Gene expression patterns of chemoresistant and chemosensitive serous epithelial ovarian tumors with possible predictive value in response to initial chemotherapy

DIMCHO BACHVAROV[1,4], SYLVAIN L'ESPERANCE[1,4], ION POPA[3,4], MAGDALENA BACHVAROVA[4], MARIE PLANTE[2,4] and BERNARD TÊTU[3,4]

Departments of [1]Medicine and [2]Obstetrics and Gynecology, [3]Division of Pathology, Faculty of Medicine, Laval University, Québec G1K 7P4; [4]Cancer Research Centre, Hôpital L'Hotel-Dieu de Québec, Centre Hospitalier Universitaire de Québec, 9 rue McMahon, Québec G1R 2J6, Canada

Received April 26, 2006; Accepted June 29, 2006

Abstract. Chemotherapy (CT) resistance in ovarian cancer is broad and encompasses diverse, unrelated drugs, suggesting more than one mechanism of resistance. We aimed to analyze the gene expression patterns in primary serous epithelial ovarian cancer (EOC) samples displaying different responses to first-line CT in an attempt to identify specific molecular signatures associated with response to CT. Initially, the expression profiles of 15 chemoresistant serous EOC tumors [time to recurrence (TTR) ≤6 months] and 10 chemosensitive serous EOC tumors (TTR ≥30 months) were independently analyzed which allowed the identification of specific sets of differentially expressed genes that might be functionally implicated in the evolution of the chemoresistant or the chemosensitive phenotype. Our data suggest that the intrinsic chemoresistance in serous EOC cells may be attributed to the combined action of different molecular mechanisms and factors linked with drug influx and efflux and cell proliferation, as possible implications of other molecular events including altered metabolism, apoptosis and inflammation cannot be excluded. Next, gene expression comparison using hierarchical clustering clearly distinguished chemosensitive and chemoresistant tumors from the 25 serous EOC samples (training set), and consecutive class prediction analysis was used to develop a 43-gene classifier that was further validated in an independent cohort of 15 serous EOC patients and 2 patients with other ovarian cancer histotypes (test set). The 43-gene predictor set properly classified serous EOC patients at high risk for early (≤22 months) versus late (>22 months) relapse after initial CT. Thus, gene expression array technology can effectively classify serous EOC tumors according to CT response. The proposed 43-gene model needs further validation.

Correspondence to: Dr Dimcho Bachvarov, CHUQ-Cancer Research Centre, Hôpital Hôtel-Dieu de Québec, 9 rue McMahon, Québec G1R 2J6, Canada
E-mail: dimtcho.batchvarov@crhdq.ulaval.ca

Key words: serous epithelial ovarian cancer, response to chemotherapy, gene expression, DNA microarray, gene predictor set

Introduction

Ovarian cancer is responsible for more cancer deaths among women in the Western world than all other gynecologic malignancies (1). If not detected early, this disease has a 5-year survival rate of <20%. Epithelial carcinoma of the ovary is characterized by presentation at an advanced stage, spreads primarily by an intraperitoneal route, and relative sensitivity to CT. An initial surgical approach is essential for aggressive cytoreduction and proper staging of the disease process, which in turn improves response to CT and survival (2). CT has had an increasingly important role in the effective treatment of ovarian cancer. Combination CT with paclitaxel (taxol) plus a platinum compound (carboplatin or cisplatin) is the current regimen of choice for the treatment of advanced EOC (3). A number of clinical issues, however, are unresolved including drug dosage and schedule, duration of treatment, and route of administration (4). Indeed, although significant proportions of women respond to CT, the majority of responders (~60-75%) eventually relapse and die from recurrent disease (5-8). CT resistance in ovarian cancer is broad and encompasses diverse, unrelated drugs, suggesting more than one mechanism of resistance. A number of cellular factors have displayed increased expression and activity in drug-resistant ovarian cancer lines and/or tumor tissues (9-18), but consecutive *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 (19-21).

To avoid unnecessarily subjecting a patient to the side effects of anticancer drugs, it is a matter of urgency to understand the molecular mechanisms of drug resistance and to establish a diagnostic method to determine sensitivity to CT in patients with advanced ovarian cancer. Recent advances in expression genomics through global transcript analysis have led to the molecular classification of cancers (22-25) and the prediction of outcome and treatment response (26-29).

In the present study, gene expression patterns were analyzed in primary tumor samples obtained upon surgery from patients with advanced serous EOC in an attempt to identify specific molecular signatures of serous EOC tumors according to their response to initial CT. The initial group comprised 15 resistant tumors from patients with progressive disease under CT or displaying recurrence in the first 6 months, and 10 sensitive tumors from patients with a TTR of at least 30 months. These conservative clinical criteria for defining first-line platinum sensitivity and resistance were employed to exclude tumors with intermediate levels of resistance. The serous EOC histotype was chosen because serous adenocarcinomas represent the most frequent type (~80%) of EOC (30,31). Moreover, we wanted to use a more homogeneous set of tumor samples for our gene expression analyses, since different ovarian cancer histotypes display distinct expression patterns (32). First, the expression profiles of all 25 serous EOC tumors were independently analyzed which allowed the identification of specific sets of differentially expressed genes that might be functionally implicated in the evolution of the chemoresistant or the chemosensitive phenotype. Next, by performing gene expression comparison of the 25 primary chemosensitive and chemoresistant serous EOC tumors we were able to clearly distinguish chemosensitive and chemoresistant tumors from the 25 serous EOC samples by hierarchical clustering, and to consecutively identify a 43-gene classifier that was further tested in an independent cohort comprising 15 serous EOC tumors, one clear cell carcinoma and one endometrioid carcinoma. Our results provide the basis for extended study to further refine our predictor gene set which could help to overcome drug resistance and ameliorate ovarian cancer treatment.

Materials and methods

Patients and tissue specimens. Primary cancer tissues were obtained for expression profiling analysis prior to initial CT from 40 patients with invasive serous papillary adenocarcinoma of the ovary (FIGO stage IIIC and IV, grade 2 and 3) at the Hotel-Dieu de Quebec Hospital, Quebec, Canada. The patients received CT following debulking surgery between 1998 and 2003. All tumors were histologically classified according to the criteria defined by the World Health Organization. The CT treatment was completed for all patients and the response to treatment was evaluated following the RECIST group guidelines (33). Time to recurrence (TTR) was added as a supplementary variable, defined as the time interval between the last cycle of the initial CT regimen and recurrence.

The 25 serous EOC patients initially included in the study were formally divided into two groups based on response to CT. The sensitive group (1S-10S) showed a TTR of at least 30 months, while in the resistant group the disease progressed under CT or during the first 6 months following CT (TTR ≤6 months) (Table IA). These 25 patients were further used for predictor marker discovery (training set), and an additional 15 serous EOC patients were analyzed as an independent validation set (test set). Two patients with different histological subtypes of ovarian cancer (one patient with clear cell carcinoma and one patient with endometrioid carcinoma) were also included in the test set (Table IB).

Tumor tissue from all patients was snap-frozen in liquid nitrogen within 1 h after surgery. A control section was cut from the bloc and stained with H&E, in order to assess the percentage of tumor tissue; only samples with >70% of tumor cells were selected. The study was approved by the Clinical Research Ethics Committee of the Hotel-Dieu de Quebec Hospital and all patients gave informed consent for voluntary participation.

Gene expression analysis. Gene expression analysis was carried out as previously described (34). Briefly, fluorescently labeled cRNA targets were generated from 0.5 μg of total RNA in each reaction using a fluorescent linear amplification kit (Agilent) and 10.0 mM Cyanine 3- or 5-labeled CTP (Perkin-Elmer, Boston, MA), following the 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. One microgram of cyanine-labeled cRNA from one ovarian tumor was mixed with the same amount of reverse-color cyanine-labeled cRNA from a pool, which contained equal amounts of each RNA from the 25 serous EOC patients included in the training set. Array hybridization, washing, scanning and data extraction were performed as previously described (34). GeneSpring software (Agilent) was used to generate lists of selected genes and for different statistical and visualization methods, as described previously (34). Additionally, class prediction analysis was performed to predict the value, or 'class', of an individual parameter in an uncharacteristic sample or set of samples. Classification was generated by the 'Support Vector Machines' algorithm of GeneSpring using the training set and the test set for the parameter 'Response to initial CT'. Fisher's exact test method was used to select the predictor set of genes.

Semi-quantitative RT-PCR (sqRT-PCR). Validation of microarray data was performed for selected differentially expressed genes by sqRT-PCR as previously described (34). Upon analysis of the microarray data, we found that the tumor-differentially expressed 1 (TDE1) gene 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).

