Serum peptidome profiling analysis for the identification of potential biomarkers in cervical intraepithelial neoplasia patients

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ABSTRACT

Cervical intraepithelial neoplasia (CIN) is a precancerous *disease of* cervical squamous cell carcinoma. We Used Mass Spectrometry based peptidome profile study to predict the transformation of CIN1, which is the primary stage of this lesion. . Serum samples of 34 Cervical squamous cell carcinoma patients, 31 healthy controls, and 29 CIN1 samples were analyzed. Peptides were purified by WCX magnetic beads (Bioyong), and analyzed by MALDI TOF (Bruker). Raw data were analyzed by BioExplorer software (Bioyong). The results showed 14 mass peaks with significant differences. The diagnosis model is established by analyzing peptide profiles of 15 SCC patients and 20 healthy women serum, with a sensitivity of 100% and specificity of 100.00%. In validation set, the SCC diagnosis model also had good performance with a sensitivity of 80%, a specificity of 100%. In addition, this model could predict 29 CIN1 patients with accuracy of 55.17%. These results would provide a new method to predict the trend of CIN1 and take effective measures for high risk group timely.

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1. Background

Cervical intraepithelial neoplasia (CIN) is a precancerous lesion of cervical squamous cancer, including low-grade squamous intraepithelial lesions (LSILs)/cervical intraepithelial neoplasia grade 1 (CIN1), high-grade squamous intraepithelial lesions (HSILs)/cervical intraepithelial neoplasia grades 2 to 3 (CIN2-3), can occur in women of all ages, and the peak age is 25—35 years old. With the application of cervical cytology, colposcopy and thin base high-risk type HPV virus detection technology, CIN and SCC can get timely diagnosed [1,2].

Nearly all cervical carcinomas result from CIN1. For CIN1 lesions, most are transient and regress spontaneously to normal epithelium and 10%—15% of them progress to CIN2-3, which are considered the immediate precursors to cervical carcinoma [3,4]. Therefore, it's meaningful to predict the evolution of CIN1 and distinguish the lesion with a higher chance of progression toward a CIN2-3, which can provide follow-up references.

From the beginning of this century, surface enhanced laser desorption ionization time of flight mass spectrometry (SELDI-TOF-MS) and matrix assisted laser desorption ionization tandem time of flight mass spectrometry technique (MALDI-TOP-MS) have been widely used for the serum peptide pattern examination of ovarian cancer, bile duct cancer, breast cancer, rheumatoid arthritis and the establishing other diseases spectrum diagnosis model [5—7]. In the present study, we investigated differences in serum polypeptide profiles of healthy women, CIN1 and SCC patients, to explore the progression of disease and predict the evolution of CIN1.

2. Materials and methods

2.1. Patients and sample collection

Serum samples were collected from patients treated between June 2007 and December 2007, at Beijing Obstetrics and Gynecology Hospital, Capital Medical University, Beijing, China. SCC was diagnosed according to FIGO staging system(1995).

All participants were recruited as controls by reference to the

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following criteria: 1) Without any treatment before blood collection, such as chemotherapy, radiotherapy, interventional therapy and so on; 2) After biopsy, pathological section diagnosis; 3) complete Clinical, pathological data; 4) Having signed the informed consent.

A total of 35 SCC patients (ages: 25–51 years old women) and a total of 20 healthy controls, (ages: 22–55 years old women) were recruited. A total of 29 CIN1 patients (ages: 19–53 years old women) were recruited.

The serum samples were prepared according to standard protocol. Fasting blood samples were collected from subjects in the morning and allowed to clot at room temperature for .5 h, the serum were then separated by centrifugation at 2000 rpm for 20 min and stored at −80 °C after split until analysis [8].

2.2. Serum pretreatment with magnetic beads

All serum samples were subjected to fractionate by using WCX (weak cation exchange) magnetic bead kit (Bioyong Tech., Beijing, China, SPE-C kit) and magnetic bead separation device for a 96-well plate format(Bioyong Tech., Beijing, China). Samples were purified and isolated through three steps: binding, washing, and elution. Firstly, 10 μl of beads, 95 μl MB-WCX binding solution (CB) and 10 μl serum were added in a tube, mixed carefully, and then incubated for 5 min. The tube of incubated serum bound beads were then placed on a magnetic bead separation device and beads collected from the tube wall for 1 min. Then, we removed the supernatant and added 100 μl of magnetic bead washing solution (CW), and mixed thoroughly. After washing three times, we removed the supernatant, added another 10 μl of magnetic bead eluting solution (CE), and collected beads from the tube wall in the separation device for 2 min. This final sample was then stored in a −20 °C freezer.

