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Glycoproteomic identification of potential glycoprotein biomarkers in ovarian cancer proximal fluids

Abstract

Background: Ovarian cancer is the leading cause of death among all gynecological disorders. Aberrant glycosylation, or more specifically, increased sialylation of proteins has been observed in ovarian cancer. Several sialyltransferase genes have been shown to be up-regulated at both the mRNA and protein levels in a number of cancers, including that of the ovary. *ST6GAL1* (β-galactosamide α2,6-sialyltranferase 1) gene expression has previously been shown to be upregulated in ovarian cancers of all major subtypes.

Methods: We have identified the sialome (i.e., sialic acid containing glycoproteins) of biological fluids from ovarian cancer patients and ovarian cancer cell lines utilizing tandem mass spectrometry as a potential pool of novel biomarker candidates. The sialoglycopeptides from four ovarian cancer cell lines, pooled ascites (n=13) and ovarian cyst (n=14) fluids from ovarian cancer patients were enriched utilizing affinity to agarose-immobilized *Elderberry* lectin (*Sambucus nigra* agglutinin) and magnetic hydrazide beads folowing periodate-mediated oxidation of sialic acids. Benign ovarian cyst (n=10) and peritoneal effusion (n=20) fluids were analyzed in the same fashion to serve as controls. PNGase F deglycosylated peptides were identified using electrospray ionization-LTQ Orbitrap tandem mass spectrometry.

Results: In all of the samples analyzed in the glycoproteomic portion of the study, we have identified 579 glycosylation sites on 333 proteins. Of these, 13 were exclusively identified in biological fluids from ovarian cancer patients, and another eight were common to these fluids and the ovarian cancer cell line supernatants.

Conclusions: The proteins identified in the present study could form the basis for future studies examining and quantifying their sialylation status as biomarkers of ovarian cancer.

Keywords: glycobiomarker; glycopeptide; lectin; mass spectrometry; N-glycosylation; ovarian cancer; sialic acid.

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Introduction

Approximately 30,000 North American women are diagnosed with ovarian cancer every year and over 50% of these women die within 5 years following diagnosis. As such, ovarian cancer has the highest mortality rate of all gynecological malignancies in the developed world. To date, only CA125, a large mucin-type glycoprotein, is widely used as an ovarian cancer biomarker in the clinical setting; for monitoring the response of patients to treatment, but has shown less promise as a screening tool since CA125 can be elevated in a number of other malignancies and benign conditions, as well as during menstruation and pregnancy [1, 2]. Therefore, there still exists a need for new ovarian cancer biomarkers.

Approximately half of all mammalian proteins are glycosylated with as much as an estimated 3000 different glycan structures, which can vary to a large degree based on differences in tissue, cell type and disease state [3]. Disruption of glycosylation pathways has been established as a common characteristic of oncologic malignancies and has been observed in almost all types of experimental and human cancers. In cells undergoing malignant transformation under-, over-, or neoexpression of glycan moieties

can occur on glycoproteins [4]. These changes most often arise from disturbances in the expression levels of different glycosyltransferases.

There is strong evidence that increased sialylation of glycoproteins occurs in ovarian tumor cells. In addition to carcinomas of the brain, breast, cervix and colon, the expression of *ST6GAL1* (a sialyltransferase responsible for attachment of α2–6-linked terminal sialic acids) is increased in ovarian tumors [5, 6]. Direct mRNA expression analysis of multiple sialyltransferases in ovarian tumor tissues has indicated the preference for the attachment of α2–6- over α2–3-linked sialic acids on glycoproteins produced by cancer cells [6]. Specifically, *ST6GAL1* levels were shown to be increased while the levels of *ST3GAL6*, a competing enzyme which normally attaches sialic acid in a α2–3 linkage, were decreased. Additionally, sialyltransferases attaching sialic acid in an α2–3 linkage to O-glycans were shown to be upregulated while enzymes with the same linkage specificity for N-glycans were downregulated [6]. Therefore, the identification, and subsequent characterization and quantification, of proteins with α2–6-linked sialic acids in ovarian cancer proximal fluids or cell lines has great potential in the identification of new and improvement of existing ovarian cancer biomarkers by including their glycosylation patterns in their measurement.

Considering the need for additional ovarian cancer biomarkers, we developed a tandem mass spectrometry-based strategy for elucidating potential biomarker candidates. Due to the well-established upregulation of sialylation in ovarian cancer, we conducted a study with the purpose of identifying the N-linked sialome (sialic acid containing glycoproteins) of ovarian cancer proximal fluids and cell line conditioned media in an effort to mine for novel biomarkers of this disease. Towards this purpose, we analyzed two types of samples from ovarian cancer patients, ascites and malignant cyst fluids, in addition to supernatants from four different ovarian cancer-derived cell lines. As non-malignant controls, we also analyzed benign ovarian cyst and peritoneal effusion fluids. Proteominer technology and size-exclusion chromatography were utilized for the enrichment of low-abundance proteins in the biological fluids analyzed. Sialylated glycopeptides in analyzed samples were enriched by two well-established methodologies, lectin affinity and hydrazide chemistry. In total, 333 proteins and 579 distinct glycosylation sites with the sialic acid moiety were identified. Of these, 21 were unique to the biological fluids from ovarian cancer patients and are candidate biomarkers worth exploring in the future.

Materials and methods

Microarray profiling

Microarray profiles of normal and cancerous ovarian tissues were extracted from previously published studies on Affymetrix HGU-133A platform. The meta-analysis on 5372 human samples representing 369 different tissue types was used to select a total of 104 samples from human ovary, including normal and pathological tissue [7]. The expression data had been previously normalized using robust multi-array analysis, and log2 intensities for each probe were used for the analysis. Probe to gene mappings based on current Affymetrix annotations were used. In cases where multiple probes hybridize to the same gene, the most informative probe was selected as the one having the largest range of expression. Ratios for comparison between groups were obtained as ratios of the means of each group. Significance analysis was performed using the limma package [8], comparing the complete set of tumor (n=87) and benign ovarian polycystic syndrome (n=5) samples to normal ovarian tissue (n=12). Tumor samples were derived from patients with clear cell (n=7), serous (n=34), mucinous (n=11) and endometrioid (n=35) subtypes of ovarian cancer.

Clinical samples

The biological fluids used in the study were collected with informed consent and institutional review board approval and were leftovers submitted for routine medical testing. All samples used were stored at −80°C prior to use. Ovarian ascites and malignant cyst fluids were collected from patients with FIGO stage III and IV of the major histological subtypes of epithelial ovarian carcinomas and pooled-based on total protein content. In total, 13 ascites fluids from ovarian cancer patients with different histological presentation were used: three serous, three endometrioid, three mucinous and four undifferentiated. Fourteen malignant ovarian cyst fluids were also pooled (4 serous, 4 endometrioid, 3 mucinous and 3 undifferentiated). As non-malignant controls, fluid from benign ovarian cysts (n=10) and peritoneal effusions from patients with peritonitis (n=20) were utilized and pooled in the same fashion. Samples analyzed were from women in the age range between 37 and 76. All pooled biological fluid samples were subjected to size exclusion chromatography or Proteominer low abundance protein enrichment (BioRad, Hercules, CA, USA). Size exclusion chromatography was performed using a 0.75×60-cm TSK-Gel G3000SW column (Tosoh Bioscience, San Francisco, CA, USA) attached to an Agilent (Santa Clara, CA, USA) 1100 HPLC system. 0.5 mL of pooled biological fluids was loaded onto the system equilibrated with PBS and run for 1 h at a flow rate of 0.5 mL/min. Fractions were collected every minute, and only sub-50 kDa fractions (to avoid the contamination by high molecular weight high abundance proteins in subsequent steps) were pooled and concentrated using centrifugal spin columns with a 3 kDa cut-off (Millipore, Billerica, MA, USA). These were used for subsequent analyses. Proteominer enrichment was performed with 1 mL of pooled biological fluids, as per the manufacturer's protocol (BioRad, Hercules, CA, USA).

Cells line supernatants

The human ovarian cancer cell lines, OVCAR-3 (HTB-161), ES-2 (CRL-1978), and TOV-112D (CRL-11731) were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). OVCAR-5 cells were obtained from the Fox Chase Cancer Centre (Philadelphia, PA, USA). ES-2, TOV-112D, and OVCAR-5 cells were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) (Thermo Scientific, Waltham, MA, USA). RPMI 1640 containing 20% FBS was used to maintain OVCAR-3 cells. All cells were cultured in a humidified incubator adjusted to 37°C with an atmosphere of 5% CO_2. Each cell line was cultured in duplicate using T-175 cm^2 flasks. Upon reaching 80% confluency, culture media was removed and the attached cells were washed gently three times using 25 mL of phosphate buffered saline (PBS). Following the washes, cells were grown in 30 mL of CD CHO chemically defined media (Invitrogen, Carlsbad, CA, USA) supplemented with 8 mM L-glutamine (Invitrogen, Carlsbad, CA, USA) for 48 h. After this period, the conditioned media were collected and centrifuged at 64×*g* for 5 min to remove cellular debris. All cell line supernatants were dialyzed using a 3.5 kDa molecular weight cut-off porous membrane (Spectrum Laboratories, Inc., Compton, CA, USA) in 4 L of 1 mM ammonium bicarbonate (NH_4HCO_3) buffer overnight at 4°C. The buffer was exchanged for a total of three times before the dialyzed samples were frozen at −80°C. Frozen samples were then lyophilized to complete dryness using a ModulyoD Freeze Dryer (Thermo Scientific, Waltham, MA, USA).

Elderberry lectin sialoglycopeptide enrichment

A total of 100 µg of total protein from all samples was brought to 300 µL volume and final 50 mM ammonium bicarbonate concentration. Ten microliters of 400 mM DTT was added and the solution was incubated at 60°C for 45 min and subsequently brought to room temperature. At this point, 11 µL of 800 mM iodoacetamide solution was added and the solution was incubated at room temperature with no exposure to light for 45 min then 2 µg of sequencing grade trypsin was added and the solution was left overnight (12 h) at 37°C. Following trypsin digestion of the samples, the resulting peptide-containing reaction mixture was heated to 90°C for 15 min. The samples were stored at −80°C until further use. One hundred and fifty microliters of agarose-bound *Elderberry* lectin slurry (Vector Labs, Burlingame, CA, USA) was added to 500 µL spin columns (Thermo Fisher, Waltham, MA, USA). The storage solution was removed by centrifugation (300×*g*) and the beads were washed three times with 1× lectin binding buffer (10 mM HEPES, 150 mM NaCl, 0.1 mM Ca++, pH 8). Trypsinized samples were diluted 1:1 in 2× lectin binding buffer (20 mM HEPES, 300 mM NaCl, 0.2 mM Ca++, pH 8) and incubated with lectin-bound agarose beads for 2 h. Flow-through was removed by centrifugation (300×*g*) and the beads were washed six times with 1× lectin binding buffer. The beads were resuspended in 300 µL of 50 mM ammonium bicarbonate containing 500 units of mass spectrometry grade PNGase F (New England Biolabs, Ipswich, MA, USA) and left overnight (12 h) at 37°C with shaking. The eluate was collected by centrifugation (300×*g*), acidified to pH 3 with formic acid (0.1%) and used for MS analysis as described below.

Hydrazide bead sialoglycopeptide enrichment

A total of 100 µg of total proteins from all biological fluid samples were brought up to 500 µL volume with phosphate buffered saline (PBS), sodium periodate (Sigma, St. Louis, MO, USA) was added to a final concentration of 2 mM and the reaction mixture was incubated on ice for 10 min. The oxidation reaction was stopped by addition of 5 µL of glycerol. The solution was concentrated to 300 µL and buffer exchanged with sodium acetete solution (100 mM sodium acetate, 100 mM NaCl, pH 5.5) using ultracentrifugal spin columns with a 3 kDa molecular weight cut-off (Millipore, Billerica, MA, USA). The sodium periodate-treated samples were added to hydrazide-conjugated magnetic beads (Invitrogen, Carlsbad, CA, USA) and incubated at room temperature for 14 h with shaking. The supernatant was removed and the magnetic beads were washed three times with a 50 mM ammonium bicarbonate solution containing 8 M urea. The beads were washed again six times with a 50 mM ammonium bicarbonate solution. Four hundred µL of ammonium bicarbonate solution were added to the beads followed by the addition of 50 µL of 50 mM DTT. The solution was incubated with shaking for 45 min at 60°C and allowed to cool to room temperature. Next, 60 µL of 100 mM iodoacetamide were added and the reaction mixture was left for 45 min at room temperature with no exposure to light. One microgram of sequencing grade trypsin was added and the solution was incubated at 37°C overnight (12 h) with shaking. The supernatant was removed from the beads and they were washed six times with 50 mM ammonium bicarbonate. The beads were resuspended in 300 µL of 50 mM ammonium bicarbonate containing 500 units of mass spectrometry grade PNGase F (New England Biolabs, Ipswich, MA, USA) and left overnight (12 h) at 37°C with shaking. The eluate was collected, acidified to pH 3 with formic acid (0.1%) and used for MS analysis as described below.

ESI-LTQ

Glycosylated tryptic peptides from the different samples described above were initially desalted by binding to ZipTip pipette tips containing C_{18} beads (Millipore, Billerica, MA, USA) and eluted in 5 µL of buffer containing 64.5% ACN, 35.4% H_2O, 0.1% formic acid, 0.02% trifluoroacetic acid. Eighty µL of a 95% H_2O, 0.1% formic acid, 5% ACN, 0.02% trifluoroacetic acid was added to the eluate. Tryptic peptides from 40 µL of each sample were then bound to a 2 cm C_{18} pre-column with a 5 mm diameter and eluted onto a resolving 5 cm analytical C_{18} column (3 mm diameter) with a 8 mm tip (New Objective). The liquid chromatography setup was connected to a Thermo LTQ Orbitrap XL mass spectrometer with a Proxeon nanoelectrospray ionization source in data dependent mode (Thermo Fisher, Waltham, MA, USA). A binary buffer system was utilized where Buffer A (running) contained 0.1% formic acid, 5% ACN, and 0.02% trifluoroacetic acid in water and Buffer B (elution) contained 90% ACN, 0.1% formic acid, and 0.02% trifluoroacetic acid in water. Each MS run was conducted over a 90 min linear gradient and eluted peptides were scanned once in the Orbitrap (450–1450 m/z range), followed by top six (by m/z peak intensity) data-dependent MS/MS scans in the LTQ. Unassigned, 1+, and 4+ charge states were ignored. Each sample was analyzed in quadruplicate.

Database searches and glycopeptide assignment

XCalibur RAW mass spectrometry files were processed using Mascot Daemon (version 2.2.0) and extract_msn, and subsequently searched with Mascot (Matrix Science, London, UK; version 2.2). The data was searched against the concatenated non-redundant IPI.Human v.3.71 database with parent and fragment tolerances of 7 ppm and 0.4 Da, respectively. Searches were performed for tryptic peptides with a fixed carbamidomethylation of cysteines, a single missed cleavage allowed, variable asparagine deamidation, and variable methionine oxidation. Resulting data files were uploaded into Scaffold (Proteome Software Inc., Portland, OR; v.2.6) and a further search using X!Tandem was conducted. Potential formerly glycosylated peptides were filtered for in several steps. Only peptides with reported N deamidation within the NxS/T consensus sequence were selected for further analysis. Due to reports of chemical deamidation during trypsin digestion of glycan-unoccupied asparagines with a glycine in the NxS/T consensus sequence, peptides with NGS or NGT sequences alone were omitted from further analysis [9]. As well, peptides with R or K in the NxS/T sequence where tryptic cleavage had occurred were also included in the analysis.

Results

Sialyltransferase expression

Although previous reports have shown the dysregulation of gene expression of some sialyltransferase genes in ovarian tumors [6, 10], these studies were limited when considering the number of studied sialyltransferase genes and the patient samples used (i.e., only the serous subtype). Therefore, we had undertaken the task of searching an existing gene expression microarray database [7] containing information from several different subtypes of ovarian cancer and examining the expression patterns of the most common sialyltransferase genes. In support of previous reports, only the *ST6GAL1* gene showed consistent overexpression across different tumor subtypes when compared to normal tissue and benign cysts (Figure 1). The expression of the majority of other sialyltransferase genes was either undisturbed or decreased in tumor samples.

