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Analysis of diferentially expressed protein from primary and recurrent ovarian cancer serum

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ABSTRACT

Objective: To study the value of the differentially expressed proteins from primary and recurrent ovarian cancer serum for early diagnosis of primary and recurrent ovarian cancer. **Methods:** WCX kit (Bruker Daltonics GraBH) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) technology were used to detect serum samples from 49 patients with primary ovarian cancer and 21 patients with recurrent disease. **Results:** In the mass range (Mr) from 1 000 to 12 000 Da, eight differentially expressed protein peaks were screened from primary ovarian cancer serum. Among them, four protein peaks with Mr 1 457, 1 857, 2 202, 7 761 were lowly expressed and the others with Mr 2 946, 5 333, 5 859, 5 901 were highly expressed. Ten diferentially expressed protein peaks were screened from recurrent ovarian cancer serum. Among them, 1 944, 1 980, 2 080, 2 661, 2 993, 4 450, 4 659, 5 359 Da protein expressions were increased significantly, and 1897, 7868 Da protein expressions were decreased significantly. The pattern of primary ovarian cancer was applied to 8 early-stage ovarian cancer serum samples, and 7 serum samples were successfully predicted with the accuracy of 87.5%. The pattern of recurrent ovarian cancer was applied to 9 without pelvic or abdominal mass recurrent ovarian cancer serum samples, and 8 serum samples were successfully predicted with the accuracy of 88.9%. **Conclusions:** Combination of MALDI-TOF-MS and WCX kit technology can directly screen the diferential expressed protein from primary and recurrent ovarian cancer serum. They have clinical significance for enhancement of sensitivity and specificity of ovarian cancer diagnosis.

1. Introduction

Ovarian cancer is one of the most common malignant cancer among women. It causes higher mortality than any other gynecological malignat tumors. About 70% of the patients are found to have been in advanced-stages (Stage III and IV) by the time of diagnosis. And 70% of the patients have recurrence eventhough they go through primary cytoreductive surgery and chemotherapy. With the alteration of life style, increasing envirenmental pollution, application of assisted reproductive technique and oversue of ovulation drugs, the morbidity of ovarian cancer is increased year by year. Hence, It is urgent to seek diagnosis methods with good sensitivity and high specificity for ovarian cancer, and to enhance the diagnosis of primary and reoccurent ovarian cancer, which in turn helps in timely and accruate detection and treatment of the disease.

At present diagnosis of ovarian cancer commonly relies on the detection of CA125 in blood serum. However, CA125 concentration is increased in only 50%-60% stage I patients[1]. As a single marker, its positive predictive value is even less than 10%, and this value could be only up to 20% in combination with ultrasonic inspection, not to mention the possibility of false positive results. Hence, to eliminate the weakness of CA125 diagnosis, the discovery of new overial tumor biomarkers with high sensitivity and

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specificity is the key for early diagnosis and monitoring.

To the best of our knowledge, it is rarely reported around the world about the application feasibility of WCX kit(Bruker Daltonics GraBH) in commbinition with matrix-assisted laser desorption/ionization time-of-fligh-t mass spectrometry (MALDI-TOF-MS) technology. For this reason, the present study combined these two technologies to detect the diferentially expressed protein peaks in the serum of control group and patients with primary and reoccurent ovarian cancer so as to build a diagnosis model with higher sensitivity and specificity, and to decrease the mortality from ovarian cancer.

2. Materials and methods

2. 1. Patients

2. 1. 1. Primary ovarian cancer group

A total of 49 primary epithelial ovarian cancer patients admitted from September 2007 and May 2009 were selected, who all underwent surgery and pathological diagnosis, and were initial cases without any other complications.

