Sains Malaysiana 50(12)(2021): 3667-3681
http://doi.org/10.17576/jsm-2021-5012-17

Proteomic Profile Mapping and Differential Expression of Protein in Ovarian Cancer

(Pemetaan Profil Proteomik dan Ungkapan Pembezaan Protein dalam Kanser Ovari)

AMBREEN TAUSEEF, ASIMA KARIM, GULFAM AHMAD, QURRATULANN AFZA GARDNER & MUHAMMAD WAHEED AKHTAR*

ABSTRACT

This study aimed to characterize differentially expressed proteins in malignant ovarian tissue to find out potential novel biomarkers in ovarian cancer (OC). We enrolled 20 ovarian cancer patients (40-65 years) and an equal number of age-matched healthy women to get malignant and healthy ovarian tissue samples for protein extraction and quantification after tissue lysis. The protein profile was analyzed using two-dimensional gel electrophoresis followed by MALDI-TOF mass spectrometry. Based on the information thus obtained, the proteins were identified using the relevant software and protein databank to analyze the malignant and non-malignant ovarian tissue samples (n = 20/group). In this proteomic analysis of the ovarian tissue, 112 proteins were detected. Based on a minimum of ≥ 1.5-fold expression difference (p-value ≤ 0.05; FDR ≤ 0.05 and PMF ≥ 79), 17 proteins were found to be upregulated while 27 were downregulated in the malignant ovarian tissue. Six of these proteins have not been previously reported in ovarian cancer. Out of these, three are upregulated while the other three are downregulated. The upregulated proteins are centrosomal protein of 290 kDa (Cep290), uncharacterized protein C1orf109 (C1orf109) and GTPase-activating Rap/Ran-GAP domain-like protein 3 (GARNL3), and the three downregulated proteins identified are actin-related protein 3 (ARP3), cytosolic carboxypeptidase 3 (AGBL3) and NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 10 (NDUFA10). This proteomic mapping not only provides data on protein profiling of ovarian cancer in Pakistani population for the first time but also reports six novel differentially expressed proteins, which have not been previously reported in ovarian cancer patients. They may serve as potential novel biomarkers after further validation for early diagnosis and prognosis of ovarian cancer. It also provides additional data to improve existing knowledge of already reported protein ovarian cancer biomarkers.

Keywords: Mass spectrometry; ovarian cancer; proteomics; two-dimensional gel electrophoresis

ABSTRAK

Kajian ini bertujuan untuk mencirikan protein yang diekspreskan secara berbeza dalam tisu ovari ganas untuk mengetahui potensi penanda biologi baru dalam barah ovari (OC). Kami mendaftarkan 20 pesakit barah ovari (40-65 tahun) dan sebilangan wanita sihat yang sesuai dengan usia untuk mendapatkan sampel tisu ovari yang ganas dan sihat untuk pengambilan dan pengukuran protein setelah lisis tisu. Profil protein dianalisis menggunakan elektroforesis gel berdimensi dua diikuti dengan jisim spektrometri MALDI-TOF. Berdasarkan maklumat yang diperoleh, protein dikenal pasti menggunakan perisian dan pangkalan data protein yang relevan untuk menganalisis sampel tisu ovari yang ganas dan tidak ganas (n = 20/kumpulan). Dalam analisis proteomik tisu ovari ini, 112 protein dikesan. Berdasarkan perbezaan ekspresi minimum ≥ 1.5 gandaan perbezaan ungkapan (nilai p ≤ 0.05; FDR ≤ 0.05 dan PMF ≥ 79), 17 protein didapati diatur secara berlebihan sementara 27 diatur dengan lebih rendah pada tisu ovari malignan. Enam daripada protein ini belum pernah dilaporkan terkena kanser ovari. Daripada jumlah tersebut, tiga diatur lebih tinggi, sementara tiga yang lain diatur. Protein yang diatur adalah protein sentrosom 290 kDa (Cep290), protein C1orf109 yang tidak dicirikan (C1orf109 dan protein seperti domain 3/Rap-Ran-GAP yang mengaktifkan GTPase (GARNL3) dan tiga protein yang tidak terkawal yang dikenal pasti adalah protein yang berkaitan dengan aktin 3 (ARP3), sitosolik karboksipeptidase 3 (AGBL3) dan NADH dehidrogenase [ubiquinone] 1 subkompleks subunit alpha 10 (NDUFA10). Pemetaan proteomik ini tidak hanya memberikan data mengenai profil protein kanser ovari pada populasi Pakistan untuk pertama kalinya, tetapi juga melaporkan enam protein yang dinyatakan secara berbeza, yang sebelumnya tidak pernah dilaporkan pada pesakit barah ovari. Mereka boleh menjadi penanda biologi baru yang berpotensi setelah pengesahan lebih lanjut untuk diagnosis awal dan prognosis kanser ovari. Ia juga memberikan data tambahan untuk meningkatkan pengetahuan sedia ada mengenai penanda biologi kanser ovari protein yang sudah dilaporkan.

Kata kunci: Elektroforesis gel berdimensi dua; jisim spektrometri; kanser ovari; proteomik

Introduction

Ovarian cancer is a silent killer and the most lethal gynecological cancer. It is a highly prevalent disease with a rising incidence of 42.4% amongst all gynecological malignancies. Worldwide, it is the fifth most commonly occurring female cancer and the fourth leading cause of death because of female pelvic malignancy. In Asian countries, identified new cases of ovarian cancer were 110,526 whereas Pakistan was among the first five countries with 3703 identified cases (Jemal et al. 2008). Ovarian cancer affects all age groups from young girls to post-menopausal (Razi et al. 2016). However, the mean age of ovarian cancer presentation is estimated to be 49.2 ± 13 years (Momenimovahed et al. 2019).

In Pakistan, this cancer is emerging with an increasing incidence as well. It has been ranked the fourth most common malignancy in the general population and stands among the first three common female malignancies with an incidence rate of 13.6% (Hashmi et al. 2016). The dilemma of the disease is associated with its identification since > 70% of patients are diagnosed at their late stages (Bhurgri et al. 2011).

One of the biggest challenges in the diagnosis of ovarian cancer is its poorly understood etiology. It has multifactorial associations with hormonal, genetic, and reproductive aspects (Badar & Mahmood 2017). Owing to the absence of specific signs or symptoms during the initial stages of the disease and the unavailability of robust early screening and diagnostic tests, most of the ovarian tumors (79.2%) are identified at advanced stages (III, IV) with high mortality rate (Girolimetti et al. 2014). As explained by the International Federation of Gynecology and Obstetrics (FIGO), if this cancer is detected at an early stages (I, II), five-year survival rates can be achieved in 90 to 95% of cases whereas, it is only 28 and 10% for advanced stages (III, IV) respectively. Hence, the timely diagnosis of cancer at early stages is critical for its fundamental control and successful treatment (Mostafa et al. 2012).

Currently accepted and widely used serum biomarker, Cancer Antigen -125 (CA-125) shows inadequate sensitivity and specificity for early-stage diagnosis (Homburg 2008). Therefore, there is an utmost need to seek early-stage protein biomarker/s through a proteomic-based approach. An algorithm measuring the risk of this malignancy, based on serial CA125 values predicted that about 20% of ovarian cancers showed either minimum or absent expression of CA-125 (Visintin et al. 2008). This gives the insight and enhances the significance of additional serum biomarker exploration, which will be capable enough to substitute or complement CA-125 for accurate detection and prompt management of ovarian cancer at the beginning of its process.

This study identifies and compares the proteomic profile between human malignant and non-malignant ovarian tissue samples and determines the potential for nominated protein biomarkers, which could serve as more specific and sensitive new diagnostic, prognostic, and therapeutic markers for this deadly disease.

Materials and Methods

This cross-sectional comparative study was conducted after getting approval from the Institutional Ethical Review Committee (UHS/REG-18/ERC/2625). This study population consisted of 20 ovarian cancer patients (40-65 years) and an equal number of age-matched healthy women scheduled for hysterectomy with bilateral salpingo-oophorectomy for reasons other than ovarian cancer. All involved registered participants from Jinnah and Services hospitals, Lahore, Pakistan, during December 2018 - January 2020, signed the written informed consent before sample collection. Based on medical records and history, participants with known conditions of pregnancy, Cushing's disease, adrenal hyperplasia, thyroid disease, hyperprolactinemia, diabetes mellitus, and benign ovarian cyst/s, any other malignancy, hepatitis B and C, and patients on chemotherapy were excluded from this study.

