486

Benson K[1]
1. *Sanford Clinic Urogynecology and Female Pelvic Medicine*

DIFFERENTIAL GENE EXPRESSION IN CELLS SLOUGHED IN THE URINE IN INTERSTITIAL CYSTITIS SUBJECTS

Hypothesis / aims of study

We hypothesize that subjects affected with Interstitial Cystitis show differential gene expression in cells sloughed in the urine compared to unaffected controls.
Study Aim #1 To determine if RNA is extractable and quantifiable in cells sloughed in the urine
Study Aim #2 To determine if RNA extracted from cells found in the urine is stable at various temperatures when suspended in TRI reagent
Study Aim #3 To determine if differential gene expression is seen in cells sloughed in the urine of Interstitial Cystitis subjects compared to unaffected controls

Study design, materials and methods

Longitudinal prospective cohort control matched study of 28 total subjects. 18 subjects with diagnosis of Interstitial Cystitis based on ICS criteria compared to 10 control subjects seeking care for management of stress urinary incontinence. Catheterized urine samples collected before cystoscopic evaluation. Urine samples spun down and cellular pellets suspended in TRI reagent. 8 test subjects' cellular pellets examined with Real time RT-PCR evaluation for quantification of RNA, Evaluation of "housekeeper" genes actin and GADPH were used for analysis. After confirmation of extractable, intact RNA was determined it was followed by DNA microarray analysis of a total of 20 subjects (10 test and 10 controls).

Results

Our study reveals with real time RT-PCR evaluation that viable RNA is indeed recoverable from cells sloughed in the urine when suspended in TRI reagent. Additionally we have shown RNA stability when suspended in TRI reagent across a variety of temperatures from -80°C to 27°C. DNA microarray analysis demonstrated a total of 21 named genes expressed differentially with greater than a 2 fold difference.

Overexpressed Genes

The most over expressed gene was Homo Sapiens A kinase (PRKA) anchor protein 6 (AKAP6). AKAP6 was over expressed 4.69 fold. This gene is involved in signal transduction. AKAP6 is a member of a larger group of proteins that has a function of binding to the regulatory subunit of protein kinase A (PKA) and confining the holoenzyme to discrete locations within the cell. This gene encodes a member of the AKAP family. The encoded protein is highly expressed in various brain regions and cardiac and skeletal muscle. It is specifically localized to the sarcoplasmic reticulum and nuclear membrane, and is involved in anchoring PKA to the nuclear membrane or sarcoplasmic reticulum. The second most over expressed gene was Engrailed homeobox 2 (EN2). EN2 was over expressed 3.78 fold compared to controls. EN2 is involved in signal transcription. EN2 may be involved in developmental processes. The third most over expressed gene was Histone Cluster 1 (HIST1H4F). Hist1H4F was over expressed 3.13 fold compared to controls. Histones are basic nuclear proteins that are responsible for the nucleosome structure of the chromosomal fiber. Two molecules of each of the four core histones (H2A, H2B, H3, and H4) form an octomer, around which 146 base pairs of DNA is wrapped in repeating units, termed nucleosomes. This gene is found as part of a larger histone cluster on chromosome 6. The fourth most over expressed gene was Integrin, Alpha 9 (ITGA9). ITGA9 was over expressed 3.07 over controls. Integrins are integral membrane glycoproteins that mediate cell-cell and cell-matrix adhesion This is consistent with previously published articles. The fifth leading over expressed gene is Calcium binding protein G (S100G). This gene is over expressed 2.7 fold compared to controls. S100G encodes for calbindin D9k a vitamin D-depenent calcium-binding protein. S100G belongs to a family of calcium-binding proteins that include calmodulin, parvalbumin, troponin-C and S100 protein. .

Under expressed genes

The five most under expressed genes were varied in functional groupings. The groups included proteases and regulators, vesicle transport, signal transduction, channels and transporters, and transcriptional regulation. No clear unifying pattern of under regulation was seen within our test group compared to controls. To our awareness there is no published data relating the underexpressed genes to the urinary tract or IC. The most under expressed gene was Plasminogen-like B1(PLGLB1) with 0.09-fold expression. PLGL-B1 plays a role in signaling apolipoproteins. The second most underexpressed gene was Huntingtin Interacting Protein-1-Related (HIP1R) with a 0.2 fold expression. This gene is involved in vesicle transport and endocytosis. The third most under expressed gene was Colony Stimulating Factor 1 (macrophage) (CSF1). CSF1 had a 0.26 fold expression. CSF1 is involved in signal transduction and may play a role in the health of the bladder. The fourth least expressed gene was Solute carrier family 2 (facilitated glucose transporter). The gene fold expression was 0.28 fold. This gene is involved as a channel transporter. The fifth least expressed gene was Zinc finger, MYM domain containing 1 (ZMYM1). ZMYM1 exhibited 0.28 fold expression compared to controls. ZMYM1 expression has been linked to the development of the urogenital tract.

Interpretation of results

Several new findings were revealed with this study. The first significant finding is that RNA is recoverable and quantifiable from cells sloughed into urine specimens. To our knowledge this was previously unknown. Second, cellular RNA of cells shed in the urine is stable at a variety of temperatures when suspended in TRI reagent. This has also not been demonstrated previously. Thirdly a large number of genes are differentially expressed in cells sloughed in the urine of Interstitial Cystitis subjects;

however the absolute differences are not dramatic when compared to controls. This suggests that, while cellular RNA is present, it may represent the fact that cellular machinery is dismantling in uroepithelial cells as they are shed into the bladder lumen and thus may not be reflective of the actual disease process occurring within the bladder at the time of shedding.

Concluding message

Our study confirms that RNA may be extracted from cells shed in the urine of IC subjects. We have also shown that cellular RNA from shed bladder cells is stable, when suspended in TRI reagent, across a wide variety of temperatures. This finding will make it potentially easier to collect and store specimens. We have also shown differential gene expression in IC patients sloughed uroepithelial cells. The differences in gene expression are not dramatic as seen in other studies of cultured IC bladder cells. This may represent a different aspect of the disease and warrants further investigation. Analysis of cells in the urine may play a defining role in identifying IC patients in the future.

Specify source of funding or grant	**University of South Dakota School of Medicine**
Is this a clinical trial?	**Yes**
Is this study registered in a public clinical trials registry?	**No**
Is this a Randomised Controlled Trial (RCT)?	**No**
What were the subjects in the study?	**HUMAN**
Was this study approved by an ethics committee?	**Yes**
Specify Name of Ethics Committee	**University of South Dakota Institutional Review Board** **Sanford Health Institutional Review Board**
Was the Declaration of Helsinki followed?	**Yes**
Was informed consent obtained from the patients?	**Yes**