Genome-wide gene expression profiles of ovarian carcinoma: Identification of molecular targets for the treatment of ovarian carcinoma

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Abstract. This study aimed to clarify the molecular mechanisms involved in ovarian carcinogenesis, and to identify candidate molecular targets for its diagnosis and treatment. The genome-wide gene expression profiles of 22 epithelial ovarian carcinomas were analyzed with a microarray representing 38,500 genes, in combination with laser microbeam microdissection. A total of 273 commonly up-regulated transcripts and 387 down-regulated transcripts were identified in the ovarian carcinoma samples. Of the 273 up-regulated transcripts, only 87 (31.9%) were previously reported as up-regulated in microarray studies using bulk cancer tissues and normal ovarian tissues for analysis. *CHMP4C* (chromatin-modifying protein 4C) was frequently overexpressed in ovarian carcinoma tissue, but not expressed in the normal human tissues used as a control. Our data should contribute to an improved understanding of tumorigenesis in ovarian cancer, and aid in the development of diagnostic tumor markers and molecular-targeting therapy for patients with the disease.

Introduction

Ovarian cancer is the second most common gynecologic malignancy and the fifth leading cause of cancer-related death in women. As there are no specific indicators or symptoms of ovarian cancer during the early stages of the disease, the majority of patients with epithelial ovarian cancer (EOC) are diagnosed at an advanced stage, with involvement of other sites such as the upper abdomen, pleural space and paraaortic lymph nodes. The cancer antigen 125 assay (CA-125) has been used to screen for ovarian cancer, but is not specific; only 50% of patients with early ovarian cancer test positive using this assay (1). The prognosis for ovarian cancer patients remains poor and largely dependant on the stage at diagnosis, with a 5-year survival rate of 45% (2). Chemotherapy is the most common choice of treatment for patients at advanced stages, but often causes severe adverse reactions without affecting the tumor. Hence, the identification of new markers for early diagnostics and molecular targets that can be applied to the development of new treatment is eagerly anticipated.

Genome-wide microarray analysis enabled us to obtain comprehensive gene expression profiles related to the phenotypic and biological information of cancer cells (3,4). We identified multiple targets that may prove useful in the development of novel anti-cancer drugs and/or diagnostic biomarkers. In ovarian carcinoma, this approach can also be applied to identify unknown molecules involved in the carcinogenic pathway.

Through the gene expression profile analysis of 22 epithelial ovarian cancers coupled with purification of the cancer cell population by laser microbeam microdissection (LMM) on a microarray consisting of approximately 38,500 transcripts, we identified a number of transcripts that were overexpressed in ovarian cancers. We report important information regarding the mechanisms of ovarian carcinogenesis, as well as the discovery of potential targets for the development of diagnostic markers and novel therapeutic strategies for ovarian cancer.

Materials and methods

Tissue samples and microdissection. Twenty-two ovarian cancer tissue samples (median patient age 54 years, range

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Abbreviations: LMM, laser microbeam microdissection; EOC, epithelial ovarian cancer

Key words: gene expression profile, microarray, ovarian carcinoma, molecular target gene

Table I. Clinicopathological features of ovarian cancer clinical samples used for microarray analysis.

No.	Age	Histology	Clinical stage
BOv.T_3	75	Clear-cell carcinoma	III
BOv.T_4	61	Serous adenocarcinoma	III
BOv.T_6	75	Serous adenocarcinoma	IV
BOv.T_7	27	Mucinous adenocarcinoma	III
BOv.T_9	57	Serous clear-cell adenocarcinoma	III
BOv.T_10	62	Poor-to-differentiated carcinoma	IV
BOv.T_11	57	Cystadenocarcinoma	II
BOv.T_14	65	Serous adenocarcinoma	IV
BOv.T_15	37	Papillary serous adenocarcinoma	IV
BOv.T_16	69	Papillary serous adenocarcinoma	IV
BOv.T_17	62	Mucinous cystadenocarcinoma	I
BOv.T_19	49	Moderate adenocarcinoma	II
BOv.T_21	39	Moderate differentiated adenocarcinoma	IV
BOv.T_23	53	Moderate adenocarcinoma	III
BOv.T_27	52	Moderate serous papillary adenocarcinoma	III
BOv.T_28	46	Moderate serous papillary adenocarcinoma	III
BOv.T_5	73	Papillary serous adenocarcinoma	III
BOv.T_6	43	Moderate papillary serous adenocarcinoma	I
BOv.T_7	30	Well-differentiated papillary serous cystadenocarcinoma	III
BOv.T_8	52	Poor-to-well differentiated papillary serous cystadenocarcinoma	III
BOv.T_9	47	Poor-to-well differentiated papillary serous adenocarcinoma	III
BOv.T_10	61	Moderate serous papillary cystadenocarcinoma	I

27-75 years; Table I) were obtained with written informed consent. The samples were collected from patients who underwent surgical procedures at the University Hospital of Obstetrics and Gynecology (Sofia, Bulgaria) and the General Hospital of Oncology (Sofia, Bulgaria). Cancer tissues were examined by professional pathologists at the hospitals. All 22 samples were determined to be epithelial ovarian carcinomas; one sample was classified as clear cell, two as mucinous carcinomas and the remaining 19 cases as serous types. Clinical information was obtained from medical records. Clinical stage was judged according to the UICC TNM classification. All specimens were embedded in TissueTek OCT medium (Sakura, Tokyo, Japan) immediately after surgical resection and stored at -80˚C until use. These frozen tissues were cut into 8-μm sections using a cryostat (Sakura, Tokyo, Japan) and then stained with hematoxylin and eosin (H&E) for histological examination. Ovarian cancer cells and corresponding normal ovarian epithelial cells were selectively collected using the EZ cut system with a pulsed ultraviolet narrow beam-focus laser (SL Microtest GmbH, Jena, Germany) according to the manufacturer's protocols. The layer of normal epithelial cells was collected from eight samples by LMM, and the mixture of RNA extracted from them served as a 'universal control' for hybridization.

Affymetrix GeneChip hybridization. The affymetrix human genome U133 Plus 2.0 GeneChip array was employed for microarray hybridization. This GeneChip comprises more than 54,000 probe sets and analyzes the expression level of 38,500 genes. For microarray hybridization, we followed the protocol described in the Affymetrix GeneChip eukaryotic two cycle target preparation protocol (Affymetrix). For the first-round synthesis of double-stranded cDNA, 100 ng of total RNA was reversely transcribed using the Two-Cycle cDNA Synthesis Kit (Affymetrix, Santa Clara, CA) and T7-oligo-dT primer according to the manufacturer's instructions, followed by IVT amplification with the MEGAscript T7 Kit (Ambion Inc., Austin, TX). After cleanup of the cRNA with a GeneChip Sample Cleanup Module IVT column (Affymetrix), second-round double-stranded cDNA was amplified using the IVT Labeling Kit (Affymetrix). A 20 μg aliquot of the labeled product was fragmented by heat and ion-mediated hydrolysis at 94˚C for 35 min in H_2O combined with 8 μl of 5x Fragmentation Buffer (Affymetrix). The fragmented cRNA was hybridized for 16 h at 45˚C in a Hybridization Oven 640 to a U133 Plus 2.0 oligonucleotide array (Affymetrix). Washing and staining of the arrays with phycoerythrin-conjugated streptavidin (Molecular Probes, Eugene, OR) was completed in a Fluidics Station 450 (Affymetrix). The arrays were then scanned using a confocal laser GeneChip Scanner 3000 (Affymetrix).

Data analysis. Global normalization at a target value of 500 was applied to all 23 arrays (22 cancer arrays and one array of the universal control) under consideration using GeneChip Operating Software (Affymetrix). Normalized data from text files were imported to a Microsoft Excel spreadsheet. Because data derived from low signal intensities are less reliable, we

Table II. Primer sequences for semi-quantitative RT-PCR experiments.

Gene	Forward primer	Reverse primer
TACSTD1	5'-TTATGATCCTGACTGCGATGAG-3'	5'-AGGCAGCTTTCAATCACAAATC-3'
CHMP4C	5'-TACTGTGTCTCTTTTGGGAGAGC-3'	5'-CCCTAAGTGGCTAAATTACACCC-3'
PROM2	5'- CCCAGGCTGGAAGTGTCTAT-3'	5'-GCTACTTCCCCCAGGTAGGT-3'
RIPK4	5'-CATAGGGTGCCTTCTGAATACTG-3'	5'-CACAACAGTAAAGGCACAATGAG-3'
TMC4	5'-CTGCTTTACAGCATCTTCCTGAT-3'	5'-GATACAAGGAAGATCACCCGAG-3'
SCNN1A	5'-GGCTAGGGCTAGAGCAGAC-3'	5'-GTTGGGAAGGGAGACACAAA-3'
FAM83H	5'-TGTAGAAAGCCCCCACTGTT-3'	5'-ACATGCCCACAAGAACATCA-3'
TMEM139	5'-GCTTTGGTCACCCTGATGAT-3'	5'-TGGGGTAGGTCACCAAATGT-3'

excluded transcripts with low intensities from further analysis when the signal intensities of both normal and cancer cells were lower than that of the cut-off value. For the other genes, we calculated the signal intensities of the cancer/normal ratio using the raw data of each sample. Up-regulated or down-regulated genes were selected based on the signal intensity of their cancer/normal ratio (r): up-regulated, r>5.0; and down-regulated, r<0.2.

Calculation of contamination proportion. GSG2 (germ cell-specific gene 2), a gene that is expressed exclusively in ovarian germ cells, was used to evaluate the proportion of germ cells present in the population of microdissected normal surface epithelial cells. Each intensity was normalized to the intensity of the ß-actin gene (*ACTB*) as follows: Ratio A, the *GSG2*/*ACTB* intensity ratio in the whole normal ovarian tissue (where some of the cells correspond to germ cells); the signal intensity of poly $(A)^+$ RNA isolated from whole normal ovarian tissue was 0.2939. Ratio B, the *GSG2*/*ACTB* intensity ratio in microdissected normal ovarian epithelial cells = 0.0036 (a mixture of normal ovarian epithelial cells from eight individuals as a universal control). The proportion of the contamination was calculated as [(Ratio B)/(Ratio A)] x 100 = 1.24%.

