RESEARCH ARTICLE

Proteomic Analysis of Serum of Women with Elevated Ca-125 to Differentiate Malignant from Benign Ovarian Tumors

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Abstract

Clinically, elevated cancer antigen 125 (CA-125) in blood predicts tumor burden in a woman's body, especially in the ovary, but cannot differentiate between malignant or benign. We here used intensive modern proteomic approaches to identify predictive proteins in the serum of women with elevated CA-125 to differentiate malignant from benign ovarian tumors. We identified differentially expressed proteins in serum samples of ovarian cancer (OC) patients, benign ovarian tumor (BT) patients, and healthy control women using mass spectrometry-based quantitative proteomics. Both the OC and BT patients had elevated CA-125. Quantitation was achieved using isobaric tags for relative and absolute quantitation. We obtained 124 quantified differential serum proteins in OC compared with BT. Two proteins, apolipoprotein A-4 (APOA4) and natural resistance-associated macrophage 1, were verified using Western blotting. Proteome profiling applied to OC cases identified several differential serum proteins in the serum of women with elevated CA-125. A novel protein, APOA4, has the potential to be a marker for malignant tumor differentiation in the serum of women with elevated CA-125.

Keywords: Quantitative proteomics - ovarian cancer - CA-125 - iTRAQ - 2D-nano - LC-ESI-OrbiTrap MS/MS

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Introduction

Ovarian cancer (OC) accounts for approximately 3% of all cancers among women and ranks second among all gynecologic cancers in the USA. It is the most lethal among all gynecologic cancers, causing more deaths than any other cancer of the female reproductive system (Alzheimer's Association, 2009). Early OC usually has no obvious symptoms. In general, OC is diagnosed when it is already in the late or even in the metastatic stage (Alzheimer's Association, 2009). At present, no sufficiently accurate screening test has been proven effective in the early detection of OC. A combination of a thorough pelvic exam, a transvaginal ultrasound, and a blood test for the tumor marker cancer antigen 125 (CA-125) may be offered to women at high risk of OC and to those with persistent and unexplained symptoms. CA-125 is currently the only tumor marker exhibiting significant effects on the clinical management of OC (Whitehouse and Solomon, 2003).

CA-125 may be elevated in the blood of several cancer patients (Bast et al., 1998). CA-125 is best known as a marker for OC, and is especially useful in detecting the recurrence of OC (Osman et al., 2008). However, CA-125 may also be elevated in many relatively benign conditions, such as endometriosis (Bagan et al., 2008), several ovarian diseases, and pregnancy (Sarandakou et al., 2007). CA-125 also tends to be elevated in the presence of any inflammatory condition in the abdominal area, both cancerous and benign. Clinically, elevated CA-125 usually predicts a tumor burden, especially in the ovary. However, determining whether a tumor is benign or malignant is difficult.

Differentially expressed proteins in the serum of patients with OC and benign ovarian tumor, both having elevated CA-125, may be involved in pathways that modulate malignant and benign tumors (BT). Therefore, they are logical candidates as markers to identify whether an ovarian tumor is malignant or benign. Mass spectrometry-based quantitative proteomics, such as isobaric tags for relative and absolute quantitation (iTRAQ) combined with 2D-nano-liquid chromatography-OrbiTrap tandem mass spectrometer, allow global identification and quantification of proteins in complex samples and are well suited for discovering potential biomarkers for human diseases (DeSouza et al., 2005; Wu et al., 2006).

In the present paper, we report a quantitative proteomic analysis of serum from women with elevated CA-125, which helps identify proteins to differentiate malignant from benign ovarian tumors. Two important differential proteins were further validated using western blot technology in serum samples.

Materials and Methods

Sample collection and preparation

Fasted blood samples were obtained from 21 OC patients, 16 BT patients, and 20 healthy women from the Cancer Hospital, Xinjiang Medical University (Urumqi,

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China). The OC and BT patients all had elevated CA-125 (> 35 units/mL). The OC patients were categorized in terms of histopathological features and stages according to the tumor-node-metastasis classification of tumors. The clinical diagnoses and pathological reports of all the patients were obtained from the hospital. Clinical data such as age, clinical assay value of CA-125, and histological staging are shown in Table 1. The protocol for the present study was approved by the Cancer Hospital Institutional Review Board, and all participants gave informed consent before they were involved in the study.

Data were analyzed anonymously

Serum from OC patients, BT patients, and healthy controls (HC) were used in our proteomic discovery and validation experiments. Serum collection and processing were according to our SOP. In brief, serum from women with OC and BT were collected prior to surgery and chemotherapy. Age-matched HC controls were recruited through an OC screening program. All serum samples, regardless of the collection source, were processed using the same protocol. Blood was collected in 10 cc Vacutainer Serum Separator Tubes and allowed to sit for 30 min at room temperature. The tubes were centrifuged at 3000 rps (1000 × g) for 5 min, split into multiple 1 mL aliquots of serum, and then stored at -80 °C until analysis.

The serum proteomic discovery experiments were performed in pooled sample from each group. The serum from the OC and BT patients was compared with that from the HC group. The pooled samples were analyzed in triplicates; each pooled sample had a total of 10 cases. The validation experiments for serum samples were performed case by case, including all the serum samples previously recruited, which are 57 in total.

Immunological interaction

We used a commercial immunoaffinity depletion method prior to proteomic analysis, multiple affinity removal system (MARS) columns (Agilent Technologies, USA) performed in Prominence LC system (Shimadzu, Kyoto, Japan), to collect flow-through fraction (low-abundant proteins). This procedure was done to reduce the dynamic range of proteins in the serum and increase the likelihood of identifying medium and low-abundance serum proteins via mass spectrometry.

After removing the major highly abundant proteins, protein concentrations were determined using the Bradford Reagent (Bio-Rad, Hemel Hempstead, UK) assay in a test tube format with albumin as a reference standard (1 μg/μL to 10 μg/μL).

Peptide labeling using the iTRAQ® chemical reagent

Depleted sera were labeled with isobaric tags for relative and absolute quantification (iTRAQ, Applied Biosystems, California, USA) of the medium and low abundance proteins recovered from the MARS column. This procedure was done to identify differentially expressed proteins in the sera. Three samples were labeled with the iTRAQ tags as follows: Sample A (OC-pooled sample), 118.1 tag; Sample B (BT-pooled sample), 119.1 tag; and Sample C (HC-pooled sample), 121.1 tag.

The labeled peptides from the three samples were combined into one tube and dried in a vacuum concentrator. A SepPac™ C18 cartridge (Waters Corporation, Milford, MA) was used to exchange the buffer and remove trypsin and hydrolyzed unbound iTRAQ reagents from the labeled peptides.