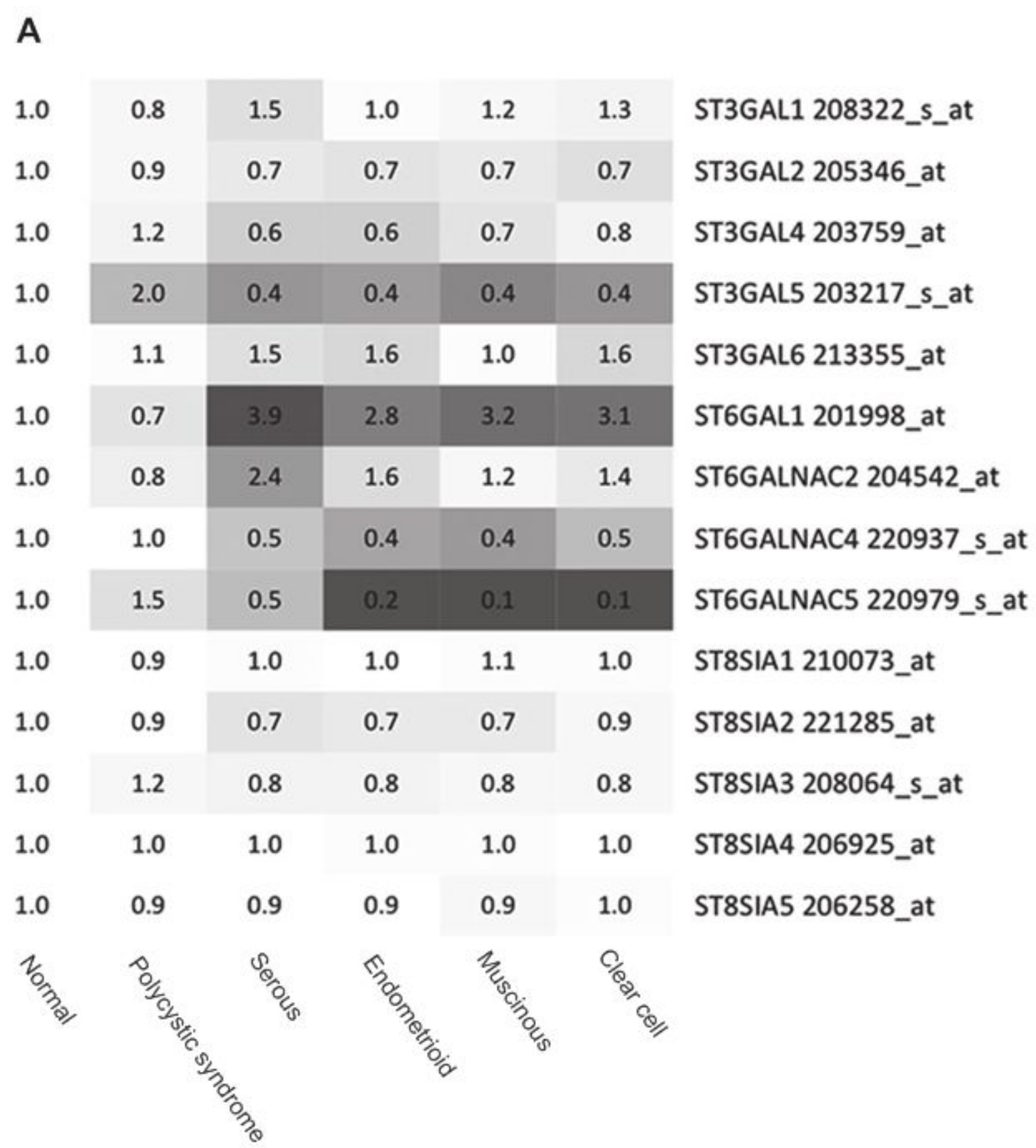

Figure 1 Sialyltransferase expression in ovarian cancer.
A heatmap of relative sialyltransferase mRNA expression in different tumor subtypes normalized against normal ovarian tissue (A). Box plot of *ST6GAL1* mRNA expression in normal and ovarian cancer tissue (B). Values are represented as the log2 corrected raw counts.

Identification of sialylated glycoproteins

Sialylated glycoproteins were identified in clinical samples from ovarian cancer patients (ascites and malignant ovarian cyst fluids), patients with no ovarian malignancy (peritoneal effusions from peritonitis patients and fluids from benign ovarian cysts), and conditioned media from four ovarian cancer cell lines (OVCAR3, OVCAR5, ES-2, and TOV-112D). To increase coverage, sialylated glycoproteins/peptides were enriched by using *Elderberry* lectin affinity (*Sambucus nigra* agglutinin) or hydrazide chemistry. The general study outline can be seen in Figure 2.

Initially, close to 700 proteins were identified in all sample types. However, data filtering steps were undertaken due to the presence of high-abundance protein contaminants, other non-specifically bound proteins, and environmental contaminants during the sample preparation process (see Materials and methods). This resulted in a final list of 333 proteins and 579 sialylated glycosylation sites identified between the different types of samples analyzed (Figure 3). Of these, 151 proteins and 291 glycosylation sites were identified in cancer associated fluids,

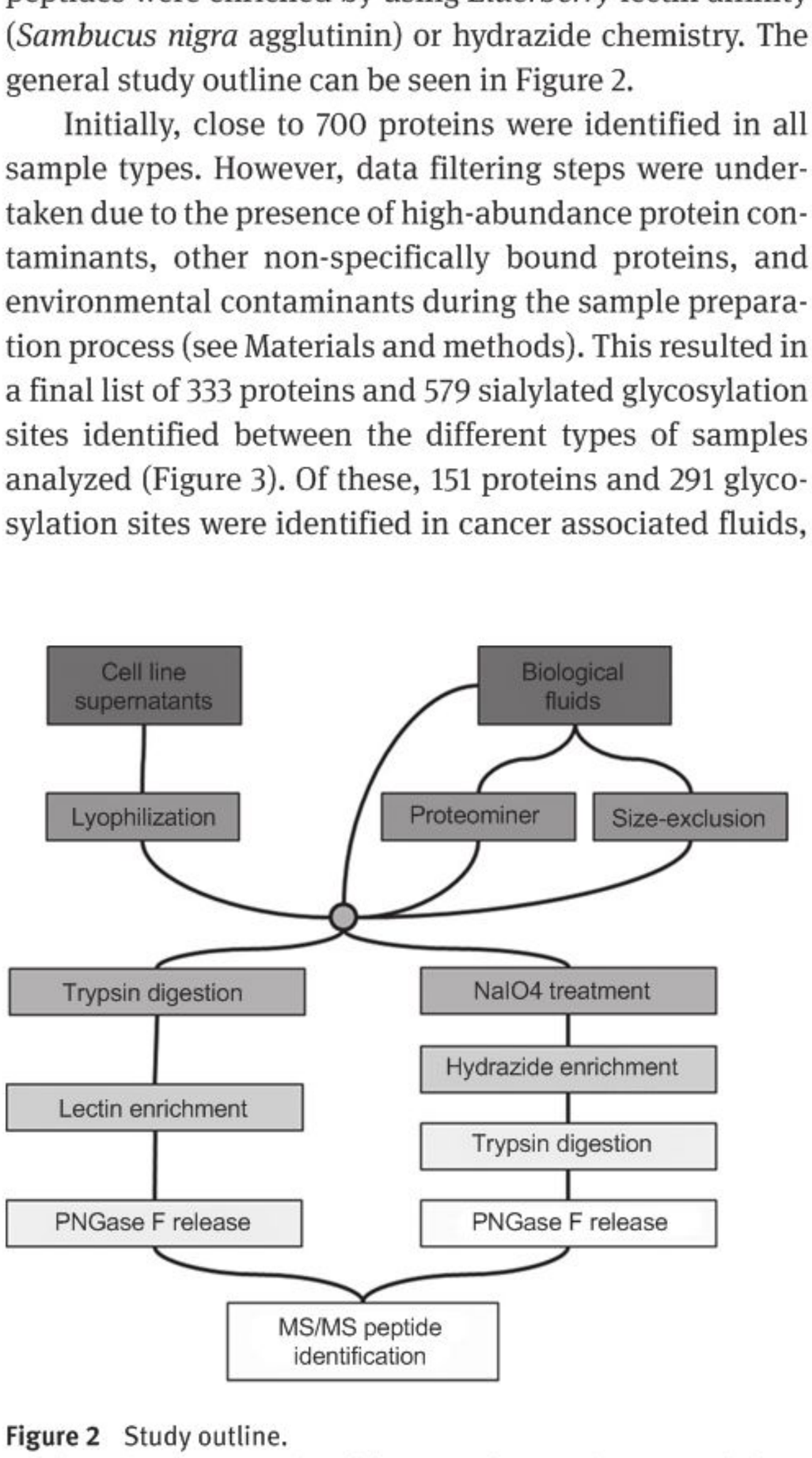

Figure 2 Study outline.
A schematic representation of the general steps taken towards the identification of sialylated glycoproteins in ovarian cancer proximal fluids and cell line supernatants. Sialylated glycopeptide were enriched separately from all sample types by utilizing hydrazide chemistry or *Elderberry* lectin affinity (see Materials and methods). Proteins from biological fluid samples were either untreated or pretreated with Proteominer or size-exclusion chromatography. Serum-free media from ovarian cancer cell lines were lyophilized prior to enrichment. Resulting peptides were subjected to tandem mass spectrometry analysis.

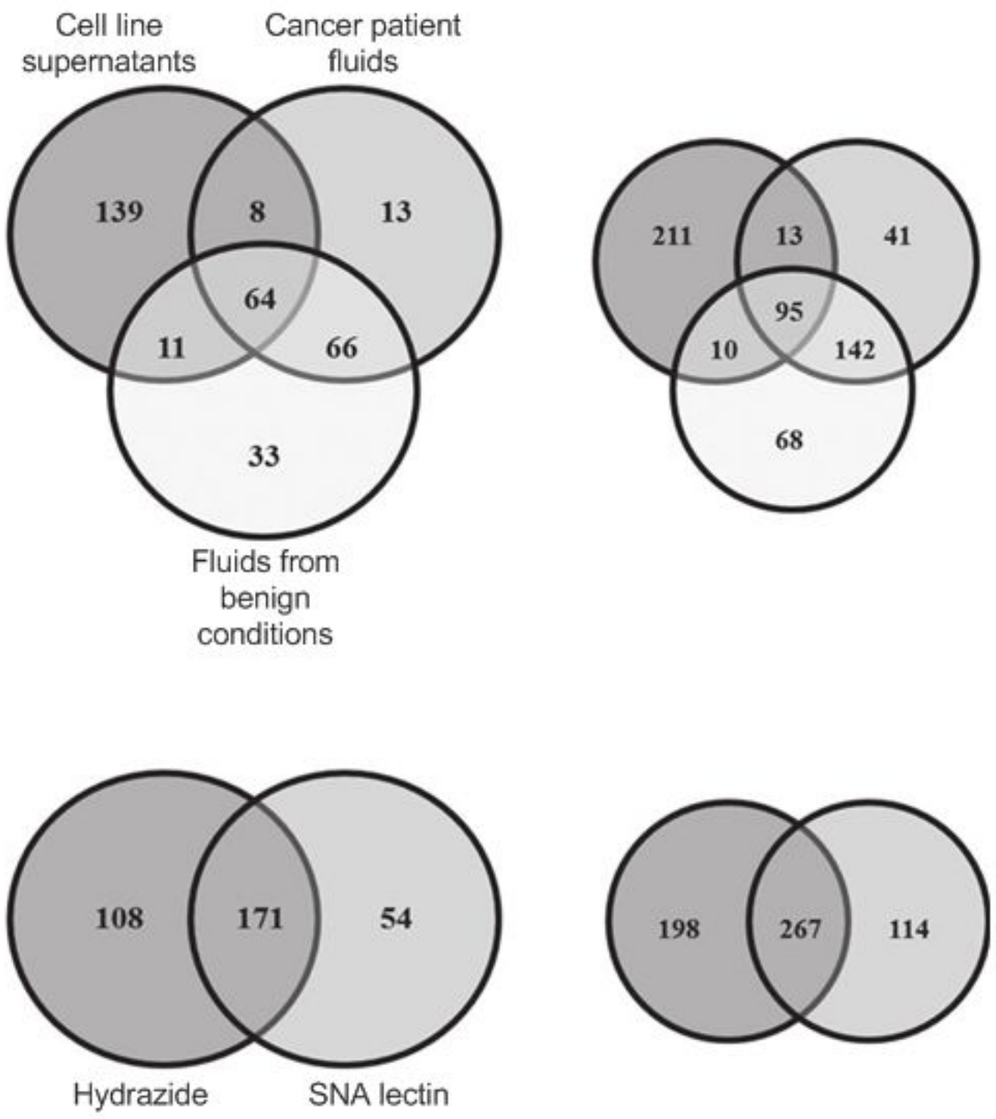

Figure 3 Identified sialylated glycoproteins.
Venn diagrams representing the number and distribution of identified sialylated glycoproteins (large circles) and glycopeptides (small circles). Comparisons for identified glycoproteins between different samples analyzed (top) and glycopeptide enrichment methods (bottom) is presented.

174 proteins and 315 sites in peritoneal effusions and benign ovarian cysts, and 222 proteins and 329 sites in conditioned media from ovarian cancer cell lines (Supplementary data, Tables S1–S3, which accompany the article at http://www.degruyter.com/view/j/cclm.2013.51.issue-6/issue-files/cclm.2013.51.issue-6.xml). In all samples analyzed, 279 proteins (465 glycopeptides) were identified using hydrazide magnetic bead pull-down methodology and 225 proteins (381 glycosylation sites) were identified by *Elderberry* lectin affinity (Figure 3). When comparing the identified proteins from the three different sample types (Figure 3), a list of 21 candidate proteins was produced (Table 1). These were proteins that were only identified in both the cell line supernatants and cancer ascites and cyst fluids, or only in the cancer-associated fluids.

Subcellular localization

For the proteins identified we also performed an analysis of subcellular localization using Gene Ontology (GO) annotation (Figure 4), secretion status (presence of signal peptide) and presence of characterized

Table 1 Proteins identified in ovarian cancer proximal fluids alone or both in cancer proximal fluids and cell line supernatants.

Gene	Description	IPI#	#aa[a]	TM[b]	SP[c]	Identified peptides
AMBP	Protein AMBP	IPI00022426	352	0	S	WNITMESYVVHTNYDEYAIFLTK
CDH6	Isoform 1 of Cadherin-6	IPI00024035	790	1	S	ETLLWHNITVIATEINNPK, EDAQINTTIGSVTAQDPDAAR
CFB	Complement factor b	IPI00947496	1115	0	S	LTDTICGVGNMSANASDQER,LGSYPVGGNVSFECEDGFILR,TMFPNLTDVR,SPYYNVSDEISFHCYDGYTLR
CFHR5	Complement factor H-related 5	IPI00006543	593	0	S	EQFCPPPPQIPNAQNMTTTVNYQDGEK
CTSD	Cathepsin D	IPI00011229	412	0	S	GSLSYLNVTR
DKK3	cDNA FLJ52545, highly similar to Dickkopf-related protein 3	IPI00002714	364	0	S	VGNNTIHVHR, ASSEVNLANLPPSYHNETNTDTK
DSG2	Desmoglein-2	IPI00028931	1118	1	S	INATDADEPNTLNSK
HGFAC	Hepatocyte growth factor activator	IPI00029193	655	1	S	DSVSVVLGQHFFNR
ICAM2	Intercellular adhesion molecule 2	IPI00009477	275	1	S	QESMNSNVSVYQPPR,AAPAPQEATATFNSTADR,GNETLHYETFGK
INHBC	Inhibin β C chain	IPI00023314	352	1	S	EQECEIISFAETGLSTINQTR
KLK6	Isoform 1 of kallikrein-6	IPI00023845	244	1	S	DCSANTTSCHILGWGK
KRT10	Keratin, type I cytoskeletal 10	IPI00009865	584	0		NVSTGDVNVEMNAAPGVDLTQLLNNMR
LBP	Lipopolysaccharide-binding protein	IPI00032311	481	0	S	LSVATNVSATLTFNTSK
LCAT	Phosphatidylcholine-sterol acyltransferase	IPI00022331	440	1	S	AELSNHTRPVILVPGCLGNQLEAK
LILRB2	Isoform 1 of Leukocyte immunoglobulin-like receptor subfamily B member 2	IPI00303952	598	1	S	QPQAGLSQANFTLGPVSR
MARCO	Macrophage receptor MARCO	IPI00009521	520	0	S	VDNFTQNPGMFR
MINPP1	Isoform 2 of multiple inositol polyphosphate phosphatase 1	IPI00028553	312	0	S	NATALYHVEAFK
POSTN	Isoform 1 of periostin	IPI00007960	836	0	S	EVNDTLLVNELK
SERPINA10	Protein Z-dependent protease inhibitor	IPI00007199	444	1	S	QLAHQSNSTNIFFSPVSIATAFAMLSLGTK,YLGNATAIFFLPDEGK,ADTHDEILEGLNFNLTEIPEAQIHEGFQELLR
SVEP1	Sushi, von Willebrand factor type A, EGF and pentraxin domain-containing protein 1 precursor	IPI00301288	3571	2	S	GAVNISACGVPCPEGK
VASN	Vasorin	IPI00395488	673	1	S	LHEITNETFR

[a]#aa, number of amino acids; [b]TM, transmembrane domain (number shown); [c]SP, signal peptide (S=present).