Tissue samples

Written informed consent to participate in the research was obtained from each participant. Immediately after surgical resection, a representative portion of the freshly excised malignant as well as healthy ovarian tissues were taken from the operation theater, immediately snapped frozen in liquid nitrogen, and then stored at -80 °C for future proteomic analysis. A histopathological report was obtained for each malignant ovarian tissue specimen for the confirmation of cancer staging (stage I-IV) and for each healthy ovarian tissue sample to ensure that they are free from any tumor cells.

Tissue protein extraction

The frozen tissue samples (0.5 - 1.0 g) were cut into small pieces and submerged into liquid nitrogen to solidify the tissue. The tissue was grounded in mortar and pestle to make a fine powder. Chilled lysis buffer (7 mol/L urea, 2 mol/L thiourea, 65 mmol/L dithiothreitol (DTT), 4% cholamidopropyldimethylammonia-propanesulfate (CHAPS), 2% servalyte) was added to the tissue and incubated on ice for 5 min. The homogenized mixture

was centrifuged at 14,000 rpm for 30 min at 4 °C and the supernatant was aliquoted. Protein concentration was estimated, using bovine serum albumin as standard by Bradford assay. Based on the total protein concentration, ~20 µg, sample protein was loaded to run the SDS-polyacrylamide gel electrophoresis (PAGE) to interpret the total protein content of the whole tissue lysates.

TWO-DIMENSIONAL GEL ELECTROPHORESIS

First-Dimensional isoelectric focusing (IEF) was carried out by loading of 18 cm, pH 3-10 immobilized linear pH gradient strips (Serva Electrophoresis, Heidelberg, Germany) with tissue lysate (800 µg total protein) and rehydration buffer (320 µL) and passively rehydrating them for 14 - 16 h at 20 °C (Biorad Protean 1-12 IEF cell). After equilibration steps, the strips were transferred onto 1.0 mm thick 12% polyacrylamide gels for the second dimension SDS PAGE analysis. The gels were kept in a fixative solution (30% ethanol and 10% acetic acid) overnight, stained first with Coomassie colloidal blue dye, and then destained in deionized water.

IDENTIFICATION OF DIFFERENTIALLY EXPRESSED SPOTS

After automatic background correction and spot normalization of two-dimensional PAGE, on average 381 ± 69 protein gel spots in normal and 406 ± 72 spots in ovarian cancer tissues were detected, respectively. Only reproducible protein spots obtained from these healthy and tumor ovarian tissues were selected and analyzed further by two-dimensional gel image analysis software Dymension (v.3.0) by Syngene (Synoptics Ltd, Cambridge, UK). To pick statistically significant gel spots, the estimation of expression change was quantified by measuring the fold change of individual spots in both groups (normal vs tumor) followed by false discovery rate (FDR) analysis (Bast et al. 2005).

IN-GEL TRYPTIC DIGESTION

The gel spots, which stained differentially between normal and malignant ovarian tissue were excised and transferred to labeled eppendorf tubes. After de-staining with 50 mM Ammonium Bicarbonate (AmBic) and 100% Acetonitrile (ACN) for 45 min at 37 °C. All gel spots were reduced in 10 mM dithiothreitol (DTT) prepared in 5 mM AmBic solution in each eppendorf at 55 °C for 30 min. Following adding 100% ACN and drying, the pellets were alkylated with 55 mM Iodoacetamide (IAM) solution at 37 °C for 30 min. After washing with AmBic and ACN and drying in a vacuum concentrator, 30 µL of sequencing grade trypsin (Promega, USA) was added to these gel pieces and incubated overnight at 37 °C. Supernatant was collected and the tryptic digests were extracted twice after adding 50 µL of 0.1% Trifluoroacetic acid (TFA), and 100 µL of ACN, concentrated and stored for further MALDI-TOF analysis (Diz et al. 2011).

MALDI-TOF MASS SPECTROMETRY

Tryptic digests (0.5 - 1 µL) were spotted on the target plate by mixing 1 µL of α-cyano-4-hydroxycinnamic acid (CHCA, Sigma-Aldrich), prepared 0.1% TFA (100 µL) and 100% ACN (50 µL) and allowed to air dry at room temperature. After complete drying, mass spectra were measured by using Autoflex™ MALDI-TOF/TOF mass spectrometer (Bruker Daltonics, Bremen, Germany). All spectra were recorded in the m/z range of 600 - 4000. Under reflection positive mode, the spectra recorded were analyzed in Bruker Daltonics flexAnalysis software (v.3.0) and the data was converted into mzXML format. The peak lists were explored using the MASCOT search engine (http://www.matrixscience.com) with the following peptide mass fingerprinting (PMF) parameters: SWISS-PROT (database); Homo Sapiens (Taxonomy); trypsin (enzyme); peptide mass tolerance: 50 - 200 ppm; one missed cleavage; cysteine carboamidomethylation (fixed modifications) and methionine oxidation (variable modifications). All protein identifications were assessed by using reproducible spots from all gels and the score hits with the highest MASCOT were sorted out with a p-value ≤ 0.05.

STATISTICAL ANALYSIS

Data analysis was done by using SPSS version 25. The expression change (over/under-expressed) was quantified by the fold change. All those protein spots that showed at least ≥ 1.5 fold change difference in expression/staining and FDR ≤ 0.05 were evaluated by statistical analysis. Mann-Whitney U test was applied to all spot intensities. A statistically significant p-value and false discovery rate (FDR) were set at ≤ 0.05. The protein identification confidence limit was considered at a 95% confidence interval (CI). The sample size was calculated by using World Health Organization (WHO) calculator as follows:

$$n = \frac{2\sigma^2 (z_{1-\alpha/2} + z_{1-\beta})^2}{(\mu_1 - \mu_2)^2}$$

by taking a difference between two means of expressed proteins in each group (control and ovarian cancer).

Population standard deviation = 6.5; Population variance = 42.25; Difference between two population means = 6.9; Sample size (n) = 20 for each group.

Results

Ovarian tissue samples of the malignant group (mean age, 53.5 ± 12.5) and controls (mean age, 46.5 ± 10.5) were processed by two-dimensional gel electrophoresis for the determination of overall profiling and their differential expression. On average, 110 ± 4.3 reproducible spots in normal and 112 ± 2.4 in tumor samples were marked, excised, and subjected to MALDI-TOF analysis (Figure 1). All these proteins were labeled based on their molecular weight (MW), isoelectric point (pI), and the number of matched peptides against the searched peptides. The other parameters, i.e. sequence coverage percentage; MASCOT accession number, and genes were also considered (Table 1).