Semi-quantitative RT-PCR. RNA from the purified populations of ovarian cancer cells and from normal epithelial cells was extracted using the RNeasy Micro Kit (Qiagen, Valencia, CA) and treated with DNase I according to the manufacturer's recommendations. Extracted RNA was subjected to two rounds of RNA amplification using T7-based *in vitro* transcription (Invitrogen), and amplified RNA were reverse-transcribed to single-stranded cDNA using random primer with Superscript II reverse transcriptase (Invitrogen). We prepared appropriate dilution of each single-stranded cDNA for subsequent PCR amplification, and monitored their reactions using *ACTB* as a quantitative control, as it showed the smallest fluctuations in the cancer/normal ratio in our ovarian cancer microarray data. PCR amplification was performed using single-stranded cDNA as a template and gene-specific primers (Table II). PCR reactions were optimized for the number of cycles to ensure product intensity within the logarithmic phase of amplification.

Northern blot analysis. Human multiple tissue Northern (MTN) blots (BD Biosciences, Palo Alto, CA) were hybridized for 16 h with ^{32}P-labeled PCR product of *CHMP4C* (chromatin modifying protein 4C) cDNA. The cDNA probe of *CHMP4C* was prepared by RT-PCR using the primers 5'-CAATGAG CAAGTTGGGCAAG-3' and 5'-CCAAGCTGCCAATTG TTTG-3'. Prehybridization, hybridization and washing were performed according to manufacturer's recommendations. The blots were autoradiographed with intensifying screens at -80˚C for 14 days.

Results

Identification of commonly up-regulated or down-regulated genes in ovarian carcinoma. To obtain the precise gene expression profiles of ovarian cancer cells, we employed LMM to purify both epithelial cancerous cells and normal epithelial cells (Fig. 1A-I). We estimated the proportion of germ cells in the microdissected population of normal ovarian epithelial cells by measuring the signal intensities of *GSG2* (germ cell-specific gene 2), which is highly and specifically expressed in human haploid germ cells (5-8). When the signal intensity of this gene was compared in whole normal ovarian tissue and microdissected normal ovarian epithelial cells, the average ratio of signal intensity was calculated to be ~98.77%, indicating that the proportion of germ cells in the microdissected normal ovarian epithelial cells was ~1.24% (described in Calculation of contamination proportion, Materials and methods) (9).

We searched for genes commonly up- and down-regulated in ovarian cancer according to the following criteria: i) genes for which expression data, defined as described in Materials and methods, was obtained in ≥50% (11/22) of the cases examined; and ii) genes whose expression ratio was >5.0 or <0.2 in ≥50% of the informative cases. According to these criteria, a total of 273 transcripts were selected as commonly up-regulated genes (Table III), and 387 as commonly down-regulated genes (Table IV). Up-regulated genes included those associated with signal transduction (*GPR39*, *FGF18*, *CXXC5*, *ARHGAP8*, *RASAL1*, *DEPDC1*, *ECT2*, *BAIAP2L1*, *SH2D3A*, *NRTN*), the regulation of transcription (*BCL11A*, *HOXB8*, *HOXB7*, *TFAP2A*, *FOXM1*, *EVI1*, *FOXQ1*, *CASZ1*, ELF3), the regulation of cell growth and the cell cycle (*DLG7*, *TPX2*, *SPC25*, *SFN*, *KIF2C*, *CDCA8*, *CHEK1*, *CCNB1*, *MKI67*, *ASPM*, *SPC24*, *AURKA*, *CEP55*, *TACSTD2*, *ESPL1*), apoptosis (*EGLN3*, *CDCA3*), proteolysis (*ST14*, *CAPN13*,

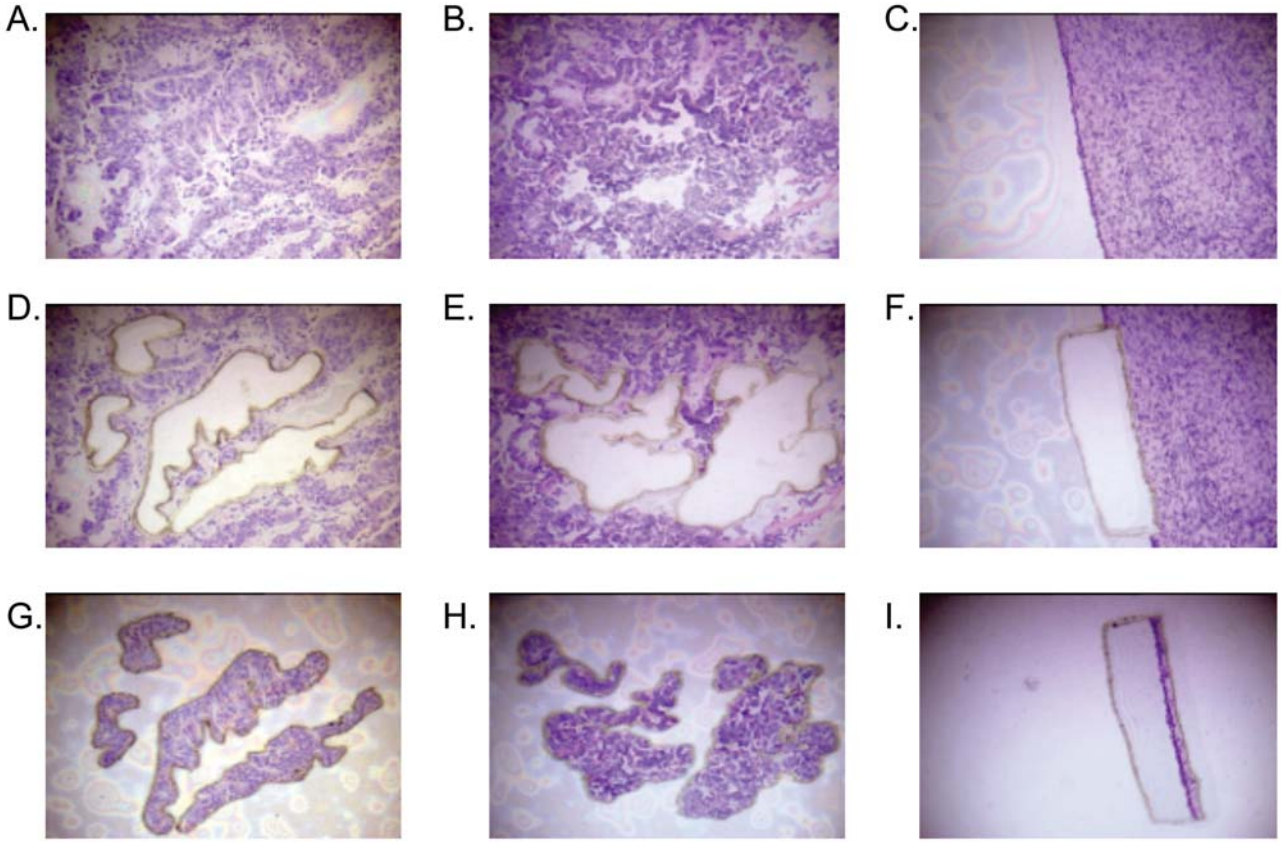

Figure 1. Laser-microbeam microdissection (LMM) of the representative mucinous (A, D and G) and serous (B, E and H) adenocarcinoma and normal surface epithelial (C, F and I) cells. (A, B and C) samples before dissection; (D, E and F) the same sections after microdissection (H&E staining); (G, H and I) the microdissected cancer cells captured on the collecting cap.

TMPRSS3, *TMPRSS4*, *WFDC2*, *KLK6*, *PRSS8*, *KLK7*), cell adhesion (*CDH6*, *MPZL2*, *GPR56*, *PKP3*, *CDH1*, *CLDN3*, *CLDN4*) and immune response (*CD24*, *ITGB6*, *CD164*). Among the up-regulated genes identified, *WAPDC2* and *SPP1* were previously reported to be genes activated in serous epithelial ovarian cancer compared with bulk normal ovarian tissue (10). *TOP2A*, *MUC1* and *SFN*, which have already been proven to be implicated in chemoresistance and tumorigenesis, are also included in the up-regulated genes (11). The FGF and WNT signaling pathways are represented by different members among the up-regulated genes. Although they were shown to have direct or indirect effects on ovarian carcinogenesis, their functional significance remains to be clarified. Some of the up-regulated genes have been found to be overexpressed not only in ovarian carcinoma, but also in a number of other malignancies. Elevated mRNA expression of *RHAMM* (hyaluronan-mediated motility receptor) was also found in patients with CML (40%), renal cell carcinoma (73%), breast carcinoma (60%) and ovarian carcinoma (50%) (12,13). *BCL11A* (b-cell cll/lymphoma 11a) is involved in both lymphoid malignancies and, with a significant amplification, in ovarian cancer (14).

Furthermore, 387 genes were identified whose expression levels were reduced to ≤20% in ovarian cancer compared with normal epithelial cells (Table IV). Certain of these were to some extent previously functionally characterized. Such genes have been implicated in the regulation of transcription (*ZFPM2*, *NR2F1*, *HOXC6*, *TCF4*), the regulation of cell growth and the cell cycle (*IGFBP5*, *HTRA1*, *GAS1*, *MCC*, *NBL1*, *NDN*), transport molecules (*COL6A3*, *COL3A1*, *SYTL4*, *FXYD6*, *DPYD*, *COL16A1*) and signal transduction (*ANK2*, *CAV1*, *RAB31*, *BNC2*). They include genes that are, it has been suggested, related to chemosensitivity (*TRA1*) and cell cycle control (*SMOC2*, *TIMP3*). The reduced expression of the genes in tumor cells may imply that they play a role in the suppression of cell growth or the inhibition of invasion. For example, *PEG3* (paternally expressed gene 3), which activates NF-κB, was suggested to have a tumor suppressive function (15). SPARC, an extracellular Ca(2+)-binding matricellular glycoprotein, was reported to be a potent antiproliferator with proapoptotic functions (16). *HTRA1* and *CAV1* also have potential antiangiogenic and antiproliferative roles, and are included in the down-regulated genes (17-20).

