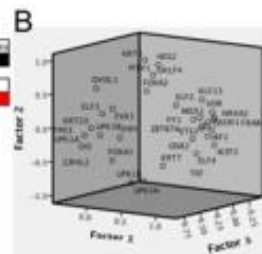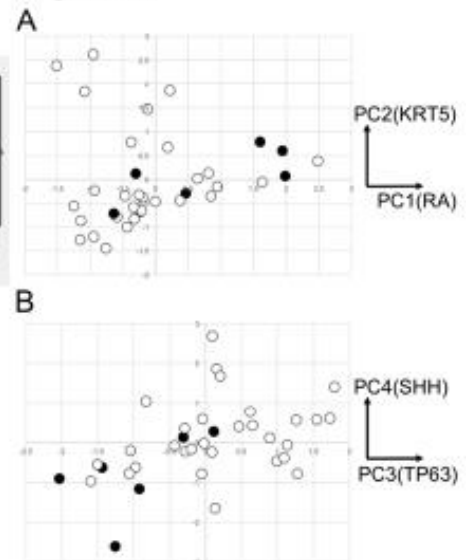## References

1. Homma Y, Ueda T, Tomoe H, Lin AT, Kuo HC, Lee MH, et al. Clinical guidelines for interstitial cystitis and hypersensitive bladder syndrome. Int. J. Urol. 2009; 16: 597-615.
2. Whyte WA, Orlando DA, Hnisz D, Abraham BJ, Lin CY, Kagey MH, et al. Master transcription factors and mediator establish super-enhancers at key cell identity genes. Cell. 2013; 153: 307-19.
3. Hnisz D, Abraham BJ, Lee TI, Lau A, Saint-Andre V, Sigova AA, et al. Super-enhancers in the control of cell identity and disease. Cell. 2013; 155: 934-47.