Basic Science Research on Bladder and Urine Markers for Interstitial Cystitis-Current and Future Directions

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INTRODUCTION

Interstitial cystitis (IC) has been estimated to affect between 1.2 and 60 cases per 100,000 female patients, depending on the different diagnostic criteria used. Characterized by bladder pain, urgency and urinary frequency, IC can have significant physical, social, economic and psychological ramifications on the lives of those afflicted.

Many etiologies have been proposed for IC, including altered bladder permeability, increased activity of mast cells, autoimmunity, occult infection, and neurogenic inflammation. However, none of these explanations apply to all IC patients [1]. IC may represent a final, common reaction of the bladder to different types of insult. Currently, there is no definitive diagnostic test for IC, so it is defined by symptom criteria. With increasing knowledge of the molecular pathophysiology of IC comes the ability to identify some promising biomarkers for the disease. A sensitive and specific marker must have the characteristics of higher level IC patients, but not of other genital-urinary tract disease, such as cancer, infection, or bladder stones. An IC marker, if found, could assist in establishing an etiologic mechanism, provide a diagnostic tool for identifying different stages of IC, and predict treatment responses.

IC is a disorder related to urothelium dysfunction or disruption, regardless of whether it is the cause or the result. The most consistent histologic findings in bladder biopsies from patients with IC are denudation or tears in the bladder epithelium, as well as thinning of the bladder epithelium. Recent studies also suggest the urothelium possesses a sensory function by modulation of receptors by proteins that have transducer properties. The molecular markers that depict the change of structure or function of urothelium in IC patients could thus be regarded as potential markers for IC.

APF-inhibition of bladder urothelium proliferation and increasing permeability

Antiproliferative factor (APF), a small glycoprotein whose peptide moiety bears 100% homology to the sixth transmembrane portion of a human receptor termed 'frizzled 8', is uniquely expressed by urothelial cells in bladders affected by IC [1]. APF has demonstrated involvement in growth inhibition and cell cycle arrest at the G_2/M phase in bladder urothelial cells. Cells explanted from IC patients, or normal cells treated with APF, reveal abnormal bladder urothelial cell gene expression identifiable by microarray and immunofluorescence analyses. The results suggest that APF causes an altered pattern of gene expression that reduces proliferation of the urothelium related to the pathogenesis of IC. APF was discovered in urine, serum, and tissue from IC patients, who showed levels very significantly different from those of asymptomatic matched controls, with a sensitivity of 94% and specificity of 95%. APF also causes downregulation of HB-EGF (heparin binding epidermoid growth factor), and possibly relates to the denudation and tears of the bladder epithelium seen in IC. Furthermore, in vitro studies show APF treatment causes a significant increase in epithelial permeability and decreases expression of tight junction protein in the human bladder epithelial monolayer. Interestingly, treatment of IC patients with bladder hydrodistension or stimulation of the third sacral nerve will normalize APF. APF is measured by bioassay as percent inhibition of thymidine incorporation and may constitute a useful marker for diagnosing IC. In addition, supplementing with recombinant HB-EGF can abrogate the effects of APF in animal studies. HB-EGF might be applicable to the treatment of IC in the future.

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GP51- a protective component of bladder uroepithelium

GP51, a glycoprotein (51 kDa molecular weight) produced mainly by bladder uroepithelial cells, is a major component of the bladder mucous layer [2]. GP51 acts as a major element of the antibacterial defense of the bladder. Decreased levels of GP51 have been noted in the urine and bladder biopsies of IC patients versus normal controls, thus GP51 may be used as a diagnostic marker for IC.

IL-6 markers for inflammatory reaction

Neuroinflammatory mechanisms, including activation of mast cells and release of their products, have been reported in association with IC. Increasing levels of IL-6 and methylhistamine have been identified in the urine of patients with IC compared to normal controls with a sensitivity of 70% and a specificity of 72.4% [3]. However, IL-6 is significantly elevated not only in urine specimens from IC patients, but also in specimens from patients with a variety of other urinary tract disorders, including bladder cancer, microhematuria, urolithiasis, and bacterial cystitis, making it unlikely to prove as useful as APF for identifying IC. However, IC symptoms are often nonspecific and may be confused with overactive bladder. If a diagnosis of IC is uncertain, IL-6 can be regarded as an adjuvant marker.

