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# GENE MICRO-ARRAY STUDIES OF BLADDER FROM WOMEN WITH INTERSTITIAL CYSTITIS

Hypothesis / aims of study

The aim of this study was to use gene micro-array techniques to identify genes with increased or reduced RNA expression in bladder biopsies taken from women diagnosed with interstitial cystitis (IC) compared to normal controls.

Study design, materials and methods

Bladder biopsies were collected by cold cup forceps biopsy from female controls undergoing stress incontinence or prolapse surgery and from women diagnosed as having interstitial cystitis based on the National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases criteria. Institutional ethics approval was obtained from Southern Health Human Research Ethics Committee C. Initially all biopsies were immediately frozen on dry ice prior to routine RNA extraction, however, subsequent evaluation of RNA quality using the Agilent Bioanalyser identified significant degradation of IC but not control samples. Subsequent to this all biopsies were collected into RNAlater (Qiagen, Germany) which resulted in improved RNA quality. Total RNA was extracted using TRIZOL™ reagent (Invitrogen, USA), followed by RNeasy micro columns with DNase treatment (Qiagen). Resulting RNA was amplified with MessageAmp™ II aRNA kit (Ambion, USA) and quality was checked using Agilent Bioanalyser. Both IC (N=4) and control (N=3) were hybridized on 8K human cDNA microarray slides purchased from Australian Genome Research Facility against common reference RNA (prepared from bladder and uterine tissues). Reference RNA was amplified in the same amplification run as experimental sample. The microarray slides were scanned using a GenePix 4000B UV laser scanner and the data were extracted using GenePixPro 5.0 software. Data were imported into Gene Spring (Silicon Genetics, USA) version 6.0 and statistical analysis was performed using default settings in this software. A control experiment was performed using normal bladder RNA amplified on 2 separate occasions arrayed against itself to ensure gene expression profiles were not altered by the amplification process.

Results

Unsupervised hierarchical clustering of gene expression profiles from control and IC biopsies revealed a high degree of similarity between the 2 groups. This similarity was confirmed when only 8 down-regulated and 4 up-regulated genes were identified in IC bladder using GeneSpring parametric ANOVA with all available error estimates. These results require confirmation by quantitative real-time PCR. We have also generated a much larger list of genes that are differentially expressed in bladder compared to uterus, many of which have relatively high RNA expression levels.

Interpretation of results

The lack of statistical difference in gene expression profiles between bladder biopsies from women with IC versus controls is probably due to the small numbers of subjects in the study to date, as well as the variability in gene expression between subjects diagnosed as having IC. Increasing the size of the study will help to overcome this problem. A surprising finding of this study was the poor quality of RNA collected from women with IC, and in particular from those with severe IC. The cause of this problem appears to be IC specific, since control biopsies treated in exactly the same manner produced high quality RNA. Identification of a large group of “bladder specific genes” (at least when compared to uterus) will help to provide insights into molecular mechanisms governing bladder structure and function.

Concluding message

Gene expression profiling of small human IC bladder biopsy samples presents significant technical challenges. Results obtained by gene array must be validated independently by quantitative RT-PCR before further work is undertaken.