Tubulin Beta 2C Chain (TBB2C), a Potential Marker of Ovarian Cancer, an Insight from Ovarian Cancer Proteome Profile

Shahzadi Noreen, Safa Akhtar, Tahira Batool, Qurratulann Afza Gardner, and Muhammad Waheed Akhtar*

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ABSTRACT: Ovarian cancer (OC) is the most lethal among female reproductive system malignancies. Depending upon the stage at presentation, the five year survival ratio varies from ~92 to ~30%. The role of biomarkers in early cancer diagnosis, including OC, is well understood. In our previous study, through an initial screening, we have analyzed eleven proteins that exhibited differential expression in OC using two-dimensional gel electrophoresis (2D-GE) and matrix-assisted laser desorption/ionization-time of flight mass spectrometric (MALDI-TOF MS) analysis. In continuation of our previous study, the present work describes analysis of twenty more proteins that showed aberrant expression in OC. Among these, six showed consistent significant deregulation in the OC false discovery rate [FDR ≤ 0.05]. Upon MS analysis, they were identified as vimentin, tubulin beta 2C chain, tubulin alpha 1C chain, actin cytoplasmic 2, apolipoprotein A-I, and collagen alpha 2(VI) chain [peptide mass fingerprint (PMF) score ≥ 79]. One of the differentially regulated proteins, tubulin beta 2C chain, was found to be significantly (fold change, 2.5) enhanced in OC. Verification by western blot and enzyme-linked immunosorbent assay (ELISA) demonstrated that the tubulin beta 2C chain may serve as a valuable marker for OC (ANOVA p < 0.0001). The assessment of the likely association of TBB2C with OC in a larger population will not only help in developing clinically useful biomarkers in the future but also improve our understanding of the progression of OC disease.

1. INTRODUCTION

Ovarian cancer (OC) remains the most lethal of all gynecological malignancies worldwide.1 In Pakistan, OC is the most common reproductive tract malignancy reported in women of all ethnic groups.2,3 Symptoms are often vague and mostly confused with other gynecological and gastrointestinal problems, resulting in late diagnosis. Over the past years, enormous efforts employing different omic technologies, i.e., proteomics,4 transcriptomics,5 genomics,6 proteogenomics,7 and metabolomics,8 have been made for identification of reliable markers for OC from a number of sampling materials including urine,9 serum,10 ascites,11 biopsy tissues,4 exosomes,12 and cell lines.4 Even though several potential markers have been identified so far, more specific and clinically significant markers of the disease are still needed for better patient outcome.13

Due to enriched presence of tumor-drawn proteins in proximity to their source, analysis of tumor tissues and proximal body fluids for identification of differentially represented proteins is an active practice to isolate diagnostic and therapeutic targets. Two-dimensional gel electrophoresis by employing two physical properties of proteins, i.e., isoelectric point and molecular weight, allows comprehensive analysis of protein expression in a biological system separating >1000 different protein spots on a single gel. Mass spectrometry in combination with two-dimensional gel electrophoresis (2D-GE) is a reliable tool in proteomics rendering protein identifications over a dynamic range of protein abundance and helps understanding the molecular complexity of diseases.14,15

In our previous study,16 by employing 2-DE and matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF-MS) analysis, we compared the proteome maps of the OC and benign disease control ovarian tissues to identify differentially represented proteins that can serve as putative biomarkers of the disease. Resultantly, we reported annexin A6, a potential indicator of OC. In the present study, in an effort to identify more potential OC protein markers, we have subjected some additional 2D gel spots to MALDI-TOF MS analysis that were found to be differentially expressed in the OC. The study resulted in...
identification of significantly upregulated levels of tubulin beta 2C chain (TBB2C) association with the progression of OC with more obvious levels observed in the advanced stages III–IV of the disease compared to the benign controls.