Identification of CHMP4C as a molecular target gene for ovarian cancer therapy. To validate the expression data obtained using microarray analysis, we performed semi-quantitative RT-PCR for a total of 20 representative genes found to be frequently overexpressed in ovarian cancer cases, using samples from 17 clinical ovarian cancer cases. These genes were confirmed as highly expressed in all or most of the ovarian cancer cases examined, while their expression could not be detected in normal ovarian surface epithelial cells (seven genes are shown in Fig. 2A). The results of RT-PCR using ovarian cancer materials were very concordant with those of the microarray analysis.

Table III. Genes commonly up-regulated in ovarian cancer.

No.	Accession no.	Gene symbol	Gene name
1	U29343	HMMR	Hyaluronan-mediated motility receptor (RHAMM)
2	NM_014750	DLG7	Discs, large homolog 7
3	AF080216	BCL11A	B-cell CLL/lymphoma 11A (zinc finger protein)
4	AI807532	ABHD7	abhydrolase domain containing 7
5	AI056699	TSPAN12	Tetraspanin 12
6	AF326731	NUF2	NUF2, NDC80 kinetochore complex component
7	N63576	GPM6B	Glycoprotein M6B
8	AI652872	EPB41L5	Erythrocyte membrane protein band 4.1 like 5
9	AV717094	GPR39	G protein-coupled receptor 39
10	U27699	SLC6A12	Solute carrier family 6
11	BC006148	OVOL2	Ovo-like 2
12	AI827972	METTL7B	Methyltransferase like 7B
13	NM_004991	MDS1	Myelodysplasia syndrome 1
14	N59856	CDNA FLJ36181	CDNA FLJ36181
15	L10343	PI3	Peptidase inhibitor 3, skin-derived (SKALP)
16	AI758950	SLC26A7	Solute carrier family 26, member 7
17	AK000168	CD24	CD24 molecule
18	AB028021	FOXA2	Forkhead box A2
19	BG327863	CD24	CD24 molecule
20	AA761181	CD24	CD24 molecule
21	AI277015	HOXB8	Homeobox B8
22	U82319	YDD19	YDD19 protein
23	BE670097	RCAN3	RCAN family member 3
24	AW102783	HOXB7	Homeobox B7
25	NM_003862	FGF18	Fibroblast growth factor 18
26	NM_005498	AP1M2	Adaptor-related protein complex 1
27	NM_000492	CFTR	Cystic fibrosis transmembrane conductance regulator
28	BF343007	TFAP2A	Transcription factor AP-2 α
29	AI684991	CP	Ceruloplasmin (ferroxidase)
30	AF098158	TPX2	TPX2, microtubule-associated, homolog
31	AI493046	MARVELD3	MARVEL domain containing 3
32	NM_016725	FOLR1	Folate receptor 1 (adult)
33	L33930	CD24	CD24 molecule
34	NM_002354	TACSTD1	Tumor-associated calcium signal transducer 1
35	AA532807	LRRC8E	Leucine rich repeat containing 8 family, member E
36	AI870547	Transcribed locus	Transcribed locus
37	U73945	DEFB1	Defensin, ß 1
38	AF225416	SPC25	SPC25, NDC80 kinetochore complex component
39	H40020		Transcribed locus
40	BG109249	CDNA FLJ34964	CDNA FLJ34964
41	AL556703	CP	Ceruloplasmin (ferroxidase)
42	BE791251	CLDN3	Claudin 3
43	U20428	ST14	Suppression of tumorigenicity 14
44	R54212	XKR4	XK, Kell blood group complex subunit-related family, member 4
45	BE674305		Transcribed locus
46	NM_005490	SH2D3A	SH2 domain containing 3A
47	NM_021953	FOXM1	Forkhead box M1
48	NM_012485	HMMR	Hyaluronan-mediated motility receptor (RHAMM)
49	AI798863	FGF18	Fibroblast growth factor 18
50	NM_024734	CLMN	Calmin (calponin-like, transmembrane)
51	AU154891	CDNA FLJ10919	CDNA FLJ10919
52	AW276866		Transcribed locus
53	AL161995	NRTN	Neurturin
54	AI821661	ILDR1	Immunoglobulin-like domain containing receptor 1
55	AK000049	CHMP4C	Chromatin modifying protein 4C

Table III. Continued.

No.	Accession no.	Gene symbol	Gene name
56	BE466525	EVI1	Ecotropic viral integration site 1
57	AA669135		CDNA FLJ25345 fis
58	AI252081		MRNA; cDNA DKFZp686L18111
59	AK026692	CAPN13	Calpain 13
60	AI676059	FOXQ1	Forkhead box Q1
61	NM_018492	PBK	PDZ binding kinase
62	AI085338	CASZ1	Castor zinc finger 1
63	BF447901	SBK1	SH3-binding domain kinase 1
64	AB037805	KLHL14	Kelch-like 14
65	X69397	CD24	CD24 molecule
66	X57348	SFN	Stratifin
67	AL138828	FAM54A	Family with sequence similarity 54, member A
68	AI571996	STAM2	Signal transducing adaptor molecule (SH3 domain and ITAM motif) 2
69	AF017307	ELF3	E74-like factor 3
70	U63743	KIF2C	Kinesin family member 2C
71	BF344237	CDH6	Cadherin 6, type 2, K-cadherin
72	AI587638	C1orf186	Chromosome 1 open reading frame 186
73	BG260086	XDH	Xanthine dehydrogenase
74	AI382146	SOX9	SRY (sex determining region Y)-box 9
75	BF001941	RBM35A	RNA binding motif protein 35A
76	NM_144707	PROM2	Prominin 2
77	AJ011712	TNNT1	Troponin T type 1
78	AI912275	BCL11A	B-cell CLL/lymphoma 11A (zinc finger protein)
79	AW271106		Transcribed locus
80	X57348	SFN	Stratifin
81	NM_001038	SCNN1A	Sodium channel, nonvoltage-gated 1 α
82	NM_024626	VTCN1	V-set domain containing T cell activation inhibitor 1
83	BC001651	CDCA8	Cell division cycle associated 8
84	NM_024680	E2F8	E2F transcription factor 8
85	NM_001274	CHEK1	CHK1 checkpoint homolog
86	BG261252	EVI1	Ecotropic viral integration site 1
87	AF133425	TSPAN1	Tetraspanin 1
88	AU159942	TOP2A	Topoisomerase (DNA) II α 170 kDa
89	AK026736	ITGB6	Integrin, ß 6
90	AL037473		MRNA; cDNA DKFZp564C1072
91	NM_002456	MUC1	Mucin 1, cell surface associated
92	AB038160	TMPRSS3	Transmembrane protease, serine 3
93	AI436813	RAB3IP	RAB3A interacting protein (rabin3)
94	NM_016425	TMPRSS4	Transmembrane protease, serine 4
95	AF191495	F11R	F11 receptor
96	N90191	CCNB1	Cyclin B1
97	AK001782	CXXC5	CXXC finger 5
98	AI341146	E2F7	E2F transcription factor 7
99	AV681807	ERBB3	v-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (avian)
100	NM_000346	SOX9	SRY (sex determining region Y)-box 9
101	BM193618	BCL11A	B-cell CLL/lymphoma 11A (zinc finger protein)
102	NM_004415	DSP	Desmoplakin
103	NM_017697	RBM35A	RNA binding motif protein 35A
104	AK026037		CDNA FLJ37609 fis, clone BRCOC2011010
105	NM_005978	S100A2	S100 calcium binding protein A2
106	NM_003177	SYK	Spleen tyrosine kinase
107	BC006245	FGF18	Fibroblast growth factor 18
108	AW242836	TMEM45B	Transmembrane protein 45B
109	AI821669	SOX9	SRY (sex determining region Y)-box 17
110	BF001806	MKI67	Antigen identified by monoclonal antibody Ki-67

Table III. Continued.

No.	Accession no.	Gene symbol	Gene name
111	U73844	ELF3	E74-like factor 3
112	AA112507	LSM4	LSM4 homolog, U6 small nuclear RNA associated
113	NM_001048	SST	Somatostatin
114	AI922198	CP	Ceruloplasmin (ferroxidase)
115	AC005954	TJP3	Tight junction protein 3 (zona occludens 3)
116	BC004863	PSAT1	Phosphoserine aminotransferase 1
117	AK021607	EME1	Essential meiotic endonuclease 1 homolog 1
118	BC006428	CXXC5	CXXC finger 5
119	S49765	HOXB7	Homeobox B7
120	AI282982	TMEM45B	Transmembrane protein 45B
121	NM_006681	NMU	Neuromedin U
122	AF019638	GDA	Guanine deaminase
123	AI339710	FAM108C1	Family with sequence similarity 108, member C1
124	AL561834	TOP2A	Topoisomerase (DNA) II α 170 kDa
125	NM_018265	C1orf106	Chromosome 1 open reading frame 106
126	AL031588	CELSR1	Cadherin, EGF LAG seven-pass G-type receptor 1
127	NM_001305	CLDN4	Claudin 4
128	NM_014736	KIAA0101	KIAA0101
129	NM_012310	KIF4A	Kinesin family member 4A
130	AV733308	ITGA6	Integrin, α 6
131	AI692426	KSR2	Kinase suppressor of ras 2
132	AK001261	DTL	Denticleless homolog (*Drosophila*)
133	NM_020639	RIPK4	Receptor-interacting serine-threonine kinase 4
134	AI378406	EGLN3	egl nine homolog 3 (*C. elegans*)
135	NM_004701	CCNB2	Cyclin B2
136	Y11339	ST6GALNAC1	ST6 (α-N-acetyl-neuraminyl-2,3-ß-galactosyl-1,3) -N-acetylgalactosaminide α-2,6-sialyltransferase 1
137	AW044646		Transcribed locus, strongly similar to XP_001102485.1 sarcospan
138	AF275945	MPZL2	Myelin protein zero-like 2
139	BF447105	SORT1	Sortilin 1
140	NM_006516	SLC2A1	Solute carrier family 2 (facilitated glucose transporter), member 1
141	NM_018123	ASPM	asp (abnormal spindle) homolog, microcephaly associated
142	AF336127	SLC4A11	Solute carrier family 4, sodium borate transporter, member 11
143	BC002490	CXXC5	CXXC finger 5
144	AA876179		Transcribed locus
145	NM_015366	ARHGAP8	Rho GTPase activating protein 8
146	NM_004237	TRIP13	Thyroid hormone receptor interactor 13
147	NM_003258	TK1	Thymidine kinase 1, soluble
148	BF437750	MPZL2	Myelin protein zero-like 2
149	X69699	PAX8	Paired box 8
150	AL554008	GPR56	G protein-coupled receptor 56
151	AI469788	SPC24	SPC24, NDC80 kinetochore complex component, homolog
152	NM_020242	KIF15	Kinesin family member 15
153	AI610869	MUC1	Mucin 1, cell surface associated
154	NM_006113	VAV3	vav 3 guanine nucleotide exchange factor
155	BF725250	FARP1	FERM, RhoGEF (ARHGEF) and pleckstrin domain protein 1
156	AI740788		CDNA FLJ33615 fis, clone BRAMY2018396
157	AF053719	PKP3	Plakophilin 3
158	M58664	CD24	CD24 molecule
159	AU144284	IRF6	Interferon regulatory factor 6
160	AF228422	C15orf48	Chromosome 15 open reading frame 48
161	AW009562	MARVELD2	MARVEL domain containing 2
162	NM_022073	EGLN3	egl nine homolog 3
163	NM_000142	FGFR3	Fibroblast growth factor receptor 3
164	U71207	EYA2	Eyes absent homolog 2 (*Drosophila*)

Table III. Continued.

