561

Korrect G[1], Blalock E[1], Stromberg A[1], Erickson D[1]
1. *University of Kentucky*

GENE EXPRESSION ANALYSIS OF URINE SEDIMENT: A NON-INVASIVE METHOD TO SAMPLE THE UROTHELIUM IN PAINFUL BLADDER/BLADDER PAIN SYNDROME?

Hypothesis / aims of study

A large amount of research has focused on bladder biopsies from patients with painful bladder/bladder pain syndrome (PBS). Abnormalities have been shown by various methods such as light and electron microscopy, immunohistochemical staining and gene expression analysis, including two comprehensive studies of PBS patients with [1] or without [2] Hunner lesions (PBS-HL or PBS-nonHL, respectively). While some controversies remain, two themes are generally accepted. First, bladder inflammation is severe in PBS-HL. Second, urothelial differentiation is abnormal in PBS-nonHL (and perhaps also in PBS-HL).

Bladder biopsies directly show the pathology and could provide useful biomarkers, but the disadvantages are invasiveness, risk, expense and need for anesthesia. The urine sediment contains shed urothelial cells, so it may provide a non-invasive way to sample the urothelium. The main barrier to date has been the small number of cells present in the sediment.

Recent technologic advances allow gene expression analysis, including microarray, with very small amounts of RNA. In this study we extracted RNA from urine sediments and performed comprehensive exon arrays. Our aim was to identify genes that would be good candidates for development as noninvasive biomarkers. Our hypotheses were (1) expression of pro-inflammatory genes would be higher in PBS-HL compared to controls and to PBS-nonHL, and (2) expression of urothelial differentiation genes (e.g. uroplakins, tight junction proteins) would be higher in controls, compared to PBS-nonHL.

Study design, materials and methods

Urine was obtained though a catheter or cystoscope used for the patient's usual care, including bladder instillation or diagnostic or therapeutic cystoscopy. This process avoided contamination with vaginal cells in women. All subjects were Caucasian. All PBS patients had active symptoms at the time of urine collection, met the International Continence Society definition for PBS and had other disorders ruled out by history, physical exam, urinalysis, urine culture and cystoscopy.

PBS-nonHL patients were five women of mean age 40.2 (range 20-60). Three were taking no PBS medicines, one was on pentosanpolysulfate and one was on pentosanpolysulfate, amitriptyline and phenazopyridine. PBS-HL patients included two women and one man age range 55-82, who were undergoing fulguration of HL. Two were taking no PBS medicines and one was on hydroxyzine. Controls were four women and one man, mean age 57.2, range 38-75, undergoing treatment for non-PBS disorders including proximal ureteral stone, stress incontinence or benign vaginal lesions. Urine (40-100 ml) was immediately placed on ice, taken to the laboratory and centrifuged at -4C for 5 minutes. Pellets were washed twice with ice-cold phosphate-buffered saline, suspended in 0.8 ml TRIzol and RNA was extracted according to the manufacturer's instructions. RNA was stored at -80C until all samples were collected, then amplified at our Microarray Core Facility using the Affymetrix GeneChip Whole Transcript (WT) Sense Target Labeling Assay protocol and added to Human Exon 1.0 ST array chips. Results were analyzed using the Exon array analysis program in Affymetrix Expression consol (Affymetrix, Santa Clara, CA).

Statistical analysis: After initial filtering of 33,297 probe sets, there remained 19,354 genes that were unique, annotated and present. The three groups (control, PBS-HL, PBS-nonHL) were compared using one-way ANOVA and using a pattern analysis algorithm we previously published [3].

Results

On pattern analysis, control and PBS-nonHL were similar to each other, while PBS-HL was different. We were surprised to see that control and PBS-nonHL were similar, because prior studies of PBS bladder biopsies showed impaired urothelial differentiation. To re-examine this unexpected result, we considered the relevant genes individually. Indeed, the p values (PBS-nonHL vs. control) were > 0.05 for *OCLN*, all uroplakins, most claudins and most cadherins/catenins. Of the few relevant genes with $p < 0.05$ for PBS-nonHL vs. control, most were slightly increased in PBS-nonHL (fold changes 1.64 to 2.22, p values 0.0042, 0.012, 0.041, 0.047, 0.0018, 0.034, 0.0134 and 0.0048 for *TJP1, TJP2, TJP3, CLDN1, CLDN7, CLDN 23, CTNNA1* and *PCDH1*, respectively). The increase was also unexpected, but in a large microarray these may have been false discoveries.

After confirming that control and PBS-nonHL really were similar, additional pairwise comparisons were made for PBS-HL vs. (control + PBS-nonHL). To keep the false discovery rate low at 0.15, a p value of 0.0025 was chosen.

Thirty-nine gene were significantly upregulated at least 2-fold (fold changes 2.01-6.14) for PBS-HL vs. (control + PBS-nonHL). Their functions are shown in the table.

GENES SIGNIFICANTLY UP-REGULATED AT LEAST 2-FOLD PBS-HL vs. (PBS-nonHL + CONTROL)	
Immune Response/	*BTN3A1,*‡ BTN3A2,* BTN3A3,*‡ CD4, CD180,‡ CYSLTR1,‡ DTX3L, GBP4, GIMAP8,‡ HLA-F,*‡ JAK2*, OAS3, PSMB8*, PSMB9,‡ PSMB10, PSME2,‡*

Inflammation	*NCKAP1L,‡ SAMHD1,‡ SP100, STAT1*, STAT2,*‡ TAP1,‡ TRAFD1, TRIM22**
Cell Cycle Regulation	*APOL6, DDX60, NUB1, PARP14,‡ PML, SAMD9L*‡*
Miscellaneous or unknown	*APOL2, IFI35*, IFI44L*, KIAA1618,‡ MPEG1,‡ N4BP2L1, NLRC5, RNF213, WDFY1‡*
* Interferon-induced	
‡Also upregulated at least 2-fold in PBS-HL vs. control, reference 1.	

Interpretation of results

Similarity of control and PBS-nonHL: Photographs of PBS bladder biopsies often show areas of absent superficial urothelium. This absence may reflect a tendency of PBS urothelial cells to shed prematurely. If this process is occurring, loss of superficial cells would cause decreased expression of differentiation markers in the cells that remain on the biopsy. However, once they are shed, the urothelial cells may not be different for PBS-nonHL and controls.

Increased inflammation in PBS-HL: These results are consistent with prior studies showing increased bladder inflammation in PBS-HL compared to controls or to PBS-nonHL. However, due to the small number of PBS-HL patients in the study, these results are preliminary and require further study with additional subjects.

Concluding message

Gene expression analysis of urine sediment is feasible. The results are similar for PBS-nonHL vs. controls. This method is unlikely to provide a noninvsive biomarker for PBS-nonHL. In contrast, PBS-HL patients had increased expression of pro-inflammatory genes, compared to controls and to PBS-nonHL. If confirmed with larger numbers of patients and controls, gene expression analyses may be developed to provide noninvasive biomarkers of PBS-HL.

References

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