Peptide fractionation

The concentrated iTRAQ-labeled sample was added to 2 mL of Diluent buffer [10 mM potassium phosphate and 25% acetonitrile (ACN)]. The pH of the buffer was adjusted below 3 using phosphoric acid. The samples were subjected to cation exchange chromatography using a PolySULFOETHYL A HPLC column [(2.1 × 100 mmi.d), PolyLC Inc, Columbia, MD, USA]. A gradient flow of 0.2 mL/min was used, and twenty-four 0.4 mL fractions were collected in 1.5 mL microfuge tubes. The pH values of buffers A (10 mM potassium phosphate with 25% ACN) and B (high-salt buffer containing 10 mM potassium phosphate, 500 mM potassium chloride, and 25% ACN) were adjusted to pH < 3 using phosphoric acid. The following gradient was applied: 5 min 100% buffer A; 5 min to 45 min increasing to 30% buffer B; 45 min to 50 min increasing to 80% buffer B, maintained for 5 min; 56 min 100% buffer A, maintained for 15 min. Fractions were collected starting at 6 min and at 2 min intervals.

Reversed phase LC-MS/MS analysis

The peptides were resuspended with 20 μL solvent A (A: water with 0.1% formic acid; B: ACN with 0.1% formic acid) and separated using a Nano Aquity UPLC system (Waters Corporation, Milford, USA) connected to an LTQ Orbitrap XL mass spectrometer (Thermo Electron Corp., Bremen, Germany) equipped with an online nanoelectrospray ion source (Michrom Bioresources, Auburn, USA). An 18 μL peptide sample was loaded onto the Captrap Peptide column (Michrom Bioresources, Auburn, USA), with a flow of 20 μL/min for 5 min. Subsequently, the sample was eluted with a three-step linear gradient as follows: starting from 5% A to 45% B for 70 min, increased to 80% B for 1 min, and then sustained on 80% B for 4 min. The column was re-equilibrated at initial conditions for 15 min. The flow rate and temperature of the column were maintained at 500 nL/min and 35 °C, respectively. The electrospray voltage of 1.9 kV versus the inlet of the mass spectrometer was used.

LTQ Orbitrap XL mass spectrometer was operated in the data-dependent mode to switch automatically between MS and MS/MS acquisition. Survey full-scan MS spectra (m/z 300-1600) were acquired in the Obitrap with a mass resolution of 60,000 at m/z 400, followed by four sequential HCD-MS/MS scans. The automatic gain control was set to 500,000 ions to prevent over-filling of the ion trap. The minimum MS signal for triggering MS/MS was set to 10,000. In all cases, one microscan was recorded. The lock mass option was enabled, and the polydimethylcyclosiloxane ion signal (protonated $(Si(CH_3)_2O))_6$; m/z 445.120025) was used for internal calibration of the mass spectra.

MS/MS scans were acquired in the Obitrap with a mass resolution of 7,500. For MS/MS, precursor ions were

activated using 45% normalized collision energy and an activation time of 30 ms.

Peptide sequencing and data interpretation

All MS/MS spectra were identified using SEQUEST [v.28 (revision 12), Thermo Electron Corp.] against the human Swiss-Prot database (Release 2010_04). The searching parameters were set as follows: partial trypsin cleavage with two missed cleavage was considered; fixed modification of cysteines by methyl methanethiosulfonate; iTRAQ modification of peptide N termini, methionine oxidation, and lysine residues was set as variable modification; peptide mass tolerance was 15 ppm; and fragment ion tolerance was 0.1 Da. The Trans Proteomic Pipeline software (revision 4.2) (Institute of Systems Biology, Seattle, WA) was used to identify the proteins and calculate protein ratios. The peptide results were filtered using Peptide Prophet1, with a p-value over 0.90, and a Protein Prophet2 probability of 0.95 was used for the protein identification results. The false positive rate was < 1% for the experiment.

Western blot

Differentially expressed serum protein markers determined via iTRAQ were validated using western blot. The total protein (30 mg) was separated through 12% gradient sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to a polyvinylidene fluoride membrane (Amersham Bioscience). The membranes were blocked with 5% bovine serum albumin in TBST buffer (137 mM NaCl, 20 mM Tris-HCl, pH 7.6, 0.1% Tween-20) and then incubated with primary antibody for 1 h at room temperature. Subsequently, the membranes were washed and incubated with corresponding horseradish peroxidase-conjugated secondary antibody. The washed membranes were developed with enhanced chemiluminescence reagent (Amersham Bioscience). The images were captured with LAS-3000 instrument (Fuji, Japan) and calculated using Grey analysis.

Results

We analyzed in triplicates the iTRAQ-labeled pooled serum samples from the three groups (OC, BT, and HC). The peptide results were filtered using Peptide Prophet1 (Keller et al., 2002) with a p-value over 0.90, and a Protein Prophet2 probability of 0.95 was used for the protein identification results. The false positive rate was < 1% for the experiment.

We obtained quantitative data on 326 proteins. Proteins with expression ratios of over 1.2-fold in increase or at least 1.5-fold in decrease were considered differentially expressed.

We identified 9 overexpressed and 75 underexpressed proteins in OC compared with HC (Table 1 in Supplemental Digital Content). We identified 32 overexpressed and 103 underexpressed proteins in BT compared with HC (Table 2 in Supplemental Digital Content). In addition, we identified 77 overexpressed and 43 underexpressed proteins (Table 2) in OC compared with BT.

Gene ontology analysis

Gene ontology analysis was operated according to the method in nature protocol (Huang et al., 2009) using DAVID bioinformatics resources (http://david.abcc.ncifcrf.gov). The gene list was submitted to DAVID. We accessed DAVID analytic modules through the tools menu page, ran "Gene Name Batch Viewer," and finally explored the results. Table 3 lists the top 10 terms that are significantly enriched ($p < 0.004$) by molecular function.

