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ScienceDirect
Clinical Biochemistry 43 (2010) 142–149
CLINICAL BIOCHEMISTRY

Plasma proteome profiling of von Hippel-Lindau disease after total and subtotal nephrectomy: A preliminary study

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Received 9 June 2009; received in revised form 5 August 2009; accepted 15 August 2009
Available online 26 August 2009

Abstract

Objectives: Common treatment of renal cell carcinoma associated with von Hippel-Lindau (VHL) disease is total (bilateral) or subtotal nephrectomy. Whereas total nephrectomy is associated with absolutely no residual renal function, subtotal nephrectomy frequently leads to chronic kidney disease (CKD) with some residual renal functions. However, molecular mechanisms underlying CKD remain unclear and the diagnosis of CKD is frequently accomplished at its late stage.

Design and methods: We performed a plasma proteomics study to compare the plasma proteome profile of VHL patient who underwent total nephrectomy to the profiles of VHL patient with subtotal nephrectomy and healthy control. Totally 100 μg proteins from each sample was resolved by two-dimensional electrophoresis (2-DE) in triplicate and visualized with SYPRO Ruby fluorescence stain.

Results: The normal plasma proteome profile markedly differed from the profiles of VHL patients. Comparative analysis between total versus subtotal nephrectomized patients revealed significant differences in levels of 20 plasma proteins. Pathway analysis revealed two important networks involving in lipid metabolism, molecular transport, carbohydrate metabolism, cellular growth and proliferation, and small molecule biochemistry, in which these identified and other proteins interplayed.

Conclusions: Our data identified potential biomarkers for CKD. Further characterization of these identified proteins might also lead to better understanding of molecular mechanisms underlying CKD.

Keywords: Chronic kidney disease; Nephrectomy; Plasma; Proteome; Proteomics; Renal cell carcinoma; Renal failure; von Hippel-Lindau

Introduction

von Hippel-Lindau (VHL) disease is an autosomal dominant hereditary disorder characterized by the presence of benign and/or malignant tumors in various organs [1]. This genetic disorder is caused by a germline mutation of the *VHL* tumor suppressor gene on the short arm of chromosome 3 [2–4]. VHL-associated tumors include hemangioblastoma of central nervous system, retinal angioma, endolymphatic sac tumor of middle ear, serous cystadenoma and neuroendocrine tumors of pancreas, papillary cystadenoma of epididymis and broad ligament, and pheochromocytoma. In addition, the kidney is invaded by cysts and multifocal or bilateral renal cell carcinoma (RCC; clear cell type), which is the leading cause of death of VHL patients [3,5]. Because VHL is a relatively rare disease [1], diagnosis of *de novo* cases is a particular challenge. The early diagnosis and timely surgical management of RCC at an early stage prior to metastasis is a life-saving procedure.

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doi:10.1016/j.clinbiochem.2009.08.010

Surgical removal of VHL-associated RCC usually involves total (bilateral) or subtotal nephrectomy. Whereas total nephrectomy results to no residual renal function, subtotal nephrectomy is frequently associated with chronic kidney disease (CKD) with some residual functions. CKD, however, will lead to end-stage renal disease (ESRD) with reduced or no residual function, which ultimately requires renal replacement therapy (i.e. chronic hemodialysis) and renal transplantation. Therefore, subtotal nephrectomy is widely used in animal models for the investigation of CKD [6–8]. However, molecular mechanisms underlying CKD remain unclear and the diagnosis of CKD is frequently accomplished at its late stage. Recently, proteomics has been introduced as an emerging technology to study a large number of proteins simultaneously [9]. Applications of proteomics to medicine would lead to better understanding of pathogenic mechanisms and pathophysiology of diseases and its strength has been already proven in many clinical settings [9–11].

In the present study, we performed plasma proteome profiling to compare the plasma proteome profiles of VHL patient who underwent total nephrectomy versus VHL patient who underwent subtotal nephrectomy and normal healthy subject. The information obtained from this study might lead to better understanding in the pathogenic mechanisms and/or pathophysiology of CKD and ESRD, and also to biomarker discovery of deteriorated renal function.

