ELSEVIER

CrossMark

Urologic Oncology: Seminars and Original Investigations 34 (2016) 5.e11–5.e25

UROLOGIC ONCOLOGY

Original article

Proteomic analysis reveals differentially secreted proteins in the urine from patients with clear cell renal cell carcinoma

Vanessa Sandim, Ph.D.[a,b,c,d], Denise de Abreu Pereira, Ph.D.[a,b,c,e], Dário Eluan Kalume, Ph.D.[a,f], Ana Lucia Oliveira-Carvalho, B.Sc.[a,b], Antonio Augusto Ornellas, Ph.D.[g,h], Marcia Regina Soares, Ph.D.[i], Gilda Alves, Ph.D.[c,d,e,1,*], Russolina Benedeta Zingali, Ph.D.[a,b,**]

[a] *Unidade de Espectrometria de Massas e Proteômica (UEMP), Instituto de Bioquímica Médica Leopoldo de Meis, Universidade Federal do Rio de Janeiro - UFRJ, Rio de Janeiro, Brazil*

[b] *Instituto Nacional de Biologia Estrutural e Bioimagem (INBEB), Universidade Federal do Rio de Janeiro — UFRJ, Rio de Janeiro, Brazil*

[c] *Laboratório de Genética Aplicada, Serviço de Hematologia, Instituto Nacional de Câncer—INCA, Rio de Janeiro, Brazil*

[d] *Laboratório de Marcadores Circulantes, Departamento de Patologia, Faculdade de Ciências Médicas, Universidade do Estado do Rio de Janeiro — UERJ, Rio de Janeiro, Brazil*

[e] *Coordenação Geral de Ensino e Pesquisa, Instituto Nacional de Câncer—INCA, Rio de Janeiro, Brazil*

[f] *Laboratório Interdisciplinar de Pesquisas Médicas (LIPMED), Instituto Oswaldo Cruz — IOC, Fundação Oswaldo Cruz — FIOCRUZ, Rio de Janeiro, Brazil*

[g] *Serviço de Urologia, Instituto Nacional de Câncer — INCA, Rio de Janeiro, Brazil*

[h] *Serviço de Urologia, Hospital Mário Kroeff, Rio de Janeiro, Brazil*

[i] *Departamento de Bioquímica, Instituto de Química, Universidade Federal do Rio de Janeiro — UFRJ, Rio de Janeiro, Brazil*

Received 20 March 2015; received in revised form 4 June 2015; accepted 26 July 2015

Abstract

Objective: The aim of this study was to evaluate the differentially secreted protein profile in the urine from patients with clear cell renal cell carcinoma (ccRCC) using mass spectrometry–based methods. Urine composition can reflect kidney physiology and can be used to detect markers for renal diseases. Moreover, characterization of the secretome is likely to assist in the investigation of new drugs for biological targets and diagnose the ccRCC at an early stage.

Methods and materials: Urine samples from patients were divided according to Fuhrman degree (FI-IV), which was associated with the cellular differentiation as good prognosis (GP) and poor prognosis (PP). Healthy individuals were used as the control group (CG). We used both qualitative and quantitative mass spectrometry–based analyses that involved the following approaches: 1-dimensional gel electrophoresis combined with liquid chromatography mass spectrometry *in tandem* (1DE LC-MS/MS), in-solution digestion combined with label-free 1-dimensional LC-MS^E (1D LC-MS^E), and bidimensional gel electrophoresis combined with matrix-assisted laser desorption/ionization time of flight *in tandem* (2DE MALDI-TOF/TOF) or combined with LC-MS/MS.

Results: All the strategies allowed the identification of 354 proteins from the CG, GP, and PP groups. Qualitative experiments using 1DE LC-MS/MS analysis detected different protein profiles, and 224 proteins were identified in all groups. The label-free MS^E quantitative analysis identified 113 proteins and generated novel information on secreted protein profiles, including 49 up-secreted proteins in the urine from patients with ccRCC and 40 down-secreted proteins related to the CG. Proteins such as kininogen-1, uromodulin, apolipoprotein D, polyubiquitin, and CD59 glycoprotein were down secreted according to the groups CG > GP > PP. In contrast, apolipoprotein A, fibrinogen, and haptoglobin were up secreted in patient groups. The same expression profile observed for kininogen-1, apolipoprotein D,

This work was finnacially supported by the following agencies: Fundação Carlos Chagas Filho de Amparo a Pesquisa do Estado do Rio de Janeiro—FAPERJ APQ-1 Brazil (Grant APQ1 E-26/170.694/2007) and CNE (Grant CNE E-26/103.163/2011); Conselho Nacional de Desenvolvimento Científico e Tecnológico—CNPQ, Brazil (fellowship Grant 143576/2008-9); and Programa de Oncobiologia—FAF/ONCO III 2010 to AAO.

[1]Present address: Laboratório de Marcadores Circulantes, Departamento de Patologia, Faculdade de Ciências Médicas, Universidade do Estado do Rio de Janeiro—UERJ.

