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Impact of uremic environment on peritoneum: A proteomic view

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ABSTRACT

Peritoneal morphology and function are abnormal in uremia patients, but the contributing mechanisms are unclear. Here we attempted to characterize the protein targets that may be related to peritoneal change in patients with uremia and have not exposed to peritoneal dialysis fluid. Protein profiles of peritoneal fluids collected from patients with uremia and patients with normal renal function receiving laparoscopic cholecystectomy were displayed by two-dimensional gel electrophoresis (2-DE). Altered protein spots were excised and subjected to tryptic digestion followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis. Sixteen 2-DE protein spots were altered between two groups. Western blots confirmed that kininogen-1, apoptosis inhibitor 2, cat eye syndrome critical region protein 1, and apolipoprotein A-I had higher expression levels in the uremia samples. In contrast, synaptic vesicle 2-related protein, glial fibrillary acidic protein, and envelope glycoprotein (C2–V5 region) showed lower levels. The increased expression may result from a change in the permeability of the peritoneal membrane to middle-sized proteins or peritoneal inflammation with proteins sloughing off. All the identified proteins may provide a novel understanding of peritoneal changes caused by uremic toxins and may function as biomarkers or drug targets.

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1. Introduction

Uremic toxins affect the peritoneal physiology and pathophysiology in a variety of ways, including increasing oxidative [1,2] and carbonyl stress [2–4] and changing the microvascular structure. Uremia may produce an environment that is more conducive to peritoneal adhesion or encapsulating peritoneal sclerosis formation [5]. It is known that peritoneal dialysis treatment has a great impact on the progress of peritoneal change. Evidences show that uremia itself can cause the peritoneum to undergo structural and morphological changes, including angiogenesis, fibrosis, and sclerosis in patients before peritoneal dialysis [6–8]. Some proteins were found to play roles in this process. Vascular endothelial growth factor (VEGF) and basic fibroblast growth factor that were involved in structural modifications of the remnant kidney [9], heart [10], and peritoneum [11] caused by experimental uremia. Thus, characterization of the differential expression of proteins in uremic peritoneal fluids can improve the basic biological understanding and predict clinical outcomes.

In recent years, proteomic analyses of biological samples have drawn great interest and provided a wealth of information. Proteomic tools, such as two-dimensional gel electrophoresis (2-DE) and mass spectrometry, have been widely applied in the study of body fluids, including cerebrospinal fluid [12], pleural and pericardial effusions [13–15], urine [16–18], and peritoneal dialysate [19–21]. For peritoneal fluid analysis, previous studies focus on proteome changes in endometriosis and ovarian cancer. Ferrero et al. used 2-DE to display the proteins in peritoneal fluids collected from patients with mild or severe endometriosis. Compared to controls, they found that the decreased level of vitamin D binding protein isoform may be relevant in the pathogenesis of endometriosis [22]. Ferrero et al. conducted another proteomic analysis of peritoneal fluid in women with endometriosis was designed to find other specific proteins. Several proteins had aberrant expression between groups and were related to immunological or inflammatory processes, providing better understanding of the pathogenesis of this disease [23]. They continued to compare the expression of proteome in peritoneal fluids in women with different severity of endometriosis by using 2-DE followed by LC-MS/MS analysis [24]. In 2009, by analyzing the peritoneal fluids from fertile and infertile women with endometriosis, several proteins were found to have aberrant expression in those with infertility and are involved in immune response [25]. Another work suggested that the administration of gonadotropin-releasing hormone analog (GnRH-a) may reduce the inflammation in the peritoneal cavity by determining the changes in the peritoneal fluid proteome of women with endometriosis and under GnRH-a treatment [26]. Differential protein expression in peritoneal fluid of patients with ovarian or peritoneal endometriosis was used to reveal the local dysregulation potentially involved in the pathogenesis [27]. Recently, peritoneal fluid was used for identifying potential diagnostic biomarkers for ovarian cancer. Amon et al. used label-free mass spectrometry to compare peritoneal fluid taken from women with ovarian cancer and those with benign tumor. Quantitative proteomic analysis with isotopic labeling was applied on serum samples. Integrative data suggested high quality biomarker candidates for ovarian cancer [28]. Honore et al. subjected peritoneal rinse fluid sediment from ovarian cancer patients and those admitted for cesarean section to 2-DE and mass spectrometry analysis. Nine proteins spots resulting in 4 proteins were considered as potential biomarkers for ovarian cancer [29]. Here, we did a first comparative proteomic analysis of uremic and normal peritoneal fluids.

2. Material and methods

2.1. Patient sample preparation

This study recruited six patients with uremia (U1-6) (i.e. chronic kidney disease, stage 5). All six patients did not receive maintenance dialysis treatment. The control group was composed of six patients with normal renal function (C1-6). All six patients were admitted for symptomatic gall bladder stones. The peritoneal fluids were collected during operation of peritoneal dialysis catheter insertion for the uremia group and elective laparoscopic cholecystectomy for the control group. The sample collections were approved by the Institutional Research Board and carried out according to the principles of the Helsinki Declaration. Written informed consent was collected from all participants. U1-3 and C1-3 samples were used for 2-DE analysis while U4-6 and C4-6 samples serve as additional set of sample to validate the selected proteins candidates. Each sample was centrifuged at $1000\times g$ at 4 °C for 10 min. The supernatants were stored at −20 °C before use. A small aliquot of each sample was used for a protein concentration assay.

2.2. Protein precipitation

Peritoneal fluids from the three patients with uremia, each containing 40 μg of protein, were pooled and served as the uremia sample. Peritoneal fluids from the three control patients, each containing 40 μg of protein, were pooled and served as the control sample. Each sample was mixed with trichloroacetic acid in cold acetone containing 0.1% dithiothreitol (1:9) and stored in a freezer overnight. Samples were next centrifuged at 8500 rpm for 30 min. Pellets were washed three times with acetone (−20°C) containing 0.1% dithiothreitol, and air dried. Each pellet was dissolved in a rehydration solution (7 M urea, 2 M thiourea, 4% CHAPS, 2% ampholytes, 120 mM dithiothreitol) and carefully sonicated for 60 min.

2.3. Two-dimensional gel electrophoresis (2-DE)

The uremia and control samples (U1-3 and C1-3) were loaded onto immobilized pH gradient (IPG) gel strips for the first dimensional gel electrophoresis. To minimize evaporation and urea crystallization, IPG cover oil (0.8 mL) was applied on top of each gel strip. Isoelectric focusing (IEF) was run following a stepwise incremental voltage program on an IPGphor™ Isoelectric Focusing System (GE Healthcare Life Sciences, Taipei, Taiwan). The program was: 30 V for 16 h, 500 V for 1 h, 500 V for 1 h, and 8000 V for 4 h. After IEF, the strips were subjected to two-step equilibration. The first step was performed in equilibration buffer with 1% dithiothreitol w/v. The second step was performed in 2.5% w/v iodoacetamide. For the second dimensional