2.3. Anchor-chip spotting and protein/peptide profiling

For the peptidomic analysis, we used a linear MALDI-TOF mass spectrometer (Microflex; Bruker Daltonics). The mass calibration was performed with the calibration mixture of peptides in a mass range of 1000–10000 Da. The system quality controls were carried out before the MS analyses, with three peptides (molecular weights of 1532.8582 Da, 2464.1989 Da, and 5729.6087 Da, Product Numbers P2613, A8346, and I6279, respectively; Sigma) used as external standard preparation where the average molecular weight deviation was within 100 ppm. After every forty-eight samples, the standard preparation was re-calibrated. A coefficient of variability less than 30% indicated that the system was within an acceptable quality. We measured 3 MALDI preparations (MALDI spots) from each magnetic bead fraction. For each MALDI spot, 400 spectra were acquired in analysis (50 laser shots at 8 different spot positions).

2.4. Screening for candidate peptides and establishing diagnostic model for SCC identification and CIN transformation prediction

The spectra of all signals with a signal-to-noise (S/N) ratio >5 in a mass range of 1000–10,000 Da obtained from all of the samples by using BioExplorer statistical package (Bioyong Tech., Beijing, China). The t test was used for comparison between the two groups and P-value <.05 was considered statistically significant. Peak intensity higher than 300 was counted as appearance. Next, we analyzed ROC of all peaks which meet above conditions by SPSS software (version 21.0; IBM Corp.).

Serum peptide profiles of 15 patients with SCC and 20 healthy controls in the training set were used for the establishment of SCC identification and CIN1 development prediction model. Finally, we tested another 110 samples, including 10 healthy persons, 29 CIN1
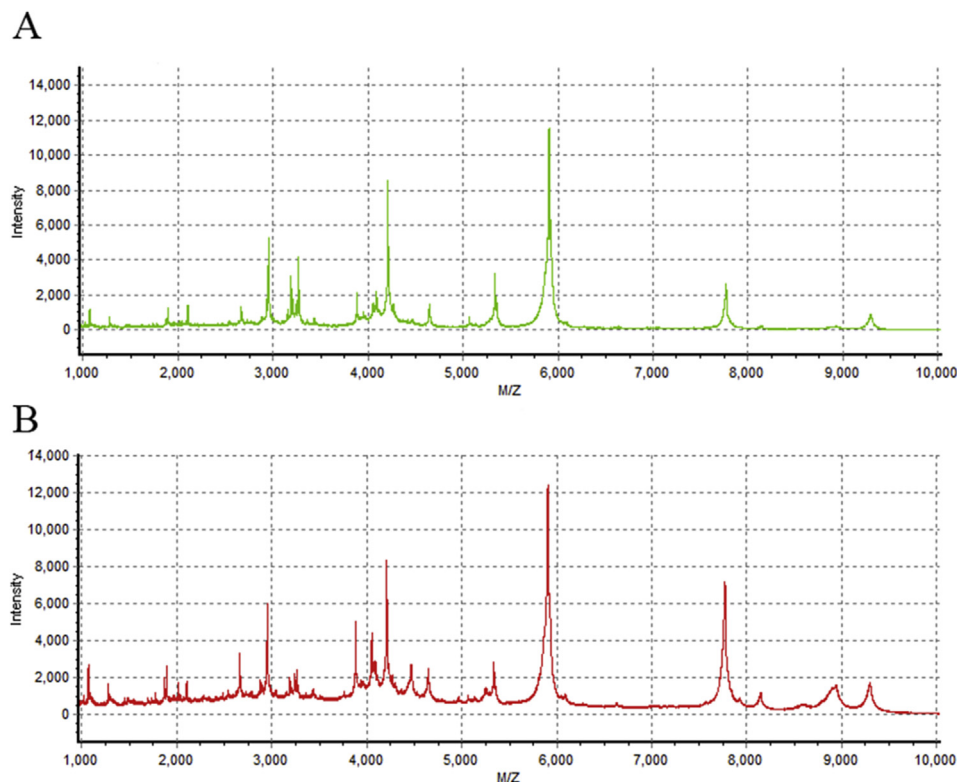

Fig. 1. Mass spectra of serum sample showing peptide fingerprints in the range of 1000–10,000 *m/z*.A. Mass spectra of a single healthy women from control group. B. Mass spectra of a single patient from SCC group.