No.	Accession no.	Gene symbol	Gene name
165	AW510657	HOXB3	Homeobox B3
166	NM_004360	CDH1	Cadherin 1, type 1, E-cadherin
167	NM_002670	PLS1	Plastin 1 (I isoform)
168	BC001060	PAX8	Paired box 8
169	NM_006103	WFDC2	WAP four-disulfide core domain 2
170	BC005238	FXYD3	FXYD domain containing ion transport regulator 3
171	AI948472	PAX8	Paired box 8
172	NM_000148	FUT1	Fucosyltransferase 1 (galactoside 2-α-L-fucosyltransferase, H blood group)
173	AL136566	C9orf58	Chromosome 9 open reading frame 58
174	R26843	TMEM139	Transmembrane protein 139
175	NM_004658	RASAL1	RAS protein activator like 1 (GAP1 like)
176	AI949095	FAM83H	Family with sequence similarity 83, member H
177	AL137725	EPPK1	Epiplakin 1
178	NM_003158	AURKA	Aurora kinase A
179	AB007935	IGSF3	Immunoglobulin superfamily, member 3
180	Z83838	ARHGAP8	Rho GTPase activating protein 8
181	BE645551	TMC4	Transmembrane channel-like 4
182	NM_002774	KLK6	Kallikrein-related peptidase 6
183	NM_005564	LCN2	Lipocalin 2 (oncogene 24p3)
184	NM_021978	ST14	Suppression of tumorigenicity 14 (colon carcinoma)
185	NM_005375	MYB	v-myb myeloblastosis viral oncogene homolog (avian)
186	AL117612	MAL2	mal, T-cell differentiation protein 2
187	BE407516	CCNB1	Cyclin B1
188	AI627262	TMEM139	Transmembrane protein 139
189	AU151483	CDH6	Cadherin 6, type 2, K-cadherin (fetal kidney)
190	BF060747	LOC130576	Hypothetical protein LOC130576
191	AF177272	UGT2B28	UDP glucuronosyltransferase 2 family, polypeptide B28
192	AW271106	IQGAP3	IQ motif containing GTPase activating protein 3
193	AA827649	C19orf46	Chromosome 19 open reading frame 46
194	AK000839		CDNA FLJ20832 fis, clone ADKA03033
195	NM_001982	ERBB3	v-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (avian)
196	AB030824	KLF5	Kruppel-like factor 5 (intestinal)
197	AA863389	EMG1	EMG1 nucleolar protein homolog
198	AK000490	DEPDC1	DEP domain containing 1
199	NM_006017	PROM1	Prominin 1
200	NM_018098	ECT2	Epithelial cell transforming sequence 2 oncogene
201	NM_018131	CEP55	Centrosomal protein 55 kDa
202	BE796148	RNF183	Ring finger protein 183
203	AU148164	KIAA1217	KIAA1217
204	J04152	TACSTD2	Tumor-associated calcium signal transducer 2
205	NM_002773	PRSS8	Protease, serine, 8
206	Z98443	WDR72	WD repeat domain 72
207	AA496034	BAIAP2L1	BAI1-associated protein 2-like 1
208	NM_002638	PI3	Peptidase inhibitor 3, skin-derived (SKALP)
209	AW874669		Full length insert cDNA clone YR23D07
210	NM_004523	KIF11	Kinesin family member 11
211	NM_003236	TGFA	Transforming growth factor, α
212	BF508634		Transcribed locus
213	BC001886	RRM2	Ribonucleotide reductase M2 polypeptide
214	AW117264		Transcribed locus
215	AI682088		Transcribed locus
216	BE966146	RAD51AP1	RAD51 associated protein 1
217	NM_006547	IGF2BP3	Insulin-like growth factor 2 mRNA binding protein 3
218	NM_001306	CLDN3	Claudin 3
219	NM_018728	MYO5C	Myosin VC

Table III. Continued.

No.	Accession no.	Gene symbol	Gene name
220	NM_007196	KLK8	Kallikrein-related peptidase 8
221	AL524035	CDC2	Cell division cycle 2, G1 to S and G2 to M
222	NM_001809	CENPA	Centromere protein A
223	NM_017780	CHD7	Chromodomain helicase DNA binding protein 7
224	BE622952	SORT1	Sortilin 1
225	AF213033	CDKN3	Cyclin-dependent kinase inhibitor 3
226	NM_004502	HOXB7	Homeobox B7
227	NM_001786	CDC2	Cell division cycle 2, G1 to S and G2 to M
228	NM_001827	CKS2	CDC28 protein kinase regulatory subunit 2
229	BC001068	FAM83D	Family with sequence similarity 83, member D
230	AL359055		MRNA full length insert cDNA clone EUROIMAGE 2344436
231	U94592	UCP2	Uncoupling protein 2 (mitochondrial, proton carrier)
232	NM_005196	CENPF	Centromere protein F, 350/400 ka (mitosin)
233	N34895	LOC653108	Similar to coxsackie virus and adenovirus receptor precursor
234	NM_024939	RBM35B	RNA binding motif protein 35B
235	NM_000343	SLC5A1	Solute carrier family 5 (sodium/glucose cotransporter), member 1
236	AA148507	SLC7A1	Solute carrier family 7 (cationic amino acid transporter, y+ system), member 1
237	NM_012101	TRIM29	Tripartite motif-containing 29
238	NM_001091	ABP1	Amiloride binding protein 1 [amine oxidase (copper-containing)]
239	AA910946	AP1M2	Adaptor-related protein complex 1, mu 2 subunit
240	AI763378	EHF	Ets homologous factor
241	NM_001878	CRABP2	Cellular retinoic acid binding protein 2
242	NM_002371	MAL	mal, T-cell differentiation protein
243	NM_002131	HMGA1	High mobility group AT-hook 1
244	NM_017763	RNF43	Ring finger protein 43
245	NM_004524	LLGL2	Lethal giant larvae homolog 2 (*Drosophila*)
246	NM_003318	TTK	TTK protein kinase
247	NM_005733	KIF20A	Kinesin family member 20A
248	D79987	ESPL1	Extra spindle pole bodies homolog 1 (*S. cerevisiae*)
249	AL137725	EPPK1	Epiplakin 1
250	NM_003710	SPINT1	Serine peptidase inhibitor, Kunitz type 1
251	AA622495	LRG1	Leucine-rich α-2-glycoprotein 1
252	AI199453	CDKL3	Cyclin-dependent kinase-like 3
253	BG290908	ATP8B1	ATPase, Class I, type 8B, member 1
254	M83248	SPP1	Secreted phosphoprotein 1
255	AF131790	SHANK2	SH3 and multiple ankyrin repeat domains 2
256	AB038160	TMPRSS3	Transmembrane protease, serine 3
257	AI802969		Transcribed locus
258	AF263279	CD164	CD164 molecule, sialomucin
259	AI928342	ASRGL1	Asparaginase like 1
260	BF248364	CASC5	Cancer susceptibility candidate 5
261	AW014155	GALNT6	UDP-N-acetyl-α-D-galactosamine:polypeptide N-acetylgalactosaminyl-transferase 6
262	N57927	MARVELD2	MARVEL domain containing 2
263	AU155415	KLK7	Kallikrein-related peptidase 7
264	BE965369	F2RL1	Coagulation factor II (thrombin) receptor-like 1
265	AK025045		CDNA: FLJ21392 fis, clone COL03505
266	NM_014584	ERO1L	ERO1-like
267	AW452823		Transcribed locus
268	NM_031299	CDCA3	Cell division cycle associated 3
269	NM_004736	XPR1	Xenotropic and polytropic retrovirus receptor
270	NM_004219	PTTG1	Pituitary tumor-transforming 1
271	NM_033514	LIMS3	LIM and senescent cell antigen-like domains 3
272	NM_012291	ESPL1	Extra spindle pole bodies homolog 1
273	BC002556	RAB3IP	RAB3A interacting protein (rabin3)

Table IV. Genes commonly down-regulated in ovarian cancer.