2. MATERIALS AND METHODS

2.1. Sample Collection and Protein Extraction. The blood and resected ovarian biopsies were taken from 34 females diagnosed with OC and 20 with benign ovarian disease (Table S1, Section S1.1) after the approval of the University Ethical Review Board [ref No. 873/12]. The proteins in tissue specimens were extracted by homogenization with liquid nitrogen. The resulting homogenate was suspended in chilled lysis buffer [2 M thiourea, 8 M urea, 65 mM dithiothreitol (DTT), 4% chomamidepropylidemethylammonio-propanesulfate (CHAPS), 2% serylate, and trace amount of protease inhibitor cocktail], vigorously vortexed, and then centrifuged at 20°C until used (Section S1.2). Protein quantification was done by the Bradford assay, and a snapshot of the total protein content of lysates was obtained by SDS-PAGE.18

2.2. 2D-GE and Mass Spectrometric (MS) Analysis. Protein fractionation by 2D-GE was performed as previously described. Briefly, the tissue lysate containing ~800 μg of proteins was mixed in rehydration buffer and applied to 17 cm linear IpG strips (pH 3–10) (Serva, Heidelberg, Germany), followed by passive rehydration and isoelectric focusing (IEF) on a PROTEAN i12 IEF cell (Bio-Rad) for a total of 60 kVhr at 20°C. Following IEF, IpG strips were equilibrated in equilibration buffers and analyzed by two-dimensional SDS-PAGE. The resulting gels were stained, scanned, and analyzed by ImageMaster 2D Platinum 7.0 (GE Healthcare Amersham Biosciences) software. For selection of differentially stained 2D gel spots, the nonparametric Mann–Whitney U test was applied on summed-up intensities of the multiple spots of the same protein followed by false discovery rate (FDR) determination.19

The proteins in 2D gel differential spots were digested in 20 ng/μL sequencing-grade trypsin (Promega, Southampton, UK). The resulting peptides were mixed with matrix solution [α-cyano-4-hydroxycinnamic acid-saturated solution in 0.1% TFA/acetonitrile], spotted on a MALDI anchrochip plate, and analyzed by MALDI-TOF MS (Ultraflex III, Bruker Daltonics, Germany). In reflecton positive mode, peptide mass fingerprints were attained with 500–5000m/z range, laser beam at 337 nm wavelength, 25 kV accelerating voltage, 6 kV lens potential, and a 2 GHz digitizer with a laser frequency of 100 Hz and intensity of 50–60%, respectively. The spectra attained following MS analysis were analyzed by MASCOT Wizard 1.1.2 (http://www.matrixscience.com/wizard.html) (15) against SwissProt and NCBIProt databases with following search parameters: trypsin as enzyme allowed missed cleavage of one; fixed modification of carbamidomethyl (cysteine); oxidation (methionine) as variable modifications; peptide mass tolerance of 50–120 ppm, and Homo sapiens taxonomy. The peptide mass fingerprint (PMF) score was calculated from MASCOT peptide mass fingerprinting (PMF) HTML report results (Section S1.3).20

2.3. Gene Ontology Enrichment, Molecular Pathway, and Network Analysis. The proteins exhibiting differential expression in OC were further categorized by gene ontology (GO) (www.geneontology.com) according to their molecular functions and biological processes and ingenuity pathway analysis (IPA, QIAGEN, www.qiagen.com/ingenuity) to identify molecular networks, canonical pathways, and the diseases and disorders associated with data set proteins. Finally, IPA findings were validated by interacting protein network construction using STRING v10.0 (string.db.org) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis.

2.4. Immunological Validation. Western blotting (WB) was performed by fractionation of 20 μg of proteins by 12% SDS-PAGE followed by transfer to a nitrocellulose membrane, blocking with 5% BSA, and incubation with the primary rabbit polyclonal antibody to the tubulin beta 2C chain (1:500 dilution, A6154, Sigma-Aldrich), vimentin (1:500 dilution, PA5-25050, Invitrogen), the anti-rabbit IgG SAB 4503083, Sigma-Aldrich), and the anti-rabbit IgG horseradish peroxidase (HRP)-conjugated goat secondary antibody (1:6000 dilution, A6154, Sigma-Aldrich). Protein bands were developed using a TMB (3,3′,5,5′-tetramethylbenzidine, Sigma) peroxidase substrate system.