Protein validation

Two differentially expressed proteins between OC and BT sera were validated via western blot: apolipoprotein A-4 (APOA4) and natural resistance-associated macrophage 1

Table 1. Clinical Data for the Serum Samples in This Study

	Ovarian cancer n=21	Ovarian benign tumor n=16	Healthy control n=20
Age (median, range)	50, 33-70	38, 25-49	37, 25-46
CA-125 (median, range)	867, 45-over 5000	57, 45-233.8	/
TNM Stage I	1	/	/
TNM Stage II	3	/	/
TNM Stage III	16	/	/
TNM Stage IV		/	/

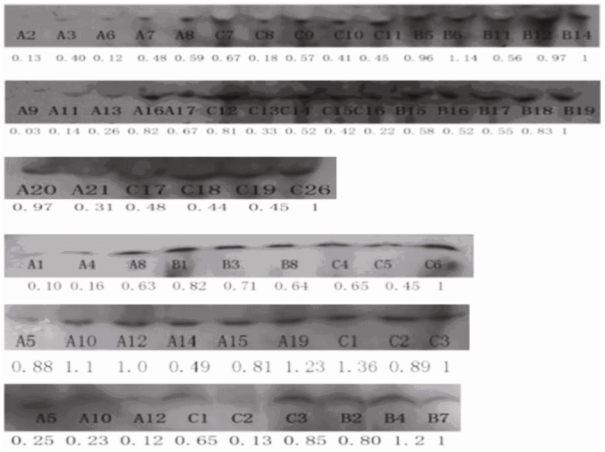

Figure 1. The Western Blotting Result for APOA4 Protein (A for ovarian cancer group; B for ovarian benign tumor group; and C for healthy group; digitals after the A, B, or C for the number of each serum sample). The value below each gel was the grey level

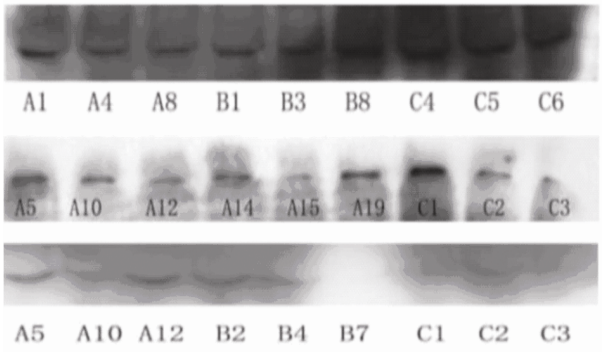

Figure 2. The Western Blotting Result for NRAM1 Protein (A for ovarian cancer group; B for ovarian benign tumor group; and C for healthy group; digitals after the A, B, or C for the number of each serum sample)

Table 2. Differentially Expressed Proteins were obtained with 77 Proteins Overexpressed, and 43 Proteins Underexpressed in Ovarian Cancers, Compared with Benign Ovarian Tumors