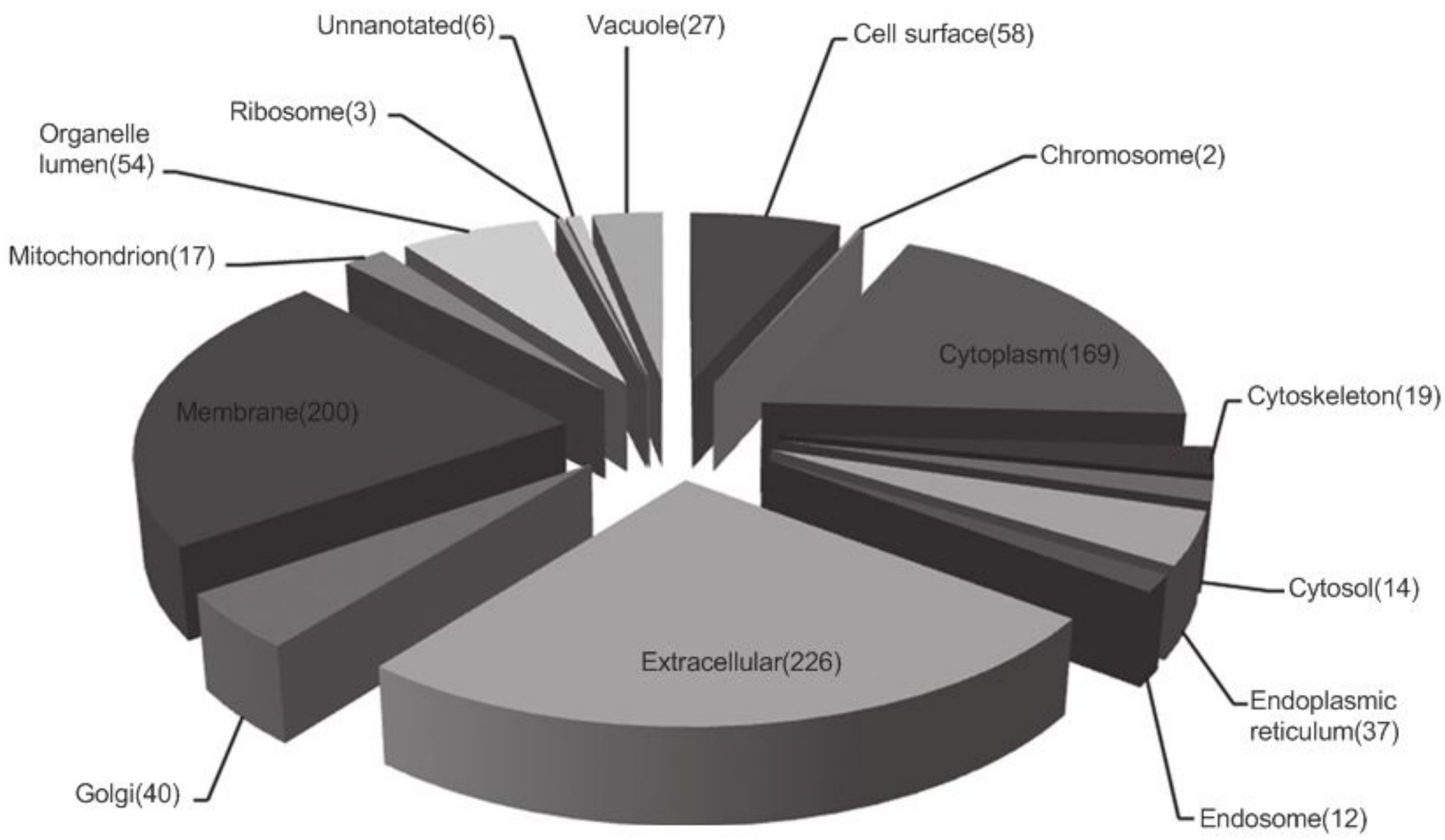

Figure 4 Subcellular localization of identified sialoglycoproteins.
Number of Gene Ontology annotations to cellular locations associated with all of the sialoglycoproteins identified in the present study. Extracted from ProteinCentre (Thermo Scientific).

transmembrane domains using the Protein Centre software (Thermo Scientific). Two hundred and eighty-eight proteins were shown to have a signal peptide, which would direct them to for the classical secretory pathway through the endoplasmic reticulum and subsequent organelles where glycosylation occurs. In total, 175 proteins were shown to have at least a single transmembrane domain. Whereas 329, or 98.8%, of the 333 proteins identified in all samples were shown to have a signal peptide and/or GO subcellular localizations to the extracellular space, cell surface, or membrane. This is significant, considering that these characteristics are highly desirable in potential protein-based cancer biomarkers, because of the heightened likelihood of their entry into the circulation.

Discussion

The majority of the most commonly clinically utilized serological biomarkers for cancer diagnosis and monitoring of malignant progression are glycoproteins. Some of these include biomarkers widely monitored in patients with prostate cancer (PSA), colon cancer (CEA), non-seminomatous testicular carcinoma (hCG-β), hepatocellular carcinoma (AFP), breast cancer (CA 15–3/MUC1) and ovarian cancer (CA125). Considering that these proteins are produced by tumor cells, it is not surprising that these proteins have shown disturbed glycosylation patterns in malignancy [4].

Disturbed glycosylation of proteins during the course of ovarian cancer progression is a well-established phenomenon [11]. Overexpression of the sialyl Lewis-X antigen has been recorded on several acute phase proteins including haptoglobin, α1-acid glycoprotein and α1-antichymotrypsin. Disturbances of normal glycosylation patterns on apolipoprotein B-100, fibronectin, immunoglobulin A1 and IgG have also been shown under malignant conditions [11].

There is strong evidence showing that sialylation of glycoproteins produced by ovarian tumor cells is increased [6, 11]. This is also supported by other data, as shown by our analysis of existing microarray experiments in Figure 1 [7]. Increased expression of α2–6-linked sialic acids generally correlates with cancer progression and metastasis [5]. The part *ST6GAL1* performs in these events is poorly understood, with possible roles in enhancing β1-integrin function [5, 12] and blocking of Fas- and TNFR1-mediated apoptosis by sialylation of these receptors [13, 14]. Although the full picture of the importance of sialic acid in the oncogenic process is not complete, it is evident that proteins produced by a subpopulation of transformed ovarian cells are enriched with the α2–6 linked sialic acid moiety. An example of this is kallikrein 6, one of the glycoproteins identified in this study, previously shown to exhibit enrichment in α2–6 linked sialic acid under malignant conditions

Table 2 Proteins identified in the ovarian cancer proximal fluids that were previously studied in ovarian cancer.

Gene, protein name	Recorded sialylation	References
A2M, α_2-macroglobulin	Yes	[18, 19]
AFM, afamin precursor	No	[20, 21]
CD59	Yes	[22, 23]
CLU, clusterin	Yes	[24, 25]
CP, ceruloplasmin	Yes	[18, 26]
CTSD, cathepsin D	No	[27]
FGB, fibrinogen	Yes	[28, 29]
HP, haptoglobin	Yes	[11, 30]
ICAM1	No	[31]
KLK6, kallikrein 6	Yes	[15, 16]
LUM, lumican	Yes	[32, 33]
MSLN, mesothelin	No	[1]
MUC16, CA125, Mucin 16	Yes	[1, 34, 35]
MUC5B	Yes	[36–38]
ORM1/2, α_1-acid glycoprotein	Yes	[39–41]
PLAUR, urokinase plasminogen activator surface receptor precursor	Yes	[42, 43]
PTGDS	No	[44]
RBP4, plasma retinol-binding protein precursor	No	[45]
S100A9	No	[19, 36, 46]
SERPINA1	No	[47]
SERPINA3	No	[48]
SPON1, spondin	No	[49]
TF, serotransferrin	Yes	[11, 50, 51]
THBS1, thrombospondin-1 precursor	No	[52]
TIMP1, metalloproteinase inhibitor 1 precursor	Yes	[53, 54]
ITIH4, isoform 1 of inter-α -trypsin inhibitor heavy chain H4 precursor	No	[22, 55]
WFDC2, isoform 1 of whey acidic protein four-disulfide core domain protein 2 precursor	No	[56, 57]

[15, 16]. Therefore, with the a priori knowledge that proteins produced by the tumor cells are enriched with a particular moiety (i.e., sialic acid), the identification of proteins with this type of glycan in biological fluids of ovarian cancer patients would enrich for the proteins produced by the transformed cells. In the subsequent studies examining these candidates, the next step would be the measurement of their protein levels in serum by ELISA or in tissue by immunohistochemistry, as is the case with other proteomic data verification studies. However, considering that a subpopulation of these proteins is uniquely produced by ovarian cancer cells with the sialic acid-containing glycans, this knowledge could be used for the development of hybrid, binary assays that can measure the levels of both protein and associated glycan.

Other sialoglycoproteins identified in this study that did not meet the criteria to be included in the candidate list (Table 1) should not be discounted. This is due to the fact that some well-known and previously studied proteins in the context of ovarian cancer were also identified (Table 2). These include the classical ovarian cancer biomarker CA125 and others such as mesothelin, *WFDC2* (HE4), α2-macroglobulin, cathepsin D, and clusterin, which have shown promise as ovarian cancer biomarkers [17]. The glycosylation status of a number of these proteins has been studied in various contexts and some were reported to be sialylated (Table 2) lending further support to our methodology.

Some of the most widely recognized and utilized cancer biomarkers exhibit a highly tissue specific expression pattern, such as PSA for prostate tissue, hCG for the placenta, and AFP for the developing fetus. Therefore, the over- or neoexpression of a protein as a result of malignant transformation can be detected and monitored with greater confidence and earlier in the progression of the malignancy in comparison to a protein produced ubiquitously or in multiple tissues. However, such proteins are rare. If it is taken into account that glycosylation patterns of individual proteins can be different between tissues, or between normal and tumor cells, the ability to detect and quantify these differences could confer tissue/tumor-specificity on a considerable number of glycoproteins. This could greatly widen the number of potential biomarkers or improve the clinical performance of existing ones. We believe that the candidate list found in this study identifies proteins with such properties. Therefore, these proteins would benefit from further development of quantitative assays capable of binary measurement of both protein and associated glycan levels that can be applied toward diagnostic purposes.

Conflict of interest statement

Authors' conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article.

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Supplemental data

Supplementary Table S1 Proteins identified in ovarian cancer-associated fluids.

Gene	Description	IPI#	#aa[a]	TM[b]	SP[c]	Identified peptides
A1BG	Isoform 1 of α-1B-glycoprotein	IPI00022895	495	0	S	EGDHEFLEVPEAQEDVEATFPVHQPGNYSCSYR
A2M	α-2-macroglobulin	IPI00478003	1474	3	S	VSNQTLSLFFTVLQDVPVR,GNEANYYSNATTDEHGLVQFSINTTNVMGTS LTVR,SLGNVNFTVSAEALESQELCGTE VPSVPEHGR
ACTL7B	Actin-like protein 7B	IPI00009126	415	0		ANKSTGILLSFANR
AFM	Afamin	IPI00019943	599	1	S	DIENFNSTQK,HNFSHCCSK,YAEDKFNETTEK
AGT	Angiotensinogen	IPI00032220	485	2	S	VYIHPFHLVIHNESTCEQLAK
AHSG	cDNA FLJ55606, highly similar to α-2-HS-glycoprotein	IPI00022431	433	1		VCQDCPLLAPLNDTR
AMBP	Protein AMBP	IPI00022426	352	0	S	WNITMESYVVHTNYDEYAIFLTK
APCS	Serum amyloid P-component	IPI00022391	223	0	S	ESVTDHVNLITPLEKPLQNFTLCFR
APOB	Apolipoprotein B-100	IPI00022229	4563	3	S	YDFNSSMLYSTAK,VNQNLVYESGSLNFSK,FNSSYLQGTNQITGR,FVEGSH NSTVSLTTK,FEVDSPVYNATWSASLK
APOC4-APOC2	Apolipoprotein C-IV	IPI00022731	127	0	S	ELLETVVNR
APOD	Apolipoprotein D	IPI00006662	189	1	S	CIQANYSLMENGK,ADGTVNQIEGEATPVNLTEPAK
APOF	Apolipoprotein F	IPI00299435	326	0	S	QGGVNATQVLIQHLR
APOH	β-2-glycoprotein 1	IPI00298828	345	0	S	VYKPSAGNNSLYR,LGNWSAMPSCK,DTAVFECLPQHAMFGNDTITCTTHG NWTK
APOM	Apolipoprotein M	IPI00030739	188	1		TELFSSSCPGGIMLNETGQGYQR
ATP6AP1	V-type proton ATPase subunit S1	IPI00784119	470	1	S	LNASLPALLLIR
ATRN	Isoform 1 of Attractin	IPI00027235	1429	6		GICNSSDVR,CINQSICEK,IDSTGNVTNELR,GCSCFSDWQGPGCSVPVPANQ SFWTR,AATCINPLNGSVCERPAN HSAK,ISNSSDTVECECSENWK
AZGP1	Zinc-α-2-glycoprotein	IPI00166729	298	0	S	DIVEYYNDSNGSHVLQGR,FGCEIENNR
AZU1	Azurocidin	IPI00022246	251	1	S	FVNVTVTPEDQCRPNNVCTGVLTR,EANLTSSVTILPLPLQNATVEAGTR
BCHE	Butyrylcholinesterase, isoform CRA_b	IPI00025864	643	1		WSDIWNATK
BTD	Biotinidase	IPI00218413	543	3	S	DVQIIVFPEDGIHGFNFTR,FNDTEVLQR,NPVGLIGAENATGETDPSHSK
C1orf151-NBL1	C1ORF15-NBL1 read-through protein isoform b	IPI00013299	181	0	S	NITQIVGHSGCEAK
C1QA	Complement C1q subcomponent subunit A	IPI00022392	245	0	S	NPPMGGNVVIFDTVITNQEEPYQNHSGR
C1S	Complement C1s subcomponent	IPI00017696	688	0	S	NCGVNCSGDVFTALIGEIASPNYPKPYPENSR
C2	cDNA FLJ55673, highly similar to Complement factor B	IPI00019591	1266	0	S	LTDTICGVGNMSANASDQER,TMFPNLTDVR,SPYYNVSDEISFHCYDGYTL R
C3	Complement C3 (Fragment)	IPI00783987	1663	1	S	TVLTPATNHMGNVTFTIPANR
C4A	Uncharacterized protein	IPI00643525	1744	4	S	FSDGLESNSSTQFEVK,NTTCQDLQIEVTVK,GLNVTLSSTGR
C4BPA	C4b-binding protein α chain	IPI00021727	597	0	S	FSLLGHASISCTVENETIGVWRPSPPTCEK,DQYVEPENVTIQCDSGYGVVGP QSITCSGNR,LSVDKDQYVEPENVTI QCDSGYGVVGPQSITCSGNR

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DOI 10.1515/cclm-2012-0642AD — Clin Chem Lab Med 2013; 51(7): Ad1–Ad18

(**Supplementary Table S1** Continued)

