Gene expression profiling of paired ovarian tumors obtained prior to and following adjuvant chemotherapy: Molecular signatures of chemoresistant tumors

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Received January 18, 2006; Accepted March 27, 2006

Abstract. Chemotherapy (CT) resistance in ovarian cancer is related to multiple factors, and assessment of these factors is necessary for the development of new drugs and therapeutic regimens. In an effort to identify such determinants, we evaluated the expression of approximately 21,000 genes using DNA microarray screening in paired tumor samples taken prior to and after CT treatment from 6 patients with predominantly advanced stage, high-grade epithelial ovarian cancer. A subset of differentially expressed genes was selected from all microarray data by initial filtering on confidence at p=0.05, followed by filtering on expression level (≥2-fold). Using these selection criteria, we found 121 genes to be commonly up-regulated and 54 genes to be down-regulated in the post-CT tumors, compared to primary tumors. Up-regulated genes in post-CT tumors included substantial number of genes with previously known implication in mechanisms of chemoresistance (TOP2A, ETV4, ABCF2, PRDX2, COX2, COX7B, MUC1, MT3, MT2A), and tumorigenesis (SCGB2A2, S100A9, YWHAE, SFN, ATP6AP1, MGC5528, ASS, TACC3, ARHGAP4, SRA1; MGC35136, PSAP, SPTAN1, LGALS3BP, TUBA4, AMY2B, PPIA, COX1, GRB2, CTSL). Down-regulated genes in post-CT samples mostly included genes implicated in chemosensitivity (GRP, TRA1, ADPRTL1, TRF4-2), cell proliferation and cell cycle control (NGFRAP1, TPD52L1, TAX1BP1) and tumor suppression and apoptosis (SMOC2, TIMP3, AXIN1, CASP4, P53SCV). Additionally, gene clustering analysis revealed the existence of two distinct expression signatures of chemoresistant tumors, which was further confirmed by assessment of some genetic (p53 gene mutation status) and clinical parameters (CT regimens). Our data suggest that intrinsic and acquired chemoresistant phenotypes of post-CT tumors may be attributed to the combined action of different factors implicated in mechanisms of chemoresistance, tumor invasion/progression and control of cell proliferation. This type of molecular profiling could have important clinical implications in resolving chemoresistance and the development of novel treatment strategies designed to prevent its emergence.

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Key words: ovarian cancer, chemoresistance, second-look laparotomy, gene expression, DNA microarray

Introduction

Epithelial carcinoma of the ovary is characterized by presentation at an advanced stage and spreads primarily by an intraperitoneal route. An initial surgical approach is essential to proper staging of the disease process and to aggressive cytoreduction, which in turn improves response to chemotherapy and survival (1). Chemotherapy (CT) has had an increasingly important role in the effective treatment of ovarian cancer. Combination CT with taxol plus a platinum compound (carboplatin or cisplatin) is the current regimen of choice for the treatment of advanced epithelial ovarian cancer (2). A number of clinical issues, however, are unresolved including drug dosage and schedule, duration of treatment, and route of administration (3). Thus, although significant proportions of women respond to CT, the majority of responders (~50-75%) eventually relapse at a median of 18-28 months (Du Bois A, *et al*, Proc ASCO 18: abs. 356, 1997; Ozols RF, *et al*, Proc ASCO 18: abs. 356, 1999). Treatment decisions at this juncture include supplementary chemotherapy with topotecan, doxorubicin, hormones, surgery, and experimental agents (4). Nonetheless, even with these additional treatments relapse rates remain high and most women with advanced ovarian cancer ultimately will die of their disease (5).

CT resistance in ovarian cancer is broad and encompasses diverse unrelated drugs, suggesting more than one mechanism of resistance. This topic has been the subject of intense

research, and previous studies on ovarian cancer chemoresistance have investigated potential involvement of molecules involved in drug transport, apoptosis, DNA repair, and detoxification pathways (6-10). However for the majority of these factors, *in vivo* studies have failed to assess their clinical importance and to translate them into recommendations for specific therapies or prognosis in ovarian cancer patients (11-13). The above investigations suggest that CT resistance in ovarian cancer is related to multiple factors, and assessment of these factors is necessary for the development of new drugs and therapeutic regimens. In an effort to identify such determinants, we evaluated the expression of ~21,000 genes using DNA microarray screening in paired tumor samples taken prior to and following CT treatment from six ovarian cancer patients. For each patient, the gene expression profile of the post-CT tumor was compared to that of the corresponding primary 'chemonaïve' tumor sample. Our results provide the basis for further extended validation of specific markers that may be involved in the molecular mechanisms of clinical multidrug resistance in ovarian cancer.

Patients and methods

Patients and tissue specimens. Cancer tissues taken prior to (primary tumor tissue), and after adjuvant CT, were obtained for expression profiling analysis from 6 ovarian cancer patients. Paired tumor samples (pre- and post-CT) from 5 patients were obtained from the ovarian tumor bank at the Cancer Research Center at the Hôtel-Dieu de Québec Hospital, Québec, Canada, and paired tumor samples from one additional ovarian cancer patient were obtained from the ovarian tumor bank at the Department of Pathology, Vancouver General Hospital, British Columbia Cancer Agency, Vancouver, Canada. All tumors were histologically classified and graded according to the criteria defined by the World Health Organization. Tumor material from all patients was snap-frozen in liquid nitrogen within 1 h after surgery. Frozen sections were stained with hematoxylin and eosin; only samples that had more than 70% tumor cells were selected. All patients provided informed consent for voluntary participation before any procedures.

Gene expression analysis. Total RNA was isolated from frozen primary ovarian tumor samples using the TRIzol reagent (Invitrogen, Burlington, ON, Canada) and finally dissolved in RNase-free H_2O. Total RNA (5-10 μg) was treated with DNase using the RNase-free DNase kit and RNeasy spin columns (Qiagen, Mississauga, ON). Total RNA treated with DNase was dissolved in RNase-free H_2O to a final concentration of 0.2-0.5 μg/μl. The quality of all RNA samples was examined by capillary electrophoresis using the Agilent 2100 Bioanalyzer (Agilent, Palo Alto, CA). Fluorescently labeled cRNA targets were generated from 0.5 μg of total RNA in each reaction using the Fluorescent Linear Amplification Kit (Agilent) and 10.0 mM Cyanine 3- or 5-labeled CTP (Perkin-Elmer, Boston, MA), and following user's manual. Labeled cRNAs were purified using the RNeasy Mini Kit (Qiagen) and applied to the Human 1A (v2) Oligonucleotide Microarray (Agilent), containing 20,174 genes. For each patient, 1 μg of cyanine-labeled cRNA from the primary (pre-CT) tumor sample was mixed with equal amount of reverse-color cyanine labeled cRNA from the post-CT tumor sample. Hybridization and washing was performed using the *in situ* Hybridization Plus Kit (Agilent) and following the manufacturer's instructions. The arrays were scanned using a dual-laser DNA microarray scanner (Agilent). The data were then extracted from images by the Feature Extraction software 6.1 (Agilent). All microarray experiments were performed in duplicates.

Data analysis. The GeneSpring software (Agilent) was used to generate lists of selected genes and for different statistical and visualization methods. An Intensity-Dependent Normalization (known as Lowess normalization) was applied to correct for artifacts caused by non-linear rates of dye incorporation as well as inconsistencies of the relative fluorescence intensity between some red and green dyes. An initial gene list of 910 genes was created by Filtering on Confidence at p=0.05 (using as measure of confidence the t-test p-value, and as multiple testing correction - the Benjamini and Hochberg false discovery rate) to eliminate genes with unreliable measurements. Consecutive lists of differentially expressed genes derived from the 910 gene list were generated considering a 1.5- to 2-fold expression and using data from all independent experiments. Moreover, based on individual gene expression profiles, two distinct groups of patients were identified and the significant differences in expressed levels between the two groups were determined using 2-fold expression difference and the One-Way ANOVA test (Welch t-test) with p-value cutoff of 0.005. Comparisons of gene expression across the two groups were performed by Cluster Analysis using the Condition Tree Algorithm. The genes in the gene lists were classified according to their function using the Gene Ontology (GO SLIMS) classification system.

Semi-quantitative RT-PCR. Validation of differential gene expression was performed for a number of genes that were differentially expressed between the pre-CT and the post-CT tumor samples in all 6 ovarian cancer patients. The actin-regulatory protein cap G gene (CAPG) displayed no change in expression levels in all tumor samples analyzed and was used as an internal standard. Primers were designed for these loci with the sequences freely available from the Entrez Nucleotide database and the Primer3 algorithm for primer design (http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi). After determination of correct cycling parameters in order to allow measurements in the exponential PCR phase, a duplex semi-quantitative RT-PCR (sqRT-PCR) was performed as previously described (14). Briefly, 2 μg of DNase I-treated RNA was reverse-transcribed into first-strand cDNA in a 20-ml reaction using the MMLV (Moloney-murine-leukaemia virus) RT (Invitrogen). cDNA templates (0.5 μg) were amplified in a 50-μl reaction containing 2.5 pmol of each primer, 200 mM dNTPs and 1.5 units of Taq polymerase (Invitrogen). Each PCR reaction contained two pairs of PCR primers needed for the simultaneous amplification of two PCR fragments: one belonging to the gene of interest and the other representing the CAPG PCR fragment, used as an internal standard. The samples were initially denatured for 3 min at 94˚C and then submitted to 21-25 cycles of PCR

Table I. Patient clinicopathological characteristics.

Patient	Age	HT	Grade	Stage	TTR (months)	Period between last CT and 2nd LAP (months)	LFU
VOA149	57	SPAC	3	IIIC	8	8	DOD
OV-111	65	SPAC	3	IV	13	13	DOD
OV-234	49	CCC	NA	IC	17	17	DOD
OV-86	63	SPAC	3	IIIC	9	40	LFU
OV-113	42	SPAC	2	IV	16	3	DOD
OV-118	41	SPAC	1	IIIC	5	12	LFU

HT, histological type; SPAC, serous papillary adenocarcinoma; CCC, clear cell carcinoma; CT, chemotherapy; TTR, time to recurrence following initial CT; LAP, laparotomy; LFU, lost from follow-up; DOD, died of disease; NA, not applicable.