Results

Gene expression signatures of primary chemoresistant and chemosensitive tumors. The clinicopathological characteristics of the patients included in this investigation are presented in Table I. In our initial study, we included tumors from 25 serous EOC patients (comprising our training set; Table IA); the tumors were either grade 2 or 3, stage IIIC and IV according to FIGO (International Federation of Obstetrics and Gynecology). All 25 patients had maximal debulking surgery, with residual disease of no more than 2 cm. In the good responders (chemosensitive) group (1S to 10S), 3 patients had a minor histology component of endometrioid, clear cell and squamous histotype respectively. In this group were

Table I. Patients' characteristics and class prediction data.

A, Clinicopathologic characteristics and class prediction analysis of the ovarian cancer patients from the training set

Chemosensitive to initial treatment patients								
No.	Patient code	Age	TTR (months)	Histological type	Stage	Grade	Adjuvant CT	Prediction[a]
1S	O-158	64	≥60	sEOC	IV	2	Carbo-Txl	S
2S	O-165	66	≥60	sEOC + EC	IIIC	2	Carbo-Txl	S
3S	O-169	68	≥60	sEOC	IIIC	3	Carbo-Txl	S
4S	O-217	55	45	sEOC	IV	2	Cis-Txl	S
5S	O-269	72	≥39	sEOC	IIIC	3	Carbo-Txl	S
6S	O-301	56	≥36	sEOC	IIIC	3	Carbo-Txl	S
7S	O-315	55	≥31	sEOC	IIIC	3	Carbo-Txl	S
8S	O-324	54	≥32	sEOC	IIIC	3	Carbo-Txl	S
9S	O-382	77	30	sEOC + SCC	IIIC	3	Carbo-Txl	S
10S	O-400	53	≥31	sEOC + CCC	IIIC	2	Carbo-Txl	S
Chemoresistant to initial treatment patients								
No.	Patient code	Age	TTR (months)	Histological type	Stage	Grade	Adjuvant CT	Prediction
1R	O-137	58	6	sEOC	IIIC	2	Carbo-Txl	R
2R	O-259	52	4	sEOC	IIIC	2	Carbo-Txl	R
3R	O-462	51	3	sEOC	IV	3	Carbo-Txl	R
4R	O-536	45	2	sEOC	IIIC	3	Carbo-Txl	R
5R	O-542	67	4	sEOC	IIIC	2	Carbo-Txl	R
6R	O-132	83	0	sEOC	IIIC	3	Carbo-Cyclph	R
7R	O-154	79	0	sEOC	IIIC	2	Carbo-Cyclph	R
8R	O-221	83	0	sEOC	IIIC	3	Carbo-Cyclph	R
9R	O-226	68	0	sEOC	IIIC	3	Carbo-Txl	R
10R	O-456	54	0	sEOC	IIIC	3	Carbo-Cyclph	R
11R	O-255	74	0	sEOC	IV	3	Carbo-Txl	R
12R	O-347	55	0	sEOC	IIIC	2	Carbo-Txl	R
13R	O-454	63	0	sEOC	IIIC	3	Carbo-Txl	R
14R	O-543	64	0	sEOC	IIIC	3	Carbo-Txl	R
15R	O-123	78	0	sEOC	IIIC	3	Carbo-Cyclph	R

B, Clinicopathologic characteristics and class prediction analysis of the ovarian cancer patients from the test set

No.	Patient code	Age	TTR (months)	Histological type	Stage	Grade	Adjuvant CT	Prediction
1A	O-527	72	0	sEOC	IIIC	2	Carbo-Txl	R
2A	O-489	86	2	sEOC	IIIB	3	Carbo-Txl	R
3A	O-487	67	3	sEOC	IV	2	Carbo-Txl	R
4A	O-332	68	4	sEOC	IV	3	Carbo-Txl	R
5A	O-130	70	8	sEOC	IIIC	2	Carbo-Txl	R
6A	O-337	53	10	sEOC	IIIC	3	Carbo-Txl	R
7A	O-73	69	14	sEOC	IIIC	3	Carbo-Txl	R
8A	O-358	62	15	sEOC	IIIC	3	Carbo-Txl	R
9A	O-122	58	18	sEOC	IIIC	3	Carbo-Txl	R
10A	O-320	68	20	sEOC	IIIC	2	Carbo-Cyclph	R
11A	O-44	44	21	sEOC	IV	3	Cis-Txl	S
12A	O-150	46	21	sEOC	IV	3	Cis-Txl	R
13A	O-127	70	22	sEOC	IIIC	3	Carbo-Txl	R
14A	O-309	60	≥30	sEOC	IIIC	3	Carbo-Txl	S
15A	O-104	66	≥50	sEOC	IIIC	2	Cis-Txl	S
16A	O-503	56	0	CCC	IIIC	X	Carbo-Txl	R
17A	O-411	54	12	EC	IIA	3	Carbo-Txl	R

sEOC, serous epithelial ovarian cancer; CCC, clear cell carcinoma; SCC, squamous cell carcinoma; EC, endometrioid carcinoma; Cis, cisplatin; Carbo, carboplatin; Txl, taxol; Cyclph, cyclophosphamide; CT, chemotherapy; TTR, time to recurrence. [a]Class prediction values are indicated for each patient following cross-validation of the training set and consecutive analysis of the test set using our 43-gene predictor model: S, chemosensitive; R, chemoresistant.

Table II. List of selected differentially expressed genes (2-fold; p=0.001) in chemoresistant or chemosensitive serous EOC tumors.
A, Selected down-regulated genes in chemoresistant serous EOC tumors

Gene symbol	Function
CDCA1, FGF18, TOPK, PPIA, FGFR3, MAD2L1, EDN3, REG1B, MIA, AKR1C3, TM4SF8, HEC, CXCL10, CKS1B, ANLN, NPM1	Cell proliferation and cell cycle control
LAMA3), LAMB3, LAMC2, PCDHB2 PCDHB5, CDH16, CDH 19, REG1B	Cell adhesion
SLC3A1, SLC35F3, KCNJ16, S100A2, SLC2A1, CACNA2D3, SLC26A9, FOLR1, SLC9A9, SLC25A4	Membrane transport
HPGD, LRIG1, NME1, NME2	Tumor (including ovarian tumor) suppression
UNG2, RFC5	DNA repair
FGFR3, PDCD5, TGM1	Apoptosis
SCGB2A1, SCGB2A2, SCGB1D1, HSPA4	Antagonists of the neoplastic phenotype
TNNI3	Angiogenesis inhibitor
AMY2B, CTSL2, TACSTD1, VIL1	Tumor marker
MMP1, TFPI2 (inhibitor of cell invasiveness)	Cancer invasion

B, Selected up-regulated genes in chemoresistant serous EOC tumors

Gene symbol	Function
ECGF1, APO E, IGF2R, CCL2, ARHGAP18, RHPN1, HDAC7A, laminin α5	Ovarian malignancy and tumor progression
NECL1, MAP3K11	Cell migration and invasion
LITAF, STAB1, CCL2	Inflammation
ABCA7, GCLC	Chemoresistance

C, Selected down-regulated genes in chemosensitive serous EOC tumors

Gene symbol	Function
HOXB13, CXCL2, CCK, FGFRL1, MMP19, VIP, gastrin, ITGBL1, MAGE-C1, MLC1, TKTL1, SELE, PPP2R1A, IL-6, IDAX, ACE, CNR1, STX11, ARRB1, MMEL2, AREG	Tumor invasion and progression
Hgß, Hgγ-G, SAA1, SAA2, CD36, ITGBL1, PLAT, KLK10, S100A1, HPSE2, ATF3, HYAL1, LOC63928, DIS3, DPEP1	Ovarian and other tumor markers
FABP4, LPL, CD36, PAFAH2, APO C-I, APO L-4, ADN, SLC27A1	Lipid metabolism and transport
SAA1, CXCL2, IL1F8, MASP2, CRLF2	Inflammation
MYCN, NPM2, RAS-D2, RAD9A	Oncogenes

10 patients with a TTR interval of ≥30 months. The poor responders (chemoresistant) group (1R to 15R) comprised 15 patients with progressive disease under CT (in this case we assigned a TTR value of 0 months) or in the first 6 months following CT. Twenty patients received 6 cycles of combined CT based on a platinum compound (cisplatin or carboplatin) and taxol, and 5 patients received a platinum compound and cyclophosphamide. The median age for the chemoresistant group was 62 (range, 45-83), and the median age for the chemosensitive group was 57 (range, 53-77).