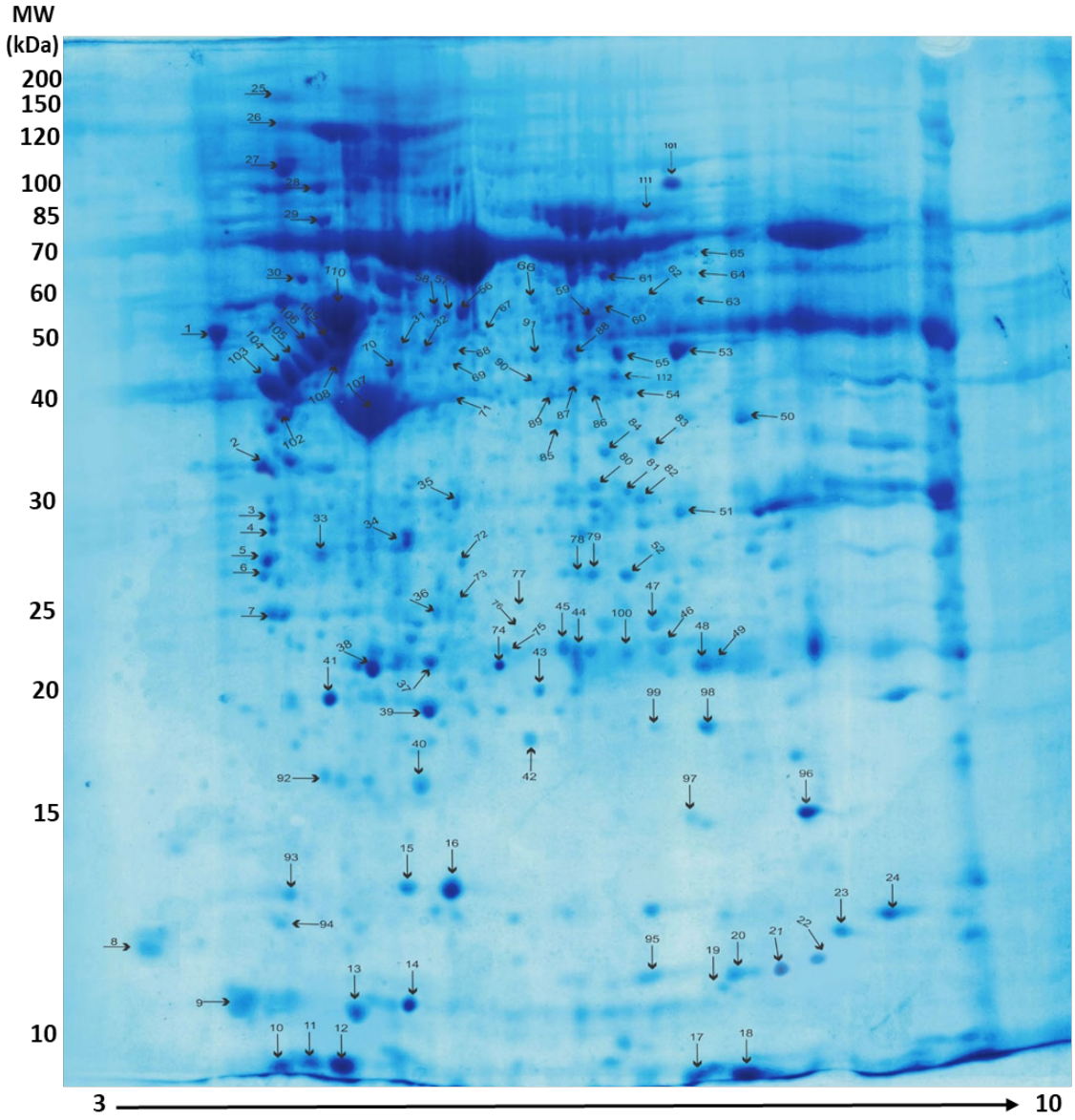

FIGURE 1. Two-dimensional gel electrophoresis proteomic profile of malignant ovarian tissue (stage II). Protein spots mapped were identified by MALDI-TOF MS showing pH range 3-10 on 1st dimension and separation with 12% SDS PAGE on 2nd dimension. Spot numbers shown here reflect the numbers listed for the proteins identified in Table 1

TABLE 1. Identified proteins by MALDI-TOF MS in ovarian cancer tissue

Spot No	Protein identified	Gene	Accession number	Locus	A	B	C	D	E	F
1	Calreticulin (CALR)	CALR	P27797	CALR_HUMAN	47	45	8	27	48.28	4.29
2	Peroxiredoxin-2 (PRDX2)	PRDX2	P32119	PRDX2_HUMAN	110	20	9	34	22.04	5.06
3	Annexin A4 (ANXA4)	ANXA4	P09525	ANXA4_HUMAN	55	12	5	24	33.75	4.64
4	Tropomyosin alpha 1 (TPM1)	TPM1	P09493	TPM1_HUMAN	72	31	9	31	32.82	4.7
5	Tropomyosin alpha-4 chain (TPM4)	TPM4	P67936	TPM4_HUMAN	59	39	10	33	28.64	4.67
6	14-3-3 Epsilon (14-3-3E)	YWHAZ	P62258	1433E_HUMAN	73	31	9	32	29.32	4.63
7	14-3-3 protein zeta/delta	YWHAZ	P63104	1433Z_HUMAN	80	27	9	38	27.89	4.73
8	Follistatin fragment(FS)	FST	P19883	FST_HUMAN	54	20	6	37	24.81	3.7
9	Myosin light polypeptide 6	MYL6	P60660	MYL6_HUMAN	58	20	6	46	17.21	4.56
10	Actin-related protein 2/3 complex subunit 3 (ARPC3)	ARPC3	O15145	ARPC3_HUMAN	49	26	5	36	9.8	5.61
11	Poly [ADP-ribose] polymerase (PARP-2)	PARP2	Q9UGN5	PARP2_HUMAN	53	36	5	36	10.5	5.7
12	Galectin-1 (Gal-1)	LGALS1	P09382	LEG1_HUMAN	64	45	7	54	15.04	5.34
13	Retinol Binding Protein (CRBP-1)	RBP1	P09455	RET1_HUMAN	87	45	10	59	11	4.64
14	Transthyretin (ATTR)	TTR	P02766	TTHY_HUMAN	103	29	9	69	16	5.52
15	Ferritin light chain (FTL)	FTL	P02792	FRIL_HUMAN	60	10	4	28	20.06	5.51
16	Myelin expression factor 2	MYEF2	Q9P2K5	MYEF2_HUMAN	61	31	7	39	20.60	5.36
17	Hemoglobin subunit beta (HBB)	HBB	P68871	HBB_HUMAN	91	32	9	74	16.1	6.75
18	Hemoglobin subunit beta HBB)	HBB	P68871	HBB_HUMAN	88	40	10	75	16.1	6.75
19	Myosin-6	MYH6	P13533	MYH6_HUMAN	48	26	17	14	22.43	5.58
20	E3 ubiquitin-protein ligase HUWE1	HUWE1	Q7Z6Z7	HUWE1_HUMAN	42	14	4	32	24.26	5.21
21	Peptidyl-prolyl Cis-trans isomerase A (PPIA)	PPIA	P62937	PPIA_HUMAN	80	38	9	58	18.22	6.68
22	A-kinase anchor protein 1, mitochondra (AKAP)	AKAP1	Q92667	AKAP1_HUMAN	54	60	8	32	20.12	7.01
23	Peptidyl-prolyl Cis-trans isomerase A (PPIA)	PPIA	P62937	PPIA_HUMAN	55	29	5	43	18.22	6.88
24	Phosphatidylethanolamine binding protein (PEBP-1)	PEBP1	P30086.3	PEBP1_HUMAN	106	27	9	50	21.15	7.01
25	Isoform 2 of collagen alpha-1	COL14A1	Q05707	COEA1_HUMAN	206	55	36	22	192.86	4.22
26	Heat shock protein HSP 90 alpha	HSP90AA1	P07900	HS90A_HUMAN	122	45	21	32	85	4.04
27	Endoplasmin	HSP90B1	P14625	ENPL_HUMAN	88	26	13	18	86.32	4.76
28	Centrosomal protein of 290 kDa (Cep290)	CEP290	O15078	CE290_HUMAN	65	20	8	55	94.42	5.09
29	Endoplasmic reticulum chaperone BiP (HSPA5)	HSPA5	P11021	BIP_HUMAN	148	32	19	33	72.4	4.07
30	Vimentin	VIM	P08670	VIME_HUMAN	114	39	17	35	54	4.06