(45 sec at 94˚C, 45 sec at 58-62˚C and 75 sec at 72˚C) followed by a 10-min final elongation step at 72˚C. One-fifth of each PCR was run on a 1.5-2% agarose gel in 1X TBE buffer (45 mM Tris/borate/1 mM EDTA) and the gel was documented using the AlphaImager 2200 gel documentation system (Alpha Innotech, San Leandro, CA) and analyzed using the publicly available NIH ImageJ 1.33u program (http://rsb.info.nih.gov/ij). Each expression value was calculated as a relative ratio between the signal of the specific PCR fragment and that of the internal standard. The data obtained were statistically analyzed by the unpaired t-test using the GraphPad InStat Software version 3.06 (San Diego, CA).

p53 gene mutation analysis. p53 mutation analysis was performed with genomic DNA extracted from all pre- and post-CT tumors using the AmpliChip p53 Test, a product which is currently under development at Roche Molecular Systems, Inc. The AmpliChip p53 Test is based on the microarray technology and is designed to re-sequence the coding region of the p53 gene and query for the presence of sequence alterations through comparative analysis of the hybridization pattern of a series of probes from sample DNA to wt reference DNA. Briefly, the test uses AmpliChip p53 microarrays, containing over 220,000 different oligonucleotide probes that are synthesized on a glass surface to analyze both sense and antisense strands of an amplified target DNA specimen. Upon PCR amplification and labeling of the p53 exons from specimen or reference samples, the biotin-labeled p53 amplicon fragments are hybridized to the AmpliChip p53 microarrays. The hybridized microarrays are washed and stained with a streptavidin-conjugated fluorescent dye (phycoerythrin) and are consecutively scanned by an Affymetrix GeneChip Scanner 3000Dx using a laser that excites the fluorescent label bound to the hybridized p53 amplicon fragments. The amount of emitted light is proportional to bound target DNA at each location on the probe microarray. Data analysis is performed by the GeneChip Operating Software (GCOS) and the AmpliChip p53 Data Analysis Software which uses p53 specific algorithms to analyze the intensity patterns of DNA samples, compare to the wt reference DNA sample and determine the sequence of p53 gene in the sample.

Results

Gene expression profiling. Our study included 6 patients with predominantly advanced stage, high-grade epithelial ovarian cancer, from whom both pre- and post-CT tumor specimens were available. The clinicopathological characteristics of these patients are presented in Table I. All 6 patients have undergone primary debulking surgery followed by standard platinum/taxol combination therapy. They have displayed different time to recurrence intervals (TTR) after initial CT (7-17 months; Table I) that are essentially below the median progression-free interval (20-22 months) estimated for ovarian cancer patients receiving similar initial treatment (15,16). The period between the last CT treatment and the second-look laparotomy was quite variable for the 6 patients, ranging between 3-40 months (Table I).

In order to look for molecular signatures of chemoresistant ovarian tumors, we performed pairwise gene expression comparison between pre- and post-CT tumor samples obtained from the same patient, as all microarray experiments were carried out in replicates. A subset of differentially expressed genes was selected from all microarray data by initial filtering on confidence at p=0.05, followed by filtering on expression level (≥2-fold). Using these selection criteria, we found 121 genes to be commonly up-regulated and 54 genes to be down-regulated in the post-CT tumors, compared to that of the primary tumors. Functional classes of the 121 up-regulated genes mainly include metabolism (14%), signal transduction (13%), tumor progression (12%), chemoresistance (7%), regulation of transcription (7%), cell growth (6%), protein biosynthesis and modification (6%), transport (5%), cellular structural component (4%), development (3%), apoptosis (1%), DNA repair (1%); the remainder (21%) have unknown function. Table IIA shows list of selected genes that were up-regulated in the post-CT tumors. As seen from Table IIA, there is substantial number of up-regulated genes with previously shown implication in mechanisms of chemoresistance, including ovarian cancer chemoresistance (TOP2A, ETV4, ABCF2, PRDX2, COX2, COX7B, MUC1, MT3, MT2A), as well as genes linked with tumor progression and ovarian malignancy (SCGB2A2, S100A9, YWHAE, SFN,

Table II. List of selected differentially expressed genes (2-fold[a]; p=0.05) in paired samples of post-CT ovarian tumors as compared to corresponding primary tumors.

A, Selected up-regulated genes in the post-CT tumors

Fold change	Gene name	Description	Functions
4.67	SCGB2A2	Secretoglobin, family 2A, member 2	Involved in breast tumorigenesis
3.74	S100A9	S100 calcium binding protein A9	Inflammation; ovarian tumor progression (42)[b]
3.42	TOP2A	Topoisomerasse (DNA) II alpha	Ovarian cancer chemoresistance (22,30,31)
2.89	YWHAE	14-3-3 epsilon protein	Cell cycle control; tumor progression
2.89	ETV4	Ets variant gene 4	Ovarian cancer cisplatin resistance (34,35)
2.76	SFN	14-3-3 sigma protein	Cell cycle control; ovarian tumor progression (43)
2.62	PPP2R4	Protein phosphatase 2A, subunit B'	Negative regulator of cell proliferation
2.61	ATP6AP1	ATPase, H+ transporting	Proton transport; tumorigenesis
2.54	MGC5528	Defective in sister chromatid cohesion 1	Chromosomal instability; cancer progression
2.52	ABCF2	ATP-binding cassette, sub-family F-2	Ovarian cancer chemoresistance (36,37)
2.45	PRDX2	Peroxiredoxin 2	Chemoresistance and radioresistance (39-41)
2.39	ASS	Argininosuccinate synthetase	Arginine and NO synthesis; tumor progression (44)
2.39	H1F0	H1 histone family, member 0	Negative regulator of cell proliferation
2.36	TACC3	Transforming, acidic coiled-coil containing protein 3	Tumorigenesis; ovarian cancer marker (45)
2.31	COX2	Cytochrome c oxidase II	Ovarian cancer chemoresistance (23,24)
2.30	ARHGAP4	Rho GTPase activating protein 4	Tumor neovascularization
2.27	SRA1	Steroid receptor RNA activator 1	Ovarian tumorigenesis (46)
2.23	APTX	Aprataxin	DNA repair; genotoxic stress
2.22	MGC35136	Hypothetical protein MGC35136	Cancer therapy; angiogenesis
2.21	PSAP	Prosaposin	Lipid transport; tumorigenesis
2.21	SPTAN1	Spectrin, alpha	Cytoskeleton; tumorigenesis
2.18	LGALS3BP	Lectin 3 binding protein	Tumor marker; tumorigenesis
2.14	TUBA4	Tubulin, alpha 4	Tumorigenesis
2.11	PHB	Prohibitin	Cell growth; DNA metabolism
2.10	IRF3	Interferon regulatory factor 3	Tumor suppressor
2.06	AMY2B	Amylase, alpha 2B; pancreatic	Carbohydrate metabolism; tumorigenesis
2.06	PPIA	Peptidylprolyl isomerase A	Protein folding; tumor progression
2.06	COX1	Cytochrome c oxidase I	Inflammation; ovarian cancer marker (47-49)
2.05	COX7B	Cytochrome c oxidase subunit VIIb	Expressed specially in post-CT breast tumors (25)
2.02	CD47	CD47 antigen	Integrin-mediated signaling; apoptosis
2.02	GRB2	Growth factor receptor-bound protein 2	Signal transduction; tumorigenesis
2.01	TP53I11	Tumor protein p53 inducible protein 11	Cell proliferation; response to stress
1.85	MUC1	Mucin 1, transmembrane	Ovarian cancer chemotherapy marker
1.68	CTSL	Cathepsin L	Tumor invasion and metastasis
1.67	MT3	Metallothionein 3	Metal ion homeostasis; chemoresistance
1.51	MT2A	Metallothionein 2A	Copper ion homeostasis; chemoresistance
1.50	UVRAG	UV radiation resistance associated gene	DNA repair

B, Selected down-regulated genes in the post-CT tumors

Fold change	Gene name	Description	Functions
-4.65	SMOC2	SPARC related modular Ca binding 2	Ovarian tumor suppression
-4.00	FHL2	Four and a half LIM domains 2	Ovarian tumor marker
-3.55	TIMP3	Tissue inhibitor of metalloproteinase 3	Inhibition of metastasis
-2.62	S100B	S100 calcium binding protein, beta	Metabolism, melanoma marker
-2.47	GRP	Gastrin-releasing peptide	Signal transduction, drug sensitivity

Table II. Continued.

B, Selected down-regulated genes in the post-CT tumors			
Fold change	Gene name	Description	Functions
-2.36	SERPING1	Serine (or cysteine) proteinase inhibitor, clade G member 1	Complement activation, putative marker in cervical cancer
-2.29	NGFRAP1	NGFR associated protein 1	Receptor activity, cell proliferation
-2.29	FMOD	Fibromodulin	Extracellular matrix, tumor-associated antigen
-2.25	PLAT	Plasminogen activator, tissue	Ovarian tumor marker
-2.16	TPD52L1	Tumor protein D52-like 1	Cell proliferation
-2.05	CSF1R	CSF 1 receptor	Receptor, ovarian cancer marker
-1.68	TRA1	Tumor rejection antigen 1	Response to stress; chemosensitivity
-1.61	ADPRTL1	PARPL	Protein ADP-ribosylation; chemosensitivity
-1.58	AXIN1	Axin 1	Signal transducer; tumor suppressor
-1.58	TAX1BP1	Tax1 binding protein 1	Cell proliferation and cell cycle control
-1.57	CASP4	Caspase 4	Apoptosis
-1.56	TFR4-2	Topoisomerase-related protein	Camptothecin sensitivity
-1.52	P53SCV	HSPC132 (p53-inducible)	Apoptosis

[a]The above gene lists contain some additional genes with lower expression values (ranging between 1.5- to 2-fold), which are functionally relevant to tumor biology and response to treatment. [b]The table contains selected references supporting the involvement of certain genes in mechanisms of chemoresistance and ovarian tumorigenesis.

ATP6AP1, MGC5528, ASS, TACC3, ARHGAP4, SRA1; MGC35136, PSAP, SPTAN1, LGALS3BP, TUBA4, AMY2B, PPIA, COX1, GRB2, CTSL). Several genes involved in negative regulation of cell proliferation (PPP2R4, H1F0, PBP, TP53I11) and DNA repair (APTX, UVRAG) were also found to be up-regulated.