According to International Federation of Gynecology and Obstetrics pathological staging, the number of cases in stage I, stage II, stage III and IV were 3, 5, 30 and 11, respectively. The age of the patients ranged from 38-83 years old, with an averange of 55.7 years. The positive control group consisted of forty patients who underwent surgery and were pathologically diagnosed as benign cystic ovarian at the same period of time with age range from 30-77 years old and averange age of 52.6 years. The normal control group consisted of forty healthy patients who underwent physical examination with age range from 32-76 years old and averange age of 52.8 year. The age of these tree groups was not significantly different (P>0. 05).

2. 1. 2. Recurrent ovarian cancer group

A total of 21 patients who had recurrence of ovarian cancer 6 months after comprehensive treatment and clinical complete response were screened. The diagnosid standards were 1) The increase of CAI; 2) the occurrence of pleural and ascitic fluid; 3) the detection of tumor by physical examination; 4) the detection of tumor by imaging technique; 5) intestinal obstruction with unknown causes[2]. Tumor would be considered to be recurrent as long as two situations above occurred. The age range of recurrent ovarian cancer group was 34-82 years old with average of 53 years. The number of cases in stage I, stage II, stage III and IV was 3, 4, 8, 6, respectively. Among them, 9 had serous cystadenocarcinoma, 4 had mucinous cystadenocarcinoma, 4 had clear cell carcinoma, 4 had undifferentiated carcinoma. And 12 cases were in midium differentiation level, 9 in low differentiation level.

All patients underwent surgery upon the diagnosis and were validated pathologically after the surgery. They had standard chemotherapy treatment for at least 6 courses. Eighteen patients with complete clinical remission who randomly visited our hospital at the same period were selected as control. The age range was 42-70 years old, with average of 61 years. There was no significant age difference between these two groups (P>0. 05).

2. 2. Chemicals and instrument

MB-WCX kit (No. 223983), target for MTP-Anchorchip800/384 and MALDI-TOFMS were all from Bruker Dahonics Ltd (Germany). Acetone, methanol, isopropanol, acetonitrile, trifluoroacetic acid, methanoic acid, *etc* were all of HPLC level and purchased from TEDIA, USA and Schadau, Spanish. Substrate was freshly prepared by dessolving HCCA (Bruker Dahonics, Germany) in ethanolalcohol: acetone (2:1) solvent to a concentration of 0. 3 mg/mL.

2. 3. Blood serum sample collection

The fasting blood was collected from patients with primary ovarian cancer, benign ovarian tumor, recurrent ovarian cancer (before the second line chemotherapy or secondary cytoreductive surgery), the normal control and patients who had an clinical complete remission. The blood was then refrigerated within 30 min at 4 ℃ for 2 h, centrifuged at 4 ℃ for 20 min at 12 000 rpm. The obtained serum was placed in EP tubes and kept in refrigerator at -80 ℃ until use.

2. 4. Blood sample processing procedure

The processing procedures of the blood sample were as the following steps: 1)The suspension of weak cationic magnetic beads were throughly mixed up by fluctuating the tube; 2) Ten μL binding solution of magnetic bead-based WCX+10 μL MB-WCX_beads was added into 200 μL PCR tube, mixed up by pipetting up and down, then 5 μL of blood serum were added, pipetting up and down for at least 5 times, inculbated the tube for 5 min; 3) The PCR tube was then placed in magnetic bead separator (MBS) for 1 min, the unbound solution were discarded without touching or sucking the magnetic beads; 4)100 μL of MB-WCX wash solution was added into PCR tube, shuttled on MBS for 10 times. The magnetic beads could be seen attached on the wall of tube, and the unbound solution were discarded. This procedure was reduplicated twice; 5) The PCR tube was then

taken down from MBS, 5 μL elution solution was added to wash the attached magnetic beads by pipetting up and down for 10 times. The tube was then put into MBS again, allowing the magnetic beads adhering onto the wall of tube, and the supernantant was then transferred in an clean centrifuge tube. 6) 5 μL of stablization solution was then added into the centrifuge tube and mixed up by pipetting up and down; 7) 1 μL of magnetic beads elution solution along with 10 μL substrate was totally mixed up and 0. 5-1 μL injectable suspension were spotted onto the target, dried at room temperature.