Gene	Description	IPI#	#aa[a]	TM[b]	SP[c]	Identified peptides
C4BPB	Isoform 1 of C4b-binding protein β chain	IPI00025862	252	0	S	EWDNTTTECR,KTLFCNASK,LGHCPDPVLVNGEFSSSGPVNVSDK,TLFCN ASKEWDNTTTECR
C8A	Complement component C8 α chain	IPI00011252	584	0	S	GGSSGWSGGLAQNR
C8B	Complement component C8 β chain	IPI00294395	591	0	S	EYESYSDFERNVTEK
C9	Complement component C9	IPI00022395	559	0	S	AVNITSENLIDDVVSLIR
CADM1	Isoform 1 of Cell adhesion molecule 1	IPI00003813	442	1	S	FQLLNFSSSELK,VSLTNVSISDEGR
CD163	Isoform 1 of Scavenger receptor cysteine-rich type 1 protein M130	IPI00104074	1156	1	S	APGWANSSAGSGR
CD59	CD59 glycoprotein	IPI00011302	128	0	S	TAVNCSSDFDACLITK
CDH6	Isoform 1 of Cadherin-6	IPI00024035	790	1	S	ETLLWHNITVIATEINNPK
CFB	Uncharacterized protein	IPI00947496	1115	0	S	LTDTICGVGNMSANASDQER,LGSYPVGGNVSFECEDGFILR,TMFPNLTDVR,SPYYNVSDEISFHCYDGYTLR
CFH	Isoform 1 of Complement factor H	IPI00029739	1231	0	S	ISEENETTCYMGK,MDGASNVTCINSR,IPCSQPPQIEHGTINSSR,SPYEMFGD EEVMCLNGNWTEPPQCK
CFHR1	Complement factor H-related protein 1	IPI00011264	330	0	S	LQNNENNISCVER,SPYEMFGDEEVMCLNGNWTEPPQCK
CFHR3	Complement factor H-related protein 3	IPI00027507	330	0	S	LGYNANTSILSFQAVCR,FVQGNSTEVACHPGYGLPK
CFHR4	Complement factor H-related protein 4	IPI00021578	331	0	S	LGYNANTSVLSFQAVCR
CFHR5	Complement factor H-related 5	IPI00006543	593	0	S	EQFCPPPPQIPNAQNMTTTVNYQDGEK
CFI	Complement factor I	IPI00291867	583	1	S	SIPACVPWSPYLFQPNDTCIVSGWGR,LSDLSINSTECLHVHCR
CILP	Cartilage intermediate layer protein 1	IPI00289275	1184	0	S	EQRPGQNCSNYTVR
CLU	Isoform 1 of Clusterin	IPI00291262	449	0	S	QLEEFLNQSSPFYFWMNGDR,MLNTSSLLEQLNEQFNWVSR,LANLTQGEDQYYLR,LKELPGVCNETMMALWEECK PCLK,EILSVDCSTNNPSQAK,HNSTGCLR,EDALNETR,ELPGVCNETMMALWEECKPCLK,KEDALNETR
CNDP1	β-Ala-His dipeptidase	IPI00064667	507	0	S	LVPHMNVSAVEK
CP	Ceruloplasmin	IPI00017601	1065	0	S	EHEGAIYPDNTTDFQR,AGLQAFFQVQECNK,ELHHLQEQNVSNAFLDK,ENLTAPGSDSAVFFEQGTTR
CPB2	Isoform 1 of Carboxypeptidase B2	IPI00329775	423	1	S	QVHFFVNASDVDNVK
CPN2	Carboxypeptidase N subunit 2	IPI00479116	545	1	S	LYLGSNNLTALHPALFQNLSK,AFGSNPNLTK
CSF1R	Macrophage colony-stimulating factor 1 receptor	IPI00011218	972	2	S	VTVQSLLTVETLEHNQTYECR
CTSD	Cathepsin D	IPI00011229	412	0	S	GSLSYLNVTR
DCD	Uncharacterized protein	IPI00847793	121	0	S	LVFGAPVNLTSIPLTSVSRP
DKK3	cDNA FLJ52545, highly similar to Dickkopf-related protein 3	IPI00002714	364	0	S	VGNNTIHVHR
DSG1	Desmoglein-1	IPI00025753	1049	2	S	TGEINITSIVDR

(**Supplementary Table S1** Continued)

Gene	Description	IPI#	#aa[a]	TM[b]	SP[c]	Identified peptides
DSG2	Desmoglein-2	IPI00028931	1118	1	S	INATDADEPNTLNSK
F11	Isoform 1 of Coagulation factor XI	IPI00008556	625	1		LETTVNYTDSQRPICLPSK,GINYNSSVAK,VYSGILNQSEIK
F12	Coagulation factor XII	IPI00019581	615	1	S	RNHSCEPCQTLAVR
F2	Prothrombin (Fragment)	IPI00019568	622	0	S	YPHKPEINSTTHPGADLQENFCR,WVLTAAHCLLYPPWDKNFTENDLLVR, SEGSSVNLSPPLEQCVPDR,SRYPHKPEINSTTHPGADLQENFCR,NFTENDLL VR
FBLN1	Isoform D of Fibulin-1	IPI00296534	703	0	S	NCQDIDECVTGIHNCSINETCFNIQGGFR,CATPHGDNASLEATFVK
FBN1	Fibrillin-1	IPI00328113	2871	0	S	VLPVNVTDYCQLVR
FCGBP	IgGFc-binding protein	IPI00242956	5405	5	S	YLPVNSSLLTSDCSER,VITVQVANFTLR
FCN3	Isoform 1 of Ficolin-3	IPI00293925	299	0	S	VELEDFNGNR
FETUB	Uncharacterized protein	IPI00552199	345	0	S	GCNDSDVLAVAGFALR,VLYLAAYNCTLRPVSK
FGB	Fibrinogen β chain	IPI00298497	491	0	S	GTAGNALMDGASQLMGENR
FGG	Isoform Gamma-B of Fibrinogen gamma chain	IPI00021891	453	0	S	DLQSLEDILHQVENK
FN1	Uncharacterized protein	IPI00845263	2421	1	S	HEEGHMLNCTCFGQGR,DQCIVDDITYNVNDTFHK,WTPLNSSTIIGYR,GGNSNGALCHFPFLYNNHNYTDCTSEGR, LDAPTNLQFVNETDSTVLVR
GOLM1	Isoform 2 of Golgi membrane protein 1	IPI00759659	391	0	S	AVLVNNITTGER
GPLD1	Isoform 1 of Phosphatidylinositol-glycan-specific phospholipase D	IPI00299503	840	0	S	NLTTSLTESVDR,LNVEAANWTVR
HGFAC	Hepatocyte growth factor activator	IPI00029193	655	1	S	DSVSVVLGQHFFNR
HP	Haptoglobin	IPI00641737	406	0	S	VVLHPNYSQVDIGLIK,NLFLNHSENATAK,MVSHHNLTTGATLINEQWLLTTAK
HPX	Hemopexin	IPI00022488	462	0	S	ALPQPQNVTSLLGCTH,NGTGHGNSTHHGPEYMR,SWPAVGNCSSALR
HRG	Histidine-rich glycoprotein	IPI00022371	525	0	S	HSHNNNSSDLHPHK,VIDFNCTTSSVSSALANTK
ICAM1	Intercellular adhesion molecule 1	IPI00008494	532	1	S	LNPTVTYGNDSFSAK,ANLTVVLLR
ICAM2	Intercellular adhesion molecule 2	IPI00009477	275	1	S	AAPAPQEATATFNSTADR
IGFBP3	Insulin-like growth factor-binding protein 3	IPI00018305	291	0	S	GLCVNASAVSR,AYLLPAPPAPGNASESEEDR
IGH@	Putative uncharacterized protein DKFZp686L19235	IPI00784950	479	0	S	LSLHRPALEDLLLGSEANLTCTLTGLR,TPLTANITK,LAGKPTHVNVSVVM AEVDGTCY
IGHA1	cDNA FLJ14473 fis, clone MAMMA1001080, highly similar to Homo sapiens SNC73 protein (SNC73) mRNA	IPI00386879	494	0	S	LSLHRPALEDLLLGSEANLTCTLTGLR,LAGKPTHVNVSVVMAEVDGTCY
IGHG1	Putative uncharacterized protein DKFZp686P15220	IPI00645363	472	0	S	EEQYNSTYR

(**Supplementary Table S1** Continued)

Gene	Description	IPI#	#aa[a]	TM[b]	SP[c]	Identified peptides
IGHG2	Putative uncharacterized protein DKFZp686I04196 (Fragment)	IPI00399007	417	0		TKPREEQFNSTFR,EEQFNSTFR
IGHG4	Putative uncharacterized protein DKFZp686M24218	IPI00930442	476	0	S	EEQFNSTYR
IGHM		IPI00941961	599	0	S	YKNNSDISSTR,THTNISESHPNATFSAVGEASICEDDWNSGER
IGHM	Ig mu chain C region	IPI00952640	452	0		GLTFQQNASSMCVPDQDTAIR,YKNNSDISSTR,THTNISESHPNATFSAVGEASICEDDWNSGER
IGHV4–31	IGH@ protein	IPI00785084	465	0	S	EEQYNSTYR
IGJ	Immunoglobulin J chain	IPI00178926	159	0	S	ENISDPTSPLR
INHBC	Inhibin β C chain	IPI00023314	352	1	S	EQECEIISFAETGLSTINQTR
ITIH1	Inter-α-trypsin inhibitor heavy chain H1	IPI00292530	911	0	S	ICDLLVANNHFAHFFAPQNLTNMNK
ITIH2	Uncharacterized protein	IPI00645038	935	1	S	GAFISNFSMTVDGK
ITIH3	Isoform 1 of Inter-α-trypsin inhibitor heavy chain H3	IPI00028413	890	1	S	NAHGEEKENLTAR
KLK6	Isoform 1 of Kallikrein-6	IPI00023845	244	1	S	DCSANTTSCHILGWGK
KLKB1	Plasma kallikrein	IPI00654888	638	0	S	LQAPLNYTEFQKPICLPSK,IYPGVDFGGEELNVTFVK,IVGGTNSSWGEWPWQVSLQVK
KNG1	Isoform HMW of Kininogen-1	IPI00032328	644	0	S	LNAENNATFYFK,ITYSIVQTNCSK,YNSQNQSNNQFVLYR,HGIQYFNNNTQHSSLFMLNEVK
KNG1	17 kDa protein	IPI00797097	154	0	S	YNSQNQSNNQFVLYR,HGIQYFNNNTQ
KRT10	Keratin, type I cytoskeletal 10	IPI00009865	584	0		NVSTGDVNVEMNAAPGVDLTQLLNNMR
KRT9	Keratin, type I cytoskeletal 9	IPI00019359	623	0		NYSPYYNTIDDLKDQIVDLTVGNNK,QGVDADINGLRQVLDNLTMEK
LBP	Lipopolysaccharide-binding protein	IPI00032311	481	0	S	LSVATNVSATLTFNTSK
LCAT	Phosphatidylcholine-sterol acyltransferase	IPI00022331	440	1	S	AELSNHTRPVILVPGCLGNQLEAK
LCN2	Isoform 1 of Neutrophil gelatinase-associated lipocalin	IPI00299547	198	0	S	SYNVTSVLFR
LGALS3BP	Galectin-3-binding protein	IPI00023673	585	0	S	EPGSNVTMSVDAECVPMVR,DAGVVCTNETR,GLNLTEDTYKPR,TVIRPFYLTNSSGVD,ALGFENATQALGR,AAIPS ALDTNSSK
LILRB2	Isoform 1 of Leukocyte immunoglobulin-like receptor subfamily B member 2	IPI00303952	598	1	S	QPQAGLSQANFTLGPVSR
LOC100133 739	Putative uncharacterized protein DKFZp686C15213	IPI00426051	464	0	S	EEQFNSTFR
LRG1	Leucine-rich α-2-glycoprotein	IPI00022417	347	0	S	LPPGLLANFTLLR,DKMFSQNDTR,MFSQNDTR
LTF	Lactotransferrin isoform 2	IPI00789477	666	0		TAGWNIPMGLLFNQTGSCK
LUM	Lumican	IPI00020986	338	0	S	LHINHNNLTESVGPLPK,LGSFEGLVNLTFIHLQHNR,AFENVTDLQWLILDHNLLENSK
LYVE1	Lymphatic vessel endothelial hyaluronic acid receptor 1	IPI00290856	322	1	S	ANQQLNFTEAK
MARCO	Macrophage receptor MARCO	IPI00009521	520	0	S	VDNFTQNPGMFR

(**Supplementary Table S1** Continued)

Gene	Description	IPI#	#aa[a]	TM[b]	SP[c]	Identified peptides
MASP1	Isoform 3 of Mannan-binding lectin serine protease 1	IPI00216882	380	0	S	FGYILHTDNR
MFAP4	Microfibril-associated glycoprotein 4	IPI00022792	255	0	S	VDLEDFENNTAYAK
MINPP1	Isoform 2 of Multiple inositol polyphosphate phosphatase 1	IPI00028553	312	0	S	NATALYHVEAFK
MMRN1	Isoform 1 of Multimerin-1	IPI00012269	1228	0	S	LQNLTLPTNASIK,FNPGAESVVLSNSTLK
MMRN2	Multimerin-2	IPI00015525	949	0	S	FNTTYINIGSSYFPEHGYFR
MPO	Isoform H17 of Myeloperoxidase	IPI00007244	745	1	S	NMSNQLGLLAVNQR,ALLPFDNLHDDPCLLTNR,DFVNCSTLPALNLASWR,SYNDSVDPR,SCPACPGSNITIR
MSLN	Isoform 2 of Mesothelin	IPI00025110	622	0	S	WNVTSLETLK,ALSQQNVSMDLATFMK
MST1	Hepatocyte growth factor-like protein	IPI00292218	711	0	S	GTGNDTVLNVALLNVISNQECNIK,GTANTTTAGVPCQR
MUC16	Mucin-16	IPI00103552	22152	8		NTSVGPLYSGCR
MUC5AC	Mucin 5AC, oligomeric mucus/gel-forming	IPI00918002	6207	6	S	AFGQFFSPGEVIYNK
ORM1	α-1-acid glycoprotein 1	IPI00022429	201	0	S	SVQEIQATFFYFTPNKTEDTIFLR,NEEYNKSVQEIQATFFYFTPNK,WFYIASAFRNEEYNK,EYQTRQDQCIYNTTYLNVQR,QDQCIYNTTYLNVQR
ORM2	α-1-acid glycoprotein 2	IPI00020091	201	0	S	QNQCFYNSSYLNVQR,SVQEIQATFFYFTPNKTEDTIFLR,NEEYNKSVQEIQATFFYFTPNK,WFYIASAFRNEEYNK
PAEP	Isoform 1 of Glycodelin	IPI00014544	180	0	S	WENNSCVEK
PGLYRP2	Isoform 1 of N-acetylmuramoyl-L-alanine amidase	IPI00163207	576	2	S	GFGVAIVGNYTAALPTEAALR,LEPVHLQLQCMSQEQLAQVAANATK
PIGR	Polymeric immunoglobulin receptor	IPI00004573	764	1	S	VPGNVTAVLGETLK,WNNTGCQALPSQDEGPSK
PIP	Prolactin-inducible protein	IPI00022974	146	0	S	TFYWDFYTNR
PLTP	45 kDa protein	IPI00022733	405	1		MLQITNASLGLR,LQITNASLGLR,GAFFPLTERNWSLPNR,VSNVSCQASVSR,IYSNHSALESLALIPLQAPLK
PLTP	cDNA FLJ39690 fis, clone SMINT2010639, highly similar to PHOSPHOLIPID TRANSFER PROTEIN	IPI00643034	513	1	S	EGHFYYNISEVK,GAFFPLTERNWSLPNR,VSNVSCQASVSR,IYSNHSALESLALIPLQAPLK
PON1	Serum paraoxonase/arylesterase 1	IPI00218732	355	0	S	HANWTLTPLK,VVAEGFDFANGINISPDGK
POSTN	Isoform 1 of Periostin	IPI00007960	836	0	S	EVNDTLLVNELK
PSAP	Isoform Sap-mu-0 of Proactivator polypeptide	IPI00012503	524	0	S	DNATEEEILVYLEK,TNSTFVQALVEHVK
PTGDS	Prostaglandin-H2 D-isomerase	IPI00013179	190	0	S	WFSAGLASNSSWLR,SVVAPATDGGLNLTSTFLR
RNASE1	Ribonuclease pancreatic	IPI00014048	156	0	S	SNSSMHITDCR
SELL	L-selectin precursor	IPI00218795	385	1		IGGIWTWVGTNK,DNYTDLVAIQNK
SEPP1	Selenoprotein P	IPI00029061	381	0	S	EGYSNISYIVVNHQGISSR,VSEHIPVYQQEENQTDVWTLLNGSK,CGNCSLTTLK
SERPINA1	Isoform 1 of α-1-antitrypsin	IPI00553177	418	1	S	QLAHQSNSTNIFFSPVSIATAFAMLSLGTK,YLGNATAIFFLPDEGK,ADTHDEILEGLNFNLTEIPEAQIHEGFQELLR
SERPINA10	Protein Z-dependent protease inhibitor	IPI00007199	444	1	S	LPYQGNATMLVVLMEK