A reference RNA pool was made by mixing equal amounts of total RNA from all 25 serous EOC samples from the training set and the gene expression pattern of each tumor sample was compared to the pooled sample. Two hybridizations were carried out for every tumor against the reference sample using a fluorescent dye reversal (dye-swap) technique.

First, we separately evaluated the gene expression profiles of the chemoresistant and the chemosensitive serous EOC tumors in search for specific markers and/or molecular mechanisms that could determine the chemoresistant or the

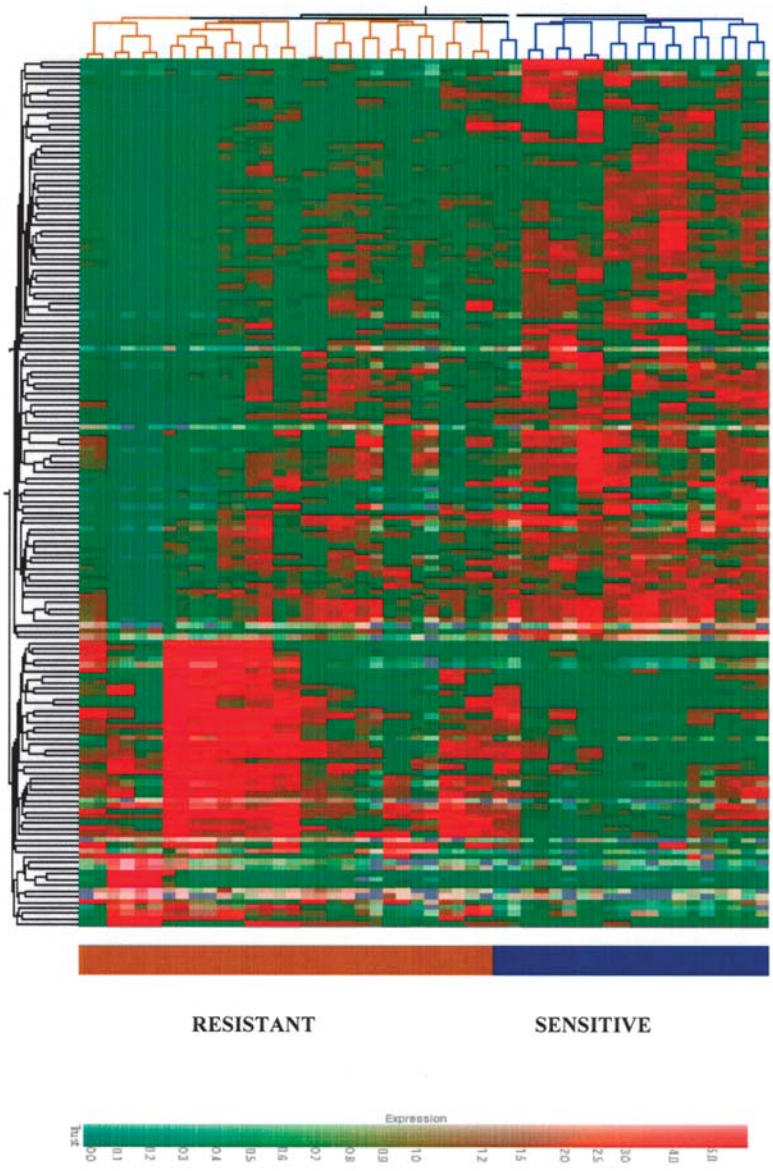

Figure 1. Hierarchical clustering of 25 tumors in duplicate included in the training set based on the 155-genes list (p-value cutoff of 0.01) that discriminates serous EOC tumors according to their response to CT. The mean appears grey, whereas red signifies up-regulation, and green signifies down-regulation (see legend bar). Sensitive tumors are indicated in blue; resistant tumors are indicated in brown.

chemosensitive phenotype. For each of the two groups, a subset of differentially expressed genes was selected by initial filtering on confidence at p=0.001, followed by filtering on expression level (≥2-fold). Using these stringent selection criteria, we found 230 genes to be down-regulated and 60 genes to be up-regulated specifically in the chemoresistant group, while 163 genes were down-regulated and 20 genes were up-regulated specifically in the chemosensitive group. Notably, those down-regulated in the chemoresistant serous EOC tumors included a substantial number of genes implicated in cellular proliferation and cell cycle control, cell adhesion, membrane transport, as well as some genes involved in tumor (including ovarian tumor) suppression, DNA repair, apoptosis, and representing antagonists of the neoplastic phenotype (Table IIA). Several markers, known to be associated with ovarian malignancy and tumor progression, as well as genes implicated in cell migration and invasion, inflammation and chemoresistance were overexpressed in the chemoresistant samples (Table IIB). Down-regulated genes in the chemosensitive serous EOC tumors included numerous genes known to enhance tumor (including ovarian tumor) progression and invasion, ovarian and other tumor markers, genes involved in lipid metabolism and transport, inflammation, as well as several oncogenes (Table IIC).

Gene expression differences between primary chemoresistant and chemosensitive tumors. Next, we evaluated whether intrinsically chemoresistant and chemosensitive serous EOC tumors could be distinguished based on their gene expression profiles. We compared the expression data in all 25 tumors from the training set in search of discriminatory genes. 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 377 genes. One-way ANOVA parametric test (Welch's t-test; variances not assumed equal)

Table III. Predictor set of 43 genes differentially expressed between ovarian cancer patients who displayed sensitivity (TTR >22 months) or resistance (TTR ≤22 months) to first-line chemotherapy.

Prognostic strength[a]	Gene symbol	Gene name	Functions[b]	Elevated levels in
18.79	MPZL1	Myelin protein zero-like 1	Signal transduction	Sensitive
18.27	TOPK	T-LAK cell-originated protein kinase	Kinase activity; increased expression in highly malignant cells (37)	Sensitive
17.79	LSM7	LSM7 homolog, U6 small nuclear RNA	mRNA processing	Sensitive
17.79	WDR12	WD repeat domain 12	Cell proliferation	Sensitive
17.00	PTGDS	Prostaglandin D2 synthase	Inflammation; role in ovarian cancer progression (38)	Resistant
17.00	PSMD14	Proteasome 26S subunit, non-ATPase, 14	Protease activity; confers chemoresistance to tumor cells (39)	Sensitive
16.53	SNRPC	Small nuclear ribonucleoprotein C	mRNA splicing	Sensitive
16.53	LDHB	Lactate dehydrogenase B	Ovarian tumor marker (40)	Sensitive
16.53	NOP5/58	Nucleolar protein NOP5/NOP58	Chaperone activity	Sensitive
16.17	LRRC59	Leucine rich repeat containing 59	Cell organization and cytoskeleton	Sensitive
16.12		Cyclophilin-related pseudogene	Unknown function	Sensitive
16.12	MRPS24	Mitochondrial ribosomal protein S24	Protein synthesis	Sensitive
15.95	HCAP-G	Chromosome condensation protein G	Cell cycle control	Sensitive
15.36	PSMD1	Proteasome 26S subunit, non-ATPase, 1	Protease activity; role in apoptosis of leukemia cells (41)	Sensitive
15.34	RSRC1	Arginine/serine-rich coiled-coil 1	Unknown function	Sensitive
15.34	ZNF155	Zinc finger protein 155 (pHZ-96)	Transcription	Sensitive
15.34	CAP2	Adenylate cyclase-assoc. protein, 2	Adenylate cyclase activation	Sensitive
15.18	BOLA3	BolA-like 3 (E. coli)	Cell proliferation and cell cycle regulation	Sensitive
15.18	DAP13	13 kDa differentiation-associated protein	Cell differentiation	Sensitive
15.18	RBBP7	Retinoblastoma binding protein 7	Cell proliferation; important role in physiology and pathology of ovarian tissue (42)	Sensitive
14.89	C6orf129	Chromosome 6 ORF 129	Unknown function	Sensitive
14.89	NDUFB4	NADH dehydrogenase 1 ß4	Electron transport; different NADH dehydrenase subunits are implicated in tumor progression (43-45) and response to treatment (46,47)	Sensitive
14.56	P53CSV	p53-inducible cell-survival factor	Apoptosis	Sensitive
14.27	C12orf11	Chromosome 12 ORF 11	Unknown function	Sensitive
14.27	CDCA2	Cell division cycle associated 2	Cell cycle regulation	Sensitive
14.27	FLJ90586	Hypothetical protein FLJ90586	Unknown function	Sensitive
14.27	Siva	CD27BP (Siva)	Apoptosis; potentiator of cisplatin-based chemotherapy (48)	Sensitive
14.23	MRPS17	Mitochondrial ribosomal protein S17	Protein synthesis	Sensitive
14.23	FLJ31751	Hypothetical protein FLJ31751	Unknown function	Sensitive
14.23	DPP7	Dipeptidylpeptidase 7	Aminopeptidase (hydrolase) activity	Resistant
14.23	COX8	Cytochrome C oxidase VIII	Electron transport	Sensitive
14.23	MRPS9	Mitochondrial ribosomal protein S9	Protein synthesis	Sensitive
14.23	ACADVL	Acyl-Coenzyme A dehydrogenase	Lipid metabolism; altered expression linked to carcinogenesis (49)	Resistant
14.17	CCT6A	Chaperonin containing TCP1, subunit 6A	Radioresistance of cancer cells (50)	Sensitive

Table III. Continued.