ATP and NGF-neurotransmitters of bladder hypersensitivity

Bladder urothelial cells (BUC) have neurosensory-like functions beyond the scope of a barrier function. Evidence supports that these cells have muscarinic receptors, purinergic receptors, and TRPV1 receptors that are commonly attributed to neurons. ATP acts as a sensory neurotransmitter and binds to purinergic receptors located on the urothelium as well as sensory nerves in the suburothelium. BUC can release ATP in response to mechanical stretching. This release was

more pronounced in patients with IC compared with controls [4]. Furthermore, in IC patients, BUC cells have a higher expression of $P2X_3$ receptors and smooth muscles have a significantly higher sensitivity to ATP in a smooth muscle organ bath as compared with controls. ATP also plays an autocrine role via augmented extracellular ATP signaling in BUC of IC patients. Therefore, ATP could possibly be used as an IC marker. In addition, development of therapeutic techniques by intravesical blocking of ATP function could be applicable to the treatment of IC.

Nerve growth factor (NGF) is a signaling protein that promotes the survival of dorsal root ganglia and sympathetic neurons during embryonic development and fetal life. In addition, more recent work indicates that NGF plays an important role in the regulation of inflammation and pain [5]. In rodents, intravesical NGF administration causes bladder hypersensitivity. In humans, IC patients have been found to have increased levels of NGF in urine and bladder tissue. However, NGF levels may also increase in patients with bladder outlet obstruction and overactive bladder, in addition to bladder inflammation. Therefore NGF can be regarded as an adjuvant marker for IC. Blocking NGF function prevents neural plasticity and bladder overactivity in experimental models. Thus NGF base therapy, either an endogenous antibody or an antibody against the NGF receptor, might provide an alternative therapy for IC.

Autoantibodies from IC patients using microarray analysis

Microarray technology was used to identify autoantibodies that might serve as potential biomarkers of IC [6]. The methodology relies on advanced bioinformatics to scrutinize information contained within mass spectrometry (MS) and high-resolution proton nuclear magnetic resonance (1H-NMR) spectral patterns to distinguish IC-affected from non-affected individuals [7]. A reverse capture autoantibody array was used to capture autoantibodies present in patients' urine samples. Captured autoantibodies from urine of patients with IC were compared to those from normal controls. Twenty-five autoantibodies significant for IC were identified; three of these bound antigens for IL-6, the chemokine CXC motif ligand 7, and the transcription factor E2F2 [8]. With such new techniques, some novel markers might emerge as IC markers.

CONCLUSIONS

IC is a complex condition for the clinician to diagnose and treat on account of multiple etiologies, the lack of objective diagnostic tests and the absence of any reliable therapy. IC biomarkers developed from understanding the various causes of IC will allow for better diagnosis, identification of subsets of patients, prediction of therapeutic responses, and personalized treatment.

REFERENCES

1. Chai TC, Keay S: New theories in interstitial cystitis. Nat Clin Pract Urol 2004; **1**:85-89.
2. Hurst RE, Moldwin RM, Mulholland SG: Bladder defense molecules, urothelial differentiation, urinary biomarkers, and interstitial cystitis. Urology 2007; **69(Suppl 4A)**:17-23.
3. Lamale LM, Lutgendorr SK, Zimmerman MB, Kreder KJ: Interleukin-6, histamine, and methylhistamine as diagnostic markers for interstitial cystitis. Urology 2006; **68**:702-706.
4. Sun Y, Chai TC: Augmented extracellular ATP signaling in bladder urothelial cells from patients with interstitial cystitis. Am J Physiol Cell Physiol 2006; **290**:27-34.
5. Steers WD, Tuttle JB: Mechanisms of Disease: The role of nerve growth factor in the pathophysiology of bladder disorders. Nat Clin Pract Urol 2006; **3**:101-110.
6. Liu BC, Ehrlich JR: Proteomics approaches to urologic diseases. Expert Rev Proteomics 2006; **3**:283-296.
7. Van QN, Klose JR, Lucas DA, et al: The use of urine proteomic and metabonomic patterns for the diagnosis of interstitial cystitis and bacterial cystitis. Dis Markers 2003-2004; **19**:169-183.
8. Caiazzo Jr RJ: Identification of Autoantibodies As Biomarkers of IC Using the "Reverse Capture" Autoantibody Microarray. Abstract in 2006 NIDDK International Symposium: Frontiers in Painful Bladder Syndrome and Interstitial Cystitis.