Materials and methods

Subjects and samples

A 37-year-old woman and 78-year-old man who were daughter and father, respectively, and had multiple cysts in both kidneys, were enrolled in this study. In addition, a 28-year-old healthy male subject was enrolled as a normal control. The study was in accordance with the ethical standards as formulated in the Helsinki Declaration of 1975 (revised in 1983). Both patients had c.349T>A mutation (p.Trp117Arg) in exon 2 of the *VHL* gene. Histopathological examination revealed clear cell type renal cell carcinoma (RCC) in both patients. The female patient underwent total (bilateral) nephrectomy, whereas the male patient received left nephrectomy and right partial nephrectomy (subtotal nephrectomy). After surgical removal, the total nephrectomized patient required renal replacement therapy and is now in queue for renal transplantation, whereas the subtotal nephrectomized patient had stable residual renal function (at stage II of CKD). EDTA-preserved plasma samples were collected from normal subject and both patients at steady state (without acute-on-top illness) and subjected to proteome profiling.

Two-dimensional electrophoresis (2-DE)

Plasma samples were diluted 1:5 with 18.2 MΩ·cm (dI) water and protein concentrations in individual samples were measured using the Bradford method [12]. Protein solutions (each with 100 μg total protein) were then premixed with a rehydration buffer containing 7 M urea, 2 M thiourea, 2% CHAPS, 120 mM DTT, 40 mM Tris–base, 2% ampholytes (pH 3–10) and a trace of bromophenol blue to make the final volume of 150 μL per sample. The mixtures were rehydrated onto Immobiline™ DryStrip (linear pH gradient of 4-7, 7-cm-long; GE Healthcare, Uppsala, Sweden) at room temperature for 10–15 h. The first dimensional separation or isoelectric focusing (IEF) was performed in Ettan IPGphor II IEF System (GE Healthcare) at 20°C, using a stepwise mode to reach 9083 Vh. After completion of the IEF, the strips were first equilibrated for 15 min in an equilibration buffer containing 6 M urea, 130 mM DTT, 112 mM Tris–base, 4% SDS, 30% glycerol and 0.002% bromophenol blue, and then in another similar buffer, which replaced DTT with 135 mM iodoacetamide, for further 15 min. The second dimensional separation was performed in 12% polyacrylamide gel using SE260 Mini-Vertical Electrophoresis Unit (GE Healthcare) at 150 V for approximately 2 h. Separated proteins were visualized with SYPRO Ruby fluorescence staining (Invitrogen–Molecular Probes; Eugene, OR). Gel images were taken using a Typhoon laser scanner (GE Healthcare). The samples were analyzed in triplicate (totally 9 gels were analyzed).

Matching and analysis of protein spots

Image Master 2D Platinum (GE Healthcare) software was used for matching and analysis of protein spots in 2-D gels. Parameters used for spot detection were (i) minimal area = 10 pixels; (ii) smooth factor = 2.0; and (iii) saliency = 2.0. A reference gel was created from an artificial gel combining all of the spots presenting in different gels into one image. The reference gel was then used for matching the corresponding protein spots between gels. Background subtraction was performed and the intensity volume of each spot was normalized with total intensity volume (summation of the intensity volumes obtained from all spots within the same 2-D gel). Significant differences in intensity levels of protein spots were defined by statistical analysis.

Statistical analysis

All the data are shown as mean ± SEM. Comparisons between two groups were performed using unpaired Student's *t*-test, whereas differences among multiple groups were determined by ANOVA using SPSS software (version 13.0). P values < 0.05 were considered statistically significant.

In-gel tryptic digestion

The protein spots whose intensity levels significantly differed between groups were excised from 2-D gels, washed twice with 200 μL of 50% acetonitrile (ACN)/25 mM NH_4HCO_3 buffer (pH 8.0) at room temperature for 15 min, and then washed once with 200 μL of 100% ACN. After washing, the solvent was removed, and the gel pieces were dried by a SpeedVac concentrator (Savant; Holbrook, NY) and rehydrated with 10 μL of 1% (w/v) trypsin (Promega; Madison,