(**Supplementary Table S1** Continued)

Gene	Description	IPI#	#aa[a]	TM[b]	SP[c]	Identified peptides
SERPINA3	cDNA FLJ35730 fis, clone TESTI2003131, highly similar to α-1-ANTICHYMOTRYPSIN	IPI00550991	448	2	S	NVIFSPLSISTALAFLSLGAHNTTLTEILK,FNLTETSEAEIHQSFQHLLR,YTGNASALFILPDQDK,TLNQSSDELQLSMGNAMFVK
SERPINA4	Kallistatin	IPI00328609	427	1	S	SQILEGLGFNLTELSESDVHR,DFYVDENTTVR,FLNDTMAVYEAK
SERPINA6	Corticosteroid-binding globulin	IPI00027482	405	3	S	AVLQLNEEGVDTAGSTGVTLNLTSKPIILR,AQLLQGLGFNLTER
SERPINA7	Thyroxine-binding globulin	IPI00292946	415	1	S	TLYETEVFSTDFSNISAAK,VTACHSSQPNATLYK
SERPINC1	Antithrombin-III	IPI00032179	464	1	S	LGACNDTLQQLMEVFK,WVSNKTEGR,SLTFNETYQDISELVYGAK
SERPIND1	SERPIND1	IPI00292950	527	2	S	NLSMPLLPADFHK
SERPINF1	Pigment epithelium-derived factor	IPI00006114	418	1	S	VTQNLTLIEESLTSEFIHDIDR
SERPING1	Plasma protease C1 inhibitor	IPI00291866	500	1	S	VGQLQLSHNLSLVILVPQNLK,VLSNNSDANLELINTWVAK,DTFVNASR
SHBG	Isoform 1 of Sex hormone-binding globulin	IPI00023019	402	1	S	LDVDQALNR
SOD3	Extracellular superoxide dismutase [Cu-Zn]	IPI00027827	240	0	S	LDAFFALEGFPTEPNSSSR
SPON1	Spondin-1	IPI00171473	807	0	S	LTFYGNWSEK
SVEP1	Sushi, von Willebrand factor type A, EGF and pentraxin domain-containing protein 1 precursor	IPI00301288	3571	2	S	GAVNISACGVPCPEGK
TF	Serotransferrin precursor	IPI00022463	698	0	S	QQQHLFGSNVTDCSGNFCLFR,CGLVPVLAENYNKSDNCEDTPEAGYFAVAVVK
TIMP1	Metalloproteinase inhibitor 1	IPI00032292	207	0	S	FVGTPEVNQTTLYQR
TMEM110	Isoform 2 of Inter-α-trypsin inhibitor heavy chain H4	IPI00218192	914	1	S	LPTQNITFQTESSVAEQEAEFQSPK,AFITNFSMIIDGMTYPGIIK
VASN	Vasorin	IPI00395488	673	1	S	LHEITNETFR
VNN1	Pantetheinase	IPI00030871	513	1	S	LTGVAGNYTVCQK
VTN	Vitronectin	IPI00298971	478	0	S	NISDGFDGIPDNVDAALALPAHSYSGR,NNATVHEQVGGPSLTSDLQAQSK
VWF	von Willebrand factor	IPI00023014	2813	0	S	ASPPSSSCNISSGEMQK
WFDC2	Isoform 1 of WAP four-disulfide core domain protein 2	IPI00291488	124	0	S	TGVCPELQADQNCTQECVSDSECADNLK

[a]#aa, number of amino acids; [b]TM, transmembrane domain (number shown); [c]SP, signal peptide (S = present).

Supplementary Table S2 List of identified proteins in benign fluids.

Gene	Description	IPI #	#aa[a]	TM[b]	SP[c]	Identified peptides
A1BG	Isoform 1 of α-1B-glycoprotein	IPI00022895	495	0	S	EGDHEFLEVPEAQEDVEATFPVHQPGNYSCSYR
A2M	α-2-macroglobulin	IPI00478003	1474	3	S	VSNQTLSLFFTVLQDVPVR
ACTL7B	Actin-like protein 7B	IPI00009126	415	0		ANKSTGILLSFANR
AFM	Afamin	IPI00019943	599	1	S	DIENFNSTQK,HNFSHCCSK,YAEDKFNETTEK
AGT	Angiotensinogen	IPI00032220	485	2	S	VYIHPFHLVIHNESTCEQLAK
AHSG	cDNA FLJ55606, highly similar to α-2-HS-glycoprotein	IPI00022431	433	1		VCQDCPLLAPLNDTR
ALPP	Alkaline phosphatase, placental type precursor	IPI00007289	550	2	S	FNQCNTTR
APCS	Serum amyloid P-component	IPI00022391	223	0	S	ESVTDHVNLITPLEKPLQNFTLCFR
APOB	Apolipoprotein B-100	IPI00022229	4563	3	S	YDFNSSMLYSTAK,VNQNLVYESGSLNFSK,QVFPGLNYCTSGAYSNASSTDSASYYPLTGDTR,FNSSYLQGT NQITGR,FVEGSHNSTVSLTTK,FEVDSPVYNATWSASLK
APOC4-APOC2	Apolipoprotein C-IV	IPI00022731	127	0	S	ELLETVVNR
APOD	Apolipoprotein D	IPI00006662	189	1	S	CIQANYSLMENGK,ADGTVNQIEGEATPVNLTEPAK
APOF	Apolipoprotein F	IPI00299435	326	0	S	QGGVNATQVLIQHLR
APOH	β-2-glycoprotein 1	IPI00298828	345	0	S	VYKPSAGNNSLYR,LGNWSAMPSCK,DTAVFECLPQHAMFGNDTITCTTHGNWTK
APOM	Apolipoprotein M	IPI00030739	188	1		TELFSSSCPGGIMLNETGQGYQR
ASGR2	Isoform 1 of Asialoglycoprotein receptor 2	IPI00011155	311	1		EAFSNFSSSTLTEVQAISTHGGSVGDK
ATP6AP1	V-type proton ATPase subunit S1	IPI00784119	470	1	S	LNASLPALLLIR
ATRN	Isoform 1 of Attractin	IPI00027235	1429	6		GICNSSDVR,IDSTGNVTNELR,ISNSSDTVECECSENWK
AZGP1	Zinc-α-2-glycoprotein	IPI00166729	298	0	S	DIVEYYNDSNGSHVLQGR,FGCEIENNR
AZU1	Azurocidin	IPI00022246	251	1	S	FVNVTVTPEDQCRPNNVCTGVLTR,EANLTSSVTILPLPLQNATVEAGTR
BCHE	Butyrylcholinesterase, isoform CRA_b	IPI00025864	643	1		YGNPNETQNNSTSWPVFK
BPIFB1	Isoform 1 of Long palate, lung and nasal epithelium carcinoma-associated protein 1	IPI00291410	484	1	S	GDQLILNLNNISSDR,DHNATSILQQLPLLSAMR
BTD	Biotinidase	IPI00218413	543	3	S	DVQIIVFPEDGIHGFNFTR,FNDTEVLQR,NPVGLIGAENATGETDPSHSK
C1orf151-NBL1	C1ORF15-NBL1 read-through protein isoform b	IPI00013299	181	0	S	NITQIVGHSGCEAK
C1QA	Complement C1q subcomponent subunit A	IPI00022392	245	0	S	NPPMGGNVVIFDTVITNQEEPYQNHSGR
C1RL	Complement C1r subcomponent-like protein	IPI00009793	487	0	S	KNQSVNVFLGHTAIDEMLK
C1S	Complement C1s subcomponent	IPI00017696	688	0	S	NCGVNCSGDVFTALIGEIASPNYPKPYPENSR
C2	cDNA FLJ55673, highly similar to Complement factor B	IPI00019591	1266	0	S	LTDTICGVGNMSANASDQER,TMFPNLTDVR,SPYYNVSDEISFHCYDGYTLR
C3	Complement C3 (Fragment)	IPI00783987	1663	1	S	TVLTPATNHMGNVTFTIPANR
C4A	Uncharacterized protein	IPI00643525	1744	4	S	FSDGLESNSSTQFEVK,NTTCQDLQIEVTVK,GLNVTLSSTGR

(**Supplementary Table S2** Continued)

Gene	Description	IPI #	#aa[a]	TM[b]	SP[c]	Identified peptides
C4BPA	C4b-binding protein α chain	IPI00021727	597	0	S	LSVDKDQYVEPENVTIQCDSGYGVVGPQSITCSGNR
C4BPB	Isoform 1 of C4b-binding protein β chain	IPI00025862	252	0	S	EWDNTTTECR,LGHCPDPVLVNGEFSSSGPVNVSDK
C8A	Complement component C8 α chain	IPI00011252	584	0	S	GGSSGWSGGLAQNR
C8B	Complement component C8 β chain	IPI00294395	591	0	S	EYESYSDFERNVTEK
C9	Complement component C9	IPI00022395	559	0	S	AVNITSENLIDDVVSLIR
CADM1	Isoform 1 of Cell adhesion molecule 1	IPI00003813	442	1	S	FQLLNFSSSELK
CD163	Isoform 1 of Scavenger receptor cysteine-rich type 1 protein M130	IPI00104074	1156	1	S	APGWANSSAGSGR,EDAAVNCTDISVQK
CD44	Isoform 12 of CD44 antigen	IPI00297160	361	1	S	AFNSTLPTMAQMEK
CD55	Decay-accelerating factor splicing variant 4	IPI00152418	525	1	S	GSQWSDIEEFCNR
CD59	CD59 glycoprotein	IPI00011302	128	0	S	TAVNCSSDFDACLITK
CEACAM5	Carcinoembryonic antigen-related cell adhesion molecule 5	IPI00027486	702	0	S	TLTLFNVTR
CFH	Isoform 1 of Complement factor H	IPI00029739	1231	0	S	ISEENETTCYMGK,MDGASNVTCINSR,IPCSQPPQIEHGTINSSR,SPYEMFGDEEVMCLNGNWTEPPQCK
CFHR1	Complement factor H-related protein 1	IPI00011264	330	0	S	LQNNENNISCVER,SPYEMFGDEEVMCLNGNWTEPPQCK
CFHR3	Complement factor H-related protein 3	IPI00027507	330	0	S	LGYNANTSILSFQAVCR,KFVQGNSTEVACHPGYGLPK,FVQGNSTEVACHPGYGLPK
CFHR4	Complement factor H-related protein 4	IPI00021578	331	0	S	LGYNANTSVLSFQAVCR
CFI	Complement factor I	IPI00291867	583	1	S	SIPACVPWSPYLFQPNDTCIVSGWGR,LSDLSINSTECLHVHCR
CILP	Cartilage intermediate layer protein 1	IPI00289275	1184	0	S	EQRPGQNCSNYTVR
CLN5	Ceroid-lipofuscinosis neuronal protein 5	IPI00026050	407	2		IFLYSGEPTYLGNETSVFGPTGNK
CLU	Isoform 1 of Clusterin	IPI00291262	449	0	S	QLEEFLNQSSPFYFWMNGDR,MLNTSSLLEQLNEQFNWVSR,LANLTQGEDQYYLR,LKELPGVCNETMMALWEECKPCLK,EILSVDCSTNNPSQAK,HNSTGCLR,EDALNETR,ELPGVCNETMMALWEECKPCLK,KEDALNETR
CNDP1	β-Ala-His dipeptidase	IPI00064667	507	0	S	LVPHMNVSAVEK
CNTN6	Contactin-6	IPI00004433	1028	0	S	AYNTAGTGPSSPPVNVTTK
COL5A1	Collagen α-1(V) chain	IPI00844090	1838	0	S	NVTLILDCK
CP	Ceruloplasmin	IPI00017601	1065	0	S	EHEGAIYPDNTTDFQR,AGLQAFFQVQECNK,ELHHLQEQNVSNAFLDK,ENLTAPGSDSAVFFEQGTTR
CPB2	Isoform 1 of Carboxypeptidase B2	IPI00329775	423	1	S	QVHFFVNASDVDNVK
CPE	Carboxypeptidase E precursor	IPI00031121	572	2		GNETIVNLIHSTR
CPM	Carboxypeptidase M	IPI00026270	443	0	S	DPEITNLINSTR,NFPDAFEYNNVSR
CPN2	Carboxypeptidase N subunit 2	IPI00479116	545	1	S	LYLGSNNLTALHPALFQNLSK,AFGSNPNLTK
CSF1R	Macrophage colony-stimulating factor 1 receptor	IPI00011218	972	2	S	VTVQSLLTVETLEHNQTYECR
DCD	Uncharacterized protein	IPI00847793	121	0	S	LVFGAPVNLTSIPLTSVSRP

(**Supplementary Table S2** Continued)

Gene	Description	IPI #	#aa[a]	TM[b]	SP[c]	Identified peptides
DMBT1	Isoform 1 of Deleted in malignant brain tumors 1 protein	IPI00099110	2413	1		AEYYSSPSNDSTNLLCLPNHMQASVSR,LVNLNSSYGLCAGR
DSG1	Desmoglein-1	IPI00025753	1049	2	S	TGEINITSIVDR
ELANE	Neutrophil elastase	IPI00027769	267	2	S	VVLGAHNLSR
F11	Isoform 1 of Coagulation factor XI	IPI00008556	625	1		LETTVNYTDSQRPICLPSK,GINYNSSVAK,VYSGILNQSEIK
F12	Coagulation factor XII	IPI00019581	615	1	S	RNHSCEPCQTLAVR
F13B	Coagulation factor XIII B chain	IPI00007240	661	1		KEHETCLAPELYNGNYSTTQK
F2	Prothrombin (Fragment)	IPI00019568	622	0	S	YPHKPEINSTTHPGADLQENFCR,NFTENDLLVR
FBLN1	Isoform D of Fibulin-1	IPI00296534	703	0	S	CATPHGDNASLEATFVK
FBLN5	Fibulin-5	IPI00294615	448	0	S	NHTCNLQQTCYNLQGGFK
FBN1	Fibrillin-1	IPI00328113	2871	0	S	VLPVNVTDYCQLVR
FCGBP	IgGFc-binding protein	IPI00242956	5405	5	S	YLPVNSSLLTSDCSER,LLISSLSESPASVSILSQADNTSK,VTVRPGESVMVNISAK,GLCVLSVGANLTTFDGAR,VVTVAALGTNISIHK,VITVQVANFTLR
FCN3	Isoform 1 of Ficolin-3	IPI00293925	299	0	S	VELEDFNGNR
FETUB	Uncharacterized protein	IPI00552199	345	0	S	GCNDSDVLAVAGFALR
FGA	Isoform 1 of Fibrinogen α chain	IPI00021885	866	0	S	MDGSLNFNR
FGB	Fibrinogen β chain	IPI00298497	491	0	S	GTAGNALMDGASQLMGENR
FGG	Isoform Gamma-B of Fibrinogen gamma chain	IPI00021891	453	0	S	DLQSLEDILHQVENK
FN1	Fibronectin isoform 4 preproprotein	IPI00845263	2330	0	S	HEEGHMLNCTCFGQGR,DQCIVDDITYNVNDTFHK,LDAPTNLQFVNETDSTVLVR
FOLR1	Folate receptor α	IPI00441498	257	1	S	KNACCSTNTSQEAHK,GWNWTSGFNK
FSTL1	Follistatin-related protein 1	IPI00029723	308	0	S	GSNYSEILDK
GFRA2	Isoform 1 of GDNF family receptor α-2	IPI00011732	464	0		ANELCAAESNCSSR
GOLM1	Isoform 2 of Golgi membrane protein 1	IPI00759659	391	0	S	AVLVNNITTGER
GP2	cDNA FLJ56017, highly similar to Pancreatic secretory granule membrane major glycoprotein GP2	IPI00299429	515	1		QDLNSSDVHSLQPQLDCGPR,DPNCSSILQTEER
GPLD1	Isoform 1 of Phosphatidylinositol-glycan-specific phospholipase D	IPI00299503	840	0	S	NLTTSLTESVDR,LNVEAANWTVR
GPR126	Isoform 1 of G-protein coupled receptor 126	IPI00217481	1221	8	S	ILSNLSCNVK
HP	Haptoglobin	IPI00641737	406	0	S	VVLHPNYSQVDIGLIK,NLFLNHSENATAK,MVSHHNLTTGATLINEQWLLTTAK
HPX	Hemopexin	IPI00022488	462	0	S	ALPQPQNVTSLLGCTH,NGTGHGNSTHHGPEYMR,SWPAVGNCSSALR
HRG	Histidine-rich glycoprotein	IPI00022371	525	0	S	HSHNNNSSDLHPHK,VIDFNCTTSSVSSALANTK
HYOU1	Hypoxia up-regulated protein 1	IPI00000877	999	0	S	VINETWAWK
ICAM1	Intercellular adhesion molecule 1	IPI00008494	532	1	S	ANLTVVLLR
IGFBP3	Insulin-like growth factor-binding protein 3	IPI00018305	291	0	S	GLCVNASAVSR,AYLLPAPPAPGNASESEEDR
IGH@	Putative uncharacterized protein DKFZp686L19235	IPI00784950	479	0	S	LSLHRPALEDLLLGSEANLTCTLTGLR,TPLTANITK,LAGKPTHVNVSVVMAEVDGTCY