Fifty-four genes were subject to at least 2-fold down-regulation in the post-CT tumors. Major classifications of these genes comprise immune response (15%), regulation of transcription (14%), metabolism (13%), signal transduction (11%), development (10%), tumor progression (8%), cellular structural component (7%), transport (6%), cellular growth (3%), and unknown function (13%). Table IIB shows list of selected genes that were down-regulated in the post-CT tumors. This list includes genes implicated in chemosensitivity (GRP, TRA1, ADPRTL1, TRF4-2), cell proliferation and cell cycle control (NGFRAP1, TPD52L1, TAX1BP1), tumor suppression and apoptosis (SMOC2, TIMP3, AXIN1, CASP4, P53SCV). Interestingly, several tumor (including ovarian tumor) markers (FHL2, S100B, PLAT, SERPING1, FMOD, CSF1R) were found to be down-regulated in the post-CT samples.

Supplemental data Table I shows the complete list of differentially expressed genes in the post-CT ovarian tumors as compared to that of the primary tumors.

Confirmation of the expression measurements. To validate microarray results, we arbitrarily selected 13 up-regulated genes and quantified their expression by sqRT-PCR in the available pre- and post-CT tumor samples, Table III summarizes the gene expression measurements of all validated genes. We found that both methods (microarray analysis and sqRT-PCR) detected similar patterns for the 13 up-regulated genes selected for validation. Mean expression values were positive for all 13 genes and significantly positive ($p \leq 0.05$) for 8 of 13 genes.

Identification of two distinct pre/post-CT expression profiles: possible link with p53 mutation status and/or CT treatment regimens. Surprisingly, upon examination of the individual gene expression profiles (pre- and post-CT) of the 6 ovarian cancer patients studied we have detected two distinct types of gene expression profiles, unraveling the possible existence of two separate groups each including 3 patients: Group 1 including patients VOA149, OV-111 and OV-234, and Group 2 including patients OV-86, OV-113 and OV-118. This observation was further confirmed by cluster analysis. First, we selected a subset of candidate genes by filtering on signal intensity (2-fold) to eliminate genes with uniformly low expression or genes whose expression did not vary significantly across the samples, retaining 3,167 genes. One-way ANOVA parametric test (Welch t-test; variances not assumed equal) was further used to select discriminatory genes. t-tests with p-value cutoff of 0.005 selected 264 genes for which expression differed between the 2 groups. Clustering analysis based on the 264 gene list was performed using the standard Condition Tree algorithm provided in GeneSpring and revealed formation of two major cluster groups (Fig. 1). The 264 gene list is presented in Supplemental data Table II.

One hundred and twenty-one genes from the 264-genes list were respectively up-regulated in Group 1 tumors and down-regulated in Group 2 tumors. Major classifications of these genes include regulation of transcription, protein biosynthesis, cell proliferation and metabolism. Genes down-regulated in Group 1 tumors and up-regulated in Group 2

Table III. Correlation of mRNA expression data from the training set with sqRT-PCR derived values.

Gene name	Fold change (microarrays)	Mean value[a] in pre-CT tumors	Mean value in post-CT tumors	Ratio post-CT/pre-CT	p-value
TOP2A	3.420	0.3395	0.6056	1.7838	0.0079
ATP6AP1	2.608	0.7968	1.1537	1.4479	0.1119
PRDX2	2.446	1.1230	1.5670	1.3954	0.0389
ASS	2.390	0.7469	1.080	1.4460	0.1352
COX2	2.310	1.5722	4.7160	2.9996	0.0471
PPP2R4	2.264	0.1113	0.2440	2.1923	0.1022
PPIA	2.060	0.4370	0.4939	1.1302	0.3495
COX1	2.060	1.2241	3.9063	3.1912	0.0476
COX7B	2.051	2.5561	2.9661	1.1604	0.5394
MUC1	1.849	0.5938	1.0560	1.7784	0.0159
CTSL	1.683	0.7038	2.1220	3.0151	0.0002
MT2A	1.505	1.1500	2.4950	2.1696	0.0344
UVRAG	1.480	0.2349	0.5782	2.4615	0.0470

[a]Mean value was calculated as the mean expression value for given marker in all pre- and post-CT tumor samples.

Table IV. Selected functional categories of differentially expressed genes (2-fold; p=0.005) that are specific for Group 1, or Group 2 post-CT ovarian tumors.

A, Specific sets of genes up-regulated in Group 1 and down-regulated in Group 2 Gene symbol	Function
TERF1, OS-9, TACC3, SMT3H1, CDK4, RPS4X, NRD1	Cell proliferation
DARS, RPL29, NACA, EIF3S6IP, RPL39, RPL23A, MRPL45, MRPL43, RPL30, MRP63, MRPS27	Protein biosynthesis

B, Specific sets of genes down-regulated in Group 1 and up-regulated in Group 2 Gene symbol	Function
IGHM, TNFRSF4, THY1, LILRB4, EDG6, HCST, LY86, LIMR, CXCL14	Inflammation and immune response
FLT3LG, DOK4, GALR3, GRIN1, SLA, GNA12, ABR, TRPV5, CBRC7TM_2, LOC90139, DRD1A, CBRC7TM_519, NTNG2, GPR14, GPR7, RAB6B	Signal transduction
COL3A1, CSPG2, ARHGAP9, NRXN2, DPT, BAIAP1	Cell adhesion

tumors (143 genes) are mainly involved in signal transduction, regulation of transcription, immune response and inflammation, protein modification, metabolism and cell adhesion. Our data indicate the presence of some functional categories of differentially expressed genes that are specific for each of the two cluster groups (Table IV).

We further checked the mutation status of the p53 gene in these tumor samples and performed more detailed analysis of the clinical charts of the six patients in order to find any parameters that could potentially explain their clustering. The p53 mutation analysis showed some differences between the groups, since two Group 1 tumors carried different p53 mutations, while two Group 2 tumors contained the wild-type p53 gene with the codon 72 polymorphism (Table V). Interestingly, patient VOA149 had a mutation at codon 85 of the p53 gene only in the post-CT tumor sample, while the primary tumor contained the wild-type p53 allele, indicating that this mutation might be acquired during/after CT treatment. Analysis of different clinical parameters, including CA125 values, initial and residual tumor size, genetic predisposition, etc., did not displayed any significant differences between the two tumor groups (data not shown). The only substantial distinction between the patients from the two groups was the intensity of CT regimens between the two surgical inter-

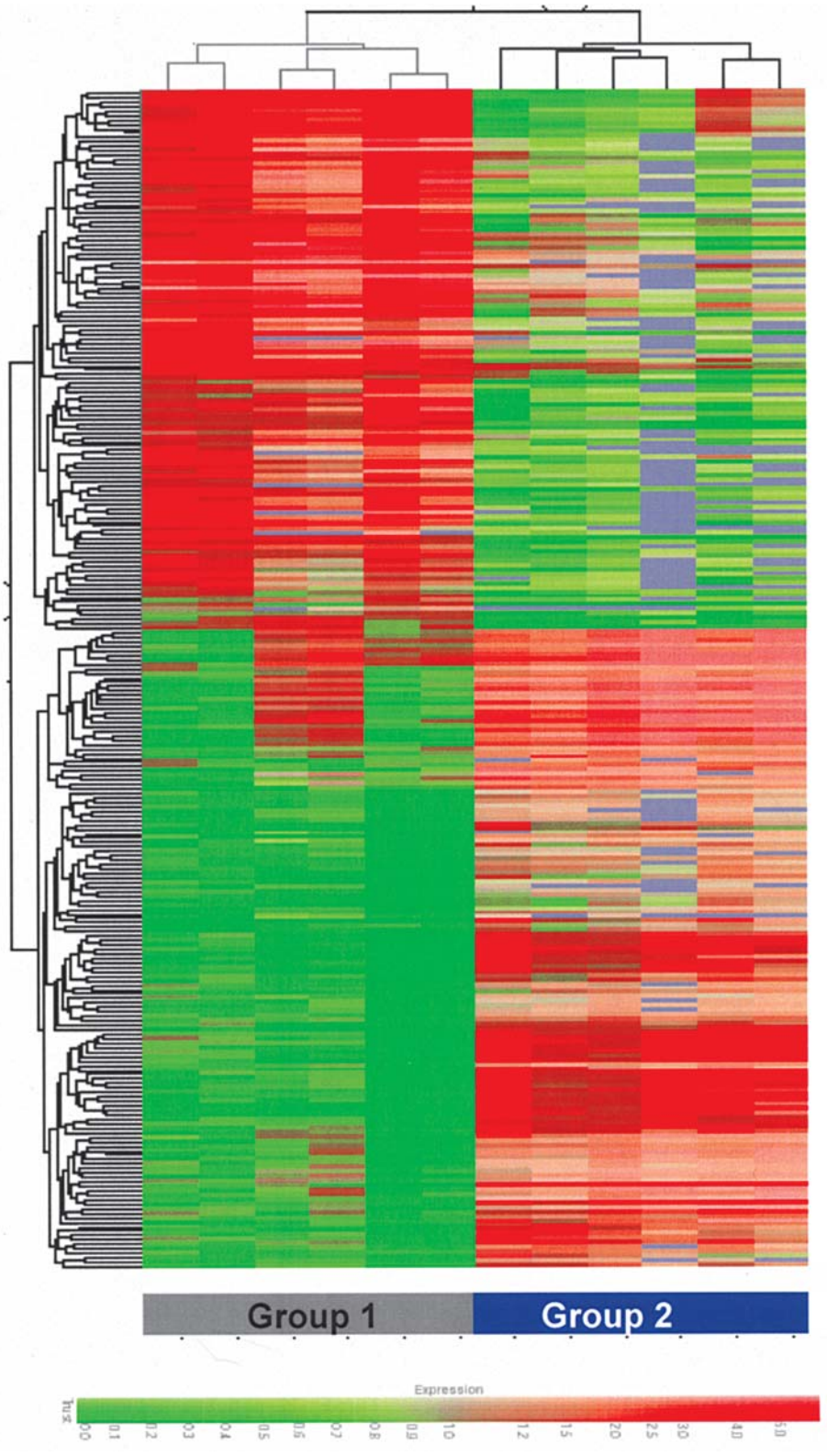

Figure 1. Hierarchical clustering of the 6 post-CT tumors in duplicate based on the 264 gene list (gene expression 2-fold; p-value cutoff of 0.005) that discriminates between Group 1 and Group 2 tumors. The mean appears grey, whereas red signifies up-regulation, and green signifies down-regulation (see legend bar). Group 1 tumors are indicated in grey; Group 2 tumors are indicated in blue.

ventions (the primary cytoreductive surgery and the second-look laparotomy). During this period, the patients from Group 1 have received only one CT regimen, while the Group 2 patients have been more heavily treated, receiving at least two CT regimens (Table V).

