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A PILOT STUDY TO ESTABLISH THE FEASIBILITY OF GENE EXPRESSION MICROARRAY ANALYSIS OF INTERSTITIAL CYSTITIS.

Background

Interstitial cystitis (IC) is a bladder disorder characterized by urinary frequency, urgency, and pelvic pain. Glycosaminoglycan layer dysfunction and neurogenic inflammation are believed to play a major role in the pathophysiology of the disease, but the aetiology remains essentially unknown.

Aim of Study

To establish the feasibility of studying gene expression differences between interstitial cystitis and normal bladder.

Methods

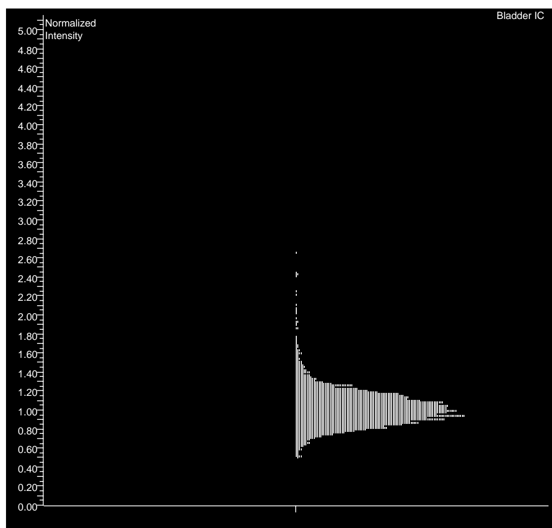
Six women who met the NIDDK criteria for IC underwent cystoscopy, hydrodistension, and bladder biopsy. Control tissue was obtained from 5 women undergoing routine cystoscopic assessment following stress incontinence or prolapse surgery with no evidence of sensory urgency or detrusor overactivity on preoperative urodynamic evaluation. Biopsies were immediately placed on dry ice, and stored at –80°C until total RNA extraction with TRIZOL reagent. RNA underwent clean-up by Qiagen RNeasy columns, and was only used if OD 260 / 280 was 1.9 – 2.0. From 30µg total RNA each from 5 IC bladders and 3 normal bladders, labeled cDNA probes were created with direct labeling by Cy3 and Cy5 labelled dCTP (normal bladder and interstitial cystitis bladder respectively). Labelled cDNA was mixed under a coverslip on a glass 5K cDNA microarray (Peter MacCallum Cancer Institute, Melbourne, Australia). After hybridization overnight at 42°C, the slide was scanned with a ScannArray 5000 UV scanner. The relative ratio of cDNA for IC versus normal bladder for 5000 genes was determined using Quantarray software. Results were normalized and visualized using Genespring (bioinformatics software, Silicon Genetics, USA) to determine genes up and down-regulated in IC bladder relative to control.

Results

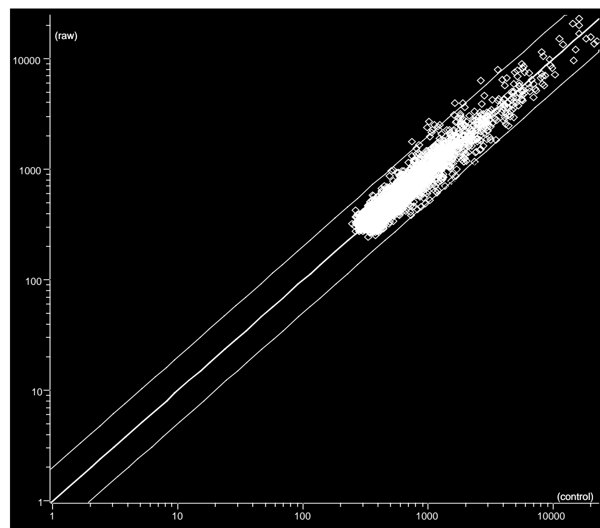
The average single biopsy weighed 15-25 mg, although the contribution of blood and mucus to the biopsy was variable and actual amount of bladder mucosa often difficult to determine. By the method described, an average of 3-5 µg total RNA was extracted per single biopsy. Between 6-10 biopsies were required for enough RNA for labeled cDNA probe creation for either IC or control. The single array performed to date was successful (Figure 1), and after normalization revealed a number of upregulated genes involved in the inflammatory process.

Figure 1: A) Histogram of gene expression ratios of IC vs normal bladder for 5000 genes on the microarray. B) scatterplot of gene expression in IC vs normal bladder controls with genes overexpressed in IC bladder biopsies compared with normal bladder biopsies by greater than a factor of 2 above the upper line, and genes underexpressed by a factor of 2 or more in IC specimens below the lower line.

A



B



Conclusions

It is possible to generate gene expression microarrays utilizing cystoscopic bladder biopsies. Confirmation of differentially expressed genes (from one microarray only at this stage) is underway. Several issues were identified in this pilot study:

1. The identification of an optimal control bladder is an issue, as patients undergoing cystoscopy are unlikely to be 'normal'.

2. Since IC is relatively uncommon, biopsies are hard to obtain. Also, a bladder cold-cup forceps biopsy is very small, generating only 3-5 µg total RNA. Pooling samples is unlikely to yield statistically significant results by gene expression microarray unless an alternative to direct cDNA labeling is used (such as linear amplification using a bacteriophage promoter). This would enable arrays to be performed from as little as 2-3 µg total RNA.

4. Whether gene expression changes will reveal clues to the aetiology of the disease, or merely reflect different composition of mucosa from IC and normal bladders, awaits confirmation.

Our initial results are promising, showing that gene expression microarrays from small biopsies may be used to uncover new genes with a role in the pathogenesis of interstitial cystitis.