(**Supplementary Table S2** Continued)

Gene	Description	IPI #	#aa[a]	TM[b]	SP[c]	Identified peptides
IGHA1	cDNA FLJ14473 fis, clone MAMMA1001080, highly similar to Homo sapiens SNC73 protein (SNC73) mRNA	IPI00386879	494	0	S	LSLHRPALEDLLLGSEANLTCTLTGLR,LAGKPTHVNVSVVMAEVDGTCY
IGHA2	Putative uncharacterized protein DKFZp686C02220 (Fragment)	IPI00423461	500	0	S	LSLHRPALEDLLLGSEANLTCTLTGLR,HYTNSSQDVTVPCR,TPLTANITK
IGHG1	Putative uncharacterized protein DKFZp686P15220	IPI00645363	472	0	S	EEQYNSTYR
IGHG2	Putative uncharacterized protein DKFZp686I04196 (Fragment)	IPI00399007	417	0		TKPREEQFNSTFR,EEQFNSTFR
IGHG4	Putative uncharacterized protein DKFZp686M24218	IPI00930442	476	0	S	EEQFNSTYR
IGHM	56 kDa protein	IPI00941837	511	0	S	EEQYNSTFR
IGHM		IPI00941961	599	0	S	STGKPTLYNVSLVMSDTAGTCY,YKNNSDISSTR,THTNISESHPNATFSAVGEASICEDDWNSGER,GLTFQQNASSMCGPDQDTAIR
IGHM	Ig mu chain C region	IPI00952640	452	0		GLTFQQNASSMCVPDQDTAIR,STGKPTLYNVSLVMSDTAGTCY,YKNNSDISSTR,THTNISESHPNATFSAVGEASICEDDWNSGER
IGHV4–31	IGH@ protein	IPI00785084	465	0	S	EEQYNSTYR
IGJ	Immunoglobulin J chain	IPI00178926	159	0	S	ENISDPTSPLR
IL6ST	Isoform 1 of Interleukin-6 receptor subunit β	IPI00297124	918	1	S	SHLQNYTVNATK
ITIH1	Inter-α-trypsin inhibitor heavy chain H1	IPI00292530	911	0	S	ICDLLVANNHFAHFFAPQNLTNMNK
ITIH2	Uncharacterized protein	IPI00645038	935	1	S	GAFISNFSMTVDGK
ITIH3	Isoform 1 of Inter-α-trypsin inhibitor heavy chain H3	IPI00028413	890	1	S	NAHGEEKENLTAR
KLKB1	Plasma kallikrein	IPI00654888	638	0	S	LQAPLNYTEFQKPICLPSK,IYPGVDFGGEELNVTFVK
KNG1	Isoform HMW of Kininogen-1	IPI00032328	644	0	S	LNAENNATFYFK,ITYSIVQTNCSK,YNSQNQSNNQFVLYR,HGIQYFNNNTQHSSLFMLNEVK
KNG1	17 kDa protein	IPI00797097	154	0	S	YNSQNQSNNQFVLYR,HGIQYFNNNTQ
KRT9	Keratin, type I cytoskeletal 9	IPI00019359	623	0		NYSPYYNTIDDLKDQIVDLTVGNNK
LCN2	Isoform 1 of Neutrophil gelatinase-associated lipocalin	IPI00299547	198	0	S	SYNVTSVLFR
LGALS3BP	Galectin-3-binding protein	IPI00023673	585	0	S	EPGSNVTMSVDAECVPMVR,DAGVVCTNETR,GLNLTEDTYKPR,TVIRPFYLTNSSGVD,ALGFENATQALGR,AAIPSALDTNSSK
LOC100133739	Putative uncharacterized protein DKFZp686C15213	IPI00426051	464	0	S	EEQFNSTFR
LOX	Protein-lysine 6-oxidase	IPI00002802	417	0	S	DPGAAVPGAANASAQQPR
LRG1	Leucine-rich α-2-glycoprotein	IPI00022417	347	0	S	LPPGLLANFTLLR,MFSQNDTR
LTF	lactotransferrin isoform 2	IPI00789477	666	0		TAGWNIPMGLLFNQTGSCK
LUM	Lumican	IPI00020986	338	0	S	LHINHNNLTESVGPLPK,AFENVTDLQWLILDHNLLENSK
LYVE1	Lymphatic vessel endothelial hyaluronic acid receptor 1	IPI00290856	322	1	S	ANQQLNFTEAK

(**Supplementary Table S2** Continued)

Gene	Description	IPI #	#aa[a]	TM[b]	SP[c]	Identified peptides
MASP1	Isoform 3 of Mannan-binding lectin serine protease 1	IPI00216882	380	0	S	FGYILHTDNR
MFAP4	Microfibril-associated glycoprotein 4	IPI00022792	255	0	S	VDLEDFENNTAYAK
MMRN1	Isoform 1 of Multimerin-1	IPI00012269	1228	0	S	LQNLTLPTNASIK,FNPGAESVVLSNSTLK
MMRN2	Multimerin-2	IPI00015525	949	0	S	FNTTYINIGSSYFPEHGYFR
MPO	Isoform H17 of Myeloperoxidase	IPI00007244	745	1	S	NMSNQLGLLAVNQR,DFVNCSTLPALNLASWR,SYNDSVDPR,SCPACPGSNITIR
MSLN	Isoform 2 of Mesothelin	IPI00025110	622	0	S	WNVTSLETLK,ALSQQNVSMDLATFMK
MST1	Hepatocyte growth factor-like protein	IPI00292218	711	0	S	GTGNDTVLNVALLNVISNQECNIK,GTANTTTAGVPCQR
MUC16	Mucin-16	IPI00103552	####	8		NTSVGLLYSGCR
MUC5AC	Mucin-5AC (Fragment)	IPI00103397	5030	2	S	NVTLHCTDGSSR,FANNTEGQCGTCTNDR
MUC5AC	Mucin 5AC, oligomeric mucus/ gel-forming	IPI00918002	6207	6	S	VVLLDPKPVANVTCVNK,AFGQFFSPGEVIYNK,QVNETWTLENCTVAR,LDG PTEQCPDPLPLPAGNCTDEEGICHR
OGN	cDNA FLJ59205, highly similar to Mimecan	IPI00025465	356	1		ANDTSYIR
OLFM4	Olfactomedin-4	IPI00022255	510	0	S	LNDTTLQVLNTWYTK,VNLTTNTIAVTQTLPNAAYNNR,KLLNLTVR,SLGSGGSVSQLFSNFTGSVDDR
OLFML3	Isoform 1 of Olfactomedin-like protein 3	IPI00024621	406	0	S	IYVLDGTQNDTAFVFPR
ORM1	α-1-acid glycoprotein 1	IPI00022429	201	0	S	SVQEIQATFFYFTPNKTEDTIFLR,WFYIASAFRNEEYNK,EYQTRQDQCIYNT TYLNVQR,QDQCIYNTTYLNVQR
ORM2	α-1-acid glycoprotein 2	IPI00020091	201	0	S	QNQCFYNSSYLNVQR,SVQEIQATFFYFTPNKTEDTIFLR,WFYIASAFRNEEYNK
OVGP1	Oviduct-specific glycoprotein	IPI00029779	678	1	S	GENLTSEVGTHPR
PAEP	Isoform 1 of Glycodelin	IPI00014544	180	0	S	WENNSCVEK
PGLYRP2	Isoform 1 of N-acetylmuramoyl-L-alanine amidase	IPI00163207	576	2	S	GFGVAIVGNYTAALPTEAALR,LEPVHLQLQCMSQEQLAQVAANATK
PIGR	Polymeric immunoglobulin receptor	IPI00004573	764	1	S	ANLTNFPENGTFVVNIAQLSQDDSGR,VPGNVTAVLGETLK,QIGLYPVLVIDSSGYVNPNYTGR,IIEGEPNLKV PGNVTAVLGETLK,WNNTGCQALPSQDEGPS K
PIK3IP1	Isoform 1 of Phosphoinositide-3-kinase-interacting protein 1	IPI00298388	263	2		CLNWLDAQSGLASAPVSGAGNHSYCR
PIP	Prolactin-inducible protein	IPI00022974	146	0	S	TFYWDFYTNR
PKHD1L1	Fibrocystin-L precursor	IPI00749489	4243	6	S	TILGEVNLTIK,EVVLNATYISLQGGR,TCQILHWNFTDIR,ILILNDSLSYTHFAEK
PLTP	45 kDa protein	IPI00022733	405	1		MLQITNASLGLR,LQITNASLGLR,GAFFPLTERNWSLPNR,VSNVSCQASVSR,IYSNHSALESLALIPLQAPLK
PLTP	cDNA FLJ39690 fis, clone SMINT2010639, highly similar to PHOSPHOLIPID TRANSFER PROTEIN	IPI00643034	513	1	S	EGHFYYNISEVK,GAFFPLTERNWSLPNR,VSNVSCQASVSR,IYSNHSALESLALIPLQAPLK
PODXL	Podocalyxin-like isoform 2 precursor	IPI00299116	528	1	S	QLVLNLTGNTLCAGGASDEK
PON1	Serum paraoxonase/arylesterase 1	IPI00218732	355	0	S	HANWTLTPLK,VVAEGFDFANGINISPDGK
PRNP	Isoform 1 of Major prion protein	IPI00022284	253	1	S	GENFTETDVK
PROC	Vitamin K-dependent protein C	IPI00021817	461	1	S	EVFVHPNYSK
PRPSAP1	Isoform 1 of Phosphoribosyl pyrophosphate synthase-associated protein 1	IPI00291578	356	2		NATVHPGLELPLMMAK

(**Supplementary Table S2** Continued)

Gene	Description	IPI #	#aa[a]	TM[b]	SP[c]	Identified peptides
PSAP	Isoform Sap-mu-0 of Proactivator polypeptide	IPI00012503	524	0	S	TNSTFVQALVEHVK
PTGDS	Prostaglandin-H2 D-isomerase	IPI00013179	190	0	S	WFSAGLASNSSWLR,SVVAPATDGGLNLTSTFLR
RNASE1	Ribonuclease pancreatic	IPI00014048	156	0	S	SNSSMHITDCR
RNASET2	Isoform 1 of Ribonuclease T2	IPI00414896	256	0	S	QDQQLQNCTEPGEQPSPK
SELL	L-selectin precursor	IPI00218795	385	1		IGGIWTWVGTNK,DNYTDLVAIQNK
SEPP1	Selenoprotein P	IPI00029061	381	0	S	EGYSNISYIVVNHQGISSR,VSEHIPVYQQEENQTDVWTLLNGSK
SERPINA1	Isoform 1 of α-1-antitrypsin	IPI00553177	418	1	S	QLAHQSNSTNIFFSPVSIATAFAMLSLGTK,YLGNATAIFFLPDEGK,ADTHDEI LEGLNFNLTEIPEAQIHEGFQELLR
SERPINA3	cDNA FLJ35730 fis, clone TESTI2003131, highly similar to α-1-ANTICHYMOTRYPSIN	IPI00550991	448	2	S	FNLTETSEAEIHQSFQHLLR,YTGNASALFILPDQDK,TLNQSSDELQLSMGNAMFVK
SERPINA4	Kallistatin	IPI00328609	427	1	S	SQILEGLGFNLTELSESDVHR,DFYVDENTTVR,FLNDTMAVYEAK
SERPINA5	Plasma serine protease inhibitor	IPI00007221	406	1	S	VVGVPYQGNATALFILPSEGK
SERPINA6	Corticosteroid-binding globulin	IPI00027482	405	3	S	AVLQLNEEGVDTAGSTGVTLNLTSKPIILR,AQLLQGLGFNLTER
SERPINA7	Thyroxine-binding globulin	IPI00292946	415	1	S	TLYETEVFSTDFSNISAAK,VTACHSSQPNATLYK
SERPINC1	Antithrombin-III	IPI00032179	464	1	S	LGACNDTLQQLMEVFK,WVSNKTEGR,SLTFNETYQDISELVYGAK
SERPIND1	SERPIND1	IPI00292950	527	2	S	NLSMPLLPADFHK
SERPINF1	Pigment epithelium-derived factor	IPI00006114	418	1	S	VTQNLTLIEESLTSEFIHDIDR
SERPING1	Plasma protease C1 inhibitor	IPI00291866	500	1	S	VGQLQLSHNLSLVILVPQNLK,VLSNNSDANLELINTWVAK,DTFVNASR
SHBG	Isoform 1 of Sex hormone-binding globulin	IPI00023019	402	1	S	LDVDQALNR
SOD3	Extracellular superoxide dismutase [Cu-Zn]	IPI00027827	240	0	S	LDAFFALEGFPTEPNSSSR
SORT1	Sortilin	IPI00217882	831	3	S	DITDLINNTFIR
SPON1	Spondin-1	IPI00171473	807	0	S	LTFYGNWSEK
TF	Serotransferrin precursor	IPI00022463	698	0	S	QQQHLFGSNVTDCSGNFCLFR,CGLVPVLAENYNKSDNCEDTPEAGYFAVAVVK
TFPI2	Tissue factor pathway inhibitor 2	IPI00009198	235	0	S	DEGLCSANVTR
THY1	Thy-1 membrane glycoprotein	IPI00022892	161	1	S	HENTSSSPIQYEFSLTR
TIMP1	Metalloproteinase inhibitor 1	IPI00032292	207	0	S	FVGTPEVNQTTLYQR,SHNRSEEFLIAGK
TMEM110	Isoform 2 of Inter-α-trypsin inhibitor heavy chain H4	IPI00218192	914	1	S	LPTQNITFQTESSVAEQEAEFQSPK,AFITNFSMIIDGMTYPGIIK
TSPAN8	Tetraspanin-8	IPI00015872	237	3	S	IVNETLYENTK
VNN1	Pantetheinase	IPI00030871	513	1	S	LTGVAGNYTVCQK,MTGSGIYAPNSSR
VTN	Vitronectin	IPI00298971	478	0	S	NISDGFDGIPDNVDAALALPAHSYSGR,NNATVHEQVGGPSLTSDLQAQSK
VWF	von Willebrand factor	IPI00023014	2813	0	S	ASPPSSSCNISSGEMQK
WFDC2	Isoform 1 of WAP four-disulfide core domain protein 2	IPI00291488	124	0	S	TGVCPELQADQNCTQECVSDSECADNLK

[a]#aa, number of amino acids; [b]TM, transmembrane domain (number shown); [c]SP, signal peptide (S=present).

Supplementary Table S3 Proteins identified in ovarian cancer cell lines.