Discussion

In this study we have used the microarray technology to examine gene expression profiles of ovarian tumor samples obtained from the same patient prior to, and following systemic

Table V. Individual CT treatment regimens and mutation status of the p53 gene in the tumor samples of the 6 ovarian cancer patients enrolled in the study.

Patient	Group	p53 gene mutation status		CT treatment between 1st and 2nd LAP
		Prior CT	Following CT	
VOA149	1	WT	Exon 4, codon 85 (M) CCT→CTT (Pro→Val)	TX-CP 9X
OV-111	1	Exon 6, codon 218 (M) GTG→GAG (Val→Glu)	Exon 6, codon 218 (M) GTG→GAG (Val→Glu)	TX-CP 6X
OV-234	1	WT	WT	TX-CP 6X
OV-86	2	Exon 4, codon 72 (P) CGC→CCC (Arg→Pro)	Exon 4, codon 72 (P) CGC→CCC (Arg→Pro)	TX-CP 6X; TX-6X
OV-113	2	WT	WT	TX-CP 6X; TX-CP 6X; DOX 3X
OV-118	2	Exon 4, codon 72 (P) CGC→CCC (Arg→Pro)	Exon 4, codon 72 (P) CGC→CCC (Arg→Pro)	TX-CP 6X; TPT 4X

CT, adjuvant chemotherapy; LAP, laparotomy; TX, taxol; CP, carboplatin; TPT, topotecan; DOX, liposomal doxorubicin; WT, wild-type (non-mutated); (M), mutation; (P), polymorphism.

CT. Such samples are quite uncommon since second-look laparotomy is a very rare practice in treatment of ovarian carcinomas, and obviously these cases refer to aggressive/metastatic cancer. Indeed, screening of our ovarian tumor bank which currently comprises more than 600 ovarian tumor specimens allowed us to find paired tumor samples (pre- and post-CT) only from 5 patients, as we have obtained paired samples from one more patient from the ovarian tumor collection in the University of British Columbia. For each patient, we performed pairwise gene expression comparison between pre- and post-CT tumor samples in order to gain insight into global changes describing the chemoresistant phenotype. To our knowledge, similar expression analyses using paired ovarian tumor samples with common origin (obtained from the same patient) have not been yet performed. The rationale for this approach was 2-fold. First, we were able to compare gene expression profiles between primary and post-CT tumor samples with identical genetic background, thus avoiding any 'noise' due to individual's genetic variations. Second, after each cycle of cytotoxic CT, the 'log kill' effect leads to a significant reduction in the number of tumor cells that are sensitive to the administered therapy (17,18). Hence, tumor samples obtained following CT are mostly derived from intrinsic resistant clones and are likely to display molecular signatures associated with chemoresistance. However, we cannot exclude the possibility that some tumor cells that survive the treatment are likely to experience changes in gene expression that allow them to withstand the selective pressure of the drugs used (acquired chemoresistance).

Using our selection criteria (expression 2-fold at p=0.05), we observed that more than twice (121 of 175) genes had higher expression in the post-CT compared with the primary tumors. A similar imbalanced distribution has been observed for other CT-based gene sets, i.e., platinum resistance in 60 NCI cell lines (19), in 7 gastric cell lines (20), in taxol-resistant breast cancer specimens (21), and also quite recently, in resistant compared with sensitive ovarian tumors (22). Thus our data confirm previous findings that overexpression of discriminatory genes is more often associated with CT resistance than sensitivity (22).

As expected, post-CT tumors displayed up-regulation of several genes with functional relevance to mechanisms of chemoresistance (Table IIA). Notably, increased COX2 expression has been repeatedly associated with CT resistance in ovarian cancer patients (23,24). Another member from the same gene family, COX7B, was also found to be up-regulated in post-CT breast tumors as compared to primary breast tumors (25). TOP2A gene overexpression has been formerly linked with mechanisms of chemoresistance (26-29), including ovarian cancer chemoresistance (22,30,31). ETV4 (E1AF or PEA3) is a member of the Ets-related transcription factor family and has been associated with high rates of cell invasion in ovarian cancer (32,33). Platinum treatment has been reported to up-regulate ETV4 (34) and ETV4 gene expression was significantly increased in cisplatin-resistant ovarian carcinoma (35). The ABCF2 protein is a member of the ABCF transporter superfamily and was found to be amplified in a chemoresistant ovarian cancer cells (36). Moreover, Tsuda *et al* recently reported that ABCF2 expression significantly correlated with gene amplification and CT response in clear cell ovarian adenocarcinomas, possibly contributing to the chemoresistant phenotype of this ovarian cancer histotype (37). PRDX2 has been known to be induced by various oxidative stimuli and to play an important protective role from oxidative damage (38). Its expression was correlated with resistance to apoptosis induced by ionizing radiation or cisplatin (39-41), highlighting

the potential clinical importance of PRDX2 in chemo- and radiation-resistance in cancer. We have also observed some increase in MUC1, MT3 and MT2A gene expression (Table IIA), which possibly contributes to the chemoresistant phenotype of the post-CT ovarian tumors. In parallel, some genes involved in chemosensitivity (GRP, TRA1, ADPRTL1, TRF4-2) were found to be down-regulated in the post-CT tumor specimens (Table IIB).

Additionally, numerous genes that have been implicated previously in tumorigenesis and more specifically in ovarian tumorigenesis were found to be up-regulated in post-CT tumors (Table IIA). For instance, S100A9 was found to be overexpressed in common cancers, including ovarian cancer (42). SFN gene expression and methylation status can characterize histological features of different types of ovarian cancer (43). ASS is an enzyme involved in the arginine biosynthesis as L-arginine represents the sole precursor for nitric oxide (NO) synthesis (44). NO enhances tumor initiation, promotion and progression, and it was suggested that arginine-degrading enzyme therapy might be beneficial for cancer patients, including ovarian cancer patients (22). TACC3 has been recently identified as a novel biomarker of ovarian cancer (45). SRA1 displayed significant up-regulation in serous ovarian tumors compared with other ovarian tumor types and normal ovary (46). Recent reports revealed that COX1 contributes to carcinoma development in the ovary through stimulation of prostanglandin E2 production and neovascularization (47,48) and thus represents a potential target for prevention and treatment of epithelial ovarian cancer (49).

All genes mentioned above provide targets for prospective investigations of intrinsic and/or acquired chemoresistance in ovarian cancer. Differences in RNA expression were confirmed by sqRT-PCR for a sample of genes.

Interestingly in post-CT tumor samples, the expression of some genes representing positive regulators of cell proliferation (NGFRAP1, TPD52L1, TAX1BP1) was suppressed, while several genes involved in negative regulation of cell proliferation (PPP2R4, H1F0, PBP, TP53I11) were found to be up-regulated (Table II). This observation supports the concept that decreased proliferation state of tumor cells may be involved in the development of acquired chemoresistance (50-54).

Unexpectedly, assessment of the individual post-CT/pre-CT gene expression profiles of the six patients enrolled in this study revealed the existence of two distinct expression signatures of chemoresistant tumors, which was further confirmed by cluster analysis. Using highly stringent selection criteria (2-fold differential gene expression; p-value cutoff of 0.005) we have identified a set of 264 discriminatory genes for which expression differed between the 2 groups (Fig. 1). Thus, 121 genes from the 264-genes list were respectively up-regulated in Group 1 tumors and down-regulated in Group 2 tumors, while 143 genes were found to be down-regulated in Group 1 tumors and correspondingly up-regulated in Group 2 tumors (Supplemental data Table I). Some functional categories of differentially expressed genes displayed group specificity. For instance, genes with functional relevance to mechanisms of cell proliferation and protein biosynthesis were mostly up-regulated in Group 1 tumors, while genes involved in inflammation and immune response, signal transduction and cell adhesion, were predominantly up-regulated in Group 2 tumors (Table IV). Based on these data we could assume the existence of two subtypes of post-CT ovarian tumors with Group 2 tumors possibly displaying more chemoresistant/aggressive phenotype than Group 1 tumors. Indeed, lower proliferation state, diminished protein biosynthesis and higher inflammation rates have been previously linked with chemoresitance (see above) and enhanced tumor progression (55,56). The more aggressive and chemoresistant phenotype of Group 2 tumors could be additionally sustained by higher expression of genes involved in signal transduction and cell adhesion, since it was shown that cell adhesion molecules not only define a tumor cell's adhesive repertoire, but also directly influence classic signal transduction pathways, thereby modulating the metastatic behavior of tumor cells (57).

The above observation for the existence of 2 subtypes of chemoresistant ovarian tumors with Group 2 tumors displaying the more chemoresistant/aggressive phenotype was further supported by analyses of some additional parameters, including the p53 gene mutation status and CT treatment regimens (Table V). Thus, two of the Group 1 tumors carried different p53 missense mutations; however, p53 activity appears to have insignificant effect on response and survival to platinum-based treatment of ovarian tumors (58). In contrast, the p53 codon 72 polymorphism was found in two Group 2 tumors, and this polymorphism was previously linked with chemoresistance (59) and poor prognosis in ovarian cancer (60). Moreover, for the period between the two surgical interventions, Group 1 patients received only one CT regimen, while the Group 2 patients were more heavily treated, receiving at least two CT regimens which supports the suggestion for the more resistant phenotype of Group 2 tumors.

In summary, gene expression profiling has evidenced for specific expression signatures of chemoresistant post-CT ovarian tumors when compared with their paired primary tumors obtained from the same ovarian cancer patient. Our data suggest that intrinsic and acquired chemoresistant phenotypes of post-CT tumors may be attributed to the combined action of different factors implicated in mechanisms of chemoresistance, tumor invasion/progression and control of cell proliferation. Additionally, gene clustering analysis revealed the existence of two distinct expression signatures of chemoresistant tumors, which was further confirmed by assessment of some genetic and clinical parameters. It will be enlightening to carry out an extended study with more paired ovarian tumor samples in order to better understand the molecular mechanisms of ovarian cancer chemoresistance and the possible existence of different subtypes of chemoresistant tumors. This type of molecular profiling could have important clinical implications in resolving chemoresistance and the development of novel treatment strategies designed to prevent its emergence.