Prognostic strength[a]	Gene symbol	Gene name	Functions[b]	Elevated levels in
13.86	ALDH9A1	Aldehyde dehydrogenase 9, member A1	Electron transport and oxidoreductase activity; implicated in ovarian carcinogenesis and chemoresistance (51)	Sensitive
13.78	STC2	Stanniocalcin 2	Signal transduction; associated with tumor ER status in breast cancer cells (52)	Sensitive
13.78	ACAT2	Acetoacetyl Coenzyme A thiolase	Lipid metabolism; biomarker for hepato-cellular carcinoma (53)	Sensitive
13.78	ZNF180	Zinc finger protein 180 (HHZ168)	Transcription	Sensitive
13.72	GPR49	G protein-coupled receptor 49	Signal transduction; involved in the development of hepatocellular carcinomas (54)	Sensitive
13.72	HSPA4	Heat shock 70 kDa protein 4	Stress response; confers resistance to apop-tosis in ovarian cancer cells (55)	Sensitive
13.72	DUSP2	Dual specificity phosphatase 2	Protein dephosphorylation	Resistant
13.23	AD024	AD024 protein	Mitosis	Sensitive
13.23	BRRN1	Barren homolog (*Drosophila*)	Chromosome condensation	Sensitive

[a]As determined by the 'Support Vector Machines' algorithm of the GeneSpring class prediction analysis. [b]Some references indicate functions relevant to cancer biology and response to treatment.

was further used to select discriminatory genes. t-test with p-value cutoff of 0.01 selected 155 genes for which expression differed in primary sensitive and resistant tumors. Clustering analysis based on the 155-genes list was performed using the standard Condition Tree algorithm provided in GeneSpring and revealed the formation of two major cluster groups that perfectly correspond with response to initial CT treatment (Fig. 1). The 155-genes list is presented in Supplementary Table I.

The 155 genes differently expressed at p-value cutoff of 0.01 were up-regulated or down-regulated at least 2-fold in chemoresistant tumors in comparison with chemosensitive tumors. Functional classes of these differently expressed genes mainly include metabolism (30%), cell growth and maintenance (18%), signal transduction (12%), immune response (12%), cell organization and biogenesis (11%), transport (9%) and apoptosis (3%); the remainder (5%) have unknown functions.

Fifty-three genes from the 155-genes list were up-regulated in chemoresistant tumors. Major classifications of these genes include signal transduction, metabolism, cell growth and maintenance and immune response. Notably, ~22% of all up-regulated genes in resistant tumors are associated with inflammatory and immune responses (including chemokine C-C and C-X-C motif ligands, several serum amyloid A family members and prostaglandin D2 synthase). Genes down-regulated in resistant tumors (102 genes) are mainly involved in metabolism, cell growth and maintenance, cell organization and biogenesis.

Cross-validation of the training set. Next, we cross-validated the 25 tumor samples of the training set in an attempt to identify the minimal list of genes that can correctly classify the tumor samples from this set as chemoresistant or chemo-sensitive. Classification was generated using the 'Support Vector Machines' algorithm of the class prediction analysis of GeneSpring. The 25 serous EOC tumor samples were cross-validated for the parameter 'Response to initial CT'. We began validation analysis using an expanded gene list of all 1.4-fold filtered (2,514) genes to avoid selection bias (35,36). Fisher's exact test method was used to select the minimal gene number with perfect class prediction score. Using this analysis we identified a 43-gene predictor gene set (Table III) that correctly classified all primary sensitive and resistant tumors included in the training set (Table IA).

Confirmation of the expression measurements. To confirm measurement of RNA concentrations, expression values derived from adjusted Agilent data were correlated with values from sqRT-PCR for 21 variably expressed genes (Table IV). Validation of differential gene expression was performed for selected genes from the 43-gene predictor set (ACAT2, ALDH9A1, PTGDS, LDHB, RBBP7, TOPK), as well as from a number of genes that were down-regulated (GSTA1, HSPE1, NDUFB3, SCGB2A1, CTSL2, ASNS, PRSS2, BMO39 SDHC) or up-regulated (ECGF1, APOE, CD36, FOSB) in the resistant serous EOC tumors, as well as 2 genes (HBB, SAA1) that were down-regulated in the sensitive serous EOC group. Mean expression values were positive for all 21 genes and significantly positive ($p \leq 0.05$) for 13 of 21 genes.

Validation in an independent cohort (test set). Consecutively, 15 serous EOC patients that exhibited more diverse TTR values ranging between 0 and ≥50 months were used as a test set for the validation of the predictive limits of the 43-gene

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

Gene name	GeneBank	Mean value chemoresistant[a]	Mean value chemosensitive	p-value
ACAT2	AF356877	1,30816668	1,30290174	0.8345
ALDH9A1	NM_000696	0,94065391	1,45774098	0.0445
ASNS	BC014621	1,90588828	0,67766433	0.0060
CD36	M98398	3,56331854	0,5092241	0.0140
FOSB	NM_006732	0,33831546	0,10256704	0.4657
GSTA1	S49975	0,17409568	0,37106005	0.0274
HSPE1	BC030260	0,2688147	0,73120117	0.6399
LDHB	BC015122	1,31411078	1,18463644	0.1551
NDUFB3	BC018183	1,36570113	3,08019435	0.0252
PTGDS	AK075333	0,71857437	0,56139953	0.0206
PRSS2	BC030260	0,57747991	0,47947217	0.1218
RBBP7	NM_002893	1,11056746	1,99232818	0.1007
SDHC	NM_003001	0,84320883	1,972385	0.0031
TOPK	AB027249	0,47959928	0,95206733	0.0073
BM039	NM_018455	0,26673712	0,49436265	0.0092
SCGB2A1	AF071219	2,1962592	3,16022742	0.0361
CTSL2	AB001928	1,29356079	2,20727353	0.1733
ECGF1	M63193	1,47680917	0,55276644	0.0100
APOE	NM_000041	1,57108933	0,78477098	0.0072
HBB	BC007075	2,86510402	1,06310064	0.0416
SAA1	NM_000331	3,84237549	2,54028984	0.1886

[a]Mean value was calculated as the mean expression value for given marker in all resistant or sensitive tumor samples of the training set.

classifier. Fourteen patients had serous EOC, stage IIIC or IV, grade 2 or 3; one patient had serous EOC stage IIIB grade 3. The median age was 66 years (range 44-86). After debulking surgery, the patients received 6 cycles of CT with a platinum compound and taxol (14 cases) or a platinum compound and cyclophosphamide (1 case). In a preliminary attempt to validate our selected predictor gene list in other histotypes of ovarian cancer, we have also included in the test set one patient with clear cell carcinoma stage IIIC and one patient with endometrioid carcinoma stage IIA, grade 3. The clinical characteristics of all 17 patients from the test set are shown in Table IB. The gene expression pattern of each tumor sample of the test set was compared to that of the pooled reference sample used for cross-validation of the training set. All serous EOC patients from the test set displaying TTR values of ≤22 months were classified as resistant to first-line CT treatment with the exception of one serous EOC patient with TTR of 21 months that was classified as sensitive, while the serous EOC patients with TTR ≥30 months were classified as sensitive. The two additional patients with different histological subtypes of ovarian cancer (TTR values of 0 and 12 months, respectively) were correctly classified as resistant with our 43-gene predictor set. All data from the validation analysis of the test set are presented in Table IB.

Discussion

In the present study, we initially analyzed the gene expression profiles in primary tumor samples of 10 serous EOC patients that displayed a good response to CT (TTR ≥30 months) and 15 serous EOC patients with a poor response to CT (TTR ≤6 months, aiming to discover specific factors or mechanisms that determine response to first-line treatment and/or disease progression. The majority of these patients were treated postoperatively with a platinum compound and taxol; five patients were treated with carboplatin and cyclophosphamide (Table IA). Our goal was to determine the profile of two definite groups of tumors with different responses to CT, and to identify those genes that best distinguish the two groups. For this reason, we chose to use a pool of equal amounts of total RNA from each tumor as reference RNA. This has two advantages: firstly, it maximizes differences among tumor samples; and secondly, it ensures accurate quantification of expression levels for genes that are not expressed or are expressed at very low levels in a universal reference sample (56). Moreover, a pooled reference design is preferable when the major goal of a microarray experiment represents clustering and class prediction analysis (57).