31	Isoform Gamma A of fibronogen gamma chain (FGG)	FGG	P02679	FIBG_HUMAN	72	32	9	26	50.09	5.7
32	Actin-related protein 3 (ACTR3)	ACTR3	P61158	ARP3_HUMAN	70	50	11	32	48	5.61
33	Annexin A5 (ANXA5)	ANXA5	P08758	ANXA5_HUMAN	156	53	19	57	35.97	4.94
34	Isoform 2 of Ras-related protein Rab-36	RAB36	O95755	RAB36_HUMAN	47	36	6	33	34.4	5.46
35	Clathrin light chain A (CLTA)	CLTA	P09496	ZN189_HUMAN	69	35	10	24	32	5.12
36	APC membrane recruitment protein (fragment)	AMER3	Q8N944	AMER3_HUMAN	52	81	10	50	20	6.02
37	Heat shock protein beta -1 (HSPB1)	HSPB1	P04792	HSPB1_HUMAN	88	45	10	48	22.82	5.98
38	Apolipoprotein A-I (Apo-A1)	APOA1	P02647.1	APOA1_HUMAN	100	59	19	56	31	5.56
39	Zinc finger protein 345	ZNF345	Q14585	ZN345_HUMAN	60	32	9	31	37.32	6.28
40	Peroxiredoxin-2 (PRDX2)	PRDX2	P32119	NM_005809	100	25	9	35	22.04	6.06
41	Rho GDP-dissociation inhibitor 1 (Rho GDI-1))	ARHGDIA	P52565	GDIR1_HUMAN	73	27	9	38	21.56	5.37
42	Parkinson disease protein 7	PARK7	Q99497	PARK7_HUMAN	72	27	8	49	20.05	6.33
43	Glutathione S transferase Mu 2 (GSTM2-2)	GSTM2	P28161	GSTM2_HUMAN	95	31	12	59	26	6
44	Glutathione S-transferase Mu 1 (GSTM-1)	GSTM1	P09488	GSTM1_HUMAN	66	39	11	50	26	6.24
45	Isoform Enoyl-CoA delta isomerase 1, mitochondrial	ECI1	P42126	ECI1_HUMAN	68	35	8	34	31.1	7.07
46	Triosephosphate isomerase (TIM)	TPI1	P60174	TPIS_HUMAN	193	47	19	76	31.05	7.65
47	Phosphoglycerate mutase 1 (PGAM-1)	PGAM1	P18669	PGAM1_HUMAN	110	43	14	51	28.90	6.67
48	Putative uncharacterized protein encoded by LINC02694	LINC02694	Q8NAA6	CO053_HUMAN	40	25	4	27	20.12	7.8
49	Hemoglobin subunit beta	HBB	P68871	HBB_HUMAN	112	40	10	81	16.1	6.75
50	Lysosomal acid phosphatase	ACP2	P11117	PPAL_HUMAN	52	19	7	21	48.71	6.28
51	Annexin A2 (ANXA-2)	ANXA2	P07355	ANXA2_HUMAN	214	32	19	69	34.56	6.91
52	Pulmonary surfactant-associated protein D	SFTPD	P35247	SFTPD_HUMAN	63	33	11	45	38.04	6.25
53	Alpha-enolase	ENO1	P06733	ENOA_HUMAN	168	41	18	50	48	6.57
54	Isocitrate Dehydrogenase [NADP]cytoplasmic (IDH)	IDH1	O75874	IDHC_HUMAN	123	39	14	41	46.91	6.53
55	Alpha-enolase	ENO1	P06733	ENOA_HUMAN	185	54	21	52	47.48	7.01
56	Protein disulfide-isomerase A3	PDIA3	P30101	PDIA3_HUMAN	209	44	22	40	57.14	5.98
57	Protein disulfide-isomerase A3	PDIA3	P30101	PDIA3_HUMAN	159	28	16	29	57.14	5.98
58	Myosin-3	MYH3	P11055	MYH3_HUMAN	66	21	11	8	22.48	5.62
59	Retinal dehydrogenase 1 (RALDH 1)	ALDH1A1	P00352	AL1A1_HUMAN	106	18	9	22	55.45	6.3
60	Fibrinogen beta chain	FGB	P02675	FIBB_HUMAN	87	58	13	30	56.57	7.54

61	Clathrin coat assembly protein AP180	SNAP91	O60641	AP180_HUMAN	60	21	4	93	62.83	7.98
62	Isoform 12 of Dystrobrevin alpha	DTNA	Q9Y4J8	DTNA_HUMAN	50	40	8	26	62.25	7.73
63	Isoform 11 of Dystrobrevin alpha	DTNA	Q9Y4J8	DTNA_HUMAN	55	31	8	23	53. 50	7.89
64	Catalase	CAT	P04040	CATA_HUMAN	61	14	6	15	60	6.9
65	Fibrinogen beta chain (FGB)	FGB	P02675	FIBB_HUMAN	86	58	14	30	56.57	8.54
66	Isoform 2 of T-complex protein 1 subunit beta	CCT2	P78371	TCPB_HUMAN	81	22	8	95	53.02	6
67	Aldehyde dehydrogenase, mitochondrial (ALDH-2)	ALDH2	P05091	ALDH2_HUMAN	72	31	9	20	57	6.63
68	Cytosolic non-specific dipeptidase	CNDP2	Q96KP4	CNDP2_HUMAN	65	44	9	25	53.18	5.66
69	Actin- related protein 3	ARP3	P61158	ARP3_HUMAN	133	31	14	37	42.26	5.41
70	Eukaryotic initiation factor 4A-II (eIF-4A-II)	EIF4A2	Q14240	IF4A2_HUMAN	81	32	10	26	46.6	5.33
71	Actin, cytoplasmic 1	ACTB	P60709	ACTB_HUMAN	91	26	9	35	42.05	5.29
72	Annexin A4 (ANXA-4)	ANXA4	P09525	ANXA4_HUMAN	82	28	10	36	36.08	5.84
73	Isoform 3 of Annexin A8	ANXA8	P13928	ANXA8_HUMAN	60	30	7	36	30.22	5.22
74	Heat shock protein beta-1 (HspB1)	HSPB1	P04792	HSPB1_HUMAN	76	27	7	41	22.82	5.98
75	Keratin, type I cytoskeletal 10	KRT10	P13645	K1C10_HUMAN	84	46	12	28	59.02	5.13
76	Phosphoglycerate mutase 1 (PGAM-1)	PGAM1	P18669	PGAM1_HUMAN	110	43	14	51	28.90	6.67
77	Transcription initiation factor TFIID subunit 1 (fragment)	TAF1	P21675	TAF1 _HUMAN	60	34	9	29	32.85	6.58
78	Delta(3,5)-Delta(2,4)-dienoyl-CoA isomerase	ECH1	Q13011	ECH1_HUMAN	57	12	5	19	36.13	8.16
79	Myosin-14	MYH14	Q6AI08	HEAT6_HUMAN	56	16	10	6	23	5.52
80	Annexin A1 (ANXA-1)	ANXA1	P04083	ANXA1_HUMAN	58	20	6	23	39	6.57
81	Proteasome subunit alpha type1	PSMA1	P25786	PSMA1_HUMAN	60	43	8	25	32	6.15
82	Activated CDC42 kinase 1 (fragment)	ACK1	Q07912	ACK1_HUMAN	37	51	7	27	35.29	6.50
83	Fructose-bisphosphate aldolase C	ALDOC	P09972	ALDOC_HUMAN	92	14	7	23	39.83	6.41
84	Growth/differentiation factor 6 (GDF-6)	GDF6	Q6KF10	GDF6_HUMAN	48	31	7	31	39.47	7.93
85	Glyceraldehyde-3-phosphate dehydrogenase	GADP	P04406	GADP_HUMAN	65	19	5	19	38.5	8.5
86	Septin-2 (Fragment)	SEPTIN2	Q15019	SEPT2_HUMAN	72	6	4	45	41.5	5.53
87	Putative uncharacterized protein PSMG3-AS1	PSMG3-AS1	Q96PY0	K1908_HUMAN	51	45	7	7	33	8.62
88	Phosphatidylinositol 5-phosphate 4-kinase	PIP4K2A	P48426	PI42A_HUMAN	45	17	5	21	40	8.51
89	Elongation factor 1-gamma	EEF1G	P26641	EEF1G_HUMAN	71	21	11	21	50.01	8.59
90	Rab GDP dissociation inhibitor beta	GDI2	P50395	GDIB_HUMAN	89	31	13	35	51.08	6.11

