Chin Med Sci J
December 2017

Vol. 32, No. 4
P. 248-252

CHINESE
MEDICAL SCIENCES
JOURNAL

ORIGINAL ARTICLE

Preliminary Application of WCX Magnetic Bead-Based Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry in Analyzing the Urine of Renal Clear Cell Carcinoma

Dexin Dong, Zhigang Ji,* Hanzhong Li, Weigang Yan, Yushi Zhang

Department of Urology, Peking Union Medical College Hospital,
Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100730, China

Key words: renal clear cell carcinoma; proteomics; magnetic beads; urine; diagnosis model

Objective To evaluate the application of weak cation exchange (WCX) magnetic bead-based Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) in detecting differentially expressed proteins in the urine of renal clear cell carcinoma (RCCC) and its value in the early diagnosis of RCCC.

Methods Eleven newly diagnosed patients (10 males and 1 female, aged 46-78, mean 63 years) of renal clear cell carcinoma by biopsy and 10 healthy volunteers (all males, aged 25-32, mean 29.7 years) were enrolled in this study. Urine samples of the RCCC patients and healthy controls were collected in the morning. Weak cation exchange (WCX) bead-based MALDI-TOF MS technique was applied in detecting differential protein peaks in the urine of RCCC. ClinProTools2.2 software was utilized to determine the characteristic proteins in the urine of RCCC patients for the predictive model of RCCC.

Results The technique identified 160 protein peaks in the urine that were different between RCCC patients and health controls; and among them, there was one peak (molecular weight of 2221.71 Da) with statistical significance (P=0.0304). With genetic algorithms and the support vector machine, we screened out 13 characteristic protein peaks for the predictive model.

Conclusions The application of WCX magnetic bead-based MALDI-TOF MS in detecting differentially expressed proteins in urine may have potential value for the early diagnosis of RCCC.

Chin Med Sci J 2017; 32(4):248-252. DOI:10.24920/J1001-9294.2017.046

Received March 17, 2017.

* Corresponding author Tel: 86-10-69152520，E-mail: jzg1129@medmail.com.cn

RENAL clear cell carcinoma (RCCC) usually does not present obvious symptoms in its early stage. Currently, we are in short of tumor markers of RCCC to make an accurate and sensitive diagnosis, and to monitor its development in clinical practice. Most tumor markers are proteins that are abnormally expressed. By means of dynamic analysis of proteins, we can detect the minor index and early symptoms of diseases. The emphasis of proteomics is to identify the proteins that are differentially expressed in cancer. Therefore, proteomics provides an ideal technology platform for screening tumor markers of renal cell carcinoma.

Protein fingerprinting is a new protein technology developed in recent years. It can capture subtle changes in protein during the process of tumor evolution, which makes early diagnosis possible.[1] Proteomics provides a novel idea and research platform for the diagnosis of renal cell carcinoma. Tested specimens of renal cell carcinoma in proteomics technology are mainly serum, urine and cell lines. In this study we used bead-based MALDI-TOF MS to identify the characteristics of protein expression in the urine of patients with renal clear cell carcinoma, in order to find the specific protein peaks that can be used for an early diagnosis and treatment of renal cell carcinoma.[2,3]

MATERIALS AND METHODS

Subjects investigated

This study has been approved by the institutional ethics review committee. From March 2010 to April 2010 in the Department of Urology, Peking Union Medical College Hospital, a total of 11 patients with newly diagnosed renal clear cell carcinoma were enrolled in the study, including 10 males and 1 female, aged from 46 to 78 years, with an average of 63.0 years old. Clinical diagnosis was established by imaging examinations such as ultrasonography, CT, or MRI. The pathological diagnosis of RCCC was confirmed by biopsy or surgery. Clinical TNM staging was as following: 3 cases of stage Ia, 7 cases of stage Ib, 1 case of stage Ⅱ, and 1 case of stage Ⅳ. Six cases had laparoscopic nephron sparing surgery, 4 cases had laparoscopic radical nephrectomy, and 1 case had targeted drug therapy of SUTENT after biopsy. The urine routine examination is completely normal.

Ten healthy volunteers were recruited as normal controls. All were males, aged from 25 to 32, with an average of 28.7 years old. They were negative in physical examination, and the routine examinations of urine were completely normal.

Urine specimen collection and preparation

We collected the second middle clean urine of every participant at 7 o’clock in the morning after fasting from solids and liquids for 9 hours. After centrifugation at a speed of 2000 rpm for 5 minutes, the supernatant was obtained and packed in Eppendorf tubes, frozen in a low-temperature refrigerator under −80℃ till use. The procedure was done within 30 minutes.

After being taken out from refrigerator, 30ul urine was put into a tube that contained magnetic bead binding buffer, magnetic beads, and magnetic beads elution buffer. They were homogeneously mixed together for at least five times, and kept at room temperature till use.

Preparation of weak cation bead

10ul bead binding buffer and 10ul beads (Bruker Daltonics Inc. USA) were mixed in a 200ul sample tube; then 5ul urine was put into the sample tube and mixed together. The sample tube was put into a magnetic bead separator, and supernatant liquid was sucked out after separation. Then added 100 ul beads in the sample tube, put into a magnetic separator, and then removed the supernatant. This procedure was repeated twice to ensure that the suspension was absorbed completely. Next, 5ul beads elution buffer was put into the sample tube, and the tube was placed in a magnetic bead separator. After the magnetic beads were fully separated from the suspension liquid, the supernatant was transferred into a 50ul sample tube. Then 5ul magnetic stabilization buffer was put into the tube and mixed for use.

Examination of standard sample

We dissolved 1ul standard sample with 10ul matrix at room temperature. 1ul mixture liquid was placed in the standard position of target Anchor chip at room temperature and in dry environment. The Anchor chip target was put into the mass spectrometer (Autoflex™, Bruker Daltonics Inc., USA), with the selection method of LP Clinprot and the acquisition range of 1000-13000Da. The composition and the mass spectrometry of the standard sample were as shown in Fig. 1.

Examination of urine samples

At room temperature in a dry environment, 1ul of sample-treated magnetic beads was placed in the sample target position of Anchor chip. American MALDI-TOF-MS mass spectrometer(Autoflex™, Bruker Daltonics Inc., USA) was applied. The Anchor chip target was placed into the mass spectrometer, with a linear mode and an acquisition range of 800-10 000 Da.

Download English Version:

https://daneshyari.com/en/article/8757130

Download Persian Version:

https://daneshyari.com/article/8757130

Daneshyari.com