The TBB2C quantification in the plasma of study subjects was performed by enzyme-linked immunosorbent assay (ELISA) in precoated ELISA plates against human TBB2C (MyBioSource, San Diego, CA) following the protocol recommended by the manufacturer. Blood samples from healthy females (n = 15) were also included as healthy controls in validation analysis by ELISA. All of the assays were performed in duplicate.
Table 1. MS-Identified 2D Gel Protein Spots

| Sr. No. | protein name/ID | accession number | spot number | fold change | p-value | FDR | observed pl | database observed pl | approx. MW (KDa) | mascot score | sequence coverage (%) | mass values searched | mass values matched | mass values observed | database observed | PMF score |
|---------|-----------------|-----------------|------------|-------------|---------|-----|-------------|-----------------------|-----------------|--------------|----------------------|-------------------|-------------------|------------------|----------------|----------|
| 1       | vimentin/VIM    | P08670          | C12/T12    | 1.51        | 0.005   | 0.04| 5.12–5.63   | 5.06                  | 51–54           | 53.61        | 73             | 24                | 62              | 11               | 100.6            |
| 2       | tubulin beta 2C chain/TBB2C | P68371 | C13/T13    | 2.50        | 0.001   | 0.03| 4.63–4.94   | 4.79                  | 50             | 50       | 58             | 40                | 127              | 19               | 145             |
| 3       | tubulin beta chain/TBR5 | P07437 | C14/T14    | 1.13        | 0.42    | 0.44| 4.47–4.83   | 4.78                  | 45–49           | 49.63        | 90             | 33                | 68              | 11               | 122             |
| 4       | tubulin alpha 1C chain/TBA1C | Q9RQX3 | C15/T15    | 1.54        | 0.015   | 0.05| 4.87–5.21   | 4.96                  | 33–36           | 49.86        | 76             | 31                | 55              | 9                | 121             |
| 5       | actin, cytoplasmic 1/ACTB | P60709 | C16/T16    | 1.57        | 0.032   | 0.06| 5.1–5.4     | 5.29                  | 41–45           | 41.71        | 157            | 46                | 113             | 12               | 89.9            |
| 6       | actin, cytoplasmic 2/ACTG  | P63261 | C17/T17    | 1.50        | 0.014   | 0.05| 5.0–5.5     | 5.31                  | 43             | 42.10        | 76             | 58                | 154             | 17               | 85.4            |
| 7       | glectin-1/LGALS1 | P09582 | C18/T18    | 1.43        | 0.034   | 0.06| 5.1–5.3     | 5.34                  | 8–12           | 15.04        | 63             | 51                | 74              | 6                | 35.8            |
| 8       | endoplasmin/ENPL  | P14625 | C19/T19    | 1.55        | 0.069   | 0.10| 4.6–4.9     | 4.76                  | 76–80           | 92.41        | 64             | 33                | 176             | 27               | 135.8           |
| 9       | retinol-binding protein 1/RBP1 | P09655 | C20/T20    | 2.02        | 0.041   | 0.07| 4.9–4.9     | 4.99                  | 14–16           | 16.01        | 62             | 51                | 38              | 8                | 109             |
| 10      | albumin, partial/AEE | F6KPG5 | C21/T21    | 1.72        | 0.024   | 0.06| 6.7–7.1     | 5.73                  | 66–71           | 66.48        | 72             | 33                | 64              | 16               | 100.3           |
| 11      | aldobumin venetia/AAA | P02768 | C22/T22    | 1.52        | 0.012   | 0.05| 6.6–6.9     | 5.99                  | 71             | 71.17        | 124            | 32                | 74              | 20               | 89.7            |
| 12      | serum albumin/ALBU | P02768 | C23/T23    | 1.55        | 0.033   | 0.06| 6.4–6.8     | 5.92                  | 71             | 71.31        | 68             | 22                | 75              | 15               | 65.6            |
| 13      | apolipoprotein A-I/APOA1 | P02647 | C24/T24    | 1.74        | 0.014   | 0.05| 4.3–5.2     | 5.56                  | 27–32           | 30.75        | 77             | 47%               | 132             | 13               | 94.2            |
| 14      | collagen alpha 2(VI) chain/COL6A2 | P12110 | C25/T25    | 1.56        | 0.013   | 0.05| 5.6–5.9     | 5.85                  | 120            | 108        | 180            | 13                | 41              | 13               | 149             |
| 15      | ATP synthase subunit beta, mitochondrial/ATPB | P06576 | C26/T26    | 2.23        | 0.034   | 0.06| 4.8–5.1     | 5.26                  | 45–50           | 56.52        | 100            | 58                | 160             | 25               | 178             |
| 16      | prohibitin/PHB   | P35232 | C27/T27    | 1.26        | 0.287   | 0.33| 6.2–6.4     | 5.57                  | 17–20           | 29.78        | 62             | 33                | 27              | 6                | 72              |
| 17      | phosphatidylethanolamine-binding protein 1/PIE1P1 | P30086 | C28/T28    | 1.29        | 0.289   | 0.28| 7.1         | 7.01                  | 17             | 21.15        | 61             | 40                | 109             | 8                | 55              |
| 18      | keratin 1/K1     | H6VRF9 | C29/T29    | 1.42        | 0.12    | 0.16| 7.2–7.8     | 7.60                  | 53             | 66.02        | 74             | 25                | 50              | 9                | 84.5            |
| 19      | keratin, type II cytoskeletal 2 epidermal/K22E | P35908 | C30/T30    | 1.31        | 0.14    | 0.19| 8.8         | 8.07                  | 52–54           | 65.39        | 58             | 22                | 53              | 9                | 81              |
| 20      | keratin, type I cytoskeletal 9/K1C9 | P35527 | C31/T31    | 1.85        | 0.14    | 0.18| 5.6         | 5.14                  | 57–62           | 62.02        | 69             | 25                | 100             | 13               | 98              |