91	Calcium-binding mitochondrial carrier protein SCaMC-3 (SCaMC-3)	SLC25A23	Q9BV35	SCMC3_HUMAN	59	12	6	15	54.23	7.24
92	D-3-phosphoglycerate dehydrogenase	PHGDH	043175	SERA_HUMAN	44	45	5	49	16.75	5.06
93	Protein NDRG1 (Fragment)	NDRG1		ZN714_HUMAN	45	26	4	31	18.31	4.58
94	Ester hydrolase C11orf54 (Fragment)	C11orf54	Q9H0W9	CK054_HUMAN	56	24	4	61	10.54	6.55
95	Ceramide-1-phosphate transfer protein (CPTP)	CPTP	Q5TA50	CPTP_HUMAN	53	16	4	36	13.68	8.69
96	Uncharacterized protein C1orf109	C1orf109	Q9NX04	CA109_HUMAN	60	36	7	45	13.06	7.79
97	Phosphatidylethanolamine-binding protein 1 (PEBP-1)	PEBP1	P30086	PEBP1_HUMAN	112	26	5	55	21.15	7.01
98	Citrate synthase, mitochondrial (fragment)	CS	075390	CISY_HUMAN	63	24	5	48	15.60	8.82
99	Transgelin	TAGLN	Q01995	TAGL_HUMAN	52	9	4	19	22.65	8.87
100	S-formylglutathione hydrolase	ESD	P10768	ESTD_HUMAN	88	19	7	33	31.95	6.54
101	GTPase-activating Rap/Ran-GAP domain-like protein 3 (GARNL3)	GARNL3	Q5VVW2	GARL3_HUMAN	60	30	13	14	111.04	7.13
102	Endoplasmic reticulum chaperone BiP	HSPA5	P11021	BIP_HUMAN	122	23	15	25	72	5.07
103	Vimentin	VIM	P08670	VIME_HUMAN	186	77	30	57	53.67	5.06
104	ATP synthase subunit beta, mitochondrial	ATP5F1B	P06576	ATPB_HUMAN	106	40	13	33	56	5.26
105	Tubulin alpha-1B chain	TUBA1B	NP_006073	NP_006073	90	61	14	42	51	4.94
106	Tubulin Beta chain	TUBB	P07437	TBB5_HUMAN	112	64	19	34	48.13	5.1
107	ATP Synthase Beta, mitochondrial	ATP5F1B	P06576	ATPB_HUMAN	99	55	17	38	56.52	5.26
108	Tubulin Alpha-1B chain	TUBA1B	P68363	TBA1B_HUMAN	102	46	14	39	51	4.94
109	Vimentin	VIM	P08670	VIME_HUMAN	149	48	20	41	54	5.06
110	Vimentin	VIM	P08670	VIME_HUMAN	199	73	37	64	54	5.06
111	Cytosolic carboxypeptidase 3	AGBL3	Q8NEM8	CBPC3_HUMAN	76	7	6	12	73.57	6.98
112	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 10, mitochondrial	NDUFA10	O95299	NDUAA_HUMAN	68	10	6	19	49.04	6.76

A: MASCOT score; B: Peptides searched; C: Peptides matched; D: Protein sequence coverage %; E: Spot MW [kDa]; F: pI

Out of the total 112 identified proteins present in all gels, 17 were found to be upregulated and 27 downregulated based on the ≥ 1.5-fold difference between the two groups (Table 2, Figure 2(a) and 2(b)). All these differentially expressed proteins have been reported in previous studies to be associated with ovarian cancer except shown in bold in Table 2 and the magnified portions of the gels in Figure 2(a) and 2(b)). Whereas, the clinical information of enrolled ovarian cancer subjects is given in Table 3.

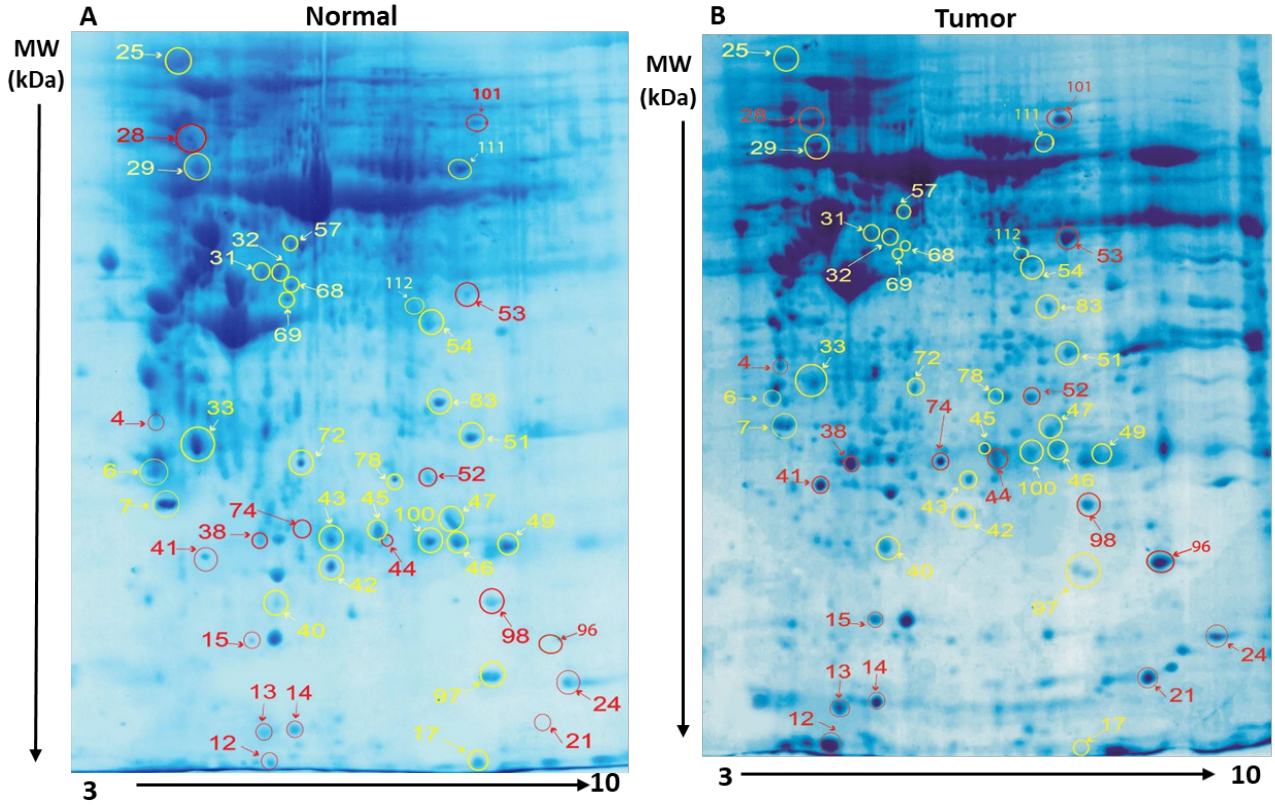

FIGURE 2. Magnified images of the gels showing the differentially expressed proteins in normal (A) and tumor (B) tissues. The proteins upregulated in the malignant tissue are encircled as red while the downregulated proteins are encircled as yellow

TABLE 2. Differentially expressed proteins in ovarian cancer tissue compared to healthy tissue

Spot No	Protein Identified	MASCOT score	Expression	Fold change	p-value	FDR	PMF score	Functions
4	Tropomyosin alpha 1	72	Upregulated	1.992	0.003	0.044	76	Cytoskeleton binding and muscle contraction
12	Galectin-1	64	Upregulated	2.295	0.001	0.022	83	Regulates apoptosis, cell proliferation and differentiation
13	Retinol Binding Protein	87	Upregulated	1.6421	0.012	0.014	97	Lipid homeostasis, retinol uptake
14	Transthyretin	103	Upregulated	1.6199	0.008	0.011	82	Cellular protein metabolic process and transports thyroxin
15	Ferritin light chain	60	Upregulated	2.0565	0.001	0.008	66	Protein and iron binding
21	Peptidyl-prolyl Cistransisomerase A	80	Upregulated	1.606	0.015	0.007	45	Accelerate the folding of proteins
24	Phosphatidylethanolamine binding protein	106	Upregulated	2.384	0.001	0.006	75	Serine type endopeptidase inhibitor
28	**Centrosomal protein of 290 kDa**	**65**	**Upregulated**	**1.9148**	**0.001**	**0.005**	**112**	**Cilium biogenesis and degradation**
38	Apolipoprotein A-I	100	Upregulated	1.7184	0.003	0.004	79	Lipid binding protein