No.	Accession no.	Gene symbol	Gene name
1	AW276078	LOC387763	Hypothetical LOC387763
2	AL042588	PEG3	Paternally expressed 3
3	NM_002345	LUM	Lumican
4	NM_004684	SPARCL1	SPARC-like 1 (mast9, hevin)
5	AI826799	EFEMP1	EGF-containing fibulin-like extracellular matrix protein 1
6	D21254	CDH11	Cadherin 11, type 2, OB-cadherin (osteoblast)
7	AF260333	C4orf18	Chromosome 4 open reading frame 18
8	AA628535	COL1A2	Collagen, type I, α 2
9	AW007532	IGFBP5	Insulin-like growth factor binding protein 5
10	NM_004369	COL6A3	Collagen, type VI, α 3
11	AL575922	SPARC	Secreted protein, acidic, cysteine-rich (osteonectin)
12	AU144167	COL3A1	Collagen, type III, α 1
13	NM_002775	HTRA1	HtrA serine peptidase 1
14	AU148057	DKK3	Dickkopf homolog 3
15	AA428240	RBMS3	RNA binding motif, single stranded interacting protein
16	AL359062		MRNA full length insert cDNA clone EUROIMAGE 1913076
17	BF449053	FAM150B	Family with sequence similarity 150, member B
18	BC005254	CLEC2B	C-type lectin domain family 2, member B
19	NM_012342	BAMBI	BMP and activin membrane-bound inhibitor homolog
20	AF130082	COL3A1	Collagen, type III, α 1
21	NM_002736	PRKAR2B	Protein kinase, cAMP-dependent, regulatory, type II, ß
22	NM_002048	GAS1	Growth arrest-specific 1
23	NM_012082	ZFPM2	Zinc finger protein, multitype 2
24	BE967311	MCC	Mutated in colorectal cancers
25	NM_020190	OLFML3	Olfactomedin-like 3
26	AL391688	SYTL4	Synaptotagmin-like 4 (granuphilin-a)
27	AF279145	ANTXR1	Anthrax toxin receptor 1
28	AI951185	NR2F1	Nuclear receptor subfamily 2, group F, member 1
29	NM_003118	SPARC	Secreted protein, acidic, cysteine-rich (osteonectin)
30	NM_018371	ChGn	Chondroitin ß1,4 N-acetylgalactosaminyltransferase
31	X56210	CFH/CFHR1	Complement factor H/complement factor H-related 1
32	NM_003247	THBS2	Thrombospondin 2
33	NM_001449	FHL1	Four and a half LIM domains 1
34	NM_022003	FXYD6	FXYD domain containing ion transport regulator 6
35	NM_000110	DPYD	Dihydropyrimidine dehydrogenase
36	M18767	C1S	Complement component 1, s subcomponent
37	NM_025208	PDGFD	Platelet derived growth factor D
38	AA788711	COL1A2	Collagen, type I, α 2
39	D28124	NBL1	Neuroblastoma, suppression of tumorigenicity 1
40	AB014737	SMOC2	SPARC related modular calcium binding 2
41	NM_004503	HOXC6	Homeobox C6
42	NM_020405	PLXDC1	Plexin domain containing 1
43	U50748	LEPR	Leptin receptor
44	NM_014668	GREB1	GREB1 protein
45	BF511276	AKAP12	A kinase (PRKA) anchor protein (gravin) 12
46	NM_014899	RHOBTB3	Rho-related BTB domain containing 3
47	AU152178	ANTXR1	Anthrax toxin receptor 1
48	AI927067	TCF4	Transcription factor 4
49	NM_000089	COL1A2	Collagen, type I, α 2
50	BF726212	ANK2	Ankyrin 2, neuronal
51	AL390170		Clone 23555 mRNA sequence
52	U35139	NDN	Necdin homolog (mouse)
53	AW303375	CCDC80	Coiled-coil domain containing 80
54	AU147399	CAV1	Caveolin 1, caveolae protein, 22 kDa
55	AL541302	SERPINE2	Serpin peptidase inhibitor, clade E, member 2

Table IV. Continued.

No.	Accession no.	Gene symbol	Gene name
56	AW514267	LOC202134	Hypothetical protein LOC202134
57	NM_014710	GPRASP1	G protein-coupled receptor associated sorting protein 1
58	AF183421	RAB31	RAB31, member RAS oncogene family
59	NM_018286	TMEM100	Transmembrane protein 100
60	AA523939		Transcribed locus
61	NM_001613	ACTA2	Actin, α 2, smooth muscle, aorta
62	AI922599	VIM	Vimentin
63	BF592782	TCF4	Transcription factor 4
64	AI761728	RNASE4	Ribonuclease, RNase A family, 4
65	BF107565	TIMP2	TIMP metallopeptidase inhibitor 2
66	H97386	BNC2	Basonuclin 2
67	W94001	NCAM1	Neural cell adhesion molecule 1
68	NM_001856	COL16A1	Collagen, type XVI, α 1
69	BE968786	TIMP2	TIMP metallopeptidase inhibitor 2
70	NM_014890	FILIP1L	Filamin A interacting protein 1-like
71	AF074331	PAPSS2	3'-phosphoadenosine 5'-phosphosulfate synthase 2
72	NM_002985	CCL5	Chemokine (C-C motif) ligand 5
73	NM_021111	RECK	Reversion-inducing-cysteine-rich protein with kazal motifs
74	NM_000898	MAOB	Monoamine oxidase B
75	BC004490	FOS	v-fos FBJ murine osteosarcoma viral oncogene homolog
76	AL022324	IGLL3	Immunoglobulin λ-like polypeptide 3
77	AL573058	C1R	Complement component 1, r subcomponent
78	NM_147174	HS6ST2	Heparan sulfate 6-O-sulfotransferase 2
79	AF055376	MAF	v-maf musculoaponeurotic fibrosarcoma oncogene homolog (avian)
80	NM_000064	LOC653879	Similar to complement C3 precursor
81	NM_000592	C4A/4B	Complement component 4A
82	NM_001552	IGFBP4	Insulin-like growth factor binding protein 4
83	AL031781	QKI	Quaking homolog, KH domain RNA binding
84	K01228	COL1A1	Collagen, type I, α 1
85	NM_018013	SOBP	Sine oculis binding protein homolog
86	AA126505	NCAM1	Neural cell adhesion molecule 1
87	NM_001759	CCND2	Cyclin D2
88	NM_003199	TCF4	Transcription factor 4
89	AF035307	PLXNC1	Plexin C1
90	K02403	C4A/4B	Complement component 4A
91	AL040051	FRMD6	FERM domain containing 6
92	NM_004530	MMP2	Matrix metallopeptidase 2
93	AF065389	TSPAN5	Tetraspanin 5
94	BC001830	TGFB1I1	Transforming growth factor ß 1 induced transcript 1
95	BF433429	TCF4	Transcription factor 4
96	AA292373	COL6A1	Collagen, type VI, α 1
97	NM_020169	LXN	Latexin
98	AL569804	PDZRN3	PDZ domain containing RING finger 3
99	L03203	PMP22	Peripheral myelin protein 22
100	AV711904	LYZ	Lysozyme (renal amyloidosis)
101	NM_000313	PROS1	Protein S (α)
102	NM_001343	DAB2	Disabled homolog 2, mitogen-responsive phosphoprotein
103	BF060767	ADAMTS5	ADAM metallopeptidase with thrombospondin type 1 motif, 5
104	NM_006317	BASP1	Brain abundant, membrane attached signal protein 1
105	NM_020353	PLSCR4	Phospholipid scramblase 4
106	N21202	DAB2	Disabled homolog 2, mitogen-responsive phosphoprotein
107	AK026674	TCF4	Transcription factor 4
108	NM_001553	IGFBP7	Insulin-like growth factor binding protein 7
109	AU157303	HNMT	Histamine N-methyltransferase
110	R15072	SLC16A14	Solute carrier family 16, member 14

Table IV. Continued.

No.	Accession no.	Gene symbol	Gene name
111	AV706522	PPM1K	Protein phosphatase 1K
112	NM_002899	RBP1	Retinol binding protein 1, cellular
113	AW888223	MXRA8	Matrix-remodelling associated 8
114	NM_014934	DZIP1	DAZ interacting protein 1
115	BE789881	RAB31	RAB31, member RAS oncogene family
116	NM_003304	TRPC1	Transient receptor potential cation channel, subfamily C, member 1
117	AL136693	CYBRD1	Cytochrome b reductase 1
118	BE620739	RHOBTB3	Rho-related BTB domain containing 3
119	AU145658	MGC24103	Hypothetical protein MGC24103
120	U79271	AKT3	V-akt murine thymoma viral oncogene homolog 3
121	NM_007005	TLE4	Transducin-like enhancer of split 4
122	NM_003186	TAGLN	Transgelin
123	NM_006614	CHL1	Cell adhesion molecule with homology to L1CAM
124	AF349719	TRO	Trophinin
125	NM_000362	TIMP3	TIMP metallopeptidase inhibitor 3
126	AB003476	AKAP12	A kinase (PRKA) anchor protein
127	NM_002923	RGS2	Regulator of G-protein signaling 2
128	AW189885	PCDH18	Protocadherin 18
129	NM_001085	SERPINA3	Serpin peptidase inhibitor, clade A
130	AF054589	MDFIC	MyoD family inhibitor domain containing
131	W73819	DDR2	Discoidin domain receptor family, member 2
132	AA788946	COL12A1	Collagen, type XII, α 1
133	NM_006379	SEMA3C	Sema domain, immunoglobulin domain (Ig), secreted, (semaphorin) 3C
134	AU145127	FBXL7	F-box and leucine-rich repeat protein 7
135	AI968085	WNT5A	Wingless-type MMTV integration site family, member 5A
136	AW574504	PECAM1	Platelet/endothelial cell adhesion molecule (CD31 antigen)
137	L35594	ENPP2	Ectonucleotide pyrophosphatase/phosphodiesterase 2 (autotaxin)
138	NM_012445	SPON2	Spondin 2, extracellular matrix protein
139	BG036514	FAM101B	Family with sequence similarity 101, member B
140	AI377043	BNC2	Basonuclin 2
141	NM_003304	TRPC1	Transient receptor potential cation channel, subfamily C, member 1
142	AL577322	SDC2	Syndecan 2
143	NM_001964	EGR1	Early growth response 1
144	NM_000633	BCL2	B-cell CLL/lymphoma 2
145	NM_005410	SEPP1	Selenoprotein P, plasma, 1
146	AW058622	WIPF1	WAS/WASL interacting protein family, member 1
147	AB014609	MRC2	Mannose receptor, C type 2
148	AU157224		CDNA FLJ11570 fis, clone HEMBA1003309
149	NM_002290	LAMA4	Laminin, α 4
150	AI338338	NLGN4X	Neuroligin 4, X-linked
151	BF732712	GPRASP2	G protein-coupled receptor associated sorting protein 2
152	NM_003613	CILP	Cartilage intermediate layer protein, nucleotide pyrophosphohydrolase
153	NM_004430	EGR3	Early growth response 3
154	NM_001554	CYR61	Cysteine-rich, angiogenic inducer, 61
155	AL583520	CALD1	Caldesmon 1
156	AL133001	SULF2	Sulfatase 2
157	AA845258	BGN	Biglycan
158	BE673665		Full-length cDNA clone CS0DD001YA12 of Neuroblastoma Cot 50
159	AI953360	LYPLAL1	Lysophospholipase-like 1
160	NM_004010	DMD	Dystrophin (muscular dystrophy, Duchenne and Becker types)
161	AW025330	NAP1L5	Nucleosome assembly protein 1-like 5
162	NM_000591	CD14	CD14 molecule
163	AW467136		Transcribed locus
164	NM_003254	TIMP1	TIMP metallopeptidase inhibitor 1
165	AA234096	MGC16121	Hypothetical protein MGC16121
166	AV707102	PDK4	Pyruvate dehydrogenase kinase, isozyme 4

Table IV. Continued.