"Six proteins with fold change ≥1.5, FDR ≤ 0.05, and PMF score >79 are shown in bold. "-" sign denotes downregulated expression in OC."
Figure 2. Gene ontology-based categorization of the differentially regulated proteins by their molecular functions (A), biological processes (B), and the top network functions related to differential proteins in OC by IPA (C). The legend explaining the network nodes and edges is given at the bottom of the figure. The color intensity of the nodes is proportional to the expression of proteins, with red presenting up- and green downregulation.
2.5. Statistical Analysis. Statistical analyses were done using SPSS version 15.0 (IBM Corporation, Armonk, NY) and/or GraphPad Prism 8.30. The Kruskal Wallace one-way analysis of variance (ANOVA) test was used for comparative analysis of the antibody titer among study cohorts with the significance level set at $p < 0.05$. All quantitative data is shown as mean ± SD. The receiver operating characteristic (ROC) curve was drawn, and the corresponding area under the ROC curve (AUC) was calculated to evaluate the diagnostic performance of TBB2C in discriminating OC patients from benign disease controls and healthy females (1).

3. RESULTS

3.1. 2D-GE Profiling and MALD-TOF Identification of Proteins. In our previous report on differentially expressed proteins in ovarian malignant tissues, we had picked 11 differential spots from the 2D gels. As a result of further screening of the differentially expressed proteins, we found another 20 candidates as potential biomarkers. Spots of these fractions on the 2D gels were different from the previously reported proteins. Thirteen of these fractions were found to be upregulated, while seven were found to be in low abundance in OC samples as compared to those in samples from control ovarian tissues (Figure 1A,B, Table 1).

The three-tier criterion ($p$-value $< 0.05$, FDR $\leq 0.05$, and PMF score $\geq 79$; Table 1) set for potential biomarker screening was reached by six protein fractions, which were VIM, TBB2C, TBA1C, ACTG, APOA1, and COL6A2 (Table 1). These proteins were considered for further analysis. Five proteins, VIM, TBA1C, ACTG, APOA1, and COL6A2, have already been reported in OC. Interestingly, TBB2C has not been reported to be directly associated with OC in the literature.