41	Rho GDP-dissociation inhibitor 1	73	Upregulated	1.6642	0.006	0.004	60	Proteins homeostasis and negative regulation of apoptosis.
44	Glutathione S-transferase Mu 1	66	Upregulated	1.5134	0.015	0.004	91	Cellular detoxification
52	Pulmonary surfactant-associated protein D	63	Upregulated	1.5726	0.018	0.007	80	Cellular protein metabolic process
53	Alpha-enolase	168	Upregulated	1.7086	0.046	0.01	147	Glycolysis and Negative regulation of cell growth
74	Heat shock protein beta-1	76	Upregulated	1.5743	0.049	0.009	65	Oxidative stress sensor
96	**Uncharacterized protein C1orf109**	**60**	**Upregulated**	**1.8489**	**0.001**	**0.008**	**55**	**May promote cancer proliferation**
98	Citrate synthase, mitochondrial	63	Upregulated	1.5442	0.01	0.008	50	Oxidative metabolism
101	**GTPase-activating Rap/ Ran-GAP domain-like protein 3**	**60**	**Upregulated**	**2.0983**	**0.001**	**0.007**	**115**	**GTPase activator activity**
6	14-3-3 Epsilon	73	Downregulated	1.9033	0.003	0.007	89	Binding and regulation of membrane proteins.
7	14-3-3 protein zeta/delta	80	Downregulated	2.266	0.003	0.006	94	Major regulator of apoptosis
17	Hemoglobin subunit beta	91	Downregulated	2.0389	0.004	0.008	90	Oxygen transportation
25	Collagen alpha-1	206	Downregulated	1.5296	0.009	0.012	91	Strengthening of tissues
29	Endoplasmic reticulum chaperone BiP	148	Downregulated	1.5062	0.013	0.014	93	Protein folding and degradation of misfolded proteins
31	Gamma A of fibronogen gamma chain	72	Downregulated	1.6625	0.007	0.013	91	Blood coagulation and cellular protein metabolic process
32	Actin-related protein 3	70	Downregulated	1.5196	0.028	0.012	87	Actin binding and structural constituent of cytoskeleton
33	Annexin A5	156	Downregulated	1.6787	0.02	0.014	146	Phospholipid-binding protein
40	Peroxiredoxin-2	100	Downregulated	1.7322	0.009	0.015	54	Redox regulation
42	Parkinson disease protein 7	72	Downregulated	1.5337	0.003	0.014	60	Oxidative stress sensor
43	Glutathione S transferase Mu 2	95	Downregulated	1.5751	0.003	0.014	93	Detoxification of electrophilic compounds
45	Isoform of Enoyl-CoA delta isomerase 1, mitochondrial	68	Downregulated	1.6416	0.001	0.015	93	Oxidation of unsaturated fatty acids
46	Triosephosphate isomerase	193	Downregulated	1.5926	0.001	0.016	154	Metabolic enzyme in glycolysis and gluconeogenesis.
47	Phosphoglycerate mutase 1	110	Downregulated	1.5276	0.009	0.017	108	Glycolysis
49	Hemoglobin subunit beta	112	Downregulated	1.7479	0.035	0.017	79	Oxygen transportation
51	Annexin A2	214	Downregulated	2.1964	0.001	0.019	89	Organize exocytosis of intracellular proteins
54	Isocitrate Dehydrogenase[NADP] cytoplasmic	123	Downregulated	1.6196	0.011	0.019	175	Glycolysis
57	Protein disulfide-isomerase A3	159	Downregulated	1.7299	0.002	0.021	134	Glucose regulation
68	Cytosolic non-specific dipeptidase	65	Downregulated	1.7245	0.03	0.022	63	Dipeptide hydrolysis
69	**Actin- related protein 3**	**133**	**Downregulated**	**1.7477**	**0.003**	**0.023**	**101**	**Actin binding and structural constituent of cytoskeleton**

72	Annexin A4	82	Downregulated	1.6571	0.007	0.032	81	Phospholipid-binding protein
78	Delta(3,5)-Delta(2,4)-dienoyl-CoA isomerase	57	Downregulated	1.517	0.03	0.033	79	Fatty acid oxidation cycle
83	Fructose-bisphosphate aldolase C	92	Downregulated	1.6717	0.001	0.033	89	Structural component
97	Phosphatidylethanolamine-binding protein 1	112	Downregulated	1.5617	0.017	0.037	70	Protease inhibitor and ATP binder
100	S-formylglutathione hydrolase	88	Downregulated	1.5168	0.056	0.048	60	Detoxification of formaldehyde and hydrolase activity
111	**Cytosolic carboxypeptidase 3**	**76**	**Downregulated**	**1.9562**	**0.001**	**0.05**	**94**	**Mediates both deglutamylation and deaspartylation of target proteins**
112	**NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 10, mitochondrial**	**68**	**Downregulated**	**1.8192**	**0.007**	**0.056**	**89**	**Electron transport respiratory chain**

Six proteins not previously reported in ovarian cancer are shown in bold with fold change ≥ 1.5, p- value ≤ 0.05, FDR ≤ 0.05, and PMF score ≥ 79

TABLE 3. Clinical information of enrolled ovarian cancer subjects

S.No	Sample. Code	Age (years)	Menstrual History	FIGO Staging	TNM Staging	Lymph node metastasis	Liver metastasis	Histology
1	C1	42	Irregular	IVA	T4N1M1	ü	✓	High grade serous adenocarcinoma
2	C2	62	Menopause	IVA	T4N2M1	ü	✓	Clear cell adenocarcinoma
3	C3	35	Irregular	IIIA	T3N1aM0	ü	û	Serous adenocarcinoma
4	C4	43	Irregular	IIA	T2aN0M0	û	û	Serous adenocarcinoma
5	C7	62	Menopause	IIA	T2aN0M0	û	û	Endometroid carcinoma
6	C10	38	Irregular	IIA	T1aN0M0	û	û	Low serous adenocarcinoma
7	C12	45	Irregular	IVA	T3N1aM1	ü	ü	Serous adenocarcinoma
8	C16	55	Menopause	IVA	T4N2M1	ü	ü	Clear cell adenocarcinoma
9	C20	45	Irregular	IVA	T4N2M1	ü	ü	Mucinous adenocarcinoma
10	C21	50	Menopause	IVA	T4N1M1	ü	ü	Mucinous adenocarcinoma
11	C23	53	Menopause	IIIB	T3bN1M0	ü	û	Mucinous cystadenocarcinoma
12	C28	60	Menopause	IIIB	T3bN1M0	ü	×	Mucinous Adenocarcinoma
13	C29	61	Menopause	IVA	T4N1M2	ü	ü	High grade serous carcinoma
14	C33	65	Menopause	IIIA	T3N1aM0	ü	û	High grade serous carcinoma
15	C34	51	Menopause	IVA	T4N1M2	ü	ü	Clear cell adenocarcinoma
16	C35	53	Menopause	IIA	T1cN0M0	û	û	High grade serous carcinoma
17	C36	37	Irregular	IIA	T2aN0M0	×	û	High grade serous carcinoma
18	C37	49	Irregular	IIIC	T3bN0M0	û	û	Mucinous cystadenocarcinoma
19	C38	48	Irregular	IIIB	T3bN1M0	ü	×	Endometroid carcinoma
20	C39	36	Irregular	IVA	T4N1aM2	ü	ü	Clear cell adenocarcinoma

These differentially expressed proteins have been evaluated with higher confidence according to their PMF score. This score was determined by (HR × 100) + MC+ (ELDP × 10) and the threshold value ≥ 79 was considered significant for positive hits. Whereas hit ratio (HR = no. of mass matched/no. of masses submitted), mass coverage (MC = % sequence coverage × protein mass in kDa/100), and excess of limit digested peptide (ELDP = no. of matched peptides with no missed cleavage – no. of matched peptides with a missed cleavage) (North et al. 2010).

Discussion

This study profiled 112 proteins including a total 44 differentially expressed proteins, of these 38 (14 upregulated, 24 downregulated) have been reported previously as ovarian cancer biomarkers and possess oncogenic characteristics. However, herein we report six new proteins, which have not been reported earlier in ovarian cancer and may serve as potential ovarian cancer biomarkers subject to validation.

Several studies on protein profiling of the normal and malignant ovarian tissues have been reported by other researchers are consistent with this study except few differences in methodology and identified proteins. A study conducted by Stead et al. (2006) was the first detailed study, reporting 165 expression profiling and quantification of proteins from the ovarian cancer cell line. Most of the proteins found in this study are similar to the proteins identified by our 2-DE proteomic analysis with their biological and cellular functions related to tumor proliferation. Our study reported one of the strongly over-expressed (fold change: 1.708) non-structural proteins; alpha enolase (spot 53) that is involved in cell glycolysis. This is inconsistent with the observation of a previous report that glycolysis pathways are overexpressed in 70% of cancer proliferation. Notably, the current study uses human tissues, whereas the above research was conducted on ovarian epithelial cell lines. Other commonly identified overexpressed proteins are apolipoprotein A-1 (spot 38), stress protein like heat shock protein beta-1 (spot 74), and glutathione S-transferase Mu (spot 44) involved in cellular detoxification and reported as proposed biomarkers. Moreover, our identified down-regulated proteins, have been nominated as biomarkers/therapeutic targets and are inconsistent with previous results (Gagné et al. 2005). These under-expressed proteins 14-3-3 epsilon (spot 6), peroxiredoxin-2 (spot 40), triosephosphate isomerase (spot 46), annexin A2 (spot 51), and protein disulfide-isomerase A3 (spot 57) have been associated with differentiation, proliferation, and detoxification in other epithelial-related cancers especially colorectal cancer (Fujii & Ikeda 2002; Le Page et al. 2004).