Protein	Protein probability	Number of unique peptides	Description	Cancer	Benign tumor	FC (C/B)
sp|Q05DH4|F16A1_HUMAN	0.9997	4	UPF0518 protein FAM160A1 OS=Homo sapiens GN=FAM160A1 PE=2 SV=2	1.21	0.33	3.62
sp|Q4KMG0|CDON_HUMAN	0.9931	1	Cell adhesion molecule-related/down-regulated by oncogenes OS=Homo sapiens GN=CDON PE=1 SV=1	1.23	0.36	3.43
sp|Q9BQ69|MACD1_HUMAN	1	2	MACRO domain-containing protein 1 OS=Homo sapiens GN=MACROD1 PE=1 SV=2	0.93	0.29	3.23
sp|Q96N67|DOCK7_HUMAN	0.9973	1	Dedicator of cytokinesis protein 7 OS=Homo sapiens GN=DOCK7 PE=1 SV=4	0.69	0.23	3
sp|Q8TD22|SFXN5_HUMAN	0.999	1	Sideroflexin-5 OS=Homo sapiens GN=SFXN5 PE=2 SV=1	0.91	0.31	2.93
sp|Q6IMN6|CAPR2_HUMAN	0.9988	1	Caprin-2 OS=Homo sapiens GN=CAPRIN2 PE=1 SV=1	0.85	0.33	2.6
sp|Q9UPW5|CBPC1_HUMAN	0.9989	1	Cytosolic carboxypeptidase 1 OS=Homo sapiens GN=AGTPBP1 PE=1 SV=3	0.75	0.33	2.25
sp|Q93033|IGSF2_HUMAN	0.9989	1	Immunoglobulin superfamily member 2 OS=Homo sapiens GN=IGSF2 PE=1 SV=2	1.16	0.54	2.15
sp|P13645|K1C10_HUMAN	0.9989	1	Keratin, type I cytoskeletal 10 OS=Homo sapiens GN=KRT10 PE=1 SV=4	0.77	0.36	2.12
sp|Q68DN6|RGPD1_HUMAN	1	4	RANBP2-like and GRIP domain-containing protein 1 OS=Homo sapiens GN=RGPD1 PE=1 SV=1	0.66	0.32	2.06
sp|Q99569|PKP4_HUMAN	1	2	Plakophilin-4 OS=Homo sapiens GN=PKP4 PE=1 SV=1	0.66	0.32	2.06
sp|O95622|ADCY5_HUMAN	0.998	1	Adenylate cyclase type 5 OS=Homo sapiens GN=ADCY5 PE=2 SV=3	0.76	0.39	1.94
sp|P49279|NRAM1_HUMAN	0.999	1	Natural resistance-associated macrophage protein 1 OS=Homo sapiens GN=SLC11A1 PE=1 SV=1	1.27	0.67	1.91
sp|O60353|FZD6_HUMAN	0.9999	2	Frizzled-6 OS=Homo sapiens GN=FZD6 PE=1 SV=2	0.64	0.34	1.88
sp|Q16720|AT2B3_HUMAN	0.9998	2	Plasma membrane calcium-transporting ATPase 3 OS=Homo sapiens GN=ATP2B3 PE=1 SV=3	0.88	0.48	1.85
sp|Q7Z3Z2|RD3_HUMAN	0.9984	1	Protein RD3 OS=Homo sapiens GN=RD3 PE=2 SV=1	0.86	0.47	1.85
sp|O60344|ECE2_HUMAN	0.9978	4	Endothelin-converting enzyme 2 OS=Homo sapiens GN=ECE2 PE=1 SV=4	0.86	0.47	1.85
sp|Q6MZM0|HPHL1_HUMAN	0.9988	1	Hephaestin-like protein 1 OS=Homo sapiens GN=HEPHL1 PE=2 SV=2	0.95	0.53	1.81
sp|Q16363|LAMA4_HUMAN	1	2	Laminin subunit alpha-4 OS=Homo sapiens GN=LAMA4 PE=1 SV=3	1.21	0.68	1.78
sp|Q9NZW5|MPP6_HUMAN	0.9987	1	MAGUK p55 subfamily member 6 OS=Homo sapiens GN=MPP6 PE=1 SV=2	0.8	0.45	1.75
sp|Q14160|LAP4_HUMAN	0.999	1	Protein LAP4 OS=Homo sapiens GN=SCRIB PE=1 SV=3	0.95	0.55	1.73
sp|P33992|MCM5_HUMAN	0.9809	1	DNA replication licensing factor MCM5 OS=Homo sapiens GN=MCM5 PE=1 SV=5	0.93	0.55	1.68
sp|A6NDL7|YM009_HUMAN	0.999	1	UPF0567 protein ENSP00000298105 OS=Homo sapiens PE=3 SV=2	0.8	0.48	1.67
sp|Q9HBJ7|UBP29_HUMAN	0.9983	1	Ubiquitin carboxyl-terminal hydrolase 29 OS=Homo sapiens GN=USP29 PE=2 SV=1	0.7	0.43	1.65
sp|Q05BV3|EMAL5_HUMAN	1	2	Echinoderm microtubule-associated protein-like 5 OS=Homo sapiens GN=EML5 PE=2 SV=3	0.86	0.52	1.64
sp|O71037|ENK3_HUMAN, sp|P61565|ENK1_HUMAN, sp|P61570|ENK17_HUMAN, sp|Q69384|ENK2_HUMAN, sp|Q902F8|ENK6_HUMAN, sp|Q902F9|ENK5_HUMAN, sp|Q9UKH3|ENK4_HUMAN, sp|Q9UKH7|ENK9_HUMAN	0.9987	1	HERV-K_19q12 provirus ancestral Env polyprotein OS=Homo sapiens PE=1 SV=2, HERV-K_12q14.1 provirus ancestral Env polyprotein OS=Homo sapiens PE=1 SV=1, HERV-K_11q22.1 provirus ancestral Env polyprotein OS=Homo sapiens PE=3 SV=1, HERV-K_7p22.1 provirus ancestral Env polyprotein OS=Homo sapiens GN=ERVK6 PE=1 SV=1, HERV-K_8p23.1 provirus ancestral Env polyprotein OS=Homo sapiens PE=1 SV=1, HERV-K_19p13.11 provirus ancestral Env polyprotein OS=Homo sapiens PE=1 SV=1, HERV-K_6q14.1 provirus ancestral Env polyprotein OS=Homo sapiens PE=1 SV=1, Putative HERV-K_5q13.3 provirus ancestral Env polyprotein OS=Homo sapiens PE=5 SV=1	0.88	0.54	1.64
sp|P20933|ASPG_HUMAN	0.9989	1	N(4)-(beta-N-acetylglucosaminyl)-L-asparaginase OS=Homo sapiens GN=AGA PE=1 SV=1	1.08	0.67	1.63
sp|Q8IZC6|CORA1_HUMAN	1	4	Collagen alpha-1(XXVII) chain OS=Homo sapiens GN=COL27A1 PE=1 SV=1	1.05	0.65	1.63
sp|Q9NVH2|INT7_HUMAN	1	2	Integrator complex subunit 7 OS=Homo sapiens GN=INTS7 PE=1 SV=1	1.05	0.65	1.63
sp|O95398|RPGF3_HUMAN	1	3	Rap guanine nucleotide exchange factor 3 OS=Homo sapiens GN=RAPGEF3 PE=1 SV=4	1	0.63	1.58
sp|P23280|CAH6_HUMAN	0.9988	2	Carbonic anhydrase 6 OS=Homo sapiens GN=CA6 PE=2 SV=3	1.03	0.65	1.58
sp|Q6BDS2|URFB1_HUMAN	1	3	UHRF1-binding protein 1 OS=Homo sapiens GN=UHRF1BP1 PE=1 SV=1	1.28	0.81	1.58
sp|Q02388|CO7A1_HUMAN	1	2	Collagen alpha-1(VII) chain OS=Homo sapiens GN=COL7A1 PE=1 SV=2	0.9	0.58	1.57
sp|Q86YJ5|MARH9_HUMAN	0.999	1	E3 ubiquitin-protein ligase MARCH9 OS=Homo sapiens GN=MARCH9 PE=1 SV=2	0.63	0.41	1.55
sp|O95918|OR2H2_HUMAN	1	3	Olfactory receptor 2H2 OS=Homo sapiens GN=OR2H2 PE=2 SV=1	0.77	0.5	1.55
sp|Q8TE23|TS1R2_HUMAN	1	2	Taste receptor type 1 member 2 OS=Homo sapiens GN=TAS1R2 PE=2 SV=2	0.97	0.63	1.54