Serous EOC displayed distinctive gene expression according to their response to CT. Thus, different genes implicated

in tumor and neoplastic phenotype suppression, cell adhesion and apoptosis were down-regulated in the resistant tumor serous EOC samples (Table IIA). Interestingly, the expression of numerous genes representing positive regulators of cell proliferation was suppressed in the resistant serous EOC tumors (Table IIA), which supports the concept of ours and others that a decreased proliferation state may be involved in the development of acquired chemoresistance (34,58-62). Down-regulated genes in the resistant group also comprised different membrane transporters and channels including amino acid (SLC3A1), nucleoside (SLC25A4) and folate transporters (FOLR1), Ca^{2+} channels and binding proteins (CACNA2D3, S100A2), and different ion transporters and channels (KCNJ16, SLC26A9, SLC9A9). Multiple types of membrane transporters and channels play important roles in sensitivity and resistance to anticancer drugs (63). Thus similar to our findings, the suppression of the S100A2 gene was previously associated with chemoresistance (64). Moreover, it was demonstrated that solute carrier (SLC) transporters, such as folate, nucleoside, and amino acid transporters, commonly increase chemosensitivity by mediating the cellular uptake of hydrophilic drugs, and the expression of different sodium, chloride, potassium, and other ion channels correlated with drug sensitivity and activity (65). Hence, the suppressed expression of the above transporter genes may contribute to the chemoresistant serous EOC phenotype. On the contrary, one member (ABCA7) of the ABC transporter family known to confer drug resistance by enhancing drug efflux (63,65), was up-regulated in the chemoresistant serous EOC tumors. Overexpressed genes in the resistant group also included various genes implicated in ovarian malignancy and tumor progression, cell migration/invasion and inflammation (Table IIB). These data confirm our recent findings that genes functionally involved in mechanisms of chemoresistance and ovarian tumor progression are commonly upregulated in post-CT (chemoresistant) ovarian tumors (34).

The sensitive serous EOC phenotype was mainly characterized by the suppression of different genes known to be functionally involved in disease progression, including genes responsible for tumor invasion, inflammatory mediators, various tumor markers and some oncogenes (Table IIC). The observed down-regulation of several oncogenes (MYCN, NPM2, RAS-D2, RAD9A; Table IIC) may additionally support the sensitive phenotype since elevated transcription of some oncogenes including c-Myc and Ras has been previously linked to multidrug resistance (66-68). In sensitive tumors, we have also monitored the down-regulation of different genes involved in control of lipid metabolism and transport. Indeed, enhanced lipid and protein degradation is a common finding among cancer patients. Alterations in the serum lipolytic activity of cancer patients correlate with response to therapy as patients who showed a positive response to CT also showed a decrease in their plasma levels of lipolytic activity (69). Ovarian cancer patients exhibit altered lipid metabolism and the degree of these alterations has been previously linked with response to therapy, as these metabolic alterations may influence disease outcome (70). Our data suggest that lower lipid metabolism rates might improve treatment response in serous EOC patients.

As a further step in the analysis of our initial data (training) set, we first looked for gene expression patterns that could discriminate good from poor responders upon initial CT of serous EOC patients. Clustering analysis, based on a set of 155 discriminatory genes, accurately distinguished primary serous EOC tumors according to their response to CT (Fig. 1). Fifty-three of these genes were overexpressed in the treatment-resistant cluster with major categories including immune and inflammatory response, regulation of transcription and hemoglobin synthesis. These results are not unexpected since: a) different inflammatory mediators, which play diverse roles such as inducing angiogenesis, invasion, autocrine growth loops and resistance to apoptosis, are shown to be elevated in ovarian carcinoma (71); b) some of the over-expressed transcription regulators (FOSB, FOS, NR4A1, NR4A2 and NR4A3) are shown to be associated with cancer progression and invasion (72,73); c) Hb-α and Hb-ß were recently characterized as ovarian cancer biomarker proteins (74). Of the 102 genes overexpressed in the good response group, major categories were signal transduction, metabolism, regulation of transcription and nucleosome assembly (almost all histone genes); cell cycle regulation, cell adhesion, cyto-skeleton structure, transport and apoptosis. In sensitive tumors, genes involved in cell cycle control (e.g., overexpression of CDCA1, CDCA7, BIRC5, BUB1 and CKS2) and apoptosis (e.g., overexpression of Siva and PDCD5) seem to contribute to sensitivity to CT. Differences in RNA expression were confirmed by sqRT-PCR for a sample of genes.

Next, an additional statistical approach (class prediction analysis) was applied to specifically search for the minimal set of genes that could predict response to first-line CT. This led to the identification of a 43-gene predictive model that correctly classified all 25 tumors based on response to initial CT. Our predictor model was subsequently validated in an independent cohort of 15 serous EOC patients and 2 patients with other ovarian cancer histotypes. The 43-gene model classified most serous EOC tumors with TTR ≤22 months as resistant to first-line CT, with the exception of one serous EOC tumor with TTR of 21 months that was classified as sensitive. The serous EOC tumors with TTR ≥30 months were classified as sensitive. Thus according to our predictive method, the 21- to 22-month TTR interval may represent the turning point for our 43-gene predictor in classifying women with advanced serous EOC destined for early (TTR ≤22 months) or late (TTR >22 months) relapse following CT treatment. Indeed, this transition period coincides with the literature data for median progression-free survival (20-22 months) of ovarian cancer patients with optimally debulked advanced-stage disease treated with platinum-paclitaxel (75,76). Similarly, a 14-gene predictive model for treatment outcome in ovarian cancer was previously developed (75) that could discriminate women at high risk for early (≤21 months) versus late (>21 months) relapse after initial CT. Furthermore, the fact that one clear cell carcinoma patient and one endometrioid carcinoma patient (TTR values of 0 and 12 months respectively) were both correctly classified as resistant with our 43-gene model, as well as the presence in the training set of three patients with mixed histotypes, is indicative for the possible application of our predictor set in other ovarian cancer histotypes.

Our 43-gene classifier list does not include many genes that have been implicated in drug resistance based on studies

in model systems but, rather, identifies expression patterns of genes that could be used as a predictive test for response to CT. In this regard, the present results additionally parallel those of Hartmann *et al*, who identified a gene expression profile in ovarian cancer patients that correlated with drug sensitivity and treatment outcome but also contained a paucity of transcripts commonly implicated in resistance (77). It is provocative to speculate on the biologic function of the 43 genes that form our current predictor. Indeed, many of these genes need not have a proximal role in the biologic function that they predict. They may be robust but distant downstream transcriptional effects of biologic events that influence drug sensitivity. Furthermore, informative gene lists can change substantially as the training set size from which they are generated increases. The rank order of genes is particularly susceptible to change from one list to another. Therefore, from the vantage point of gaining mechanistic insight into the biology of CT sensitivity or resistance, these results should be regarded as hypothesis-generating only. However, it was encouraging to see that different genes in our marker set of 43 have been shown previously to be involved in disease progression (TOPK, PTGDS, LDHB, RBBP7, NDUFB4, ACADVL, ALDH9A1, ACAT2, GPR49), apoptosis (PSMD1, Siva, P53CSV, HSPA4), or response to treatment (PSMD14, NDUFB4, CCT6A, ALDH9A1) in different cancer types including ovarian carcinoma (see Table III for details). It is interesting to note that some of the above listed and several other genes from our predictive gene list are involved in the regulation of cellular proliferation or cell cycle control (TOPK, PTGDS, WDR12, HCAP-G, BOLA3, RBBP7, CDCA2, ADO24, BRRN1) which supports once more the concept that the modulation of the cellular proliferative rate could be determinative for CT response (34,56-60).

Several recent studies have used gene expression profiling to predict disease prognosis and survival (78,79) and response to CT (62,77,80,81) in ovarian cancer patients. These studies have identified different prognostic and predictor gene sets which can distinguish early from late relapse or disease progression; however, no significant overlap was found between the individual predictor lists. Our 43-gene predictor set also does not display any evident similarity with the predictor sets identified so far. These differences could be due to several reasons including various criteria of patient selection and the utilization of different gene expression platforms (nylon cDNA arrays, Affymetrix chips, Agilent oligonucleotide microarrays). Additionally, our analysis was based mostly on primary serous EOC tumors, while the other groups have used mixed histotypes of ovarian tumors, including recurrent and metastatic tumors. Although all of these studies, including ours, suggest that gene expression profiling is capable of defining prognosis and response to treatment, additional validation is required to determine the ultimate value of this approach in clinical practice. Also, combining the different predictor gene sets, while posing challenging informatics problems, might be much more informative.