No.	Accession no.	Gene symbol	Gene name
167	NM_001450	FHL2	Four and a half LIM domains 2
168	NM_003759	SLC4A4	Solute carrier family 4, sodium bicarbonate cotransporter, member 4
169	AI420144		Full length insert cDNA clone YX37E06
170	NM_001873	CPE	Carboxypeptidase E
171	N21138	RHOBTB3	Rho-related BTB domain containing 3
172	NM_000062	SERPING1	Serpin peptidase inhibitor, clade G (C1 inhibitor), member 1
173	NM_001553	IGFBP7	Insulin-like growth factor binding protein 7
174	NM_006195	PBX3	Pre-B-cell leukemia homeobox 3
175	N48299	APCDD1	Adenomatosis polyposis coli down-regulated 1
176	AV734646	FAM26F	Family with sequence similarity 26, member F
177	AW025980		Transcribed locus
178	BG495771	TCF4	Transcription factor 4
179	N21643		CDNA FLJ39585 fis, clone SKMUS2006633
180	NM_016608	ARMCX1	Armadillo repeat containing, X-linked 1
181	BE535746	REEP1	Receptor accessory protein 1
182	AA934610		Transcribed locus
183	AI004009		Transcribed locus, moderately similar to XP_517655.1
184	BC001745	D4S234E	DNA segment on chromosome 4
185	AI123815	FLJ21963	FLJ21963 protein
186	AU153866	GNAI1	Guanine nucleotide binding protein, α inhibiting activity polypeptide 1
187	T68445	AR	Androgen receptor
188	AI718937	KCTD12	Potassium channel tetramerisation domain containing 12
189	NM_006931	SLC2A3	Solute carrier family 2
190	AK021804		CDNA FLJ38472 fis, clone FEBRA2022148
191	AL571375	SCD5	Stearoyl-CoA desaturase 5
192	AL137364		Hypothetical protein MGC24039
193	NM_144573	NEXN	Nexilin (F actin binding protein)
194	NM_003570	CMAH	CMP-N-acetylneuraminate monooxygenase
195	AU146418		CDNA FLJ10237 fis, clone HEMBB1000438
196	NM_003020	SCG5	Secretogranin V (7B2 protein)
197	D87811	GATA6	GATA binding protein 6
198	NM_024426	WT1	Wilms tumor 1
199	NM_014705	DOCK4	Dedicator of cytokinesis 4
200	M16276	HLA-DQB1	Major histocompatibility complex, class II, DQ ß 1
201	AW290940		MRNA (clone ICRFp507I1077)
202	AL136861	CRISPLD2	Cysteine-rich secretory protein LCCL domain containing 2
203	NM_003507	FZD7	Frizzled homolog 7 (*Drosophila*)
204	NM_002135	NR4A1	Nuclear receptor subfamily 4, group A, member 1
205	BC036488	CDNA clone IMAGE:5303689	CDNA clone IMAGE:5303689
206	N66393	C21orf34	Chromosome 21 open reading frame 34
207	NM_018495	CALD1	Caldesmon 1
208	AW500180	LIX1L	Lix1 homolog (mouse)-like
209	AI651603	SPG20	Spastic paraplegia 20
210	NM_006102	PGCP	Plasma glutamate carboxypeptidase
211	AF100751	FKBP7	FK506 binding protein 7
212	BF724558		Transcribed locus
213	BE644830	ARHGAP18	Rho GTPase activating protein 18
214	NM_001753	CAV1	Caveolin 1, caveolae protein, 22 kDa
215	NM_006705	GADD45G	Growth arrest and DNA-damage-inducible, γ
216	AA570453		MRNA from chromosome 5q31-33 region
217	NM_002727	SRGN	Serglycin
218	NM_003919	SGCE	Sarcoglycan, ε
219	AK025444	PHLDB2	Pleckstrin homology-like domain, family B, member 2
220	NM_015310	PSD3	Pleckstrin and Sec7 domain containing 3
221	AW151360	TNFSF13B	Tumor necrosis factor (ligand) superfamily, member 13b

Table IV. Continued.

No.	Accession no.	Gene symbol	Gene name
222	M64497	NR2F2	Nuclear receptor subfamily 2, group F, member 2
223	AK024784	C10orf56	Chromosome 10 open reading frame 56
224	NM_002395	ME1	Malic enzyme 1, NADP(+)-dependent, cytosolic
225	AF247704	NKX3-1	NK3 homeobox 1
226	NM_000076	CDKN1C	Cyclin-dependent kinase inhibitor 1C (p57, Kip2)
227	NM_002305	LGALS1	Lectin, galactoside-binding, soluble, 1 (galectin 1)
228	AI650848	TBC1D4	TBC1 domain family, member 4
229	NM_005086	SSPN	Sarcospan
230	N30138	FRMD6	FERM domain containing 6
231	AW006185	LOC283666	Hypothetical protein LOC283666
232	Z95331	FBLN1	Fibulin 1
233	NM_006895	HNMT	Histamine N-methyltransferase
234	BG413606	LOC400120	Hypothetical LOC400120
235	M31159	IGFBP3	Insulin-like growth factor binding protein 3
236	NM_006159	NELL2	NEL-like 2
237	AL037401	NR2F2	Nuclear receptor subfamily 2, group F, member 2
238	AL559122	TRBC1,TRBV19	T cell receptor ß variable 19,T cell receptor ß constant 1
239	AA479286	SGCD	Sarcoglycan, δ
240	R78668	CDKN1C	Cyclin-dependent kinase inhibitor 1C (p57, Kip2)
241	BE220209	ST7L	Suppression of tumorigenicity 7 like
242	N95363	CDKN1C	Cyclin-dependent kinase inhibitor 1C (p57, Kip2)
243	NM_022912	REEP1	Receptor accessory protein 1
244	AL136756	SSPN	Sarcospan
245	W72694	FAM26B	Family with sequence similarity 26, member B
246	AI160540	KLHDC8B	Kelch domain containing 8B
247	AI638611	STAMBPL1	STAM binding protein-like 1
248	NM_018422	PSD3	Pleckstrin and Sec7 domain containing 3
249	NM_018004	TMEM45A	Transmembrane protein 45A
250	AL037998		MRNA; cDNA DKFZp564E143 (from clone DKFZp564E143)
251	AI913749	PLEKHH2	Pleckstrin homology domain containing, family H member 2
252	AL049437	DPY19L2P2	dpy-19-like 2 pseudogene 2
253	AF134715	TNFSF13B	Tumor necrosis factor (ligand) superfamily, member 13b
254	NM_005824	LRRC17	Leucine rich repeat containing 17
255	NM_001386	DPYSL2	Dihydropyrimidinase-like 2
256	AW173504	AR	Androgen receptor
257	NM_004417	DUSP1	Dual specificity phosphatase 1
258	NM_000597	IGFBP2	Insulin-like growth factor binding protein 2, 36 kDa
259	BG484552	RASSF8	Ras association (RalGDS/AF-6) domain family 8
260	AU144247	CLIP4	CAP-GLY domain containing linker protein family, member 4
261	AI658662	SYNPO2	Synaptopodin 2
262	NM_016315	GULP1	GULP, engulfment adaptor PTB domain containing 1
263	AI807023	RAB8B	RAB8B, member RAS oncogene family
264	AV734646	FAM26F	Family with sequence similarity 26, member F
265	BE048919	PLA2R1	Phospholipase A2 receptor 1, 180 kDa
266	AI972496	IGF1	Insulin-like growth factor 1 (somatomedin C)
267	AW021102	GLI3	GLI-Kruppel family member GLI3
268	NM_000129	F13A1	Coagulation factor XIII, A1 polypeptide
269	AI459194	EGR1	Early growth response 1
270	NM_002742	PRKD1	Protein kinase D1
271	BC005916	PTN	Pleiotrophin
272	AI961778	FAM101B	Family with sequence similarity 101, member B
273	NM_005032	PLS3	Plastin 3
274	AW299958	PAPSS2	3'-phosphoadenosine 5'-phosphosulfate synthase 2
275	AW340588	MAN1C1	Mannosidase, α, class 1C, member 1
276	D42043	RFTN1	Raftlin, lipid raft linker 1
277	N90870		CDNA FLJ38472 fis, clone FEBRA2022148

Table IV. Continued.