sp|Q06418|TYRO3_HUMAN	1	3	Tyrosine-protein kinase receptor TYRO3 OS=Homo sapiens GN=TYRO3 PE=1 SV=1	0.71	0.47	1.52
sp|Q8NF91|SYNE1_HUMAN	1	7	Nesprin-1 OS=Homo sapiens GN=SYNE1 PE=1 SV=2	0.71	0.47	1.52
sp|Q502W6|VWA3B_HUMAN	0.9949	2	von Willebrand factor A domain-containing protein 3B OS=Homo sapiens GN=VWA3B PE=2 SV=2	1.29	0.87	1.48
sp|O14523|C2C2L_HUMAN	1	3	C2 domain-containing protein 2-like OS=Homo sapiens GN=C2CD2L PE=1 SV=3	0.95	0.64	1.48
sp|O75914|PAK3_HUMAN	0.9998	2	Serine/threonine-protein kinase PAK 3 OS=Homo sapiens GN=PAK3 PE=1 SV=2	0.83	0.56	1.48
sp|Q8NB46|ANR52_HUMAN	0.9989	1	Serine/threonine-protein phosphatase 6 regulatory ankyrin repeat subunit C OS=Homo sapiens GN=ANKRD52 PE=1 SV=2	0.79	0.53	1.48
sp|P48739|PIPNB_HUMAN	0.999	1	Phosphatidylinositol transfer protein beta isoform OS=Homo sapiens GN=PITPNB PE=1 SV=2	0.65	0.44	1.48
sp|Q9NY26|S39A1_HUMAN	0.9998	2	Zinc transporter ZIP1 OS=Homo sapiens GN=SLC39A1 PE=1 SV=1	0.92	0.64	1.44
sp|Q9N2J9|ENH2_HUMAN	0.999	1	HERV-H_3q26 provirus ancestral Env polyprotein OS=Homo sapiens PE=2 SV=1	0.92	0.64	1.44
sp|O75128|COBL_HUMAN	0.9988	1	Protein cordon-bleu OS=Homo sapiens GN=COBL PE=1 SV=2	0.92	0.64	1.44
sp|Q14686|NCOA6_HUMAN	1	2	Nuclear receptor coactivator 6 OS=Homo sapiens GN=NCOA6 PE=1 SV=3	0.77	0.53	1.43
sp|P16219|ACADS_HUMAN	0.9987	1	Short-chain specific acyl-CoA dehydrogenase, mitochondrial OS=Homo sapiens GN=ACADS PE=1 SV=1	0.77	0.53	1.43
sp|Q6T423|S22AP_HUMAN	0.9935	1	Solute carrier family 22 member 25 OS=Homo sapiens GN=SLC22A25 PE=2 SV=1	0.77	0.53	1.43
sp|Q9BXW9|FACD2_HUMAN	1	2	Fanconi anemia group D2 protein OS=Homo sapiens GN=FANCD2 PE=1 SV=1	0.66	0.47	1.41
sp|Q7Z589|EMSY_HUMAN	0.9989	1	Protein EMSY OS=Homo sapiens GN=EMSY PE=1 SV=2	0.66	0.47	1.41
sp|Q03692|COAA1_HUMAN	1	3	Collagen alpha-1(X) chain OS=Homo sapiens GN=COL10A1 PE=1 SV=2	0.88	0.63	1.4
sp|Q96EK2|PF21B_HUMAN	0.9999	2	PHD finger protein 21B OS=Homo sapiens GN=PHF21B PE=2 SV=1	0.88	0.63	1.4
sp|O43379|WDR62_HUMAN	1	2	WD repeat-containing protein 62 OS=Homo sapiens GN=WDR62 PE=1 SV=2	0.79	0.57	1.38
sp|P13500|CCL2_HUMAN	0.9983	1	C-C motif chemokine 2 OS=Homo sapiens GN=CCL2 PE=1 SV=1	0.77	0.56	1.38
sp|Q2LD37|K1109_HUMAN	1	3	Uncharacterized protein KIAA1109 OS=Homo sapiens GN=KIAA1109 PE=1 SV=1	0.88	0.65	1.35
sp|P42702|LIFR_HUMAN	0.999	1	Leukemia inhibitory factor receptor OS=Homo sapiens GN=LIFR PE=1 SV=1	0.73	0.55	1.33
sp|Q14993|COJA1_HUMAN	1	3	Collagen alpha-1(XIX) chain OS=Homo sapiens GN=COL19A1 PE=1 SV=3	0.74	0.56	1.33
sp|Q01955|CO4A3_HUMAN	0.9976	2	Collagen alpha-3(IV) chain OS=Homo sapiens GN=COL4A3 PE=1 SV=3	0.74	0.56	1.33
sp|P19827|ITIH1_HUMAN	1	3	Inter-alpha-trypsin inhibitor heavy chain H1 OS=Homo sapiens GN=ITIH1 PE=1 SV=3	0.8	0.61	1.32
sp|O76024|WFS1_HUMAN	1	2	Wolframin OS=Homo sapiens GN=WFS1 PE=1 SV=1	0.87	0.67	1.31
sp|P06741|MLE3_HUMAN, sp|P12829|MYL4_HUMAN	0.999	1	Myosin light chain 1, skeletal muscle isoform OS=Homo sapiens GN=MYL1 PE=1 SV=3,Myosin light chain 3, skeletal muscle isoform OS=Homo sapiens GN=MYL1 PE=2 SV=2,Myosin light chain 4 OS=Homo sapiens GN=MYL4 PE=1 SV=3	0.85	0.65	1.31
sp|A0AVK6|E2F8_HUMAN	0.9961	1	Transcription factor E2F8 OS=Homo sapiens GN=E2F8 PE=1 SV=1	0.87	0.67	1.31
sp|Q9BSW7|SYT17_HUMAN	0.9749	1	Synaptotagmin-17 OS=Homo sapiens GN=SYT17 PE=2 SV=1	0.64	0.49	1.3
sp|P30153|2AAA_HUMAN	1	2	Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform OS=Homo sapiens GN=PPP2R1A PE=1 SV=4	0.69	0.53	1.29
sp|Q14119|VEZF1_HUMAN	0.9996	2	Vascular endothelial zinc finger 1 OS=Homo sapiens GN=VEZF1 PE=2 SV=1	0.69	0.53	1.29
sp|O43464|HTRA2_HUMAN	0.999	1	Serine protease HTRA2, mitochondrial OS=Homo sapiens GN=HTRA2 PE=1 SV=2	0.52	0.4	1.29
sp|Q02880|TOP2B_HUMAN	0.9994	2	DNA topoisomerase 2-beta OS=Homo sapiens GN=TOP2B PE=1 SV=3	0.82	0.64	1.28
sp|Q9Y5P3|RAI2_HUMAN	1	2	Retinoic acid-induced protein 2 OS=Homo sapiens GN=RAI2 PE=2 SV=2	0.67	0.52	1.27
sp|O14686|MLL2_HUMAN	1	6	Histone-lysine N-methyltransferase MLL2 OS=Homo sapiens GN=MLL2 PE=1 SV=1	0.85	0.67	1.27
sp|P37837|TALDO_HUMAN	1	2	Transaldolase OS=Homo sapiens GN=TALDO1 PE=1 SV=2	0.83	0.65	1.27
sp|Q8NGC7|O11H6_HUMAN	0.999	1	Olfactory receptor 11H6 OS=Homo sapiens GN=OR11H6 PE=2 SV=1	0.72	0.58	1.24
sp|Q06495|NPT2A_HUMAN	1	2	Sodium-dependent phosphate transport protein 2A OS=Homo sapiens GN=SLC34A1 PE=1 SV=1	0.76	0.62	1.23
sp|Q9Y616|IRAK3_HUMAN	1	3	Interleukin-1 receptor-associated kinase 3 OS=Homo sapiens GN=IRAK3 PE=1 SV=1	0.78	0.63	1.23
sp|Q5T4S7|UBR4_HUMAN	0.9977	3	E3 ubiquitin-protein ligase UBR4 OS=Homo sapiens GN=UBR4 PE=1 SV=1	0.82	0.67	1.23
sp|Q9NXV6|CARF_HUMAN	1	3	CDKN2A-interacting protein OS=Homo sapiens GN=CDKN2AIP PE=1 SV=2	0.83	0.68	1.22
sp|Q9BZZ2|SN_HUMAN	0.9919	1	Sialoadhesin OS=Homo sapiens GN=SIGLEC1 PE=1 SV=2	0.83	0.68	1.22