Acknowledgements

This study was supported by grant from the Cancer Research Society (CRS) of Canada (The Strategic Research Program on Genomics and Proteomics of Metastasic Cancer) and by financial support of the Cancer Research Network of the 'Fonds de la Recherche en Santé du Québec', Québec, Canada.

We are thankful to Dr Blake Gilks (Department of Pathology and Laboratory Medicine, Vancouver General Hospital) for sending us paired pre-/post-CT tumor samples and corresponding clinical data from one ovarian cancer patient.

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Supplementary Table I. List of differentially expressed genes (2-fold; p=0.05) in post-CT ovarian tumors compared to pre-CT tumors.

Normalized data	Common	GenBank	Description
4.67	SCGB2A2	U33147	Secretoglobin, family 2A, member 2
3.74	S100A9	AF086362	S100 calcium binding protein A9 (calgranulin B)
3.53	TRAP95	AF121228	Thyroid hormone receptor-associated protein, 95-kDa subunit
3.34	TTK	BC032858	TTK protein kinase
3.06	RERE	NM_012102	Arginine-glutamic acid dipeptide (RE) repeats
3.03	KRT6A	BC014152	Keratin 6A
2.99	MELK	D79997	Maternal embryonic leucine zipper kinase
2.95	NPL4	AK000664	Hypothetical protein FLJ20657
2.93	MRPL12	NM_002949	Mitochondrial ribosomal protein L12
2.89	YWHAE	BC001440	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, epsilon
2.89	ETV4	NM_001986	Ets variant gene 4 (E1A enhancer binding protein, E1AF)
2.86	FKBP8	NM_012181	FK506 binding protein 8, 38 kDa
2.83	PRSS2	BC030260	Protease, serine, 2 (trypsin 2)
2.80	UGCG	D50840	UDP-glucose ceramide glucosyltransferase
2.80		AF023860	Cercopithecus aethiops cyclophilin A
2.76		AF023861	Macaca mulatta cyclophilin A mRNA
2.76	SFN	AF029081	14-3-3 sigma protein promoter and gene
2.75	P4HB	BC029617	Procollagen-proline, 2-oxoglutarate 4-dioxygenase (proline 4-hydroxylase), beta
2.72	KRT6E	NM_173086	Keratin 6E
2.67	EPN3	NM_017957	Epsin 3
2.67		AF023860	Cercopithecus aethiops cyclophilin A
2.65	HSPB1	Z23090	28 kDa heat shock protein

Supplementary Table I. Continued.

Normalized data	Common	GenBank	Description
2.62	PPP2R4	BC002545	Protein phosphatase 2A, regulatory subunit B' (PR 53)
2.61	ATP6AP1	BC000724	ATPase, H+ transporting, lysosomal accessory protein 1
2.58	PP3111	NM_022156	PP3111 protein
2.58	DCXR	AF113123	Dicarbonyl/L-xylulose reductase
2.56	UQCRC1	L16842	Ubiquinol-cytochrome c reductase core protein I
2.54	MGC5528	NM_024094	Defective in sister chromatid cohesion homolog 1 (*S. cerevisiae*)
2.54	CLTB; LCB	M20469	Clathrin light-chain b; human brain-type clathrin light-chain b mRNA, complete cds
2.54	MGC2198	NM_138820	Hypothetical protein MGC2198
2.52	ABCF2	NM_005692	ATP-binding cassette, sub-family F (GCN20), member 2
2.50	COPE	AL136928	Coatomer protein complex, subunit epsilon
2.49	ZNF141	NM_003441	Zinc finger protein 141 (clone pHZ-44)
2.48	USP5	U47927	Ubiquitin specific protease 5 (isopeptidase T)
2.46	CAPNS2	BC005397	Calpain small subunit 2
2.45			Protein containing three C2H2 type zinc finger domains
2.45	DAPK3	AB007144	Death-associated protein kinase 3
2.45	PRDX2	BC039428	Peroxiredoxin 2
2.44	FBXW5	NM_018998	F-box and WD-40 domain protein 5
2.44	TP53TG3	NM_015369	TP53TG3 protein
2.42	CBARA1	NM_006077	Calcium binding atopy-related autoantigen 1
2.39	ASS	X01630	Agininosuccinate synthetase
2.39	FLJ21125	NM_024627	Hypothetical protein FLJ21125
2.39	H1F0	BC000145	H1 histone family, member 0
2.39	NCOA3	NM_006534	Nuclear receptor coactivator 3
2.38	EIF2AK3	AF110146	Eukaryotic translation initiation factor 2-alpha kinase 3
2.37	LOC283820	BC041131	Hypothetical protein LOC283820
2.36	PSMD3	D67025	Proteasome (prosome, macropain) 26S subunit, non-ATPase, 3
2.36	TACC3	AF093543	Transforming, acidic coiled-coil containing protein 3
2.35	FLII	NM_002018	Flightless I homolog (*Drosophila*)
2.34	IDH3G	U40272	Isocitrate dehydrogenase 3 (NAD+) gamma
2.32	SLC25A5	NM_001152	Solute carrier family 25 member 5
2.31	COX2	NM_173705	Cytochrome c oxidase II
2.30	ARHGAP4	X78817	Rho GTPase activating protein 4
2.28	TRIM26	BC032297	Tripartite motif-containing 26
2.28	CDC42EP1	BC009356	CDC42 effector protein (Rho GTPase binding) 1
2.28	PDE9A	AF048837	Phosphodiesterase 9A
2.27	MGC20806	NM_144999	Hypothetical protein MGC20806
2.27	SRA1	AF293024	Steroid receptor RNA activator 1
2.26	LMAN2	U10362	Lectin, mannose-binding 2
2.25	HARS	AK000498	Histidyl-tRNA synthetase
2.24	CASKIN2	NM_020753	CASK interacting protein 2
2.24	DAP4	AB023181	Disks large-associated protein 4
2.24	MGC2641	BC000755	Hepatoma-derived growth factor-related protein 2
2.23	APTX	NM_175073	Aprataxin
2.22	PP1201	AK090618	PP1201 protein
2.22	TNRC5	BC004423	Trinucleotide repeat containing 5
2.22	ACY1	L07548	Aminoacylase 1
2.22	MGC35136	NM_152427	Hypothetical protein MGC35136
2.21	PSAP	D00422	Prosaposin
2.21	ZSIG11	AK023997	Putative secreted protein ZSIG11
2.21	RFWD1	NM_032271	Ring finger and WD repeat domain 1
2.21	FLJ20641	NM_017915	Hypothetical protein FLJ20641

Supplementary Table I. Continued.

Normalized data	Common	GenBank	Description
2.21	SPTAN1	NM_003127	Spectrin, alpha, non-erythrocytic 1 (alpha-fodrin)
2.20	RGS19IP1	BC012810	Regulator of G-protein signalling 19 interacting protein 1
2.20	SORT1	NM_002959	Sortilin 1
2.20	GMPPA	NM_013335	GDP-mannose pyrophosphorylase A
2.20	CDA017	NM_032024	CDA017 protein
2.20	SIAT7E	AK056241	Sialyltransferase 7E
2.19	HSU79266	NM_013299	Protein predicted by clone 23627
2.18	LGALS3BP	BC015761	Lectin, galactoside-binding, soluble, 3 binding protein
2.18	ARHGEF15	BC036749	Rho guanine nucleotide exchange factor (GEF) 15
2.17	GPRC5C	NM_018653	G protein-coupled receptor, family C, group 5, member C
2.16	TCF15	NM_004609	Transcription factor 15 (basic helix-loop-helix)
2.16	GDI1	BC012201	GDP dissociation inhibitor 1
2.15	STMN4	BC011520	Stathmin-like 4
2.15		X52858	Human cyclophilin-related processed pseudogene
2.14		X52855	Human cyclophilin-related processed pseudogene
2.14	C20orf129	NM_030919	Chromosome 20 open reading frame 129
2.14	TUBA4	NM_025019	Tubulin, alpha 4
2.12	NICE-4	D63478	NICE-4 protein
2.12	CRIP2	BC034151	Cysteine-rich protein 2
2.11	PHB	S85655	Prohibitin
2.11	C19orf4	BC010446	Chromosome 19 open reading frame 4
2.11	RHO	S76579	Macaca fascicularis opsin mRNA
2.10	DHX8	D50487	DEAH (Asp-Glu-Ala-His) box polypeptide 8
2.10	EST1B	NM_015327	Est1p-like protein B
2.10	IRF3	Z56281	Interferon regulatory factor 3
2.09	PSMD11	AF001212	Proteasome (prosome, macropain) 26S subunit, non-ATPase, 11
2.08	GCN5L1	D64007	GCN5 general control of amino-acid synthesis 5-like 1 (yeast)
2.07	TMPRSS3	NM_024022	Transmembrane protease, serine 3
2.07	MT	BC042195	Malonyl-CoA:acyl carrier protein transacylase (malonyltransferase)
2.07	LOC55974	BC005943	Stromal cell protein
2.06	AMY2B	NM_020978	Amylase, alpha 2B; pancreatic
2.06	PPIA	BC013915	Peptidylprolyl isomerase A (cyclophilin A)
2.06	COX1	NM_173704	Cytochrome c oxidase I
2.05	NLGN2	AF376802	Neuroligin 2
2.05	COX7B	Z14244	Cytochrome c oxidase subunit VIIb
2.04	FLJ20989	NM_023080	Hypothetical protein FLJ20989
2.04	PAFAH1B2	BC000398	Platelet-activating factor acetylhydrolase, isoform Ib, beta subunit 30 kDa
2.02	CD47	X69398	CD47 antigen (Rh-related antigen, integrin-associated signal transducer)
2.02	PGLS	BC014006	6-phosphogluconolactonase
2.02	MGC13102	NM_032323	Hypothetical protein MGC13102
2.02	C14orf59	NM_174976	Chromosome 14 open reading frame 59
2.02	GRB2	NM_002086	Growth factor receptor-bound protein 2
2.01	HUMPPA	BC047534	Paraneoplastic antigen
2.01	ET	AK000233	Hypothetical protein ET
2.01	C40	NM_017546	Hypothetical protein C40
2.01	RNF44	NM_014901	Ring finger protein 44
2.01	TP53I11	NM_006034	Tumor protein p53 inducible protein 11
2.00	TRAP100	NM_014815	Thyroid hormone receptor-associated protein (100 kDa)
-2.00	HT011	NM_018472	Uncharacterized hypothalamus protein HT011
-2.00	GYPB	X08055	Glycophorin B (includes Ss blood group)
-2.01	MORC	AF084946	Microrchidia homolog (mouse)

Supplementary Table I. Continued.

