IDENTIFICATION OF NUCLEAR PROTEINS IN THE CHRONIC CYSTITIC RAT MODEL

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INTRODUCTION AND OBJECTIVE: Interstitial cystitis (IC) is a chronic inflammatory disease of the bladder characterized by bladder pain and urinary frequency and urgency. Diagnosis of IC is primarily based on symptoms, as there are no currently available blood or urine tests due to the lack of demonstrated biological markers. Therefore, we propose to use a proteomic approach to identify specific new markers related to chronic cystitis in two rat IC models.
METHODS: Two rat models of chronic cystitis were utilized: a) A one time intravesical instillation of 0.2 ml 0.4N Hydrochloric Acid (HCl) and b) Intraperitoneal injection of 75 mg/kg cyclophosphamide (CYP) every 3rd day for 14 days. The NE-PER Kit (Pierce Biotechnology) was used to isolate both the nuclear and cytoplasmic extracts from each bladder tissue and then run on the Protean® IEF System with immobilized pH gradients (BioRad) for 2-Dimensional (2D) SDS-PAGE gel analyses. After electrophoresis, the gels were initially stained with Silver Stain Plus (BioRad) and analyzed. Differential protein spots were compared between the normal and each IC model. Once the proteins of interest were identified, samples were repeated on the 2D gel system and subsequently stained with Sypro Ruby (Invitrogen). Gel were placed in a gel imaging device with an integral gel cutting tool and imaged at two excitation wavelengths (545±10 nm for Cy3 and 635±15 nm for Cy5) using a cooled CCD camera with a 16-bit CCD chip. Gel plugs were excised using an automated picker into a microtiter plate containing 1% acetic acid. Proteins in the gel plugs were then identified by peptide mass fingerprinting (PMF).
RESULTS: The silver stained gels were compared between the normal versus diseased states. First, we found little difference between the normal with the CYP-induced tissues. However, many differences were seen between the normal and HCl tissues. We found that 15 nuclear proteins were differentially expressed compared to the normal rat bladders. Among these fifteen, six nuclear proteins were down-regulated and nine proteins were up-regulated in these samples. Through PMF, one down-regulated protein was identified as SM-22 (transgelin), a 22-kD protein.
CONCLUSIONS: The identification of new markers for IC would be valuable in the accurate diagnosis and early prediction of the disease. These results could also identify new molecular targets for drug therapy. Using 2D SDS-PAGE analysis we have identified three nuclear proteins that are unique in chronic cystitis rat model.

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