[*] Corresponding author. Tel.: + 55-21-3207-1805.

[**] Corresponding author. Tel./fax: +55-21-3938-6782.

E-mail addresses: galvesbrown@gmail.com (G. Alves), lzingali@bioqmed.ufrj.br (R.B. Zingali).

http://dx.doi.org/10.1016/j.urolonc.2015.07.016

fibrinogen, and haptoglobin was corroborated by 2DE LC-MS/MS or 2DE MALDI-TOF/TOF analyses. These 2 strategies also showed 13 differentially secreted proteins among the 3 groups.

Conclusions: The proteins kininogen-1, apolipoprotein D, fibrinogen, and haptoglobin presented similar quantitative protein profiles according to MS^E and 2DE approaches. The latter proteins were up secreted and the former ones were down-regulated. The strategies used proved to be valuable in identifying proteins that were differentially secreted in urine from patients with RCC.

Keywords: Renal cell carcinoma; Urine; Label-free MS^E; Quantitative proteomics; Secreted proteins

1. Introduction

Renal cell carcinomas (RCCs) represent approximately 85% to 90% of all renal tumors [1]. It is the seventh most common type of cancer in men and 12th most common in women, accounting for 3% of the causes of death due to cancer. Tumor characteristics such as the histopathological subtype and tumor stage and grade seem to have limited value in predicting the clinical outcomes. The most common histological subtype is clear cell RCC (ccRCC) that comprises approximately 70% of all RCCs [1]. In patients with early-stage tumors and good prognostic indicators, surgery increases the survival rate. However, patients with advanced-stage or metastatic disease and poor prognostic indicators have a low 5-years survival rate [2,3]. The tumor shows resistance to chemotherapy and radiotherapy, and this complicates the treatment [4]. Currently, the initial diagnosis of RCC is most often accomplished using noninvasive imaging techniques [5]. However, these techniques are not able to effectively detect RCC in the early stages of the disease nor are they useful as predictive diagnostic tools [6].

Accurate methods are needed not only to provide early diagnosis but also for the identification of occult metastatic disease [7]. Proteomic analyses using mass spectrometry methods have proven to be useful as powerful tools in the diagnostic, prognostic, and patient follow-up [8]. Among those techniques, 2-dimensional gel electrophoresis as well as shotgun proteomics with sample prefractionation followed by LC-MS/MS analysis have been widely used [9,10]. Recently, more sensitivity and accurate label-free mass spectrometric quantitative analysis has been introduced in urine proteomics studies [9].

Urine is an interesting biological sample to investigate kidney physiology and detection of markers for renal diseases [11]. A significant variance in the secreted levels of constitutive proteins and peptides in urine could reflect a malfunction of the kidney and could be used as a diagnostic tool. However, studies on RCC are yet limited to a small number of differentially expressed proteins. Here, we investigate the proteins in the urine from patients with ccRCC who had been classified into 2 distinct grades and compare them with healthy donors. Firstly, a qualitative approach using 1DE LC-MS/MS was performed to evaluate the urine protein profile. Then, we performed a label-free 1D LC-MS^E quantitative analysis and 2DE MALDI-TOF/TOF or 2DE LC-MS/MS. The results from these strategies allowed us to detect a set of differentially secreted proteins that could be potentially related to the progression of the disease.

2. Materials and methods

2.1. Patient selection

Patients from the National Cancer Institute were recruited between 2005 and 2010. Patients signed an informed consent that had been reviewed and approved by the National Cancer Institute Ethics Committee (CEP 38/05) and the National Committee for Research Ethics (CONEP 12536). The selected patients included persons of both sexes and older than 18 years at diagnosis and excluded those with any genetic conditions, HIV infection, mental illness, previous cancer treatment, or previous kidney diseases. The control group (CG) samples were collected from healthy donor volunteers. Urine from patients with histological-type ccRCC was analyzed and was distributed according to Fuhrman grade in the good prognosis (GP) and the poor prognosis (PP) groups. GP represents patients with Fuhrman grades I and II, whereas PP corresponds to patients with Fuhrman grades III and IV. Urine sample pools for the CG, GP, and PP patient groups consisted of 30 individuals (13 women and 17 men) and their mean age ± standard deviation was 37 ± 18.4 (range: 24–50), 56 ± 36.1 (33–86), and 52.5 ± 37.5 (27–78), respectively (Table 1).

The methodology used to analyze the ccRCC sample is schematically shown in Fig. 1, and the steps were divided into sample preparation and qualitative and quantitative analysis by mass spectrometry–based techniques.

2.2. Urine collection and storage

Samples of the second urine in the morning were collected before surgery in sterile cups containing 1 mM of phenylmethanesulfonyl fluoride (Sigma, St. Louis, MO) to inhibit proteases. The samples were centrifuged at 2000 *g* for 10 minutes at 4 °C to remove cells and debris, and the supernatants were stored at −80 °C.