No.	Accession no.	Gene symbol	Gene name
278	NM_000165	GJA1	Gap junction protein, α 1, 43 kDa
279	X03348	NR3C1	Nuclear receptor subfamily 3, group C, member 1
280	BF059276		CDNA clone IMAGE:5276765
281	AI333651	FZD7	Frizzled homolog 7
282	AB030655	EFEMP2	EGF-containing fibulin-like extracellular matrix protein 2
283	NM_002121	HLA-DPB1	Major histocompatibility complex, class II, DP ß 1
284	NM_016205	PDGFC	Platelet derived growth factor C
285	NM_006200	PCSK5	Proprotein convertase subtilisin/kexin type 5
286	L22431	VLDLR	Very low density lipoprotein receptor
287	U82670	ZNF275	Zinc finger protein 275
288	NM_002229	JUNB	jun B proto-oncogene
289	BG401568	SLC16A9	Solute carrier family 16, member 9
290	NM_006561	CUGBP2	CUG triplet repeat, RNA binding protein 2
291	NM_001233	CAV2	Caveolin 2
292	AL046979	TNS1	Tensin 1
293	AL518391	AQP1	Aquaporin 1
294	NM_000014	A2M	α-2-macroglobulin
295	NM_004335	BST2	Bone marrow stromal cell antigen 2
296	BE299456	C16orf45	Chromosome 16 open reading frame 45
297	AA398569	LOC91316	Similar to bK246H3.1
298	AU152579	PCSK5	Proprotein convertase subtilisin/kexin type 5
299	U69546	CUGBP2	CUG triplet repeat, RNA binding protein 2
300	AB040120	SLC39A8	Solute carrier family 39 (zinc transporter), member 8
301	NM_018589	FOXN3	Forkhead box N3
302	NM_007286	SYNPO	Synaptopodin
303	BC006236	MAG1	Lung cancer metastasis-associated protein
304	AI826437		Transcribed locus
305	AI452457	C1orf168	Chromosome 1 open reading frame 168
306	AA847654	TCEAL3	Transcription elongation factor A (SII)-like 3
307	AL049265	IL6ST	Interleukin 6 signal transducer
308	NM_003392	WNT5A	Wingless-type MMTV integration site family, member 5A
309	AA526844	MYLK	Myosin, light chain kinase
310	BF674349	WWTR1	WW domain containing transcription regulator 1
311	AA173223	LOC493869	Similar to RIKEN cDNA 2310016C16
312	AI692523	SLIT2	Slit homolog 2
313	AU157932	PALLD	Palladin, cytoskeletal associated protein
314	AK024256	CACHD1	Cache domain containing 1
315	AL136550	TMEM47	Transmembrane protein 47
316	NM_005965	MYLK	Myosin, light chain kinase
317	BE856546	IL6ST	Interleukin 6 signal transducer
318	L27624	TFPI2	Tissue factor pathway inhibitor 2
319	AB029026	TACC1	Transforming, acidic coiled-coil containing protein 1
320	NM_004445	EPHB6	EPH receptor B6
321	BC003629		CDNA: FLJ23438 fis, clone HRC13275
322	AW511319	DSC96	Mesenchymal stem cell protein DSC96
323	NM_000132	F8	Coagulation factor VIII, procoagulant component
324	AL554245	NR2F2	Nuclear receptor subfamily 2, group F, member 2
325	AI660245		Transcribed locus, moderately similar to XP_001162191.1
326	BF672019	DPF3	D4, zinc and double PHD fingers, family 3
327	AI803181	TMEM47	Transmembrane protein 47
328	BC002416	BGN	Biglycan
329	NM_020217	RPL23AP13	Ribosomal protein L23a pseudogene 13
330	AL049933	GNAI1	Guanine nucleotide binding protein, α inhibiting activity polypeptide 1
331	BC041391	TACC1	transforming, acidic coiled-coil containing protein 1
332	AB032963	ATP8B2	ATPase, Class I, type 8B, member 2
333	M81635	STOM	Stomatin

Table IV. Continued.

No.	Accession no.	Gene symbol	Gene name
334	NM_006404	PROCR	Protein C receptor, endothelial
335	AA927670		Transcribed locus
336	NM_003243	TGFBR3	Transforming growth factor, ß receptor III
337	AI129320	ZAK	Sterile α motif and leucine zipper containing kinase AZK
338	AW024656		*Homo sapiens*, clone IMAGE:3632683, mRNA
339	AL571684	LOC401152	HCV F-transactivated protein 1
340	M27487	HLA-DPA1	Major histocompatibility complex, class II, DP α 1
341	M27487	HLA-DPA1	Major histocompatibility complex, class II, DP α 1
342	BF512748	JAK3	Janus kinase 3 (a protein tyrosine kinase, leukocyte
343	AW966474	SUSD3	Sushi domain containing 3
344	AW451999	LOC286191	Hypothetical protein LOC286191
345	AW612657	LYPLAL1	Lysophospholipase-like 1
346	AL042088	TUB	Tubby homolog
347	AB020707	WASF3	WAS protein family, member 3
348	AF325503	C2orf40	Chromosome 2 open reading frame 40
349	BC005810	CLEC11A	C-type lectin domain family 11, member A
350	AL046979	TNS1	Tensin 1
351	AI636080	MCOLN3	Mucolipin 3
352	NM_016250	NDRG2	NDRG family member 2
353	AI452595	GLT8D4	Glycosyltransferase 8 domain containing 4
354	D64137	CDKN1C	Cyclin-dependent kinase inhibitor 1C (p57, Kip2)
355	BF528878	LOC91461	Hypothetical protein BC007901
356	T16544		Transcribed locus
357	NM_016938	EFEMP2	EGF-containing fibulin-like extracellular matrix protein 2
358	AI271425	LOC339483	Hypothetical LOC339483
359	AL047908	JAZF1	JAZF zinc finger 1
360	NM_001155	ANXA6	Annexin A6
361	AI806583		Transcribed locus
362	NM_006207	PDGFRL	Platelet-derived growth factor receptor-like
363	BF195608	TBC1D2B	TBC1 domain family, member 2B
364	AI096375	TSPYL5	TSPY-like 5
365	AF200715	GULP1	GULP, engulfment adaptor PTB domain containing 1
366	AL575735	COL5A2	Collagen, type V, α 2
367	AA083478	TRIM22	Tripartite motif-containing 22
368	AA917899	MPDZ	Multiple PDZ domain protein
369	BF059159	ROBO1	Roundabout, axon guidance receptor, homolog 1
370	NM_003078	SMARCD3	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, Subfamily d, member 3
371	NM_006674	HCP5	HLA complex P5
372	AI123555	ADAMTS5	ADAM metallopeptidase with thrombospondin type 1 motif, 5 (aggrecanase-2)
373	BF514079	KLF4	Kruppel-like factor 4
374	AI680541	LIFR	Leukemia inhibitory factor receptor α
375	N73970	SNED1	Sushi, nidogen and EGF-like domains 1
376	BE501862	ARHGAP18	Rho GTPase activating protein 18
377	AU157017	ZNF711	Zinc finger protein 711
378	NM_022117	TSPYL2	TSPY-like 2
379	BG287153	MAN1A1	Mannosidase, α, class 1A, member 1
380	AL110191	TSC22D3	TSC22 domain family, member 3
381	AF109161	CITED2	Cbp/p300-interacting transactivator, 2
382	NM_002526	NT5E	5'-nucleotidase, ecto (CD73)
383	NM_016081	PALLD	Palladin, cytoskeletal associated protein
384	NM_001387	DPYSL3	Dihydropyrimidinase-like 3
385	AI159874	C1orf21	Chromosome 1 open reading frame 21
386	BC002654	TUBB6	Tubulin, ß 6
387	NM_000820	GAS6	Growth arrest-specific 6

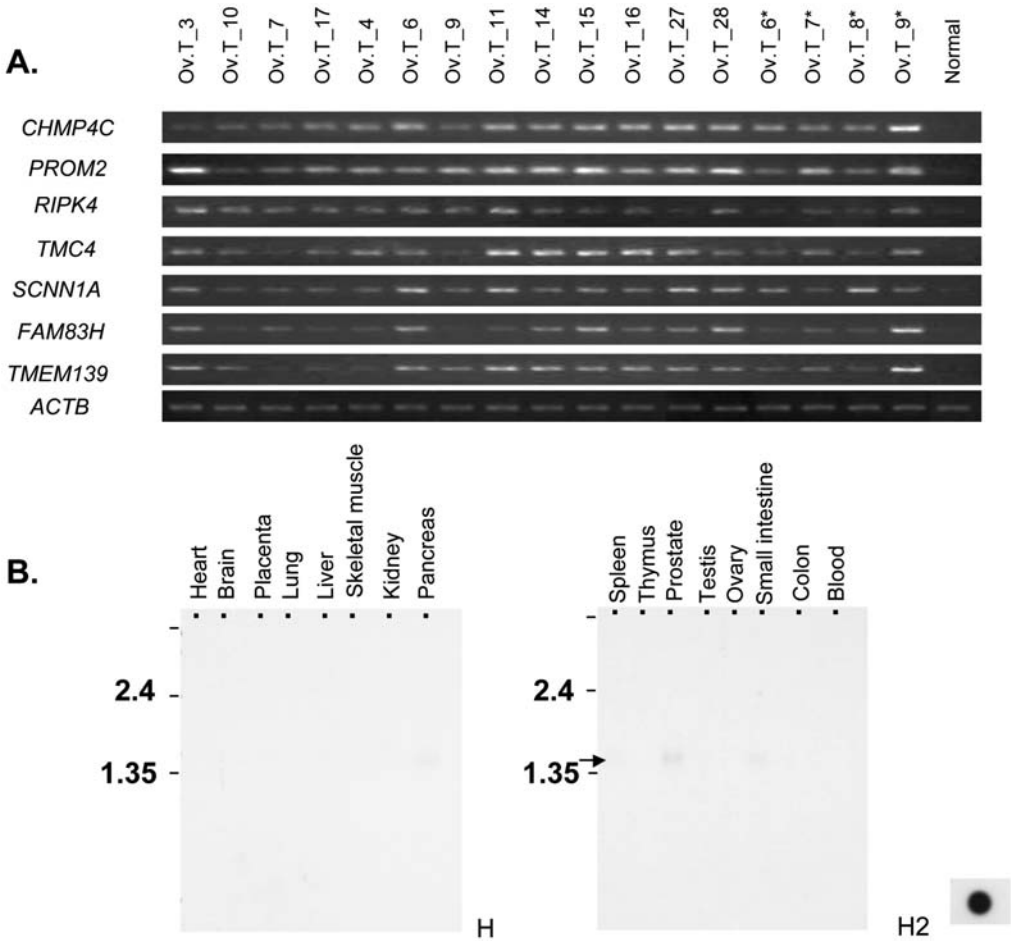

Figure 2. (A) Validation of the expression levels of seven representative genes by semi-quantitative RT-PCR analysis. RNA from 17 ovarian clinical samples and normal ovarian epithelial cells is shown. The integrity of each cDNA template was controlled through amplification of ACTB used as internal control. (B) Northern blot analysis of the expression of *CHMP4C* in normal human tissues.

Among the genes up-regulated in ovarian cancer (Fig. 2A), we focused on *CHMP4C* (chromatin modifying protein 4C) for further biological analysis as it was commonly overexpressed in clinical ovarian cancer samples but not expressed in any of the normal human tissues examined. Subsequent Northern blot analysis using a *CHMP4C* cDNA fragment as a probe confirmed that an ~1.9 kb transcript was hardly detectable in normal human tissues, with the exception of the prostate and a very weak band in the small intestine (Fig. 2B).