2. 5. MALDI-ToF-MS detection

Autoflex control parameters were set as follows: LP-Clinprot. par as the selection methods, MTP-Anchorchip800/384 as the target, the energy of the laser was adjusted to about 60%. The detection mass range (Mr) is 1 k-12 kDa; The resulting images were analyzed by Autoflex analysis and ClinProTools software.

3. Results

3. 1. Construction of diagnosis model of spectra peak pattern for ovarian cancer

3. 1. 1. primary ovarian cancer

The blood serum protein patterns obtained from patients with primary ovarian cancer, benign ovarian tumor and the heathy control were analysed and compared using ClinProTools software. The group of the neutral/benign tumor patiens and the healthy control were combined into one group as the expressed blood serum protein patterns from this two groups showed no significant variations. In the mass range (Mr) from 1 000 to 12 000 Da, eight differentially expressed protein peaks were screened ($P<0.01$ or $P<0.05$) (Table 1). Among them, four protein peaks with Mr 1 457, 1 857, 2 202, 7 761 were down-regulated and the others with Mr 2 946, 5 333, 5 859, 5 901 were up-regulated. These 8 differential pxpressed protein peaks were built into primary ovarian cancer diagnosis model which showed sensitivity and speciality for primary ovarian cancer at 95.92% (47/49) and 93.75% (75/80), respectively.

3. 1. 2. recurrent ovarian cancer

Comparing the recurrent group with the complete clinical remission group, ten differentially expressed protein peaks were detected in the Mr of 1 000-12 000 Da (Table 2). Among them, protein peaks with Mr 1 944, 1 980, 2 080, 2 661, 2 993, 4 450, 4 659, 5 359 were significantly highly expressed ($P<0.01$, $P<0.05$), the protein with Mr 1 897, 7 868, were down-regulated significantly ($P<0.01$). These ten differentially expressed protein peaks were built into peak spectral diagnosis model for recurrent ovarian cancer. The sensitivity and speciacificity of this model is 90.48% (19/21), 83.33% (15/18), respectively.

3. 2. Application of peak spectral diagnosis model for ovarian cancer

3. 2. 1. Primary ovarian cancer

Blood serum of 8 early stage primary ovarian cancer patients were treated with the same experimental methods and the mass spectra were obtained. Seven cases were predicted as ovarian cancer using the diagnosis model showing a accuracy of 87.50%.

3. 2. 2. Recurrent ovarian cancer

Blood serum of 9 recurrent ovarian cancer patients without pelvic or abdominal mass were treated with the same experimental methods and the mass spectra were obtained. Eight cases were predicted as recurrent ovarian cancer using the diagnosis model showing an accuracy of 88.89%.

Table 1
Mass spectra analysing results of primary ovarian cancer.

Mr (Da)	Average value of the control group	Average value of the primary ovarian cancer	Significance and trend
1 457.0	49.88	16.74	<0. 05 ↓
1 857.0	252.68	57.59	<0. 01 ↓
2 202.0	112.51	44.73	<0. 05 ↓
2 946.0	211.27	427.79	<0. 01 ↑
5 333.0	46.84	122.09	<0. 05 ↑
5 859.0	49.35	149.25	<0. 05 ↑
5 901.7	149.86	3 026.35	<0. 01 ↑
7 761.0	159.65	53.82	<0. 05 ↓

Table 2
The mass spectral analysis results of recurrent ovarian cancer.