In conlusion, gene expression profiling could discriminate serous EOC tumors according to their response to CT. Our data suggest that the intrinsic chemoresistance in serous EOC cells may be attributed to the combined action of different molecular mechanisms and factors linked with drug influx and efflux and cell proliferation, as the possible implications of other molecular events, including altered metabolism, apoptosis and inflammation, cannot be excluded. We have additionally developed a 43-gene classifier model that predicts early or late relapse to first-line CT in patients with advanced ovarian serous adenocarcinomas. Our data lend support to the suggestion that gene expression array technology can effectively classify serous EOC tumors according to response or resistance. To ultimately define the molecular portrait of ovarian cancers sensitive or resistant to first-line CT, these results should be validated in a study with a large prospective cohort including patients with different histological types of ovarian cancer. Further patient recruitment and analysis will refine the predictor gene list for classifying tumors based on response to initial therapy. This type of molecular profiling could have important clinical implications in resolving chemoresistance and defining the optimum treatment for an individual patient, thus reducing the use of unproductive treatments, unnecessary toxicity, and overall cost.

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 from the Cancer Research Network of the 'Fonds de la Recherche en Santé du Québec', Québec, Canada.

References

1. Wingo PA, Tong T and Bolden S: Cancer statistics. CA Cancer J Clin 45: 8-30, 1995.
2. Ozols RF: Current status of CT for ovarian cancer. Semin Oncol 22: 61-66, 1995.
3. Dunton CJ: New options for the treatment of advanced ovarian cancer. Semin Oncol 24: S5-2-S5-11, 1997.
4. Markman M: Current status and future directions of platinum/paclitaxel-based CT of ovarian cancer. Semin Oncol 24: S11-24-S11-27, 1997.
5. Bookman MA, McGuire WP III, Kilpatrick D, Keenan E, Hogan WM, Johnson SW, O'Dwyer P, Rowinsky E, Gallion HH and Ozols RF: Carboplatin and paclitaxel in ovarian carcinoma: a phase I study of the Gynecologic Oncology Group. J Clin Oncol 14: 1895-1902, 1996.
6. McGuire WP, Hoskins WJ, Brady MF, Kucera PR, Partridge EE, Look KY, Clarke-Pearson DL and Davidson M: Cyclophosphamide and cisplatin compared with paclitaxel and cisplatin in patients with stage III and stage IV ovarian cancer. N Engl J Med 334: 1-6, 1996.
7. Ozols RF: Optimum CT for ovarian cancer. Int J Gynecol Cancer 10: 33-37, 2000.
8. Du Bois A, Luck HJ, Pfisterer J, Schroeder W, Blohmer JU, Kimmig R, Moebus V and Quaas J: Second-line carboplatin and gemcitabine in platinum sensitive ovarian cancer - a dose-finding study by the Arbeitsgemeinschaft Gynakologische Onkologie (AGO) Ovarian Cancer Study Group. Ann Oncol 12: 1115-1120, 2001.
9. Kita T, Kikuchi Y, Hirata J and Nagata I: Prognosis of ovarian cancer today. Cancer J 11: 201-207, 1998.
10. Pegg AE and Byers TL: Repair of DNA containing O6-alkylguanine. FASEB J 6: 2302-2310, 1992.
11. Eastman A and Schulte N: Enhanced DNA repair as a mechanism of resistance to cis-diamminedichloroplatinum(II). Biochemistry 27: 4730-4734, 1998.
12. Yao KS, Godwin AK, Johnson SW, Ozols RF, O'Dwyer PJ and Hamilton TC: Evidence for altered regulation of γ-glutamylcysteine synthetase gene expression among cisplatin-sensitive and cisplatin-resistant human ovarian cancer cell lines. Cancer Res 55: 4367-4374, 1995.