MS analysis showed that some proteins were represented by multiple spots (Figures 1A,B & S1-A) with a slight change in MW and/or pl, suggesting their post-translational modifications (PTMs), as shown in Table 1. To address this variation, MS-identified data proteins were subjected to PTM analysis using NetNGlyc 1.0 (http://www.cbs.dtu.dk/services/NetNGlyc/) and Protter (http://wlab.ethz.ch/protter/) servers. These analyses revealed single or multiple glycosylation sites in many of the proteins including VIM, TBB2C, TBB5, ACTG, COL6A2, etc., with significantly high scores (threshold $\geq 0.5$) and other PTMs, i.e., acetylation, phosphorylation, S-nitrosylation, and ubiquitination.

3.2. In Silico Analysis by IPA and GO. Differentially expressed proteins were categorized by GO according to their molecular functions and biological processes (Figure 2A,B). Using IPA, the data proteins were correlated for their cellular functions, canonical pathways, networks, and diseases. IPA revealed involvement of these proteins in a number of biological processes relevant to tumor biology involving cellular motility, function, and maintenance and cell-to-cell signaling and interaction (Table 2). Proteins identified in this study were clustered together with those reported in the previous study to get an overview of their network relationships. This analysis showed that ANXA6 and TBB2C are in direct relationship to each other (Figure 2C). These proteins showed links to the KEGG pathways including apoptosis, proteoglycans in cancer and phagocytosis (Table S2, Figure S2). Overrepresented canonical pathways associated with data set proteins were 14–3-3-mediated signaling, phagosome maturation, and death receptor signaling (Figure S3). Cancer, organismal injury and abnormalities, reproductive system disease, and endocrine system disorders were among the top ten diseases linked with proteins identified in our data (Figure S4).

3.3. Validation Analysis. Since high abundance of the protein TBB2C had not previously been linked with OC, its expression level was further validated by WB. In agreement with the 2D-GE data, the TBB2C levels were found to be significantly higher in OC as compared to those in the control tissue samples (Figures 3A and 5S). In the present study, vimentin was found in high abundance in 2D gels of OC. Given that vimentin had formerly been reported to be linked with OC by researchers where some reported its up-21,22 and others downregulation; its expression was further confirmed by WB. In line with 2-DE results, WB validated enhanced expression of VIM in OC (Figure 3A).

Quantitative validation of TBB2C (fold change $> 2.5$; FDR $< 0.05$; and PMF $\geq 79$) in the plasma of subjects was done through ELISA. The TBB2C expression was found to be upregulated in OC patients in comparison to the benign disease control and healthy females. The plasma levels of TBB2C in OC samples increased with the progression of the disease. These levels were found to be 47.60 ± 8.99, 62.80 ± 7.50, and 82.70 ± 16.80 ng/mL at stages II, III, and IV, respectively. Thus, the level of TBB2C increased almost 8-fold at stage IV of OC as compared to the level in the benign disease (10.25 ± 5.13 ng/mL) (Figure 3B).

ROC curves were obtained for evaluation of TBB2C sensitivity and specificity in distinguishing OC cases from benign controls and healthy cohort. The AUC value for the curves was 1.00. At a cutoff value of 4.6 ng/mL, the sensitivity and specificity values were 85 and 93%, respectively, to discriminate healthy individuals from benign controls. The differentiation of the OC cases from benign controls and healthy females showed a sensitivity of 100% and specificity of 95% at a cutoff value of 20 ng/mL (Figure 3C).

# DISCUSSION

Ovarian cancer (OC) remains the most lethal among female genital tract cancers, mostly diagnosed at advanced metastatic stages. Identification of robust biomarkers may reduce morbidity and mortality associated with this disease by aiding in its early diagnosis.28,29

CA-125 and HE4, the two currently used markers for OC diagnosis, are rather nonspecific in asymptomatic early-stage diagnosis.30,31 In our earlier study,16 we reported annexin A6, a potential differentially regulated indicator of OC. As an extension of our previous work, we spotted more differentially abundant spots on 2D-GE gel. These fractions after pooling from multiple gels were subjected to MALDI-TOF MS analysis.