Mr (Da)	Average value of the control group	Average value of the recurrent ovarian cancer	Significance and trend
1 897	29.29	17.68	<0. 01 ↓
1 944	29.59	282.30	<0. 01 ↑
1 980	79.55	148.67	<0. 05 ↑
2 080	31.80	69.83	<0. 01 ↑
2 661	22.78	48.69	<0. 01 ↑
2 993	19.63	73.76	<0. 05 ↑
4 450	13.24	22.01	<0. 01 ↑
4 659	105.76	206.79	<0. 01 ↑
5 359	47.82	156.52	<0. 01 ↑
7 868	2.58	1.42	<0. 01 ↓

4. Discussion

Present researches show that the development of tumor is a muti-stage process affected by several factors (including envirenmental factor, genetic factor, *etc*), leading to the activation of proto-oncogene and the inactivation of cancer suppressor gene. In the invasive development of tumor cells, a large number of protein molecules are involved in the regulation, apoptosis and transfer. At the early stage of tumor without any symtom, the protein level has been changed, which could be used as clinical early diagnosis inde. Hence, analysing and detecting tumor protein could greatly enhance the early detection rate of tumor.

Besides recurrent ovarian cancer, the inducement and development of malignant tumour is an muti-stage process involving a lot of genes. The proto-oncogene is activated and the canser suppressor is inactivated under the effect of muti-factors. In the aggressive development of tumor cells, there is an decrease in cell division regulation machanism and increase in the ability to invade towards the surrounding tissue and to transfer distantly[3,4].

In the present research, MB-WCX and MALDI-TOF MS technologies were employed together to detect the serum of 49 primary and 21 recurrent ovarian cancer patients. Eight differentially expressed protein peaks were screened ($P<0.01$, $P<0.05$) from primary ovarian cancer patients compared with the control, showing a sensitivity and specificity of 95.92% and 93.75%, respectively. This protein pattern was applied in the prediction of 8 early primary ovarian cancer cases, resulting in an accuracy of 87.50%. Ten differentially expressed protein peaks were screened ($P<0.01$, $P<0.05$) from recurrent ovarian cancer patients compared with the control, showing a sensitivity and specificity of 90.48% and 83.33%, respectively. The pattern applied to 9 without pelvic or abdominal mass recurrent ovarian cancer serum samples, and 8 were successfully predicted with the accuracy of 88.90%. We could conclude that MB-WCX in conbinition with MALDI-TOF MS could have some clinical significance in enhancing the diagnosis sensitivity and specificity for primary and recurrent overrian cancer.

The results from present study is different from other studies, in which combined technologies like SELDI-TOF-MS, protein chic and SVM was applied to some extent[5,6]. It may be due to the experimental error, but the specific machanism remains to be discussed. For the existence of such error among studies, some researchers[7] think that it is necessary for large-scaled, forwardlooking, multicentric clinical experiments so as to validate and standardise this technology.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Brazert J, Pietryga M, Szablo ń ski W, Persona-Sliwi ń ska A, Biczysko R. Diagnostic value of the morphological ultrasound score system and the serum concentration of CA125 in the diagnosis of malignant ovarian cancer. *Gineko1 Po1* 2003; **74**: 1542-1547.

[2] Xue FX, Zhao J. The diagnosisn of recurrent ovarian cancer. *Chin J Pract Gynecol Obsterics* 2005; **21**(7): 385-386.

[3] Compagni A, Christofori G. Recent advances in research on multistage tumorigenesis. *Br J Cancer* 2000; **83**(1): 1-5.

[4] Livingston DM, Shivdasani R. Toward mechanism-based cancer care. *JAMA* 2001; **285**(5): 588-593.

[5] Kong F, Nicole WC, Xiao X, He D, Zhang Z, Yu Y, et al. Using proteomic approaches to identify new biom-arkers for detection and monitoring of ovarian cancer. *Gynecol Oncol* 2006; **100**(2): 247-253.

[6] Wang J, Zhang X, Ge X, Guo H, Xiong G, Zhu Y. Proteomic studies of early-stage and advanced ovarian cancer patients. *Gynecol Oncol* 2008; **111**(1): 111-119.

[7] van der Merwe DE, Oikonomopoulou K, Marshall J, Diamandis EP. Mass spectrometry:uncovering the cancer proteome for diagnostics. *Adv Cancer Res* 2007; **96**: 23-5O.