13. Vanderzee AGJ, Vanommen B, Meijer C, Hollema H, Vanbladeren PJ and Devries EGE: Glutathione-S-transferase activity and isoenzyme composition in benign ovarian tumours, untreated malignant ovarian tumours and malignant ovarian tumours after platinum/cyclophosphamide CT. Br J Cancer 66: 930-936, 1992.
14. Shridhar V, Bible KC, Staub J, Avula R, Lee YK, Kalli K, Huang H, Hartmann LC, Kaufmann SH and Smith DI: Loss of expression of a new member of the DNAJ protein family confers resistance to chemotherapeutic agents used in the treatment of ovarian cancer. Cancer Res 61: 4258-4265, 2001.
15. Hengstler JG, Lange J, Kett A, Dornhofer N, Meinert R, Arand M, Knapstein PG, Becker R, Oesch F and Tanner B: Contribution of c-erbB-2 and topoisomerase IIα to chemoresistance in ovarian cancer. Cancer Res 59: 3206-3214, 1999.
16. Liu JR, Fletcher B, Page C, Hu C, Nunez G and Baker V: Bcl-xL is expressed in ovarian carcinoma and modulates CT-induced apoptosis. Gynecol Oncol 70: 398-403, 1998.
17. Sasaki H, Sheng Y, Kotsuji F and Tsang BK: Down-regulation of X-linked inhibitor of apoptosis protein induces apoptosis in chemoresistant human ovarian cancer cells. Cancer Res 60: 5659-5666, 2000.
18. Li J, Feng Q, Kim JM, Schneiderman D, Liston P, Li M, Vanderhyden B, Faught W, Fung MF, Senterman M, Korneluk RG and Tsang BK: Human ovarian cancer and cisplatin resistance: possible role of inhibitor of apoptosis proteins. Endocrinology 142: 370-380, 2001.
19. Germain I, Têtu B, Brisson J, Mondor M and Cherian G: Markers of chemoresistance in ovarian carcinomas; an immunohistochemical study of 86 cases. Int J Gynecol Pathol 15: 54-62, 1996.
20. Eltabbakh GH and Awtrey CS: Current treatment for ovarian cancer. Expert Opin Pharmacother 2: 109-124, 2001.
21. Goff BA, Paley PJ, Greer BE and Gown AM: Evaluation of chemoresistance markers in women with epithelial ovarian carcinoma. Gynecol Oncol 81: 18-24, 2001.
22. Schmidt U and Begley CG: Cancer diagnosis and microarrays. Int J Biochem Cell Biol 35: 119-124, 2003.
23. Liu ET: Classification of cancers by expression profiling. Curr Opin Genet Dev 13: 97-103, 2003.
24. Raetz EA and Moos PJ: Impact of microarray technology in clinical oncology. Cancer Invest 22: 312-320, 2004.
25. Cuperlovic-Culf M, Belacel N and Ouellette RJ: Determination of tumour marker genes from gene expression data. Drug Discov Today 10: 429-437, 2005.
26. Mariadason JM, Augenlicht LH and Arango D: Microarray analysis in the clinical management of cancer. Hematol Oncol Clin North Am 17: 377-387, 2003.
27. Wulfkuhle J, Espina V, Liotta L and Petricoin E: Genomic and proteomic technologies for individualisation and improvement of cancer treatment. Eur J Cancer 40: 2623-2632, 2004.
28. Alaoui-Jamali MA, Dupre I and Qiang H: Prediction of drug sensitivity and drug resistance in cancer by transcriptional and proteomic profiling. Drug Resist Update 7: 245-255, 2004.
29. Wang Y: Gene expression-driven diagnostics and pharmacogenomics in cancer. Curr Opin Mol Ther 7: 246-250, 2005.
30. Auersperg N, Wong AS, Choi KC, Kang SK and Leung PC: Ovarian surface epithelium: biology, endocrinology, and pathology. Endocr Rev 22: 255-288, 2001.
31. Jemal A, Murray T, Ward E, Samuels A, Tiwari RC, Ghafoor A, Feuer EJ and Thun MJ: Cancer statistics, 2005. CA Cancer J Clin 55: 10-30, 2005.
32. Marquez RT, Baggerly KA, Patterson AP, Liu J, Broaddus R, Frumovitz M, Atkinson EN, Smith DI, Hartmann L, Fishman D, Berchuck A, Whitaker R, Gershenson DM, Mills GB, Bast RC Jr and Lu KH: Patterns of gene expression in different histotypes of epithelial ovarian cancer correlate with those in normal fallopian tube, endometrium, and colon. Clin Cancer Res 11: 6116-6126, 2005.
33. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, Verweij J, van Glabbeke M, van Oosterom AT, Christian MC and Gwyther SG: New guidelines to evaluate the response to treatment in solid tumors. J Natl Cancer Inst 92: 205-216, 2000.
34. L'Esperance S, Popa I, Bachvarova M, Plante M, Patten N, Wu L, Têtu B and Bachvarov D: Gene expression profiling of paired tumor samples obtained prior to and following adjuvant chemotherapy: molecular signatures of chemoresistant tumors. Int J Oncol 29: 5-24, 2006.
35. Ambroise C and McLachlan GJ: Selection bias in gene extraction on the basis of microarray gene-expression data. Proc Natl Acad Sci USA 99: 6562-6566, 2002.
36. Simon R, Radmacher MD, Dobbin K and McShane LM: Pitfalls in the use of DNA microarray data for diagnostic and prognostic classification. J Natl Cancer Inst 95: 14-18, 2003.
37. Simons-Evelyn M, Bailey-Dell K, Toretsky JA, Ross DD, Fenton R, Kalvakolanu D and Rapoport AP: PBK/TOPK is a novel mitotic kinase which is upregulated in Burkitt's lymphoma and other highly proliferative malignant cells. Blood Cells Mol Dis 27: 825-829, 2001.
38. Su B, Guan M, Zhao R and Lu Y: Expression of prostaglandin D synthase in ovarian cancer. Clin Chem Lab Med 39: 1198-1203, 2001.
39. Spataro V, Toda T, Craig R, Seeger M, Dubiel W, Harris AL and Norbury C: Resistance to diverse drugs and ultraviolet light conferred by overexpression of a novel human 26 S proteasome subunit. J Biol Chem 272: 30470-30475, 1997.
40. Yuce K, Baykal C, Genc C, Al A and Ayhan A: Diagnostic and prognostic value of serum and peritoneal fluid lactate dehydrogenase in epithelial ovarian cancer. Eur J Gynaecol Oncol 22: 228-232, 2001.
41. Wang H, Liu S, Lu X, Zhao X, Chen S and Li X: Gene expression profile changes in NB4 cells induced by realgar. Chin Med J 116: 1074-1077, 2003.
42. Burns KH, Owens GE, Ogbonna SC, Nilson JH and Matzuk MM: Expression profiling analyses of gonadotropin responses and tumor development in the absence of inhibins. Endocrinology 144: 4492-4507, 2003.
43. Cheng Q, Lau WM, Tay SK, Chew SH, Ho TH and Hui KM: Identification and characterization of genes involved in the carcinogenesis of human squamous cell cervical carcinoma. Int J Cancer 98: 419-426, 2002.
44. Tarantul VZ and Hunsmann G: Mitochondrial polypeptides of the oxidative phosphorylation pathway as potential new targets for anti-cancer therapy. Med Hypotheses 56: 386-387, 2001.
45. Perumal SS, Shanthi P and Sachdanandam P: Therapeutic effect of tamoxifen and energy-modulating vitamins on carbohydrate-metabolizing enzymes in breast cancer. Cancer Chemother Pharmacol 56: 105-114, 2005.
46. Zhu NX, Zheng S, Xu RZ, Gao RL, Shen JP and Yu RX: Identification of multidrug resistance related genes in leukemia by suppression subtractive hybridization. Zhonghua Xue Ye Xue Za Zhi 24: 14-17, 2003.
47. Shen XY, Zacal N, Singh G and Rainbow AJ: Alterations in mitochondrial and apoptosis-regulating gene expression in photodynamic therapy-resistant variants of HT29 colon carcinoma cells. Photochem Photobiol 81: 306-313, 2005.
48. Chu F, Barkinge J, Hawkins S, Gudi R, Salgia R and Kanteti PV: Expression of Siva-1 protein or its putative amphipathic helical region enhances cisplatin-induced apoptosis in breast cancer cells: effect of elevated levels of BCL-2. Cancer Res 65: 5301-5309, 2005.
49. Yeh CS, Wang JY, Cheng TL, Juan CH, Wu CH and Lin SR: Fatty acid metabolism pathway play an important role in carcinogenesis of human colorectal cancers by Microarray-Bioinformatics analysis. Cancer Lett 233: 297-308, 2006.
50. Higo M, Uzawa K, Kouzu Y, Bukawa H, Nimura Y, Seki N and Tanzawa H: Identification of candidate radioresistant genes in human squamous cell carcinoma cells through gene expression analysis using DNA microarrays. Oncol Rep 14: 1293-1298, 2005.
51. Tanner B, Hengstler JG, Dietrich B, Henrich M, Steinberg P, Weikel W, Meinert R, Kaina B, Oesch F and Knapstein PG: Glutathione, glutathione S-transferase alpha and pi, and aldehyde dehydrogenase content in relationship to drug resistance in ovarian cancer. Gynecol Oncol 65: 54-62, 1997.
52. Bouras T, Southey MC, Chang AC, Reddel RR, Willhite D, Glynne R, Henderson MA, Armes JE and Venter DJ: Stanniocalcin 2 is an estrogen-responsive gene coexpressed with the estrogen receptor in human breast cancer. Cancer Res 62: 1289-1295, 2002.
53. Song BL, Wang CH, Yao XM, Yang L, Zhang WJ, Wang ZZ, Yang JB, Qi W, Yang XY, Inoue K, Lin ZX, Zhang HZ, Kodama T, Chang CC, Liu YK, Chang TY and Li BL: Human acyl-CoA: cholesterol acyltransferase 2 gene expression in intestinal Caco-2 cells and in hepatocellular carcinoma. Biochem J 394: 617-626, 2006.