Protein	Protein probability	Number of unique peptides	Description	Cancer	Benign tumor	FC (C/B)
sp|Q8TEW0|PARD3_HUMAN	0.9989	1	Partitioning defective 3 homolog OS=Homo sapiens GN=PARD3 PE=1 SV=2	1	3.39	0.3
sp|Q9Y6V0|PCLO_HUMAN	0.9994	2	Protein piccolo OS=Homo sapiens GN=PCLO PE=1 SV=3	0.56	1.81	0.31
sp|Q8N7S6|CC071_HUMAN	0.999	1	Uncharacterized protein C3orf71 OS=Homo sapiens GN=C3orf71 PE=2 SV=1	0.83	2.48	0.33
sp|P52292|IMA2_HUMAN	1	4	Importin subunit alpha-2 OS=Homo sapiens GN=KPNA2 PE=1 SV=1	0.95	2.81	0.34
sp|A6NNH2|F90AL_HUMAN	0.999	1	Putative protein FAM90A-like OS=Homo sapiens PE=3 SV=2	0.92	2.17	0.42
sp|P00734|THRB_HUMAN	0.9995	2	Prothrombin OS=Homo sapiens GN=F2 PE=1 SV=2	0.84	1.8	0.47
sp|Q01167|FOXK2_HUMAN	0.9985	1	Forkhead box protein K2 OS=Homo sapiens GN=FOXK2 PE=1 SV=3	1.14	2.41	0.47
sp|Q14494|NF2L1_HUMAN	0.9994	2	Nuclear factor erythroid 2-related factor 1 OS=Homo sapiens GN=NFE2L1 PE=1 SV=1	0.62	1.29	0.48
sp|Q9ULD4|BRPF3_HUMAN	1	2	Bromodomain and PHD finger-containing protein 3 OS=Homo sapiens GN=BRPF3 PE=1 SV=2	0.69	1.41	0.49
sp|Q9Y5Y9|SCNAA_HUMAN	1	2	Sodium channel protein type 10 subunit alpha OS=Homo sapiens GN=SCN10A PE=1 SV=2	0.69	1.38	0.5
sp|Q86X10|K1219_HUMAN	0.999	1	Protein KIAA1219 OS=Homo sapiens GN=KIAA1219 PE=1 SV=1	1.29	2.48	0.52
sp|Q5HYC2|K2026_HUMAN	1	3	Uncharacterized protein KIAA2026 OS=Homo sapiens GN=KIAA2026 PE=2 SV=1	0.65	1.21	0.54
sp|P49005|DPOD2_HUMAN	0.9989	1	DNA polymerase delta subunit 2 OS=Homo sapiens GN=POLD2 PE=1 SV=1	0.53	0.95	0.55
sp|O14994|SYN3_HUMAN	0.9993	2	Synapsin-3 OS=Homo sapiens GN=SYN3 PE=1 SV=2	0.4	0.71	0.56
sp|Q96K37|S35E1_HUMAN	0.999	1	Solute carrier family 35 member E1 OS=Homo sapiens GN=SLC35E1 PE=1 SV=2	0.79	1.41	0.56
sp|Q8TF64|GIPC3_HUMAN	0.9986	1	PDZ domain-containing protein GIPC3 OS=Homo sapiens GN=GIPC3 PE=2 SV=1	0.71	1.21	0.59
sp|Q08211|DHX9_HUMAN	1	3	ATP-dependent RNA helicase A OS=Homo sapiens GN=DHX9 PE=1 SV=4	0.31	0.54	0.59
sp|Q14244|MAP7_HUMAN	0.9938	1	Ensconsin OS=Homo sapiens GN=MAP7 PE=1 SV=1	0.93	1.57	0.59
sp|Q8N807|PDILT_HUMAN	1	3	Protein disulfide-isomerase-like protein of the testis OS=Homo sapiens GN=PDILT PE=1 SV=2	0.55	0.93	0.59
sp|Q9C0G0|ZN407_HUMAN	0.9635	1	Zinc finger protein 407 OS=Homo sapiens GN=ZNF407 PE=2 SV=2	0.78	1.31	0.6
sp|P06727|APOA4_HUMAN	1	2	Apolipoprotein A-IV OS=Homo sapiens GN=APOA4 PE=1 SV=3	0.71	1.18	0.6
sp|Q9NR09|BIRC6_HUMAN	1	5	Baculoviral IAP repeat-containing protein 6 OS=Homo sapiens GN=BIRC6 PE=1 SV=1	0.74	1.21	0.61
sp|O60645|EXOC3_HUMAN	1	2	Exocyst complex component 3 OS=Homo sapiens GN=EXOC3 PE=1 SV=2	0.52	0.86	0.61
sp|Q8NHU2|CT026_HUMAN	1	3	Uncharacterized protein C20orf26 OS=Homo sapiens GN=C20orf26 PE=2 SV=3	0.93	1.52	0.61
sp|Q13474|DRP2_HUMAN	1	2	Dystrophin-related protein 2 OS=Homo sapiens GN=DRP2 PE=2 SV=2	0.84	1.35	0.62
sp|Q8N8J6|ZN615_HUMAN, sp|Q9GZX5|ZN350_HUMAN	0.9987	2	Zinc finger protein 615 OS=Homo sapiens GN=ZNF615 PE=1 SV=2,Zinc finger protein 350 OS=Homo sapiens GN=ZNF350 PE=1 SV=2	0.58	0.93	0.62
sp|P23258|TBG1_HUMAN, sp|Q9NRH3|TBG2_HUMAN	0.9999	2	Tubulin gamma-1 chain OS=Homo sapiens GN=TUBG1 PE=1 SV=2,Tubulin gamma-2 chain OS=Homo sapiens GN=TUBG2 PE=1 SV=1	0.59	0.95	0.62
sp|P05186|PPBT_HUMAN	0.9556	1	Alkaline phosphatase, tissue-nonspecific isozyme OS=Homo sapiens GN=ALPL PE=1 SV=4	0.71	1.14	0.63
sp|Q6UB98|ANR12_HUMAN	0.9999	2	Ankyrin repeat domain-containing protein 12 OS=Homo sapiens GN=ANKRD12 PE=1 SV=3	0.79	1.24	0.63
sp|O43296|ZN264_HUMAN	0.9989	2	Zinc finger protein 264 OS=Homo sapiens GN=ZNF264 PE=1 SV=1	0.58	0.9	0.64
sp|Q92611|EDEM1_HUMAN	0.9999	3	ER degradation-enhancing alpha-mannosidase-like 1 OS=Homo sapiens GN=EDEM1 PE=1 SV=1	0.87	1.35	0.64
sp|Q9HC52|CBX8_HUMAN	0.9999	2	Chromobox protein homolog 8 OS=Homo sapiens GN=CBX8 PE=1 SV=3	0.63	0.97	0.65
sp|Q14573|ITPR3_HUMAN	0.9992	2	Inositol 1,4,5-trisphosphate receptor type 3 OS=Homo sapiens GN=ITPR3 PE=1 SV=2	0.62	0.95	0.65
sp|Q8WXI7|MUC16_HUMAN	1	12	Mucin-16 OS=Homo sapiens GN=MUC16 PE=1 SV=2	0.65	1	0.65
sp|Q9NP78|ABCB9_HUMAN	1	2	ATP-binding cassette sub-family B member 9 OS=Homo sapiens GN=ABCB9 PE=1 SV=1	0.73	1.12	0.65
sp|Q8TDW7|FAT3_HUMAN	1	6	Protocadherin Fat 3 OS=Homo sapiens GN=FAT3 PE=2 SV=2	0.79	1.21	0.65
sp|Q9P265|DIP2B_HUMAN	1	3	Disco-interacting protein 2 homolog B OS=Homo sapiens GN=DIP2B PE=1 SV=3	0.79	1.21	0.65
sp|A6NES4|YB046_HUMAN	1	2	Protein LOC339766 OS=Homo sapiens PE=4 SV=2	0.56	0.85	0.66
sp|Q5T7J7|TDR12_HUMAN	0.999	1	Tudor domain-containing protein 12 OS=Homo sapiens GN=TDRD12 PE=2 SV=2	0.74	1.12	0.66
sp|Q5JZY3|EPHAA_HUMAN	0.999	1	Ephrin type-A receptor 10 OS=Homo sapiens GN=EPHA10 PE=2 SV=1	0.69	1.06	0.66
sp|Q9H344|O51I2_HUMAN	0.9998	2	Olfactory receptor 51I2 OS=Homo sapiens GN=OR51I2 PE=2 SV=1	0.68	1.03	0.66
sp|Q16851|UGPA_HUMAN	1	2	UTP---glucose-1-phosphate uridylyltransferase OS=Homo sapiens GN=UGP2 PE=1 SV=5	0.9	1.37	0.66
sp|P62508|ERR3_HUMAN	0.9965	1	Estrogen-related receptor gamma OS=Homo sapiens GN=ESRRG PE=1 SV=1	0.74	1.11	0.67