Normalized data	Common	GenBank	Description
-2.02	SAA4	M81349	Serum amyloid A4, constitutive
-2.03	ABCA6	AY028898	ATP-binding cassette, sub-family A (ABC1), member 6
-2.03	ADAMTS5	AF142099	A disintegrin-like and metalloprotease with thrombospondin type 1 motif 5
-2.03	FLJ14927	NM_032863	Protein of unknown function
-2.04	BTF3	X53280	Basic transcription factor 3
-2.04	LOC139231	AK091221	Hypothetical protein BC016683
-2.05	CSF1R	X03663	Colony stimulating factor 1 receptor
-2.06	C1R	BC035220	Complement component 1, r subcomponent
-2.06	ANKRD15	NM_015158	Ankyrin repeat domain 15
-2.08	FLJ10652	NM_018169	Hypothetical protein FLJ10652
-2.10	KCTD12	NM_138444	Potassium channel tetramerisation domain containing 12
-2.11	MAGP2	U37283	Microfibril-associated glycoprotein-2
-2.13	SH3BGR	NM_007341	SH3 domain binding glutamic acid-rich protein
-2.13	POGZ	AB007930	Pogo transposable element with ZNF domain
-2.14	LMX1A	NM_177398	LIM homeobox transcription factor 1, alpha
-2.15	ASXH2	NM_018263	Polycomb group protein ASXH2
-2.16	TPD52L1	U44427	Tumor protein D52-like 1
-2.16	SHBG	X05403	Sex hormone-binding globulin
-2.16	KIR2DL1	NM_014218	Killer cell immunoglobulin-like receptor
-2.16	EEF1B2	X60489	Eukaryotic translation elongation factor 1 beta 2
-2.18	RPS7	BC002866	Ribosomal protein S7
-2.19		AK025719	*Homo sapiens* cDNA: FLJ22066 fis, clone HEP10611
-2.19	EPB42	NM_000119	Erythrocyte membrane protein band 4.2
-2.19	DKFZp434G118	NM_032266	Hypothetical protein DKFZp434G118
-2.20	MATN3	AJ224741	Matrilin 3
-2.25	PLAT	BC007231	Plasminogen activator, tissue
-2.26	FLJ30213	NM_145008	Hypothetical protein FLJ30213
-2.26	GUCY2D	M92432	Guanylate cyclase 2D, membrane (retina-specific)
-2.27	FLJ10210	NM_018027	Hypothetical protein FLJ10210
-2.28	LPA	NM_005577	Lipoprotein, Lp(a)
-2.28	EAR3	X12795	v-erbA related ear-3 gene
-2.29	FMOD	BC035281	Fibromodulin
-2.29	NGFRAP1	M38188	Nerve growth factor receptor (TNFRSF16) associated protein 1
-2.33	PAP	BC036776	Pancreatitis-associated protein
-2.33	CDC26	NM_139286	Cell division cycle 26
-2.36	SERPING1	BC011171	Serine (or cysteine) proteinase inhibitor, clade G member 1
-2.44	F2RL1	BC018130	Coagulation factor II (thrombin) receptor-like 1
-2.47	GRP	BC004488	Gastrin-releasing peptide
-2.54	KYNU	U57721	Kynureninase (L-kynurenine hydrolase)
-2.58	LOC57151		Member of the alpha-lactalbumin or lysozyme C family
-2.62	S100B	BC001766	S100 calcium binding protein, beta (neural)
-2.63	ARSF	NM_004042	Arylsulfatase F
-2.70	FCGR1A	NM_000566	Fc fragment of IgG, high affinity Ia, receptor for (CD64)
-2.79	FLJ13710	NM_024817	Hypothetical protein FLJ13710
-2.85	DKFZp547H025	NM_020161	Hypothetical protein DKFZp547H025
-2.87	SGCD	NM_000337	Sarcoglycan, delta
-3.18	FERD3L	NM_152898	Fer3-like (*Drosophila*)
-3.55	TIMP3	S78453	Tissue inhibitor of metalloproteinase 3
-4.00	FHL2	L42176	Four and a half LIM domains 2
-4.65	SMOC2	AJ420521	SPARC related modular calcium binding 2
-4.76	OLFM3	AF397394	Olfactomedin 3

Supplementary Table II. Genes selected upon cluster analysis from condition Group 1 that are greater or less than those in condition Group 2 by a factor of 2-fold at p-value cutoff = 0.005.

Fold change	Common	GenBank	Product	GO biological process
10.13	FLJ10637	BC003081	Hypothetical protein FLJ10637	Unknown function
6.96	BAT1	BC004350	Unknown (protein for MGC:1518)	Unknown function
6.81	FLJ12895	NM_023926	Hypothetical protein FLJ12895	Unknown function
5.93	BECN1	NM_003766	Beclin 1	Anti-apoptosis
5.66	LOC163233	BC015765		Unknown function
5.33	C21orf59	AK098577	Chromosome 21 open reading frame 59	Unknown function
5.06	CDK4	M14505	Cyclin-dependent kinase 4 isoform 1; cyclin-dependent kinase 4 isoform 2	Cell proliferation
5.02	TGIF	NM_173207	TG-interacting factor isoform c; TG-interacting factor isoform a; TG-interacting factor isoform b; TG-interacting factor isoform d	Development
4.86	TGIF	NM_170695	TG-interacting factor isoform c; TG-interacting factor isoform a; TG-interacting factor isoform b; TG-interacting factor isoform d	Development
4.79	TERF1	U40705	Telomeric repeat binding factor 1 isoform 2; telomeric repeat binding factor 1 isoform 1	Cell proliferation
4.70	HES1	NM_005524	Hairy and enhancer of split 1	Regulation of transcription
4.65	SMT3H1	BC000036	SMT3 suppressor of mif two 3 homolog 1	Cell proliferation
4.50	OAZIN			Protein catabolism; ubiquitin cycle
4.23	KIAA0121	D50911	KIAA0121 protein	Unknown function
3.93	MYST3	NM_006766	MYST histone acetyltransferase (monocytic leukemia) 3	Chromosome organization
3.88	MRPL43	NM_032112	Mitochondrial ribosomal protein L43 isoform a; mitochondrial ribosomal protein L43 isoform b; mitochondrial ribosomal protein L43 isoform c; mitochondrial ribosomal protein L43 isoform d	Protein biosynthesis
3.84				Retired
3.83	IGBP1	BC004137	Immunoglobulin (CD79A) binding protein 1	Signal transduction
3.78	MGC42493	NM_178549	Hypothetical protein MGC42493	Unknown function
3.74	WBP11	AB029309	WW domain binding protein 11	Regulation of transcription
3.71	UBE2N	NM_003348	Ubiquitin-conjugating enzyme E2N	DNA repair
3.68	ZNF430	NM_025189	Zinc finger protein 430	Regulation of transcription
3.67	RNF5	BC004155	Ring finger protein 5	Cell motility inhibitor
3.67	MGC2396	NM_052852		Unknown function
3.60	NARG2	NM_024611	NMDA receptor-regulated gene 2	Development
3.49	KIAA1002; PR01365	NM_014925		Retired
3.46	ZNF493	NM_175910	Zinc finger protein 493	Unknown function
3.44	HSPCB	M16660	Heat shock 90 kDa protein 1, beta	Protein folding
3.44	FLJ10853	NM_018246	Hypothetical protein FLJ10853	Unknown function
3.43	EIF3S6IP	BC029265	Eukaryotic translation initiation factor 3, subunit 6 interacting protein	Protein biosynthesis
3.35	LOC285458	NM_174948	Hypothetical protein LOC285458	Unknown function
3.34	RPL30	AK026528		Protein biosynthesis
3.34	RPS4X	M58458	Ribosomal protein S4, X-linked X isoform	Cell proliferation
3.31	FTSJ3	AK000069	FtsJ homolog 3	Regulation of translation
3.29	RPL29	BC008926	Ribosomal protein L29	Protein biosynthesis
3.25	FLJ22490	AK096205		Unknown function
3.23	STAT3	BC014482	Signal transducer and activator of transcription 3 isoform 2; signal transducer and activator of transcription 3 isoform 1	Cell motility
3.23	RNF34; RFI; RIFF; FLJ21786	AK096994	Ring finger protein 34 isoform 2; ring finger protein 34 isoform 1	Unknown function
3.22				Retired

Supplementary Table II. Continued.