Discussion

Recent progress in genomic and molecular analysis has improved our understanding of the genesis of a wide range of human neoplasms. For ovarian carcinoma, several groups have reported the results of microarray-based expression profile analysis and identified so-called candidate genes that may be applicable as diagnostic markers (21-26). However, tumor tissue is generally a mixture of various cell types, such as inflammatory cells, stromal cells, endothelial cells and fibroblasts, in addition to cancer cells, and the proportions of each cell type vary significantly from one individual to another. Hence, expression profile data using mRNA isolated from bulk tumor tissue are very unlikely to exactly reflect changes during the course of ovarian carcinogenesis. In addition, normal ovarian tissue is also a mixture of different cell types. Since epithelial ovarian carcinomas (EOCs) are considered to originate from ovarian epithelial cells on the surface of the ovaries, it is preferable to use normal ovarian epithelial cells as a control to determine differences in expression levels related to the process of ovarian carcinogenesis. Therefore, in this study, we performed laser microbeam microdissection (LMM) to enrich populations of ovarian cancer cells as well as control ovarian surface epithelial cells. Upon comparison of our data and the data of a previous expression profile of ovarian cancer using RNA from bulk tumor tissues (24), only 87 of the 273 transcripts that we identified to be commonly up-regulated in over 50% of informative ovarian cancer cases, shown in Table III, overlapped with the bulk expression profile data. We assume that this discrepancy is attributable to the difference in the sample preparation process used, and that our data more precisely demonstrate the expression changes during the carcinogenesis process from ovarian epithelial to malignant cells. To evaluate the purity of microdissected cell populations, we analyzed the expression of *GSG2* (germ cell specific gene 2), which is abundantly expressed in germ cells in our gene expression profiling. By careful application of the microdissection procedure, we were able to minimize the proportion of contaminating germ cells to as few as 1.24% in preparations of normal ovarian epithelial cells. Indeed, upon comparison of our data with the previous expression profile data of ovarian cancer obtained by means of

microarray (21-26), only 46 of the 387 transcripts identified as commonly down-regulated in over 50% of informative ovarian cancer cases (Table IV) overlapped with the bulk expression profile data. Therefore, 341 genes that had not previously been identified as down-regulated were identified as down-regulated genes in this study. Among these 341 down-regulated genes, we found *GAS1* (growth arrest-specific 1), *ZFPM2* (zinc finger protein, multitype 2) and *NBL1* (neuroblastoma, suppression of tumorigenicity 1), which are reported to be a negative regulator of epithelial cell proliferation, a DNA-dependent regulator of transcription and a transcription factor with tumor-suppressive activity, respectively (27-30). This suggests that the majority of down-regulated genes, including genes with a tumor-suppressive function, were not detected in previous studies. We believe that it is crucial to apply the LMM system to purify populations of cancerous and normal epithelial cells obtained from surgical specimens to the greatest extent possible, in order to improve our understanding of the genes involved in carcinogenesis.

Certain of the genes with altered expression in most ovarian cancers may play causal roles in ovarian carcinogenesis, and may also serve as molecular diagnostic markers and candidate targets for the development of novel therapeutic drugs for ovarian cancer. Among the up-regulated genes, *CD24* (*CD24* antigen), *EVI1* (ecotropic viral integration site 1), *MDS1* (myelodysplastic syndrome 1), *HOXB7* (homeobox B7), *GPR39* (G protein-coupled receptor 39) and *CP* (ceruloplasmin) were previously reported to be up-regulated in ovarian cancer (21-26). Many studies have demonstrated the significance of the cytoplasmic membranous expression of *CD24* in the prediction of the prognosis of ovarian cancer patients (31-35). Protein levels of *EVI1* and *MDS1* were reported to be increased in both ovarian cancer tissues and ovarian cancer cell lines (36). Nanjundan *et al* (36) recently revealed that DNA copy number increases of both genes were associated with at least 5-fold-increased RNA transcript levels in 83 and 98% of advanced ovarian cancer cases, respectively. The high expression level of homeobox member gene *HOXB7* mRNA reveals its possible involvement in the invasive characteristics of ovarian cancer cells (37,38). *GPR39* was found to be a novel inhibitor of cell death, which might represent a therapeutic target with implications for processes involving apoptosis and endoplasmic reticulum stress in cancer (39). *CP* is associated with an unfavorable prognosis and poor outcome in patients with ovarian cancer (40). Its mRNA expression levels were found to be elevated in 17 of the 22 clinical tumor cases in the present study (77.3%).

In addition to genes that have been characterized broadly or partially in tumor cell growth and development, we found several novel genes, which deserve to be investigated as molecular markers for diagnostics and treatment of ovarian cancer. *CASZ1* (castor zinc finger 1) was recently cloned and found to be up-regulated during cell differentiation, and was expressed in a number of human tumors (41). *STAM2* (signal transducing adaptor molecule 2) is a regulator of receptor signaling and trafficking (42). *KIF2C* (kinesin family member 2c) has so far been connected with mammary carcinogenesis (43) and was found to be overexpressed in human gastric cancer (44). Its role in ovarian carcinogenesis remains to be investigated. *RAB3IP* (rabin), *F11R* (F11 receptor), *TMEM45B* (transmembrane protein 45B), *FAM108C1* (family with sequence similarity 108, member C1), *C1orf106* (chromosome 1 open reading frame 106), MPZL2 (myelin protein zero-like 2), *TRIP13* (thyroid hormone receptor interactor 13), *IRF6* (interferon regulatory factor 6), *FAM83H* (family with sequence similarity 83, member H), *MYO5C* (myosin 5C) are some of the novel genes identified in the present study that are significantly overexpressed in the majority of clinical cases. *RIPK4* (receptor-interacting serine-threonine kinase 4) and *CHMP4C* (chromatin modifying protein 4C) were very highly expressed in the majority of the clinical samples according to RT-PCR, and were not detected in the normal ovarian tissues. RIPK4 is an ankyrin-repeat containing protease, while CHMP4C belongs to the chromatin-modifying protein/charged multivesicular body protein (CHMP) family. Neither have been analyzed in relation to cancer, and both warrant future investigation as potential therapeutic targets for ovarian cancer therapy.

Among the 387 commonly down-regulated genes, *PEG3* (paternally expressed 3), *HTRA1* (*HtrA* serine peptidase 1), *CAV1* (caveolin 1), *Dab2* (disabled homolog 2), *SPARC* (secreted protein, acidic, cysteine-rich) were detected. Certain of these were previously suggested to have possible functions as tumor suppressors. *PEG3* was shown to be silenced by hypermethylation in endometrial and cervical cancer cell lines (15). *HTRA1* was reported to be down-regulated in ovarian tumors and cancer cell lines, suggesting that its loss plays a role in the progression of cancer (45). A microarray study of advanced stage ovarian carcinomas revealed down-regulation of the *CAV1 (*caveolin-1) gene in ovarian carcinomas. Immunohistochemical analysis confirmed loss of *CAV1* expression in serous ovarian carcinomas (18). By studying *Dab2* in 50 primary ovarian tumors and in 50 metastases by immunohistochemistry, it was shown that Dab2 was absent in the majority of ovarian tumors at both primary and metastatic sites (46). Certain of the down-regulated genes are supposed to be silenced by hypermethylation in tumors and implemented in the process of tumor cell growth. Down-regulation of *SPARC* is thought to be essential for ovarian carcinogenesis, as cancer cells become sensitized to the apoptotic activity of *SPARC* during malignant transformation (24,47). *TGFBR3* (transforming growth factor ß, receptor III) and *THBS2* (thrombospodin 2) have also been found to be highly expressed in normal ovarian epithelia and underexpressed in ovarian serous papillary carcinomas (24).

Novel down-regulated genes in the present study included *CLEC2B* (C-type lectin domain family 2, member B) and *MCC* (mutated in colorectal cancers). *CLEC2B* is preferentially expressed in lymphoid tissues, and its transcription is transiently up-regulated during lymphocyte activation by PMA (paramethoxyamphetamine). *MCC* is a known tumor suppressor gene in colorectal cancers (48), but its role in ovarian cancer remains unknown. *EFEMP1* (egf-containing fibulin-like extracellular matrix protein 1) is related with retinal dystrophy, and is also inactivated in lung cancer by promoter hypermethylation (49,50). Its role in reducing tumor angiogenesis (51) could possibly explain how the development of ovarian tumors is connected with a reduced expression of this gene. *RBMS3* (rna-binding motif protein, single strand-interacting, 3) is a non-transcriptional regulator that binds to

ssDNA and A/U rich stretches of RNA. It is activated in liver fibrosis and can connect the transcriptional factor Prx1 (52).

Among the up-regulated genes, we identified CHMP4C as a possible molecular target for ovarian cancer therapy due to its frequent transactivation in ovarian cancers and its undetectable levels of expression in any human adult normal tissues. CHMP4C belongs to the CHMP family, a family of small coiled-coil proteins involved in multivesicular body sorting through its participation in ESCRT-III (endosomal sorting complex required for transport III). CHMP4C contains an Snf7 domain which is a conserved 132-amino acid sequence among all of the 10 family members (CHMP1A, 1B, 2A, 2B, 3, 4A, 4B, 4C, 5, and 6) (53). Following its cloning in 2004, the interaction of CHMP4C with Alix was reported after co-immunoprecipitation experiments were performed using lysates of HEK293 cells (54). A more recent article indicates interaction between CHMP4C and CC2D1A (53). So far, the gene has not been mentioned in relation to ovarian carcinogenesis. Its role as a potential therapeutic target remains to be evaluated.

We demonstrated that the use of microarray represents a powerful approach to identifying key molecules in the development and progression of ovarian cancer. Additionally, we identified several genes whose elevated or decreased expression has not been previously observed in ovarian cancer, confirmed the validity of several existing markers, and provided a foundation for future studies in the understanding and management of this disease. A number of candidates reported here should provide new markers that may contribute to the precise and timely diagnosis of ovarian cancer. Observed changes in the expression profiles of tumor and normal ovarian tissue are due to the combined action of different classes of genes implicated in the mechanisms of transcription, tumor invasion/progression and the regulation of cell the cycle and growth. In summary, gene expression profiling has demonstrated the specific expression signatures of epithelial ovarian tumors as compared to their normal counterparts. An extended study of some of the identified ovarian candidate genes will ensure an improved understanding of the mechanisms of ovarian carcinogenesis and the existence of different subtypes of ovarian cancer.

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