Table 3. Gene Ontology Analysis of the Differentially Expressed Proteins: the 32 Terms that are Significantly Enriched (p<0.05) by Molecular Function

Term	Count	%	PValue	Genes
Extracellular matrix structural constituent	9	2.786	1.71E-04	Q01955, Q14055, Q17RW2, P15502, Q6W4X9, Q14993, Q16363, Q8IZC6, P02462
Adenyl nucleotide binding	48	14.86	4.79E-04	Q15477, Q9C0G6, Q86WJ1, Q8WZ42, P54132, Q01973, P31512, Q06418, P09622, O95398, Q02763, Q08211, Q58FF7, Q9NRK6, Q2VIQ3, Q96AC6, O95622, Q5JZY3, P51575, Q9UPY3, O75914, Q02880, Q9Y616, P59047, Q9NP78, Q9NQ11, P41250, Q6JQN1, Q96J66, Q16720, P16219, Q96MN2, P33992, P06576, Q13085, Q13535, O43933, P42356, O14994, Q04912, Q9NZJ5, Q9BXU1, P14616, Q8TD57, Q86UK0, P40937, Q96L91, Q96Q04
Purine nucleoside binding	48	14.86	6.66E-04	Q15477, Q9C0G6, Q86WJ1, Q8WZ42, P54132, Q01973, P31512, Q06418, P09622, O95398, Q02763, Q08211, Q58FF7, Q9NRK6, Q2VIQ3, Q96AC6, O95622, Q5JZY3, P51575, Q9UPY3, O75914, Q02880, Q9Y616, P59047, Q9NP78, Q9NQ11, P41250, Q6JQN1, Q96J66, Q16720, P16219, Q96MN2, P33992, P06576, Q13085, Q13535, O43933, P42356, O14994, Q04912, Q9NZJ5, Q9BXU1, P14616, Q8TD57, Q86UK0, P40937, Q96L91, Q96Q04
Calcium ion binding	32	9.907	7.03E-04	Q14573, Q96RW7, Q8WZ42, P19827, Q13474, Q7Z7M0, Q9Y251, Q9BXS9, P05976, Q6P587, P12830, P06741, Q03692, Q9Y6V0, Q8TDW7, P00734, P51571, Q86XX4, Q02641, Q9BSW7, P12259, Q92736, Q16720, P06576, O14815, Q9BZD6, O14994, P12829, P20702, O43497, Q92611, Q8NI22, P13611
Nucleoside binding	48	14.86	7.72E-04	Q15477, Q9C0G6, Q86WJ1, Q8WZ42, P54132, Q01973, P31512, Q06418, P09622, O95398, Q02763, Q08211, Q58FF7, Q9NRK6, Q2VIQ3, Q96AC6, O95622, Q5JZY3, P51575, Q9UPY3, O75914, Q02880, Q9Y616, P59047, Q9NP78, Q9NQ11, P41250, Q6JQN1, Q96J66, Q16720, P16219, Q96MN2, P33992, P06576, Q13085, Q13535, O43933, P42356, O14994, Q04912, Q9NZJ5, Q9BXU1, P14616, Q8TD57, Q86UK0, P40937, Q96L91, Q96Q04
Purine nucleotide binding	54	16.72	0.0012	Q15477, Q86WJ1, Q01973, O95398, Q02763, Q08211, Q9NRK6, O95622, P51575, Q9P0V9, O75914, Q02880, P59047, Q9NQ11, P23258, P41250, Q5H913, Q6JQN1, P16219, Q96MN2, O43933, P42356, O14994, Q8TD57, Q86UK0, Q96L91, Q9C0G6, Q8WZ42, Q96N67, P54132, P31512, Q06418, P09622, Q9NRH3, Q58FF7, Q2VIQ3, Q96AC6, Q5JZY3, Q9UPY3, Q9Y616, Q8WXF7, Q9NP78, Q96J66, Q16720, P33992, P06576, Q13085, Q13535, Q9NZJ5, Q04912, Q9BXU1, P14616, P40937, Q96Q04
ATPase activity	16	4.954	0.0012	Q15477, Q9C0G6, Q86WJ1, Q9NP78, Q9NQ11, P54132, Q96J66, Q16720, P06576, O43933, Q08211, Q9NRK6, Q8TD57, Q86UK0, P40937, Q9UPY3
Adenyl ribonucleotide binding	44	13.62	0.0018	Q15477, Q9C0G6, Q8WZ42, Q86WJ1, P54132, Q01973, Q06418, O95398, Q02763, Q08211, Q58FF7, Q9NRK6, Q2VIQ3, Q96AC6, O95622, Q5JZY3, P51575, Q9UPY3, O75914, Q02880, Q9Y616, P59047, Q9NP78, Q9NQ11, P41250, Q96J66, Q16720, Q96MN2, P33992, P06576, Q13085, Q13535, O43933, P42356, O14994, Q04912, Q9NZJ5, Q9BXU1, P14616, Q8TD57, Q86UK0, P40937, Q96L91, Q96Q04
ATP binding	43	13.31	0.0024	Q15477, Q9C0G6, Q8WZ42, Q86WJ1, P54132, Q01973, Q06418, Q02763, Q08211, Q58FF7, Q9NRK6, Q2VIQ3, Q96AC6, O95622, Q5JZY3, P51575, Q9UPY3, O75914, Q02880, Q9Y616, P59047, Q9NP78, Q9NQ11, P41250, Q96J66, Q16720, Q96MN2, P33992, P06576, Q13085, Q13535, O43933, P42356, O14994, Q04912, Q9NZJ5, Q9BXU1, P14616, Q8TD57, Q86UK0, P40937, Q96L91, Q96Q04
Purine ribonucleotide binding	50	15.48	0.0038	Q15477, Q86WJ1, Q01973, O95398, Q02763, Q08211, Q9NRK6, O95622, P51575, O75914, Q9P0V9, Q02880, P59047, Q9NQ11, P23258, P41250, Q5H913, Q96MN2, O43933, P42356, O14994, Q8TD57, Q86UK0, Q96L91, Q9C0G6, Q8WZ42, Q96N67, P54132, Q06418, Q9NRH3, Q58FF7, Q2VIQ3, Q96AC6, Q5JZY3, Q9UPY3, Q9Y616, Q8WXF7, Q9NP78, Q96J66, Q16720, P33992, P06576, Q13085, Q13535, Q9NZJ5, Q04912, Q9BXU1, P14616, P40937, Q96Q04

(NRAM1).