In another similar proteomic profiling, expression study carried out in Japan by Stierum et al. (2003) that used ovarian cultured cell lines, reported 18 upregulated and 31 downregulated spots. Most of the identified proteins were also found in our study. Unlike previously cited studies conducted on cell lines, a Chinese study conducted by Morita et al. (2006) on blood sera of ovarian cancer women identified 1200 serum proteins, among which 57 proteins were upregulated and 10 were downregulated. They also found retinol binding protein (spot 13) upregulated similar to the current study. However, comparative protein expression mapping was also carried out by a study conducted on the Italian population by Cheng et al. (2014) using biopsies and interstitial fluid of advanced-stage ovarian cancer showing three down-regulated proteins. These results are in contrast to our 2-DE expressed up-regulated proteins identified as retinol binding protein (spot 13, fold change: 1.642), phosphatidylethanolamine binding protein (spot: 24, fold change: 2.384), and glutathione S-transferase Mu (spot: 44, fold change: 1.513). However, annexin-A-5 (spot: 33, fold change: 1.678) was found to be downregulated and consistent with this study results. Moreover, two up-regulated identified proteins; transthyretin (spot 14) and apolipoprotein A-1 (spot 38) showed similar patterns as evidenced by another previously reported study that characterized serum biomarkers for detection of early-stage ovarian cancer. However, our identified down-regulated protein, hemoglobin subunit beta (spot 49) was in contrast to the findings of Cortesi et al. (2011).

These mentioned studies have identified and analyzed the proteomic expression profiles of ovarian cancer cell lines, serum/plasma, and interstitial/ascitic fluid mostly. To the best of our knowledge, this is the first comparative proteomic research showing a quantitative and comprehensive proteomic profile and protein expression in OCs and their healthy counterparts in Pakistani population. We employed freshly excised postoperatively ovarian tissues only depicting the real condition of disease progression, but in contrast, most studies used cell lines as a research tool, which lacked tumor-host relation and did not predict a true *in vivo* microenvironment (Kozak et al. 2005; Morita et al. 2006).

Among these differentially found proteins, almost all of them remained the object of interest as a biomarker in ovarian cancer except few ones. These above-mentioned already explored proteins have been involved in different functions and metabolic oncogenic pathways leading to

disrupted cellular, impaired protein functions, apoptosis, and tumorigenesis. Despite they have been in one way or another associated with cancer in previous studies, proteomic expression profiling variation was also noticed among different populations.

Moreover, we found six proteins, which were identified with higher confidence (PMF score ≥ 79 and FDR ≤ 0.05) and have not been previously reported in ovarian cancer. Among these, three proteins are downregulated, named actin-related protein 3 (spots 69 and 32), cytosolic carboxypeptidase 3 (spot 111), and NADH dehydrogenase 1 alpha subcomplex subunit 10 (spot 112). Along with these, two upregulated proteins including centrosomal protein of 290 kDa (spot2 8) and GTPase-activating Rap/Ran-GAP domain-like protein 3 (spot 101). Interestingly, their possible role in ovarian cancer pathogenesis and induction of oncogenic pathways nominate them as candidate biomarkers for prompt diagnosis of ovarian cancer. Hence, these potential proteins need to be validated and further elucidated for their relationship with disease representation.

Among these, actin-related protein 3 is a subunit of Arp 2/3 protein complex, which promotes homologous recombination repair in response to DNA damage by enhancing nuclear actin polymerization and positive regulation of transcription by RNA polymerase II. Its downregulation might raise the suspicion for its oncogenic pathogenesis (Cheng et al. 2014). The second protein, cytosolic carboxypeptidase 3 (AGBL3), a protease enzyme is also not reported earlier in ovarian cancer. However, another type of carboxypeptidase (AGBL2) is associated with the promotion of hepatocellular cancer cell growth as well as in gastric and breast cancer (Hurst et al. 2019; Wang et al. 2018). Third down-regulated protein NADH dehydrogenase 1 alpha subcomplex subunit 10, involved in electron transport respiratory chain. Zhang et al. (2014) significantly found its association with the downregulation of mRNA expression levels in human squamous cell carcinoma.

The first upregulated protein, centrosomal protein of 290 kDa is an integral part of centrosome and cilia but its role in ovarian cancer is still has to be elucidated. Retinal and renal diseases have been associated with its's genetic mutations and the presence of antibodies against this protein may be linked with numerous types of cancer (Teh et al. 2012). Moreover, Moradi et al. (2011) suggested that cancer can be initiated by amplification of centrosomes. After this revolutionary research, centrosomal proteins are again the focus of attention for carcinogenesis.

Our second overexpressed, uncharacterized protein *C1orf109* is a novel protein, encoded by a gene *C1orf109* that is consistent with a study that found it to be overexpressed and involved in the proliferation of cancer cells in melanoma and 11 human breast cancer cell lines mainly by stepping up from G1 to S phase conversion (Boveri 2008). In contrast, a recently conducted research by Liu et al. (2012) observed *C1orf109* as an inhibitor of cell growth in various cancer cell lines and immortalized cells.

The third upregulated protein, GTPase-activating Rap/Ran-GAP domain-like protein 3, is a regulatory protein, resulting in signal termination after binding with G proteins and stimulating their GTPase activity. As found recently by Dou et al. (2020), the Ran component has been involved in cancer initiation and acts as a key player in progression by showing its overexpression in the stomach, pancreas colon, lung, breast, and kidney cancers.

Conclusion

This proteomic mapping comprehensively profiles and identifies the differential proteins possessing oncogenic characteristics. Further validation of these potential proteins will unmask their hidden evolutionary structural-function relationship involved in disease pathogenesis. Since excised malignant tissues/samples are considered as the best source for the candidate biomarkers hence, these tissues also facilitate tumor-associated cellular proteins to be subsequently secreted/shed into the circulation from where these can be measured by implementing minimally invasive and inexpensive blood tests i.e. CA-125. As such, subject to further validation of the identified proteins, clinicians could get benefit by determining serum protein levels, which will give health providers an insight not only for the early detection but also for monitoring the effect of treatment, prediction of prognosis, and for the therapeutic targets.

LIMITATIONS

This study may be extended to a larger cohort of patients to elucidate the role of these potential biomarkers for prompt diagnosis and the development of a screening tool for the early detection of ovarian cancer in a general population. Further, the serum/plasma profiling of the patients' needs to be correlated with the tissue-based data in this study.

ACKNOWLEDGEMENTS

The authors acknowledge the support provided by the University of Health Sciences, Lahore and School of Biological Sciences, University of the Punjab, Lahore, Pakistan. This publication has been extracted from Ph.D thesis of Dr. Ambreen Tauseef.

REFERENCES

Badar, F. & Mahmood, S. 2017. Epidemiology of cancers in Lahore, Pakistan, among children, adolescents and adults, 2010-2012: A cross-sectional study part 2. *BMJ Open* 7(12): e016559. doi:10.1136/bmjopen-2017-016559.

Bast, R.C., Badgwell, D., Lu, Z., Marquez, R., Rosen, D., Liu, J. & Lu, K. 2005. New tumor markers: CA125 and beyond. *International Journal of Gynecologic Cancer* 15(Suppl 3): 274. doi:10.1136/ijgc-00009577-200511001-00015.

Bhurgri, Y., Shaheen, Y., Kayani, N., Nazir, K., Ahmed, R., Usman, A. & Zaidi, S.M. 2011. Incidence, trends and morphology of ovarian cancer in Karachi (1995-2002). *Asian Pacific Journal of Cancer Prevention* 12(6): 1567-1571.

Boveri, T. 2008. Concerning the origin of malignant tumours by Theodor Boveri. Translated and annotated by Henry Harris. *Journal of Cell Science* 1(Suppl 1): 1-84. doi:10.1242/jcs.025742.