### Table 2. Molecular and Cellular Functions of the MS-Identified Data Proteins by IPA

| molecular and cellular function | $p$-value range | no. of molecules |
|---------------------------------|----------------|-----------------|
| cellular movement               | $9.79 \times 10^{-1} - 1.45 \times 10^{-2}$ | 19 |
| cellular function and maintenance| $2.04 \times 10^{-3} - 2.59 \times 10^{-7}$ | 12 |
| cell-to-cell signaling and interaction | $1.09 \times 10^{-5} - 1.09 \times 10^{-3}$ | 5 |
| free radical scavenging         | $1.66 \times 10^{-5} - 1.66 \times 10^{-3}$ | 7 |
| cellular morphology             | $2.04 \times 10^{-5} - 2.04 \times 10^{-3}$ | 5 |
| protein synthesis               | $1.52 \times 10^{-5} - 1.52 \times 10^{-3}$ | 4 |
to look for additional potential makers for the disease. This screening resulted in the identification of 20 more protein fractions, which were expressed differentially. Of these, six proteins (Table 1, shown in bold) showed differential expression with better FDR ≤ 0.05 (95% accuracy confidence). Of these, tubulin beta 2C chain (TBB2C), also known as tubulin beta 4B chain (TBB4B), was found in statistically significant higher abundance in the OC tissue samples compared to the benign disease controls. Class IVB β-tubulin is one of the 9 tubulin β-isotypes that heterodimerize with alpha tubulins forming microtubules in the metaphase-to-anaphase transition of mitotically dividing cells, an important event in survival of tumor cells. The tubulin isotype composition may alter microtubule dynamics, tumor budding grade, and invasion of cancer cells, leading to early metastasis. It is of relevance that upregulation of TBB2C has been reported in sentinel lymph nodes of colorectal cancer. Furthermore, downregulation of TBB2C was reported in taxane-resistant breast cancer in comparison to taxane-sensitive tumors, and this protein was found most commonly missense-mutated relative to the other beta tubulin members.

Chemoresistance is a major hurdle in the treatment of OC. Previously, a correlation between enhanced expression and mutations in beta tubulins was suggested as a possible mechanism of chemoresistance in OC. A study on the expression of β-tubulin isotypes in nontreated and taxol-resistant tumor ascites described significant rise of class I (3.6-fold), III (4.4-fold), and IVa (7.6-fold) tubulins in resistant tumors relative to the untreated primary ovarian tumors, while levels of class IVb and VI β-tubulins remained unchanged. Another study upon analysis of relative expression of various tubulin isotypes (class I, II, III, IVa, and IVb) by quantitative PCR found that class IVb tubulin is a more prevalent isotype in the paclitaxel-sensitive 1A9 OC parental cell line than in resistant sub-lines. However, no association was found between the induced overexpression of tubulin βI, βII, or βIVb and sensitivity to paclitaxel in Chinese hamster ovary cells.

Given that high abundance of TBB2C had not previously been linked with OC, its expression level was further validated by WB. In concordance with the 2-DE results, immunoblotting confirmed significantly upregulated TBB2C levels in the OC tissues compared to the benign tissue samples (Figures 3A and S5). Quantitative validation of TBB2C in the plasma of subjects showed its potential to distinguish the healthy cohort and benign subjects from OC patients with Kruskal Wallace ANOVA p-value < 0.0001.

An overview of the biological roles and interactive links between differentially expressed proteins in OC as reported in our previous study and the current study was analyzed by the STRING software program. Interactive associations between the proteins reported in both studies (total 31) could be traced (Figure S2-A). The pathways in which these differential proteins were involved are shown in Table S2. In our previous study, we reported ANXA6 as an upregulated marker of OC. Analysis of network functions by Ingenuity software showed that ANXA6 and TBB2C are in direct relationship in the network involved in cancer development, organismal injury, abnormalities, and reproductive system disease (Figure 2C, Table S3). KEGG pathways also revealed involvement of the TBB2C and ANXA6 in pathways regulating apoptotic growth, cellular proliferation, microtubule dynamics, adhesion, angiogenesis, and metastasis that are important characteristics of the growing tumor cells and their migration (Table S2, Figure S2-B,C). Hence, the upregulation of TBB2C and ANXA6, observed in OC, may be a direct effect of these disturbed pathways.