Gene	Description	IPI#	#aa[a]	TM[b]	SP[c]	Identified peptides
A2M	α-2-macroglobulin	IPI00478003	1474	3	S	VSNQTLSLFFTVLQDVPVR
ACE	Isoform Somatic-1 of Angiotensin-converting enzyme	IPI00437751	1306	5	S	VTNDTESDINYLLK
ADAM17	Isoform B of Disintegrin and metalloproteinase domain-containing protein 17	IPI00029606	694	1		SEDIKNVSR,VVVVDGKNESEYTVK
ADAM9	Isoform 1 of Disintegrin and metalloproteinase domain-containing protein 9	IPI00440932	819	2	S	GYVEGVHNSSIALSDCFGLR
ADAMTS1 5	A disintegrin and metalloproteinase with thrombospondin motifs 15	IPI00152637	950	0	S	GAEYVISPLPNASAPAAQR
AHSG	cDNA FLJ55606, highly similar to α-2-HS-glycoprotein	IPI00022431	433	1		VCQDCPLLAPLNDTR
AKNA	Isoform 1 of AT-hook-containing transcription factor	IPI00377187	1439	0		LSEHSEVNPSVELSPAR
ALCAM	Isoform 1 of CD166 antigen	IPI00015102	583	1	S	IIISPEENVTLTCTAENQLER,LNLSENYTLSISNAR,NAIKEGDNITLK,LGD CIS EDSYPDGNITWYR
APLP2	Isoform 1 of Amyloid-like protein 2	IPI00031030	763	1	S	RNQSLSLLYK
APOD	Apolipoprotein D	IPI00006662	189	1	S	ADGTVNQIEGEATPVNLTEPAK
APOH	β-2-glycoprotein 1	IPI00298828	345	0	S	VYKPSAGNNSLYR,LGNWSAMPSCK
ASPH	Isoform Junctin-1 of Aspartyl/asparaginyl β-hydroxylase	IPI00024572	225	0	S	YNLSEVLQGK
ATP1B1	Isoform 1 of Sodium/potassium-transporting ATPase subunit β-1	IPI00747849	303	0	S	LEWLGNCSGLNDETYGYK
ATP6AP1	V-type proton ATPase subunit S1	IPI00784119	470	1	S	LNASLPALLLIR
ATRN	Isoform 1 of Attractin	IPI00027235	1429	6		GICNSSDVR,ISNSSDTVECECSENWK
AXL	Isoform Long of Tyrosine-protein kinase receptor UFO	IPI00296992	894	1	S	SLHVPGLNK
AZGP1	Zinc-α-2-glycoprotein	IPI00166729	298	0	S	DIVEYYNDSNGSHVLQGR,FGCEIENNR
B3GNT2	Isoform 2 of UDP-GlcNAc:βGal β-1,3-N-acetylglucosaminyltransferase 2	IPI00217345	393	1		LSNISHLNYCEPDLR,ESWGQESNAGNQTVVR
B7H6	B7 homolog 6	IPI00398918	454	1	S	NMDGTFNVTSCLK,LNSSQEDPGTVYQCVVR
BCAM	Basal cell adhesion molecule	IPI00002406	628	1	S	TQNFTLLVQGSPELK,VLSLPLNSSAVVNCSVHGLPTPALR
BMP1	Isoform BMP1–3 of Bone morphogenetic protein 1	IPI00009054	986	0	S	AAVPGNTSTPSCQSTNGQPQR,IILNFTSLDLYR
BSG	Isoform 2 of Basigin	IPI00019906	269	1	S	ILLTCSLNDSATEVTGHR
BTD	Biotinidase	IPI00218413	543	3	S	DVQIIVFPEDGIHGFNFTR,FNDTEVLQR,NPVGLIGAENATGETDPSHSK
C1orf151-NBL1	C1ORF15-NBL1 read-through protein isoform b	IPI00013299	181	0	S	NITQIVGHSGCEAK
C1S	Complement C1s subcomponent	IPI00017696	688	0	S	NCGVNCSGDVFTALIGEIASPNYPKPYPENSR
C2	cDNA FLJ55673, highly similar to Complement factor B	IPI00019591	1266	0	S	SPYYNVSDEISFHCYDGYTLR
C4A Uncharacterized protein		IPI00643525	1744	4	S	FSDGLESNSSTQFEVK,GLNVTLSSTGR
C9	Complement component C9	IPI00022395	559	0	S	AVNITSENLIDDVVSLIR
CADM1	Isoform 1 of Cell adhesion molecule 1	IPI00003813	442	1	S	FQLLNFSSSELK
CALU	Calumenin isoform c precurosr	IPI00789155	323	0	S	NATYGYVLDDPDPDDGFNYK
CD109	Isoform 1 of CD109 antigen	IPI00152540	1445	3	S	INYTVPQSGTFK,TQDEILFSNSTR,TASNLTVSVLEAEGVFEK
CD276	Isoform 2 of CD276 antigen	IPI00019275	316	1	S	TALFPDLLAQGNASLR
CD44	Isoform 12 of CD44 antigen	IPI00297160	361	1	S	AFNSTLPTMAQMEK
CD46	Isoform A of Membrane cofactor protein	IPI00012035	392	1	S	NHTWLPVSDDACYR
CD47	Isoform OA3–293 of Leukocyte surface antigen CD47	IPI00216514	292	3	S	DIYTFDGALNK
CD55	Decay-accelerating factor splicing variant 4	IPI00152418	525	1	S	GSQWSDIEEFCNR

(**Supplementary Table S3** Continued)

Gene	Description	IPI#	#aa[a]	TM[b]	SP[c]	Identified peptides
CD59	CD59 glycoprotein	IPI00011302	128	0	S	TAVNCSSDFDACLITK
CDCP1	Isoform 1 of CUB domain-containing protein 1	IPI00290039	836	1	S	ASVSFLNFNLSNCER,TCSSNLTLTSGSK
CDH6	Isoform 1 of Cadherin-6	IPI00024035	790	1	S	EDAQINTTIGSVTAQDPDAAR
CFH	Isoform 1 of Complement factor H	IPI00029739	1231	0	S	ISEENETTCYMGK,MDGASNVTCINSR,IPCSQPPQIEHGTINSSR
CFHR1	Complement factor H-related protein 1	IPI00011264	330	0	S	LQNNENNISCVER
CHRDL1	Isoform 1 of Chordin-like protein 1	IPI00478414	450	0	S	AFGIVECVLCTCNVTK
CLIC1	Chloride intracellular channel protein 1	IPI00010896	241	1		GVTFNVTTVDTK
CLU	Isoform 1 of Clusterin	IPI00291262	449	0	S	MLNTSSLLEQLNEQFNWVSR,LANLTQGEDQYYLR,HNSTGCLR, EDALNETR
COL12A1	Isoform 2 of Collagen α-1(XII) chain	IPI00221384	1899	1	S	EAGNITTDGYEILGK
COL6A1	Collagen α-1(VI) chain	IPI00291136	1028	0	S	NFTAADWGQSR,ENYAELLEDAFLKNVTAQICIDK,NVTAQICIDK
COL6A2	Isoform 2C2A of Collagen α-2(VI) chain	IPI00073454	828	0	S	GTFTDCALANMTEQIR,NMTLFSDLVAEK
COLEC12	Isoform 1 of Collectin-12	IPI00414467	742	0	S	ETLENNSFLITTVNK
CP	Ceruloplasmin	IPI00017601	1065	0	S	EHEGAIYPDNTTDFQR,AGLQAFFQVQECNK,ELHHLQEQNVSNAFLDK, ENL TAPGSDSAVFFEQGTTR
CPA4	Carboxypeptidase A4	IPI00008894	421	0	S	NWNASFAGK
CPD	Carboxypeptidase D	IPI00027078	1380	1	S	FANEYPNITR
CPVL	Probable serine carboxypeptidase CPVL	IPI00301395	476	0	S	SYAGFLTVNK,QAIHVGNQTFNDGTIVEK
CREG1	Protein CREG1	IPI00021997	220	0	S	LNITNIWVLDYFGGPK
CSF1	Isoform 3 of Macrophage colony-stimulating factor 1	IPI00218202	256	1	S	NCNNSFAECSSQGHER
CTBS	Di-N-acetylchitobiase	IPI00007778	385	0	S	QINSSISGNLWDK
CTSA	Lysosomal protective protein	IPI00021794	480	0	S	MDPPCTNTTAASTYLNNPYVR
CTSC	Isoform 1 of Dipeptidyl peptidase 1	IPI00022810	463	0	S	DVNCSVMGPQEK
CTSD	Cathepsin D	IPI00011229	412	0	S	GSLSYLNVTR
CTSL1	Cathepsin L1	IPI00012887	333	0	S	YSVANDTGFVDIPK
CTSL2	Cathepsin L2	IPI00000013	334	0	S	NLDHGVLVVGYGFEGANSNNSK,YRPENSVANDTGFTVVAPGK
DAG1	Dystroglycan	IPI00028911	895	1	S	NCSTITLQNITR
DCBLD2	Uncharacterized protein	IPI00185191	379	1	S	YCGLGLQMNHSIESK
DKK3	cDNA FLJ52545, highly similar to Dickkopf-related protein 3	IPI00002714	364	0	S	VGNNTIHVHR,ASSEVNLANLPPSYHNETNTDTK
DSG1	Desmoglein-1	IPI00025753	1049	2	S	TGEINITSIVDR
DSG2	Desmoglein-2	IPI00028931	1118	1	S	INATDADEPNTLNSK
ECE1	Isoform B of Endothelin-converting enzyme 1	IPI00002478	770	3		LGGWNITGPWAK
EFNA5	Ephrin-A5	IPI00005517	228	0	S	YAVYWNSSNPR
EFNB3	Ephrin-B3	IPI00019501	340	1	S	ENLPGDPTSNATSR
EPHB2	Isoform 1 of Ephrin type-B receptor 2	IPI00021275	1055	2	S	ELSEYNATAIK
F2R	Proteinase-activated receptor 1	IPI00296869	425	7	S	ATNATLDPR
F3	Tissue factor	IPI00010338	295	1	S	VNVTVEDER
FBLN1	Isoform D of Fibulin-1	IPI00296534	703	0	S	NCQDIDECVTGIHNCSINETCFNIQGGFR
FBN2	Isoform 1 of Fibrillin-2	IPI00019439	2912	0	S	SYNGTTCENELPFNVTK
FGFRL1	Fibroblast growth factor receptor-like 1	IPI00296561	504	1	S	HNSTIDVGGQK
FGG	Isoform γ-B of Fibrinogen gamma chain	IPI00021891	453	0	S	DLQSLEDILHQVENK

(**Supplementary Table S3** Continued)

Gene	Description	IPI#	#aa[a]	TM[b]	SP[c]	Identified peptides
FN1	Uncharacterized protein	IPI00845263	2421	1	S	WTPLNSSTIIGYR,LDAPTNLQFVNETDSTVLVR
FST	Isoform 1 of Follistatin	IPI00021081	344	0	S	SDEPVCASDNATYASECAMK
FSTL1	Follistatin-related protein 1	IPI00029723	308	0	S	FVEQNETAINITTYPDQENNK,GSNYSEILDK
GAA	Lysosomal α-glucosidase	IPI00293088	952	2	S	NNTIVNELVR,GVFITNETGQPLIGK
GBA	cDNA FLJ56157, highly similar to Glucosylceramidase	IPI00021807	621	1		DLGPTLANSTHHNVR
GDF15	Growth/differentiation factor 15	IPI00306543	308	0	S	ANQSWEDSNTDLVPAPAVR
GNS	N-acetylglucosamine-6-sulfatase	IPI00012102	552	0	S	YYNYTLSINGK,YPHNHHVVNNTLEGNCSSK
GOLM1	Isoform 2 of Golgi membrane protein 1	IPI00759659	391	0	S	AVLVNNITTGER
GPR126	Isoform 1 of G-protein coupled receptor 126	IPI00217481	1221	8	S	EANEVANQILNLTADGQNLTSANITNIVEQVK,GFNASYIR
GRN	cDNA FLJ13286 fis, clone OVARC1001154, highly similar to Homo sapiens clone 24720 epithelin 1 and 2 mRNA	IPI00181753	413	0		DVECGEGHFCHDNQTCCR,ENATTDLLTK
HEG1	Isoform 1 of Protein HEG homolog 1	IPI00297263	1381	1	S	SYSESSSTSSSESLNSSAPR,SHAASDAPENLTLLAETADAR
HLA-A	HLA class I histocompatibility antigen, A-69 α chain	IPI00760554	365	1	S	GYYNQSEAGSHTVQR
HLA-B	HLA class I histocompatibility antigen, B-58 α chain	IPI00472138	362	1	S	YYNQSEAGSHIIQR
HLA-B	HLA class I histocompatibility antigen, Cw-18 α chain	IPI00472162	366	1	S	
HLA-B	HLA class I histocompatibility antigen, B-82 α chain	IPI00472210	362	1	S	GYYNQSEAGSHTLQR
HMCN1	Hemicentin-1 precursor	IPI00045512	5636	5	S	GSVIGNINDVEFGIAFLNATITDSPNSDTR,LFTIDGISIPYTWNHTVFYDQ AQG R,GGNVTTDISVLINSLIK,GGPQSLVILLNK
HP	Haptoglobin	IPI00641737	406	0	S	VVLHPNYSQVDIGLIK,NLFLNHSENATAK
HPX	Hemopexin	IPI00022488	462	0	S	ALPQPQNVTSLLGCTH,NGTGHGNSTHHGPEYMR,SWPAVGNCSSALR
HRG	Histidine-rich glycoprotein	IPI00022371	525	0	S	VIDFNCTTSSVSSALANTK
HYOU1	Hypoxia up-regulated protein 1	IPI00000877	999	0	S	VFGSQNLTTVK,VINETWAWK,AEPPLNASASDQGEK
ICAM1	Intercellular adhesion molecule 1	IPI00008494	532	1	S	LNPTVTYGNDSFSAK,ANLTVVLLR,DHHGANFSCR
ICAM2	Intercellular adhesion molecule 2	IPI00009477	275	1	S	QESMNSNVSVYQPPR,AAPAPQEATATFNSTADR,GNETLHYETFGK
ICOSLG	Isoform 1 of ICOS ligand	IPI00219131	302	1	S	TVVTYHIPQNSSLENVDSR,LFNVTPQDEQK
IDS	Isoform 2 of Iduronate 2-sulfatase	IPI00006121	343	0	S	VHAGNFSTIPQYFK
IGFBP3	Insulin-like growth factor-binding protein 3	IPI00018305	291	0	S	GLCVNASAVSR,AYLLPAPPAPGNASESEEDR
IGFBPL1	Insulin-like growth factor-binding protein-like 1	IPI00291987	278	0	S	SVHNVTGAQVGLSCEVR
IGHG2	Putative uncharacterized protein DKFZp686I04196 (Fragment)	IPI00399007	417	0		EEQFNSTFR
IGHV4–31	IGH@ protein	IPI00785084	465	0	S	EEQYNSTYR
IL6ST	Isoform 1 of Interleukin-6 receptor subunit β	IPI00297124	918	1	S	SHLQNYTVNATK
ITGA2	Integrin α-2	IPI00013744	1181	3	S	TASCSNVTCWLK
ITGA3	Isoform 1 of Integrin α-3	IPI00215995	1051	2	S	AHCVWLECPIPDAPVVTNVTVK
ITGB1	Isoform β-1C of Integrin β-1	IPI00217561	825	1	S	NPCTSEQNCTSPFSYK,DTCTQECSYFNITK
KERA	Keratocan	IPI00013049	352	0	S	IWYLYLQNNLIETIPEKPFENATQLR
KIRREL	Isoform 1 of Kin of IRRE-like protein 1	IPI00470360	757	1	S	IDGGPVILLQAGTPHNLTCR
KITLG	Isoform 1 of Kit ligand	IPI00009450	273	2		FSNISEGLSNYSIIDK
KLKB1	Plasma kallikrein	IPI00654888	638	0	S	IYPGVDFGGEELNVTFVK
KNG1	Isoform HMW of Kininogen-1	IPI00032328	644	0	S	LNAENNATFYFK,ITYSIVQTNCSK
KRT10	Keratin, type I cytoskeletal 10	IPI00009865	584	0		NVSTGDVNVEMNAAPGVDLTQLLNNMR
KRT9	Keratin, type I cytoskeletal 9	IPI00019359	623	0		NYSPYYNTIDDLKDQIVDLTVGNNK