Fold change	Common	GenBank	Product	GO biological process
3.22	MGC32124	BC020263	Hypothetical protein MGC32124	Unknown function
3.18	KIAA1737	NM_033426	KIAA1737 protein	Unknown function
3.14	MGC32124	NM_144611	Hypothetical protein MGC32124	Unknown function
3.10	Raptor	NM_020761	Raptor	Signal transduction
3.09	ZNF43	X59244	Zinc finger protein 43 (HTF6)	Regulation of transcription
3.08	C7orf30	BC012331	Chromosome 7 open reading frame 30	Unknown function
3.08	GDI1	BC012201	GDP dissociation inhibitor 1	Protein transport
3.07	MASA	AK022656	E-1 enzyme	Metabolism
3.07	ZNF430	NM_025189	Zinc finger protein 430	Regulation of transcription
3.05	H-plk	M55422	Krueppel-related zinc finger protein	Unknown function
3.03	ZNF85	U35376	Zinc finger protein 85 (HPF4, HTF1)	Regulation of transcription
3.01	KIAA1924	NM_145294	Similar to RIKEN cDNA 3230401M21 (*Mus musculus*)	Unknown function
3.01	SMARCA5	BC023144	SWI/SNF-related matrix-associated actin-dependent regulator of chromatin a5	Chromosome organization
3.00	KIAA1279	NM_015634	KIAA1279	Unknown function
2.99				Regulation of transcription
2.97	PRNP	M13899	Prion protein preproprotein	Immune response
2.96	ZNF43	X59244	Zinc finger protein 43 (HTF6)	Regulation of transcription
2.95	ZNF161	NM_007146	Zinc finger protein 161	Defense response
2.88	TIZ	NM_138330	TRAF6-inhibitory zinc finger protein	Regulation of transcription
2.88	MLL4	NM_014727	Myeloid/lymphoid or mixed-lineage leukemia 4	Regulation of transcription
2.80	TACC3	AF093543	Transforming, acidic coiled-coil containing protein 3	Cell proliferation
2.80	TP53BP2	U58334	Tumor protein p53 binding protein 2	Apoptosis
2.79	RPL39	BC001019	Ribosomal protein L39	Protein biosynthesis
2.79	C21orf108	AB011111	KIAA0539 protein	Unknown function
2.77	DPH2L1; OVCA1	BC003099	Diptheria toxin resistance protein required for diphthamide biosynthesis-like 1	Tumor suppressor
2.74	FKSG17	NM_032031	FKSG17	Unknown function
2.71	FBXL5	NM_012161	F-box and leucine-rich repeat protein 5 isoform 1; F-box and leucine-rich repeat protein 5 isoform 2	Proteolysis and peptidolysis
2.62	CEBPD; CELF; CRP3; C/EBP-delta; NF-IL6-beta	S63168	CCAAT/enhancer binding protein delta	Regulation of transcription
2.61	C6orf37	NM_017633	Chromosome 6 open reading frame 37	Unknown function
2.61	ADD3	D67031	Adducin 3 isoform a; adducin 3 isoform b	Cytoskeleton
2.58	NRD1	X93207	Nardilysin	Cell proliferation
2.58	RAI1	NM_017574	Retinoic acid induced 1 isoform 1; retinoic acid induced 1 isoform 3; retinoic acid induced 1 isoform 2	Unknown function
2.58	PTK2	NM_005607	PTK2 protein tyrosine kinase 2 isoform b; PTK2 protein tyrosine kinase 2 isoform a	Cell adhesion
2.57	FLJ10496	NM_018114	Hypothetical protein FLJ10496	Unknown function
2.57	MGC2744	NM_025267	Hypothetical protein MGC2744	Unknown function
2.56				Retired
2.55	NICE-4	D63478	NICE-4 protein	Regulation of transcription
2.52	RPL23AP7	BC000596	RPL23AP7 protein	Development
2.49	PEX14	BC006327	Peroxisomal biogenesis factor 14	Metabolism
2.49	NPEPPS	AJ132583	Aminopeptidase puromycin sensitive	Proteolysis and peptidolysis
2.48	PACE-1	BC014662	Ezrin-binding partner PACE-1 isoform 1	Unknown function
2.48		BC047949	Similar to expressed sequence AI426465	Unknown function
2.46	MGC26610	NM_144647	Hypothetical protein MGC26610	Unknown function
2.45	LTA4H	J03459	Leukotriene A4 hydrolase	Proteolysis and peptidolysis
2.43	XTP2	NM_015172	HBxAg transactivated protein 2	Apoptosis

Supplementary Table II. Continued.

Fold change	Common	GenBank	Product	GO biological process
2.43	EPHX2	BC011628	Epoxide hydrolase 2, cytoplasmic	Defense response
2.43	UACA	NM_018003	Uveal autoantigen with coiled-coil domains and ankyrin repeats	Unknown function
2.43	MTA3	BC004227	MTA3 protein	Regulation of transcription
2.42	NCOA6	AF171667	Nuclear receptor coactivator 6	Regulation of transcription
2.41	NACA	AK090650	Nascent-polypeptide-associated complex alpha polypeptide	Protein biosynthesis
2.40	JWA	BC005143	Cytoskeleton related vitamin A responsive protein	Metabolism
2.40	FLJ20531	NM_017865	Hypothetical protein FLJ20531	Unknown function
2.38	MRP63	BC023616	Mitochondrial ribosomal protein 63	Protein biosynthesis
2.38	MRPS27	NM_015084	Mitochondrial ribosomal protein S27	Protein biosynthesis
2.37	LASS2	NM_013384	LAG1 longevity assurance homolog 2 isoform 2; LAG1 longevity assurance homolog 2 isoform 1	Metabolism
2.37	DARS	NM_001349	Aspartyl-tRNA synthetase	Protein biosynthesis
2.35	STHM	U14550	Sialyltransferase	Protein glycosylation
2.34	CN2	AK001692	Cytosolic nonspecific dipeptidase (EC 3.4.13.18)	Proteolysis and peptidolysis
2.33	SFRS2IP	AF030234	Splicing factor, arginine/serine-rich 2, interacting protein	mRNA processing
2.29	RPL23A	BC014459	Ribosomal protein L23a	Protein biosynthesis
2.25	FLJ22955	BC018132	Hypothetical protein FLJ22955	Unknown function
2.23	OS-9	U41635	Amplified in osteosarcoma	Cell proliferation
2.23	MRPL45	NM_032351	Mitochondrial ribosomal protein L45	Protein biosynthesis
2.23	SPPL3	NM_139015	SPPL3 protein	Unknown function
2.21	TCEA2	BC018896	Transcription elongation factor A protein 2 isoform a; transcription elongation factor A protein 2 isoform b	Regulation of transcription
2.20	ZNF281	NM_012482	Zinc finger protein 281	Regulation of transcription
2.16	SUPT5H	Y12790	Suppressor of Ty 5 homolog	Regulation of transcription
2.16	FDXR	NM_004110	Ferredoxin reductase isoform 2 precursor; ferredoxin reductase isoform 1 precursor	Metabolism
2.14	ELAC2	BC001939	elaC homolog 2	mRNA processing
2.11	EIF4A1	BC009585	Eukaryotic translation initiation factor 4A, isoform 1	Regulation of translation
2.10	MTATP8	NM_173703	ATP synthase 8	Ion transport
2.10	PIK4CB	NM_002651	Phosphatidylinositol 4-kinase, catalytic, beta polypeptide	Signal transduction
2.08		NM_024521		Unknown function
2.07	LOC90522	BC007644	Similar to putative transmembrane protein; homolog of yeast Golgi membrane protein Yif1p (Yip1p-interacting factor)	Unknown function
2.06	SDBCAG84	NM_015966	Serologically defined breast cancer antigen 84 isoform b; serologically defined breast cancer antigen 84 isoform a	Breast cancer antigen
2.06	MGC2491	BC000262	Hypothetical protein MGC2491	Unknown function
2.04	USP11	NM_004651	Ubiquitin specific protease 11	Protein catabolism; ubiquitin cycle
2.03	PPP1CC	X74008	Protein phosphatase 1, catalytic subunit, gamma isoform	Metabolism
2.03	RBMX	BC006550	RNA binding motif protein, X chromosome	Regulation of transcription
2.03	PRC	AF325193	PGC-1 related co-activator	Unknown function
2.03	FLJ20315	NM_017763	Hypothetical protein FLJ20315	Regulation of transcription
2.03	LOC284361	AY194293	Hypothetical protein LOC284361	Unknown function
-2.00	GPD1	NM_005276	Glycerol-3-phosphate dehydrogenase 1 (soluble)	Metabolism
-2.00				Unknown function
-2.02	CBRC7TM_519	AB065956	Seven transmembrane helix receptor	Signal transduction
-2.03	FLJ36666	NM_152482	Hypothetical protein FLJ36666	Unknown function
-2.04	PIASY	AF077952	Protein inhibitor of activated STAT protein PIASy	Regulation of transcription
-2.04	SLC12A7	AF105365	Solute carrier family 12 (potassium/chloride transporters), member 7	Ion transport
-2.06	DRD1; DADR; DRD1A	S58541	Dopamine receptor D1	Signal transduction

Supplementary Table II. Continued.

Fold change	Common	GenBank	Product	GO biological process
-2.06	LILRB4	BC026309	Leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 4	Immune response
-2.06	FLJ22202	NM_024883	Hypothetical protein FLJ22202	Unknown function
-2.07	DKFZp434L192	NM_152746	Hypothetical protein DKFZp434L192	Unknown function
-2.08	FN5	NM_020179	FN5 protein	Unknown function
-2.12	NEF3; NFM; NEFM; NF-M	Y00067	Neurofilament 3 (150kDa medium)	Cytoskeleton
-2.15	SERPINB2	Y00630	Serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 2	Anti-apoptosis
-2.15				Retired
-2.16	COH1	NM_017890	Cohen syndrome 1 protein isoform 3; Cohen syndrome 1 protein isoform 5; Cohen syndrome 1 protein isoform 1; Cohen syndrome 1 protein isoform 4; Cohen syndrome 1 protein isoform 2	Transport
-2.19	IGF2AS	NM_016412	Insulin-like growth factor 2, antisense	Regulation of transcription
-2.20	PIPPIN	NM_014460	RNA-binding protein pippin	mRNA processing
-2.20	LOC90139	AK027715	Tetraspanin similiar to uroplakin 1	Signal transduction
-2.20	BAIAP1	AB010894	BAI1-associated protein 1	Cell adhesion
-2.20	JDP2	NM_130469	Jun dimerization protein	Regulation of transcription
-2.21	GYG2	U94362	Glycogenin 2	Metabolism
-2.21	MGC40084	NM_152769	Hypothetical protein MGC40084	Unknown function
-2.23	SIAT8A	NM_003034	Sialyltransferase 8A	Metabolism
-2.23	NPPC	NM_024409	Natriuretic peptide precursor C	Regulation of transcription
-2.27	COXVIB2	NM_144613	Cytochrome c oxidase subunit VIb, testis-specific isoform	Regulation of transcription
-2.27				Unknown function
-2.28	DPT	BC033736	Dermatopontin precursor	Cell adhesion
-2.28	DKFZp434C0923	NM_017598	Hypothetical protein DKFZp434C0923	Unknown function
-2.28	LY86	BC038846	MD-1, RP105-associated	Inflammatory response
-2.30	CBRC7TM_2	AB065439	Seven transmembrane helix receptor	Signal transduction
-2.30	KIAA0937	AK074166	FLJ00239 protein	Unknown function
-2.31	ORM1	BC015964	Orosomucoid 2	Transport
-2.31	HIPK2	AF208291	Homeodomain interacting protein kinase 2	Transcription co-repressor
-2.31	KIAA1201	AB033027	KIAA1201 protein	Unknown function
-2.32	CD163	NM_004244	CD163 antigen	Retired
-2.33	ABR	U01147	Active breakpoint cluster region-related protein isoform b; active breakpoint cluster region-related protein isoform a	Signal transduction
-2.33	TRPV5	AF304464	Transient receptor potential cation channel, subfamily V, member 5	Signal transduction
-2.33				Unknown function
-2.35	GJB4	NM_153212	Gap junction protein, beta 4	Cell communication
-2.36	NRXN2	AB035266	Neurexin 2 isoform alpha-1 precursor; neurexin 2 isoform alpha-2 precursor; neurexin 2 isoform beta precursor	Cell adhesion
-2.38	STK10	AB015718	Serine/threonine kinase 10	Protein phosphorylation
-2.39	FBXW8	NM_153348	F-box and WD-40 domain protein 8 isoform 2; F-box and WD-40 domain protein 8 isoform 1	Protein catabolism
-2.40	FLT3LG	NM_001459	Fms-related tyrosine kinase 3 ligand	Signal transduction
-2.42				Retired
-2.42	RAB6B	AF166492	RAB6B, member RAS oncogene family	Signal transduction
-2.43	NRN1	BC042019	Neuritin precursor	Neuritogenesis
-2.44	ARHGAP9	BC006107	Rho GTPase activating protein 9	Cell adhesion
-2.44	SPTBN4	AF082075	Spectrin, beta, non-erythrocytic 4	Cytoskeleton
-2.44	GNA12	AF493901	Guanine nucleotide binding protein (G protein) alpha 12	Signal transduction
-2.44	MT1X	BC032338	Metallothionein 1X	Response to metal ion

Supplementary Table II. Continued.