The previously aberrant expression of many of the tubulin and annexin protein family isotypes is found to be associated with different types of tumors. Upregulation of tubulin beta 2C

Figure 3. Validation analysis of the candidate proteins. Immunoblots of TBB2C and VIM. (A) Relative expression of TBB2C in different OC stages compared to benign controls and healthy subjects (ns, non significant; *p-value, 0.01; ***p-value, 0.0004; ****p-value < 0.0001). (B) ROC curve displaying TBB2C sensitivity to distinguish OC from benign controls and healthy subjects, and benign controls from healthy subjects (C).
and Annexin A1 has been found to be associated with colorectal cancer and downregulation of annexin II and beta 2-tubulin with nasopharyngeal cancer formation. Both of these protein family members have been described to be associated with increased tumor cell differentiation, proliferation, apoptosis, motility, invasion, and budding grade. In our study, an increase in levels of TBB2C and ANXA6 with advancement of OC suggests their role in the formation, invasion, and metastasis of OC and may represent potential markers for further investigative assessment of the disease stage. Moreover, availability of two marker proteins should add to the accuracy level of OC diagnosis.

Vimentin, a constituent of intermediate filament family of proteins, is involved in the epithelial—mesenchymal transition (EMT), growth, invasion, progression, and metastatic spread of tumors. Previous studies have reported variable expression of VIM in OC; some reported its high abundance while others showed negative correlation with OC. We found elevated expression of VIM in OC tissue as compared to the benign controls. This dysregulated expression of vimentin was further validated by WB, which supported our 2-DE data regarding expression of VIM (Figure 3A).

CONCLUSIONS
The differential TBB2C protein profile linked with OC may provide a better understanding of the molecular mechanism involved in tumor biogenesis and progression. Furthermore, such biomarkers can improve patient outcomes in future if linked to novel molecular therapies targeting pathways and key molecules involved in growth and progression of tumor.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c03262.

Details about sample collection (Section S1.1), tissue lysis and protein extraction (Section S1.2), and Mascot analysis of the mass spectrometric data (Section S1.3); three tables providing information of subjects enrolled in the study (Table S1), pathways involved by differentially abundant proteins in OC (Table S2) and important interacting network functions of the differentially regulated data set proteins identified in ovarian cancer (Table S3); five figures showing information regarding 3D views and MALDI-TOF/TOF MS analysis of representative 2D gel spots (Figure S1), pathway analysis of the differentially represented proteins in OC by STRING (Figure S2), important canonical pathways of differentially expressed proteins in OC by IPA (Figure S3), ingenuity analyzed top diseases and disorders linked with proteins identified in OC (Figure S4) and immunoblot of TBB2C (Figure S5) (PDF)

AUTHOR INFORMATION

Corresponding Author
Muhammad Waheed Akhtar – School of Biological Sciences, University of the Punjab, Lahore 54590, Pakistan; orcid.org/0000-0003-2377-3754; Phone: 92-42-99230-970; Email: mwa.sbs@pu.edu.pk; Fax: 92-42-99230-980

Authors
Shahzadi Noreen – School of Biological Sciences, University of the Punjab, Lahore 54590, Pakistan
Safa Akhtar – School of Biological Sciences, University of the Punjab, Lahore 54590, Pakistan
Tahira Batool – School of Biological Sciences, University of the Punjab, Lahore 54590, Pakistan
Qurratulann Afza Gardner – School of Biological Sciences, University of the Punjab, Lahore 54590, Pakistan

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.0c03262

Notes
The authors declare no competing financial interest.

The proteomics data was deposited to the ProteomeXchange Consortium via PRIDE partner repository (http://www.ebi.ac.uk/pride/) with the data set identifier PXD014352, and the details are as follows.

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ABBREVIATIONS USED
OC: ovarian cancer; FIGO: International Federation of Gynecology and Obstetrics; IPA: ingenuity pathway analysis; 2D-GE: 2-dimensional gel electrophoresis

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