(**Supplementary Table S3** Continued)

Gene	Description	IPI#	#aa[a]	TM[b]	SP[c]	Identified peptides
L1CAM	Isoform 2 of Neural cell adhesion molecule L1	IPI00334532	1253	2	S	VPGNQTSTTLK
LAIR1	Isoform 2 of Leukocyte-associated immunoglobulin-like receptor 1	IPI00028015	270	1	S	STYNDTEDVSQASPSESEAR
LAMA2	Laminin subunit α-2 isoform b precursor	IPI00218725	3118	3	S	YIGGGVCINCTQNTAGINCETCTDGFFRPK
LAMA3	Isoform 1 of Laminin subunit α-3	IPI00003951	1713	0	S	EGSLPGNSTISIR,EVIDTNLTTLR
LAMA5	Laminin subunit α-5	IPI00783665	3695	4	S	LVGGPVAGGDPNQTIR,LNASIADLQSQLR
LAMB1	Laminin subunit β-1	IPI00013976	1786	0	S	VNASTTEPNSTVEQSALMR,LSDTTSQSNSTAK
LAMC1	Laminin subunit γ-1	IPI00298281	1609	0	S	KIPAINQTITEANEK,TANDTSTEAYNLLLR,VNNTLSSQISR,TLAGENQTAF EIEELNR,VNDNKTAAEEALR
LAMP1	LAMP1 protein variant (Fragment)	IPI00556655	392	1		ENTSDPSLVIAFGR,SSCGKENTSDPSLVIAFGR,NMTFDLPSDATVVLNR, GHT LTLNFTR
LAMP2	Isoform LAMP-2A of Lysosome-associated membrane glycoprotein 2	IPI00009030	410	1	S	VASVININPNTTHSTGSCR,IAVQFGPGFSWIANFTK
LCN2	Isoform 1 of Neutrophil gelatinase-associated lipocalin	IPI00299547	198	0	S	SYNVTSVLFR
LFNG	Isoform 2 of β-1,3-N-acetylglucosaminyltransferase lunatic fringe	IPI00219477	250	2		HTGNVVITNCSAAHSR
LGALS3B P	Galectin-3-binding protein	IPI00023673	585	0	S	EPGSNVTMSVDAECVPMVR,DAGVVCTNETR,GLNLTEDTYKPR,TVIRP FYL TNSSGVD,ALGFENATQALGR,AAIPSALDTNSSK
LIF	Leukemia inhibitory factor	IPI00009720	202	0	S	LCGPNVTDFPPFHANGTEK
LIPA	Isoform 1 of Lysosomal acid lipase/cholesteryl ester hydrolase	IPI00007207	399	2	S	YDLPASINFILNK,NYFHYNQSYPPTYNVK
LOC10013 3739	Putative uncharacterized protein DKFZp686C15213	IPI00426051	464	0	S	EEQFNSTFR
LOX	Protein-lysine 6-oxidase	IPI00002802	417	0	S	DPGAAVPGAANASAQQPR
LRG1	Leucine-rich α-2-glycoprotein	IPI00022417	347	0	S	LPPGLLANFTLLR
LUM	Lumican	IPI00020986	338	0	S	LHINHNNLTESVGPLPK,LGSFEGLVNLTFIHLQHNR,AFENVTDLQWLIL DHN LLENSK,LSHNELADSGIPGNSFNVSSLVELDLSYNK
LYPD3	Ly6/PLAUR domain-containing protein 3	IPI00004310	346	0	S	EACQGTSPPVVSCYNASDHVYK
MAN2A1	α-mannosidase 2	IPI00003802	1144	2		DSVINLSESVEDGPK
MAN2B1	Lysosomal α-mannosidase	IPI00012989	1011	0	S	WWHQQTNATQEVVR,LNQTEPVAGNYYPVNTR
MBTPS1	Membrane-bound transcription factor site-1 protease	IPI00021569	1052	3	S	YAESDPTVPCNETR
MEGF8	Isoform 1 of Multiple epidermal growth factor-like domains protein 8	IPI00027310	2845	4	S	ALLTNVSSVALGSR
MICA	MHC class I polypeptide-related sequence A	IPI00107380	383	1	S	SEASEGNITVTCR
MILR1	Isoform 1 of Allergin-1	IPI00175654	343	1		YDREPAEFNLTK
MINPP1	Isoform 2 of Multiple inositol polyphosphate phosphatase 1	IPI00028553	312	0	S	NATALYHVEAFK
MMP1	Interstitial collagenase	IPI00008561	469	0	S	AFQLWSNVTPLTFTK
MSLN	Isoform 2 of Mesothelin	IPI00025110	622	0	S	WNVTSLETLK
MUC16	Mucin-16	IPI00103552	22,152	8		NTSVGPLYSGCR
NAGLU	α-N-acetylglucosaminidase	IPI00008787	743	1	S	SVYNCSGEACR
NEO1	Isoform 1 of Neogenin	IPI00023814	1461	1	S	GSSVILNCSAYSEPSPK,TLSDVPSAAPQNLSLEVR,TPASDPHGDNLTY SVFY TK
NID2	NID2 protein	IPI00293033	969	1	S	TNIQGQVPYVPANFTAHISPYK,IHQNITYQVCR
NOV	Protein NOV homolog	IPI00011140	357	0	S	NCTSLHTYKPR
NRP1	Uncharacterized protein	IPI00165438	641	0	S	GPECSQNYTTPSGVIK
NRP2	Isoform A22 of Neuropilin-2	IPI00029693	931	3	S	NFTSPNGTIESPGFPEK

(**Supplementary Table S3** Continued)

Gene	Description	IPI#	#aa[a]	TM[b]	SP[c]	Identified peptides
NT5E	5′-nucleotidase	IPI00009456	574	0	S	LDNYSTQELGK
ORM1	α-1-acid glycoprotein 1	IPI00022429	201	0	S	SVQEIQATFFYFTPNKTEDTIFLR,QDQCIYNTTYLNVQR
ORM2	α-1-acid glycoprotein 2	IPI00020091	201	0	S	QNQCFYNSSYLNVQR,SVQEIQATFFYFTPNKTEDTIFLR
OSMR	Isoform 1 of Oncostatin-M-specific receptor subunit β	IPI00022674	979	1		NIQNNVSCYLEGK
PAEP	Isoform 1 of Glycodelin	IPI00014544	180	0	S	WENNSCVEK
PCDH7	Isoform A of Protocadherin-7	IPI00001893	1069	1	S	IDNLTGELSTSER
PDGFB	Platelet-derived growth factor subunit B	IPI00000044	241	0	S	LLHGDPGEEDGAELDLNMTR
PIGR	Polymeric immunoglobulin receptor	IPI00004573	764	1	S	VPGNVTAVLGETLK
PIP	Prolactin-inducible protein	IPI00022974	146	0	S	TFYWDFYTNR
PLA2G15	Group XV phospholipase A2	IPI00301459	412	1	S	VFVQTPTINYTLR
PLAU	Isoform 1 of Urokinase-type plasminogen activator	IPI00296180	431	0	S	ENSTDYLYPEQLK
PLOD3	Procollagen-lysine,2-oxoglutarate 5-dioxygenase 3	IPI00030255	738	1	S	SAEFFNYTVR
PLTP	45 kDa protein	IPI00022733	405	1		LQITNASLGLR,VSNVSCQASVSR,IYSNHSALESLALIPLQAPLK
PLTP	cDNA FLJ39690 fis, clone SMINT2010639, highly similar to PHOSPHOLIPID TRANSFER PROTEIN	IPI00643034	513	1	S	EGHFYYNISEVK,VSNVSCQASVSR,IYSNHSALESLALIPLQAPLK
PLXNA1	Plexin-A1	IPI00552671	1896	1	S	LSGNLTLLR
PODXL	Podocalyxin-like isoform 2 precursor	IPI00299116	528	1	S	QLVLNLTGNTLCAGGASDEK
PPT1	Palmitoyl-protein thioesterase 1	IPI00002412	306	0	S	FLNDSIVDPVDSEWFGFYR,NHSIFLADINQER
PRNP	Isoform 1 of Major prion protein	IPI00022284	253	1	S	GENFTETDVK
PROCR	Endothelial protein C receptor precursor	IPI00009276	275	1	S	ALWQADTQVTSGVVTFTLQQLNAYNR
PRSS35	Inactive serine protease 35	IPI00451450	413	0	S	VQDLVLEPTQNITTK
PSAP	Isoform Sap-mu-0 of Proactivator polypeptide	IPI00012503	524	0	S	DNATEEEILVYLEK,TCDWLPKPNMSASCK,TNSTFVQALVEHVK
PTGDS	Prostaglandin-H2 D-isomerase	IPI00013179	190	0	S	SVVAPATDGGLNLTSTFLR
PTPRG	Isoform 1 of Receptor-type tyrosine-protein phosphatase gamma	IPI00011651	1445	4		VGEEYQELQLDGFDNESSNK
PTPRJ	Receptor-type tyrosine-protein phosphatase eta precursor	IPI00290328	1392	3		SNDTAASEYK,IHVAGETDSSNLNVSEPR,VSDNESSSNYTYK
PTPRK	Isoform 1 of Receptor-type tyrosine-protein phosphatase kappa	IPI00015756	1439	2	S	IAVDWESLGYNITR
PTPRM	Receptor-type tyrosine-protein phosphatase mu	IPI00293849	1452	2	S	FIASFNVVNTTK
PXDN	Isoform 1 of Peroxidasin homolog	IPI00016112	1479	2	S	ILCDNADNITR,LSTTECVDAGGESHANNTK
QPCT	Isoform 1 of Glutaminyl-peptide cyclotransferase	IPI00003919	361	1	S	NYHQPAILNSSALR,YFQNYSYGGVIQDDHIPFLR
QSOX1	Isoform 2 of Sulfhydryl oxidase 1	IPI00465016	604	1	S	SPTNTTPHVPAEGPELI
RNASE1	Ribonuclease pancreatic	IPI00014048	156	0	S	SNSSMHITDCR
RNASET2	Isoform 1 of Ribonuclease T2	IPI00414896	256	0	S	QDQQLQNCTEPGEQPSPK
ROBO1	Uncharacterized protein	IPI00219798	1655	3		NYLGEAVSHNASLEVAILR
SELL	L-selectin precursor	IPI00218795	385	1		IGGIWTWVGTNK
SEMA3A	Semaphorin-3A	IPI00031510	771	0	S	IIYGVENSSTFLECSPK
SEMA4B	Semaphorin-4B precursor	IPI00419724	837	1	S	INSSLQLPDR
SEMA4C	Semaphorin-4C	IPI00073763	833	3	S	NNQTECFNFIR
SERPINA1	Isoform 1 of α-1-antitrypsin	IPI00553177	418	1	S	QLAHQSNSTNIFFSPVSIATAFAMLSLGTK,YLGNATAIFFLPDEGK
SERPINA3	cDNA FLJ35730 fis, clone TESTI2003131, highly similar to α-1-ANTICHYMOTRYPSIN	IPI00550991	448	2	S	YTGNASALFILPDQDK
SERPINA6	Corticosteroid-binding globulin	IPI00027482	405	3	S	AQLLQGLGFNLTER

(**Supplementary Table S3** Continued)

Gene	Description	IPI#	#aa[a]	TM[b]	SP[c]	Identified peptides
SERPINB2	Plasminogen activator inhibitor 2	IPI00007117	415	1		ANFSGMSER,SLSSAINASTGNYLLESVNK
SERPINC1	Antithrombin-III	IPI00032179	464	1	S	LGACNDTLQQLMEVFK,SLTFNETYQDISELVYGAK
SERPINE2	Isoform 1 of Glia-derived nexin	IPI00009890	398	2	S	NASEIEVPFVTR
SERPINF1	Pigment epithelium-derived factor	IPI00006114	418	1	S	VTQNLTLIEESLTSEFIHDIDR
SERPING1	Plasma protease C1 inhibitor	IPI00291866	500	1	S	VLSNNSDANLELINTWVAK
SERPINI1	Neuroserpin	IPI00016150	410	2	S	DANLTGLSDNK,WVENNTNNLVK
SEZ6L2	Isoform 3 of Seizure 6-like protein 2	IPI00018276	853	1	S	IVSPEPGGAVGPNLTCR,LLANSSMLGEGQVLR
SGCE	Epsilon-sarcoglycan isoform 1	IPI00414984	462	1		LNAINITSALDR
SIAE	Isoform 1 of Sialate O-acetylesterase	IPI00010949	523	0	S	NLTFEGPLPEK
SIRPA	Isoform 1 of Tyrosine-protein phosphatase non-receptor type substrate 1	IPI00332887	504	1	S	AENQVNVTCQVR,GTANLSETIR,IGNITPADAGTYYCVK,LQLTWLENG NVS R
SLC3A2	Isoform 2 of 4F2 cell-surface antigen heavy chain	IPI00027493	529	2		SLVTQYLNATGNR,DASSFLAEWQNITK,LLIAGTNSSDLQQILSLLESNK
SPINT2	Kunitz-type protease inhibitor 2	IPI00011662	252	1	S	CATVTENATGDLATSR
SPON1	Spondin-1	IPI00171473	807	0	S	LTFYGNWSEK
ST3GAL1	CMP-N-acetylneuraminate-β-galactosamide-α-2,3-sialyltransferase 1	IPI00009629	340	0	S	KPNNLNDTIK
SULF1	Extracellular sulfatase Sulf-1	IPI00293203	871	1	S	TFAVYLNNTGYR,FYNYTVCR
SULF2	Isoform 1 of Extracellular sulfatase Sulf-2	IPI00297252	870	1	S	FYNYTLCR
TF	Serotransferrin	IPI00022463	698	0	S	QQQHLFGSNVTDCSGNFCLFR
TFPI	Isoform α of Tissue factor pathway inhibitor	IPI00021834	304	0	S	YFYNNQTK
TFPI2	Tissue factor pathway inhibitor 2	IPI00009198	235	0	S	DEGLCSANVTR
THBD	Thrombomodulin	IPI00010737	575	1	S	GFQWVTGDNNTSYSR
THBS1	Thrombospondin-1	IPI00296099	1170	0	S	VVNSTTGPGEHLR
THBS3	Thrombospondin-3	IPI00329535	956	0	S	LGFLGNQSQGCLPAR
TIMP1	Metalloproteinase inhibitor 1	IPI00032292	207	0	S	FVGTPEVNQTTLYQR
TMEM110	Isoform 2 of Inter-α-trypsin inhibitor heavy chain H4	IPI00218192	914	1	S	LPTQNITFQTESSVAEQEAEFQSPK
TNC	Isoform 1 of Tenascin	IPI00031008	2201	0	S	NTTSYVLR,GPNCSEPECPGNCHLR,VEAAQNLTLPGSLR,GNFSTEGCGC VCE PGWK,LLETVEYNISGAER
TNFRSF11B	Tumor necrosis factor receptor superfamily member 11B	IPI00298362	401	0		GNATHDNICSGNSESTQK
TNFRSF1A	Isoform 1 of Tumor necrosis factor receptor superfamily member 1A	IPI00018880	455	1	S	YIHPQNNSICCTK
TNFRSF21	Tumor necrosis factor receptor superfamily member 21	IPI00004413	655	1	S	VLSSIQEGTVPDNTSSAR,CPAGTYVSEHCTNTSLR
TPP1	cDNA FLJ56402, highly similar to Tripeptidyl-peptidase 1	IPI00298237	572	0	S	FLSSSPHLPPSSYFNASGR
TXNDC15	Isoform 1 of Thioredoxin domain-containing protein 15	IPI00151990	360	1	S	NITGLENFTLK
TYRO3	Tyrosine-protein kinase receptor TYRO3	IPI00030887	890	2	S	LSSSNASVAWMPGADGR
VASN	Vasorin	IPI00395488	673	1	S	LHEITNETFR
VTN	Vitronectin	IPI00298971	478	0	S	NNATVHEQVGGPSLTSDLQAQSK
WFDC2	Isoform 1 of WAP four-disulfide core domain protein 2	IPI00291488	124	0	S	TGVCPELQADQNCTQECVSDSECADNLK

[a]#aa, number of amino acids; [b]TM, transmembrane domain (number shown); [c]SP, signal peptide (S=present).