Cheng, Y., Liu, C., Zhang, N., Wang, S. & Zhang, Z. 2014. Proteomics analysis for finding serum markers of ovarian cancer. *BioMed Research International* 2014: 179040. doi:10.1155/2014/179040.

Cortesi, L., Rossi, E., Casa, L.D., Barchetti, A., Nicoli, A., Piana, S. & Iannone, A. 2011. Protein expression patterns associated with advanced stage ovarian cancer. *Electrophoresis* 32(15): 1992-2003. doi:https://doi.org/10.1002/elps.201000654.

Diz, A.P., Carvajal-Rodríguez, A. & Skibinski, D.O.F. 2011. Multiple hypothesis testing in proteomics: a strategy for experimental work. *Molecular Cellular Proteomics* 10(3): M110.004374. doi:10.1074/mcp.M110.004374.

Dou, P., Li, Y., Sun, H., Xie, W., Zhang, X., Zhang, X. & Li, Y. 2020. C1orf109L binding DHX9 promotes DNA damage depended on the R-loop accumulation and enhances camptothecin chemosensitivity. *Cell Proliferation* 53(9): e12875. doi:10.1111/cpr.12875.

Fujii, J. & Ikeda, Y. 2002. Advances in our understanding of peroxiredoxin, a multifunctional, mammalian redox protein. *Redox Report* 7(3): 123-130. doi:10.1179/135100002125000352.

Gagné, J.P., Gagné, P., Hunter, J.M., Bonicalzi, M.E., Lemay, J.F., Kelly, I. & Poirier, G.G. 2005. Proteome profiling of human epithelial ovarian cancer cell line TOV-112D. *Molecular and Cellular Biochemistry* 275(1-2): 25-55. doi:10.1007/s11010-005-7556-1.

Girolimetti, G., Perrone, A.M., Santini, D., Barbieri, E., Guerra, F., Ferrari, S. & Turchetti, D. 2014. BRCA-associated ovarian cancer: From molecular genetics to risk management. *BioMed Reserach International* 2014: 787143. doi:10.1155/2014/787143.

Hashmi, A.A., Hussain, Z.F., Bhagwani, A.R., Edhi, M.M., Faridi, N., Hussain, S.D. & Khan, M. 2016. Clinicopathologic features of ovarian neoplasms with emphasis on borderline ovarian tumors: An institutional perspective. *BMC Research Notes* 9: 205. doi:10.1186/s13104-016-2015-5.

Homburg, R. 2008. Polycystic ovary syndrome. *Best Practice & Research: Clinical Obstetrics & Gynaecology* 22(2): 261-274. doi:10.1016/j.bpobgyn.2007.07.009.

Hurst, V., Shimada, K. & Gasser, S.M. 2019. Nuclear actin and actin-binding proteins in DNA repair. *Trends Cell Biol.* 29(6): 462-476. doi:10.1016/j.tcb.2019.02.010.

Jemal, A., Siegel, R., Ward, E., Hao, Y., Xu, J., Murray, T. & Thun, M.J. 2008. Cancer statistics, 2008. *CA: A Cancer Journal for Clinicians* 58(2): 71-96. doi:10.3322/ca.2007.0010.

Kozak, K.R., Su, F., Whitelegge, J.P., Faull, K., Reddy, S. & Farias-Eisner, R. 2005. Characterization of serum biomarkers for detection of early stage ovarian cancer. *Proteomics* 5(17): 4589-4596. doi:10.1002/pmic.200500093.

Le Page, C., Provencher, D., Maugard, C.M., Ouellet, V. & Mes-Masson, A.M. 2004. Signature of a silent killer: Expression profiling in epithelial ovarian cancer. *Expert Review of Molecular Diagnostics* 4(2): 157-167. doi:10.1586/14737159.4.2.157.

Liu, S.S., Zheng, H.X., Jiang, H.D., He, J., Yu, Y., Qu, Y.P. & Li, Y. 2012. Identification and characterization of a novel gene, C1orf109, encoding a CK2 substrate that is involved in cancer cell proliferation. *Journal of Biomedical Science* 19(1): 49. doi:10.1186/1423-0127-19-49.

Momenimovahed, Z., Tiznobaik, A., Taheri, S. & Salehiniya, H. 2019. Ovarian cancer in the world: Epidemiology and risk factors. *International Journal Womens Health* 11: 287-299. doi:10.2147/IJWH.S197604.

Moradi, P., Davies, W.L., Mackay, D.S., Cheetham, M.E. & Moore, A.T. 2011. Focus on molecules: Centrosomal protein 290 (CEP290). *Experimental Eye Research* 92(5): 316-317. doi:10.1016/j.exer.2010.05.009.

Morita, A., Miyagi, E., Yasumitsu, H., Kawasaki, H., Hirano, H. & Hirahara, F. 2006. Proteomic search for potential diagnostic markers and therapeutic targets for ovarian clear cell adenocarcinoma. *Proteomics* 6(21): 5880-5890. doi:10.1002/pmic.200500708.

Mostafa, M.F., El-Etreby, N. & Awad, N. 2012. Retrospective analysis evaluating ovarian cancer cases presented at the clinical oncology department, Alexandria University. *Alexandria Journal of Medicine* 48(4): 353-360.

North, S.J., Jang-Lee, J., Harrison, R., Canis, K., Ismail, M.N., Trollope, A. & Haslam, S.M. 2010. Mass spectrometric analysis of mutant mice. *Methods in Enzymology* 478: 27-77. doi:10.1016/s0076-6879(10)78002-2.

Razi, S., Ghoncheh, M., Mohammadian-Hafshejani, A., Aziznejhad, H., Mohammadian, M. & Salehiniya, H. 2016. The incidence and mortality of ovarian cancer and their relationship with the Human Development Index in Asia. *Ecancermedicalscience* 10: 628. doi:10.3332/ecancer.2016.628.

Stead, D.A., Preece, A. & Brown, A.J. 2006. Universal metrics for quality assessment of protein identifications by mass spectrometry. *Molecular Cellular Proteomics* 5(7): 1205-1211. doi:10.1074/mcp.M500426-MCP200.

Stierum, R., Gaspari, M., Dommels, Y., Ouatas, T., Pluk, H., Jespersen, S. & Ommen, B.V. 2003. Proteome analysis reveals novel proteins associated with proliferation and differentiation of the colorectal cancer cell line Caco-2. *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics* 1650(1-2): 73-91. doi:10.1016/s1570-9639(03)00204-8.

Teh, M.T., Gemenetzidis, E., Patel, D., Tariq, R., Nadir, A., Bahta, A.W. & Hutchison, I.L. 2012. FOXM1 induces a global methylation signature that mimics the cancer epigenome in head and neck squamous cell carcinoma. *PLoS ONE* 7(3): e34329. doi:10.1371/journal.pone.0034329.

Visintin, I., Feng, Z., Longton, G., Ward, D.C., Alvero, A.B., Lai, Y. & Mor, G. 2008. Diagnostic markers for early detection of ovarian cancer. *Clinical Cancer Research* 14(4): 1065-1072. doi:10.1158/1078-0432.Ccr-07-1569.

Wang, L.L., Jin, X.H., Cai, M.Y., Li, H.G., Chen, J.W., Wang, F.W. & Xie, D. 2018. AGBL2 promotes cancer cell growth through IRGM-regulated autophagy and enhanced Aurora A activity in hepatocellular carcinoma. *Cancer Letter* 414: 71-80. doi:10.1016/j.canlet.2017.11.003.

Zhang, H., Ren, Y., Pang, D. & Liu, C. 2014. Clinical implications of AGBL2 expression and its inhibitor latexin in breast cancer. *World J. Surg. Oncol.* 12: 142. doi:10.1186/1477-7819-12-142.

Ambreen Tauseef & Asima Karim
Department of Physiology and Cell Biology
University of Health Sciences
Lahore
Pakistan

Gulfam Ahmad
School of Medical Sciences
Faculty of Medicine and Health
Sydney University
Australia

Qurratulann Afza Gardner & Muhammad Waheed Akhtar*
School of Biological Sciences
University of the Punjab
Lahore
Pakistan

*Corresponding author; email: mwa.sbs@pu.edu.pk

Received: 30 January 2021
Accepted: 30 March 2021