The western blot result of APOA4 verified the quantitative proteomic results. APOA4 protein exhibited a good stability for individuals in each group. In the proteomic profiling, APOA4 protein showed 0.71 and 1.18 in OC and BT sera, respectively, compared with the value of 1 in HC. The western blot result for the protein was consistent with the profiling result. The mean grey level values of the OC, BT, and HC groups were 0.54, 0.83, and 0.61, respectively (Figure 1).

Based on the western blot result, NRAM1 protein was roughly consistent with the profiling result but was not very acceptable at the stability in individual. It fluctuated for each serum sample in each group (Figure 2).

Discussion

Recently, proteomic profiling of serum or plasma has been widely performed to discover cancer biomarkers (Amon et al., 2010; Gao et al., 2011; Qiu et al., 2011). In OC proteomic research, Amon et al. found 54 quantified serum proteins and 358 peritoneal fluid proteins, in which 17 proteins are quantified in both materials and 14 are extracellular (DeSouza et al., 2005; Wu et al., 2006; Amon et al., 2010). Pan et al. demonstrated that extracellular matrix proteins, catenins, and P53 binding protein 1 are important for chemotherapy response in OCs (Pan et al., 2009).

One successful aspect of Amon's study is the design of the serum experiment, which included three different types of subjects: OC patients, BT patients, and healthy volunteers. In the aforementioned research, proteins up-regulated in cancer relative to the healthy but not relative to the benign were eliminated from consideration. The same was true for proteins up-regulated relative to the benign but not to the healthy. Only proteins in the cancer versus the benign comparison became the key point (Amon et al., 2010). In the present study, we used a similar strategy to focus on differentially expressed proteins in the comparison between OC and BT, both having elevated CA-125. Our goal was to identify proteins to differentiate malignant from benign tumors in serum with elevated CA-125. We obtained 80 overexpressed and 44 underexpressed proteins in the comparison (Table 2) between OC and BT. For further validation, we selected two important proteins among the differential proteins: APOA4 and NRAM1.

APOA4 is a member of the APOA1/C3/A4/A5 gene cluster located on the long arm of the human chromosome 11 (Lai et al., 2005; Dieplinger et al., 2009). Members of this cluster are all involved in lipid and lipoprotein metabolism and thus in many ways associated with cardiovascular disease. APOA4 is a 46 kDa glycoprotein that is almost exclusively produced in intestinal enterocytes and secreted into the lymph. APOA4 was first identified as a component of chylomicrons and high-density lipoproteins (Beisiegel and Utermann, 1979). Dieplinger found that APOA4 has a decreasing tendency from the sera in HC to those in BT and OC (Dieplinger et al., 2009). In the present study, we observed a decreased APOA4 in the OC serum both in the results of proteomic profiling and western blot. However, we observed an elevated APOA4 (1.18 in the BT serum in the proteomic profiling) in the BT serum along with elevated CA-125. This result is consistent with the data of Dieplinger and other previous studies. The protein validation of APOA4 in the BT serum through western blot was consistent with the profiling result, with a mean grey level value of 0.83, compared with the value of 0.61 for HC. At present, the reason for the regulations of APOA4 in the serum of OC or BT is still unknown. However, the regulations of APOA4 in OC and BT made it possible to be a marker for malignant and benign tumor differentiation in serum with elevated CA-125.

NRAMP1 protein, also named the solute carrier family 11 member 1 protein, is localized to the acidic endosomal and lysosomal compartment of the macrophage (Searle et al., 1998). NRAMP1 exerts pleiotropic effects on macrophage function, including enhanced chemokine KC, tumor necrosis factor-a, interleukin-1b, inducible nitric oxide synthase, and MHC class II expression. All of which are important in the induction and maintenance of autoimmunity and cancer as well as in the resistance to intramacrophage pathogens, such as tuberculosis (Awomoyi, 2007). Under normal physiological conditions, NRAMP1 delivers bivalent metal cations from the cytosol into acidic late endosomal and lysosomal compartment where Fenton and/or Haber–Weiss reactions generate toxic antimicrobial radicals for direct antimicrobial activity

against phagocytosed microorganisms (Goswami et al., 2001). However, prolonged accumulation of toxic radicals can have detrimental effects, causing cell or tissue damage and contributing to the development or progression of numerous diseases, including cancer and autoimmunity. Toxic radicals generated due to macrophage activation in response to stimuli are reactive oxygen (generically referred to as oxidants: superoxide, hydrogen peroxide, hypochlorous acid, singlet oxygen, and hydroxyl radical) and nitrogen (nitric oxide by the inducible nitric oxide synthase) intermediates (Schumacker, 2006). In humans, there are two predominant NRAMP1 (GT) n Z-DNA promoter alleles: allele 2 and allele 3. These alleles have been reported to account for opposing levels of NRAMP1 gene expression; that is, allele 3 drives high gene expression, whereas allele 2 drives low gene expression (Searle and Blackwell, 1999). Allele 3 is associated with autoimmunity and cancer but protects against infectious diseases, whereas allele 2 is associated with infections, such as tuberculosis, but protects against autoimmunity and cancer (Searle and Blackwell, 1999). These observations suggest that chronic hyperactivation of macrophages associated with allele 3 is functionally linked to cancer and autoimmune disease susceptibility, whereas the poor level of NRAMP1 expression promoted by allele 2 contributes to infectious disease susceptibility (Searle and Blackwell, 1999).

In the present proteomic profiling, NRAM1 protein was elevated in OC (1.27) and decreased in BT (0.67). This result is consistent with previous studies. The western blot verification on the protein in the sera showed that the protein was roughly consistent with the profiling result but was unstable on the value in individuals and fluctuated for each individual serum sample in each group. The reason for this phenomenon needs further investigation.

In summary, the quantitative proteomic profiling found on the serum of women with elevated CA-125 helped identify proteins that can differentiate malignant from benign ovarian tumors. A novel protein, APOA4, has the potential to be a marker for malignant and benign tumor differentiation in serum with elevated CA-125.

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