Fold change	Common	GenBank	Product	GO biological process
-2.45	EDG6	BC014970	Endothelial differentiation, G protein coupled receptor 6 precursor	Immune response
-2.47	MGC33486	NM_153266	Hypothetical protein MGC33486	Unknown function
-2.48	MGC4645	NM_024515	Hypothetical protein MGC4645	Retired
-2.51	ZMYND15	NM_032265	Zinc finger, MYND domain containing 15	Unknown function
-2.53	SLA	U44403	Src-like-adaptor	Signal transduction
-2.62	MGC10986	AK092301	Hypothetical protein MGC10986	Electron transport
-2.62	HEM1	NM_005337	Hematopoietic protein 1	Hematopoietic differentiation
-2.73	SRRM2	AB016092	Splicing coactivator subunit SRm300	mRNA splicing
-2.73	FLJ12587	AK092231	Hypothetical protein FLJ12587	Unknown function
-2.75	HIST1H3F	NM_021018	H3 histone family, member I	Chromosome organization
-2.75	SPATA11	BC004393	Spermatogenesis associated 11	Unknown function
-2.78	C7orf10	NM_024728	Chromosome 7 open reading frame 10	Unknown function
-2.79	THY1	AK057865	Thy-1 cell surface antigen	Immune response
-2.81	ATSV	NM_138483	Axonal transport of synaptic vesicles	Unknown function
-2.82	CSPG2	NM_004385	Chondroitin sulfate proteoglycan 2 (versican)	Cell adhesion
-2.88	FLJ11017	BC037906	Hypothetical protein FLJ11017	Unknown function
-2.89	HCST	AL050163	DNAX-activation protein 10	Immune response
-2.89	DPF3	AK024141	Cer-d4 (mouse) homolog	Regulation of transcription
-2.93	LIMR	AF260728	Lipocalin-interacting membrane receptor	Inflammatory response
-2.94	HOXB8	NM_024016	Homeo box B8	Regulation of transcription
-3.01	LOC150236			Unknown function
-3.03	ZNF358	NM_018083	Zinc finger protein 358	Regulation of transcription
-3.04	QSCN6	NM_152662	Hypothetical protein FLJ23867	Unknown function
-3.07	EVX1	NM_001989	Even-skipped homeo box 1	Regulation of transcription
-3.09	KIAA0469	NM_014851	KIAA0469 gene product	Unknown function
-3.10	TP53TG3	NM_015369	TP53TG3 protein; TP53TG3a protein	p53 target gene
-3.12	NTNG2	NM_032536	Netrin G2	Signal transduction
-3.12	TRAPPC3	BC007662	BET3 homolog	Transport
-3.13	ADAMTS7	AF140675	A disintegrin and metalloprotease with thrombospondin motifs-7 preproprotein	Cell invasion, metastasis
-3.13	TNFRSF4	X75962	OX40 homologue	Immune response
-3.14		Z79611		Proteolysis and peptidolysis
-3.21	GALGT	M83651	UDP-N-acetyl-alpha-D-galactosamine:(N-acetylneuraminyl)-galactosylglucosylceramide N-acetylgalactosaminyltransferase	Metabolism
-3.26	SKIV2L	U09877	Superkiller viralicidic activity 2-like homolog	Regulation of translation
-3.28	POU2F2	M36653		Regulation of transcription
-3.30	HOXA3	NM_030661	Homeobox A3 protein isoform a; homeobox A3 protein isoform b	Regulation of transcription
-3.38	FOXA3	BC016024	Forkhead box A3	Regulation of transcription
-3.39	MEF2C	NM_002397	MADS box transcription enhancer factor 2, polypeptide C (myocyte enhancer factor 2C)	Development
-3.39	GMFG	BC032819	Glia maturation factor, gamma	Protein phosphorylation
-3.41	NOVA2	NM_002516	Neuro-oncological ventral antigen 2	Oncogene
-3.53	LOC90353	NM_145232	LOC90353	Electron transport
-3.58	STK17A	BC047696	Serine/threonine kinase 17a (apoptosis-inducing)	Apoptosis
-3.61	MYOD1	NM_002478	Myogenic factor 3	Development
-3.68	CPNE7	AJ133798	Copine 7 isoform b; copine 7 isoform a	Metabolism
-3.69	C14orf169	NM_024644	Chromosome 14 open reading frame 169	Unknown function
-3.72	GRIN1	NM_007327	NMDA receptor 1 isoform NR1-1 precursor; NMDA receptor 1 isoform NR1-3 precursor; NMDA receptor 1 isoform NR1-2 precursor	Signal transduction
-3.73	GRIN1	NM_021569	NMDA receptor 1 isoform NR1-1 precursor; NMDA receptor 1 isoform NR1-3 precursor; NMDA receptor 1 isoform NR1-2 precursor	Signal transduction

Supplementary Table II. Continued.

Fold change	Common	GenBank	Product	GO biological process
-3.80	H2AFB	AF254576	H2A-Bbd	Chromosome organization
-3.89	GPR7	U22491	G protein-coupled receptor 7	Signal transduction
-3.91	HCN2	NM_001194	Hyperpolarization activated cyclic nucleotide-gated potassium channel 2	Ion transport
-3.98	SRP	NM_033199	Stresscopin-related peptide	Response to stress
-4.03	FOXC2	NM_005251	Forkhead box C2	Development
-4.10	FLJ14464	NM_032789	Hypothetical protein FLJ14464	Unknown function
-4.13	FXR2	BC020090	Fragile X mental retardation syndrome related protein 2	Regulation of transcription
-4.13	GALR3	AF073799	Galanin receptor 3	Signal transduction
-4.15	FLJ36874	NM_152716	Hypothetical protein FLJ36874	Unknown function
-4.17	HEYL	BC006087	Hairy/enhancer-of-split related with YRPW motif-like	Regulation of transcription
-4.26	ACP5	BC025414	Tartrate resistant acid phosphatase 5 precursor	Protein dephosphorylation
-4.27	UPK3B	NM_030570	Uroplakin 3B isoform a; uroplakin 3B isoform c; uroplakin 3B isoform b	Metastasis
-4.27	SOLH	NM_005632	Small optic lobes homolog	Proteolysis and peptidolysis
-4.27	FLJ90805	AK075286	Hypothetical protein FLJ90805	Unknown function
-4.29				Unknown function
-4.31	RBP4	BC020633	Retinol-binding protein 4, plasma precursor	Metabolism
-4.37	BAG5	AF095195	BCL2-associated athanogene 5	Apoptosis
-4.37	IGHM	L01278		Immune response
-4.55	GLTSCR1	NM_015711	Glioma tumor suppressor candidate region gene 1	Tumor supressor
-4.59	PADI4	AB017919	Peptidyl arginine deiminase, type IV	Protein modification
-4.61	CXCL14	BC003513	Small inducible cytokine B14 precursor	Inflammatory response
-4.74	CAM-KIIN	NM_033259	CaM-KII inhibitory protein	Cell growth
-4.78	C15orf16	NM_130901	Chromosome 15 open reading frame 16	Unknown function
-4.81	CACNG6	AF361352	Voltage-dependent calcium channel gamma-6 subunit isoform c; voltage-dependent calcium channel gamma-6 subunit isoform a; voltage-dependent calcium channel gamma-6 subunit isoform b	Ion transport
-4.83	EVX1	X60655	Even-skipped homeo box 1	Regulation of transcription
-4.93	DUSP9	Y08302	Dual specificity phosphatase 9	Protein dephosphorilation
-5.03	MGC27165	S55736		Unknown function
-5.03	FLJ22184	NM_025094		Unknown function
-5.08	C21orf56	AL136871	Chromosome 21 open reading frame 56	Unknown function
-5.18	COL3A1	X14420	Alpha 1 type III collagen	Cell adhesion
-5.21	PTP4A3	BC003105	Protein tyrosine phosphatase type IVA, member 3 isoform 2; protein tyrosine phosphatase type IVA, member 3 isoform 1	Protein dephosphorylation
-5.26	FLJ22795	AF316855	Hypothetical protein FLJ22795	Unknown function
-5.38	DOK4	BC003541	Downstream of tyrosine kinase 4	Signal transduction
-5.46				Retired
-5.52	CASKIN1	AF451977	CASK interacting protein 1	Chromosome organization
-5.56				Unknown function
-5.62	GPR14; UTR; UTR2	AF140631	G protein-coupled receptor 14	Signal transduction
-5.62	LOC255783	NM_178511	Hypothetical protein LOC255783	Unknown function
-5.62	MECT1	NM_025021	Mucoepidermoid carcinoma translocated 1	Unknown function
-5.65	CREBL1	NM_004381	cAMP responsive element binding protein-like 1	Regulation of transcription
-5.78	LRP6	NM_002336	Low density lipoprotein receptor-related protein 6	Metabolism
-5.81	CBX6	BC012111	Chromobox homolog 6	Chromosome organization
-5.99	FOXD3; HFH2; Genesis	AF197560	Forkhead box D3	Development
-6.21	DUX4	NM_033178	Double homeobox, 4	Development
-6.67	LOC90313	AK075341	Hypothetical protein BC004507	Unknown function
-7.14	SYMPK	BC006536	Symplekin	mRNA processing
-7.63	WNT10A	AK024363	Wingless-type MMTV integration site family, member 10A precursor	Development