PROTEOMICS APPROACHES TO INTERSTITIAL CYSTITIS.

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INTRODUCTION AND OBJECTIVE: Our overall goal is to identify potential disease-associated proteins in urine specimens from patients with interstitial cystitis (IC). The technical challenge to analysis of urinary proteome is that the proteins are present at unequal concentrations. A few are so dominant that they mask detection of other proteins. Because of these high abundance proteins, current technologies cannot analyze the lower abundance proteins that may be the next biomarkers and/or drug targets. To facilitate the identification of low abundance proteins, we devised a strategy for fractionating urine samples from patients with IC and from normal controls using reversed-phase high-performance liquid chromatography (RP-HPLC). When followed by two-dimensional difference gel electrophoresis (2-D DIGE), this strategy facilitates the visualization of low abundance proteins and improved the resolution of the urine proteome.
METHODS: RP-HPLC was performed with the Beckman-Coulter ProteomeLab PF 2D multi-dimensional liquid chromatography system. A linear gradient elution was performed using buffer A (0.1% TFA in water) and buffer B (0.08% TFA in acetonitrile). Fractions were collected automatically every minute. Fractionated proteins were labeled with fluorescence dyes Cy3 or Cy5, mixed and separated by 2-D gel electrophoresis. Differentially expressed proteins were quantitatively analyzed using the DeCyder analysis software. Spots showing over 3-fold differences were picked for further LC-MS/MS on-line sequence analysis and database searching.
RESULTS: Results demonstrate improved resolution of urinary proteins on 2-D DIGE following fractionation. Several proteins that were differentially expressed in urine specimens from patients with IC were identified by mass spectrometry to be involved with inflammation. These proteins include various cell surface adhesion molecules, bradykinin, prostaglandin D2, and beta-2-glycoprotein I. Of interest is that beta-2-glycoprotein I may bind to endothelial cell surface and leads to endothelial cell activation, which is manifested by upregulation of cell surface adhesion molecules and increased secretion of IL-6 and prostaglandins. The expression of these proteins may contribute to the clinical symptoms found in IC.
CONCLUSIONS: Our results demonstrate that the use of proteomics approaches to IC, coupled with reducing the complexity of the urine proteome, may facilitate the identification of low abundance proteins and add to our ability to find biomarkers that may be useful for drug discovery and/or diagnostic tools.

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