54. Yamamoto Y, Sakamoto M, Fujii G, Tsuiji H, Kenetaka K, Asaka M and Hirohashi S: Overexpression of orphan G-protein-coupled receptor, Gpr49, in human hepatocellular carcinomas with beta-catenin mutations. Hepatology 37: 528-533, 2003.
55. Hu W, Wu W, Verschraegen CF, Chen L, Mao L, Yeung SC, Kudelka AP, Freedman RS and Kavanagh JJ: Proteomic identification of heat shock protein 70 as a candidate target for enhancing apoptosis induced by farnesyl transferase inhibitor. Proteomics 10: 1904-1911, 2003.
56. Cao QJ, Belbin T, Socci N, Balan R, Prystowsky MB, Childs G and Jones JG: Distinctive gene expression profiles by cDNA microarrays in endometrioid and serous carcinomas of the endometrium. Int J Gynecol Pathol 23: 321-329, 2004.
57. Dobbin K and Simon R: Comparison of microarray designs for class comparison and class discovery. Bioinformatics 18: 1438-1445, 2002.
58. Itamochi H, Kigawa J, Sugiyama T, Kikuchi Y, Suzuki M and Terakawa N: Low proliferation activity may be associated with chemoresistance in clear cell carcinoma of the ovary. Obstet Gynecol 100: 281-287, 2002.
59. Muller V, Stahmann N, Riethdorf S, Rau T, Zabel T, Goetz A, Janicke F and Pantel K: Circulating tumor cells in breast cancer: correlation to bone marrow micrometastases, heterogeneous response to systemic therapy and low proliferative activity. Clin Cancer Res 11: 3678-3685, 2005.
60. Linn SC, Pinedo HM, van Ark-Otte J, van der Valk P, Hoekman K, Honkoop AH, Vermorken JB and Giaccone G: Expression of drug resistance proteins in breast cancer, in relation to CT. Int J Cancer 71: 787-795, 1997.
61. Yang KP, Liang YF and Samaan NA: Intrinsic drug resistance in a human medullary thyroid carcinoma cell line: association with overexpression of mdrl gene and low proliferation fraction. Anticancer Res 11: 1065-1068, 1991.
62. Jazaeri AA, Awtrey CS, Chandramouli GV, Chuang YE, Khan J, Sotiriou C, Aprelikova O, Yee CJ, Zorn KK, Birrer MJ, Barrett JC and Boyd J: Gene expression profiles associated with response to CT in epithelial ovarian cancers clinical cancer. Clin Cancer Res 11: 6300-6310, 2005.
63. Huang Y, Anderle P, Bussey KJ, Barbacioru C, Shankavaram U, Dai Z, Reinhold WC, Papp A, Weinstein JN and Sadee W: Membrane transporters and channels: role of the transportome in cancer chemosensitivity and chemoresistance. Cancer Res 64: 4294-4301, 2004.
64. Schmidt WM, Kalipciyan M, Dornstauder E, Rizovski B, Steger GG, Sedivy R, Mueller MW and Mader RM: Dissecting progressive stages of 5-fluorouracil resistance *in vitro* using RNA expression profiling. Int J Cancer 112: 200-212, 2004.
65. Huang Y and Sadee W: Membrane transporters and channels in chemoresistance and -sensitivity of tumor cells. Cancer Lett 239: 336-349, 2006 (Epub 2005 Oct 5).
66. Chin K-V, Ueda K, Pastan I and Gottesman MM: Modulation of activity of the promoter of the human MDR1 gene by Ras and p53. Science 255: 459-562, 1992.
67. Volm M, Kastel M, Mattern J and Efferth T: Expression of resistance factors (P-glycoprotein, glutathione S-transferase-pi, and topoisomerase II) and their interrelationship to proto-oncogene products in renal cell carcinomas. Cancer 71: 3981-3987, 1993.
68. Sabbatini ARM, Basolo F, Valentini P, Mattii L, Calvo S, Fiore L, Ciardiello F and Petrini M: Induction of multidrug resistance (MDR) by transfection of MCF-10A cell line with c-Ha-ras and c-erbB-2 oncogenes. Int J Cancer 59: 208-211, 1994.
69. Beck SA, Groundwater P, Barton C and Tisdale MJ: Alterations in serum lipolytic activity of cancer patients with response to therapy. Br J Cancer 62: 822-825, 1990.
70. Gercel-Taylor C, Doering DL, Kraemer FB and Taylor DD: Aberrations in normal systemic lipid metabolism in ovarian cancer patients. Gynecol Oncol 60: 35-41, 1996.
71. Altinoz MA and Korkmaz R: NF-kappaB, macrophage migration inhibitory factor and cyclooxygenase-inhibitions as likely mechanisms behind the acetaminophen- and NSAID-prevention of the ovarian cancer. Neoplasma 51: 239-247, 2004.
72. Hennigan RF, Hawker KL and Ozanne BW: Fos-transformation activates genes associated with invasion. Oncogene 9: 3591-3600, 1994.
73. Wansa KD and Muscat GE: TRAP220 is modulated by the anti-neoplastic agent 6-Mercaptopurine, and mediates the activation of the NR4A subgroup of nuclear receptors. J Mol Endocrinol 34: 835-848, 2005.
74. Woong-Shick A, Sung-Pil P, Su-Mi B, Joon-Mo L, Sung-Eun N, Gye-Hyun N, Young-Lae C, Ho-Sun C, Heung-Jae J, Chong-Kook K, Young-Wan K, Byoung-Don H and Hyun-Sun J: Identification of hemoglobin-alpha and -beta subunits as potential serum biomarkers for the diagnosis and prognosis of ovarian cancer. Cancer Sci 96: 197-201, 2005.
75. Ozols RF, Bundy BN, Greer BE, Fowler JM, Clarke-Pearson D, Burger RA, Mannel RS, De Geest K, Hartenbach EM and Baergen R: Phase III trial of carboplatin and paclitaxel compared with cisplatin and paclitaxel in patients with optimally resected stage III ovarian cancer: a Gynecologic Oncology Group study. J Clin Oncol 21: 3194-3200, 2003.
76. Markman M, Bundy BN, Alberts DS, Fowler JM, Clark-Pearson DL, Carson LF, Wadler S and Sickel J: Phase III trial of standard-dose intravenous cisplatin plus paclitaxel versus moderately high-dose carboplatin followed by intravenous paclitaxel and intraperitoneal cisplatin in small-volume stage III ovarian carcinoma: an intergroup study of the Gynecologic Oncology Group, Southwestern Oncology Group, and Eastern Cooperative Oncology Group. J Clin Oncol 19: 1001-1007, 2001.
77. Hartmann LC, Lu KH, Linette GP, Cliby WA, Kalli KR, Gershenson D, Bast RC, Stec J, Iartchouk N, Smith DI, Ross JS, Hoersch S, Shridhar V, Lillie J, Kaufmann SH, Clark EA and Damokosh AI: Gene expression profiles predict early relapse in ovarian cancer after platinum-paclitaxel CT. Clin Cancer Res 11: 2149-2155, 2005.
78. Berchuck A, Iversen ES, Lancaster JM, Pittman J, Luo J, Lee P, Murphy S, Dressman HK, Febbo PG, West M, Nevins JR and Marks JR: Patterns of gene expression that characterize long-term survival in advanced stage serous ovarian cancers. Clin Cancer Res 11: 3686-3896, 2005.
79. Spentzos D, Levine DA, Kolia S, Otu H, Boyd J, Libermann TA and Cannistra SA: Unique gene expression profile based on pathologic response in epithelial ovarian cancer. J Clin Oncol 23: 7911-7918, 2005.
80. Spentzos D, Levine DA, Ramoni MF, Joseph M, Gu X, Boyd J, Libermann TA and Cannistra SA: Gene expression signature with independent prognostic significance in epithelial ovarian cancer. J Clin Oncol 22: 4700-4710, 2004.
81. Helleman J, Jansen MP, Span PN, van Staveren IL, Massuger LF, Meijer-van Gelder ME, Sweep FC, Ewing PC, van der Burg ME, Stoter G, Nooter K and Berns EM: Molecular profiling of platinum resistant ovarian cancer. Int J Cancer 118: 1963-1971, 2006.

Supplementary Table I. List of 155 genes used for cluster analysis.

P-value	Common	Genbank	Description
0.00922			Retired, similar to human GSTA2
0.00914	CTSL2	AB001928	Cathepsin L2
0.00904	ATF3	BC006322	Activating transcription factor 3
0.00904	CDT1	NM_030928	DNA replication factor
0.0085	PCDHB2	AK027526	Protocadherin ß 2
0.00845	DPEP1	J05257	Dipeptidase 1 (renal)

Supplementary Table I. Continued.

P-value	Common	Genbank	Description
0.00776	VAMP4	NM_003762	Vesicle-associated membrane protein 4
0.00769	GEM	U10550	GTP binding protein overexpressed in skeletal muscle
0.00683	MGC13024	NM_152288	Hypothetical protein MGC13024
0.00683	ST6GalII	NM_032528	ß-galactoside α-2,6-sialyltransferase II
0.00671	SOX2	NM_003106	SRY (sex determining region Y)-box 2
0.0062	KLF2	AF134053	Kruppel-like factor 2 (lung)
0.00615	GABRE	U66661	γ-aminobutyric acid (GABA) A receptor, epsilon
0.00585	SCGB2A1	AF071219	Secretoglobin, family 2A, member 1
0.00577	HMGB2	X62534	High-mobility group box 2
0.00564	TLR8	AF246971	Toll-like receptor 8
0.00555	CXCL2	BC015753	Chemokine (C-X-C motif) ligand 2
0.00551	CD36	M98398	CD36 antigen
0.00548		NM_152535	Protein of unknown function [133-aa form]
0.00503	KIAA1084	NM_014910	KIAA1084 protein
0.00484	ATP5G3	U09813	ATP synthase, H^+ transporting, subunit c, isoform 3
0.00484	CXCL12	NM_000609	Chemokine (C-X-C motif) ligand 12
0.00484	FOS	BC004490	v-fos FBJ murine osteosarcoma viral oncogene
0.00478	FLJ39553	NM_173549	Hypothetical protein FLJ39553
0.00477	SCGB1D2	AJ224172	Secretoglobin, family 1D, member 2
0.00456	PDCD5	BC015519	Programmed cell death 5
0.00445	PRG1	BC015516	Proteoglycan 1, secretory granule
0.00429	HBG2	NM_000184	Hemoglobin, γ G
0.00426	LAMC2	X73902	Laminin, γ 2
0.00419			Protein of unknown function
0.00416	MAGEF1	BC010056	Melanoma antigen, family F, 1
0.00415	MGC12538	NM_032746	Hypothetical protein MGC12538
0.00415	PRSS16	AF052514	Protease, serine, 16 (thymus)
0.0038	MGC1842	BC002924	Hypothetical protein MGC1842
0.0036	PSPH	Y10275	Phosphoserine phosphatase
0.00347	KCP3	NM_173853	Keratinocytes associated protein 3
0.00341	RARRES1	U27185	Retinoic acid receptor responder (tazarotene induced) 1
0.00338	AMELY	M86933	Amelogenin, Y-linked
0.00328	UBL5	AF313915	Ubiquitin-like 5
0.00318	Z39IG	AJ132502	Ig superfamily protein
0.00302	PCDHB5	BC001186	Protocadherin ß 5
0.0028	DKFZP434B195	AK075560	Hypothetical protein DKFZp434B195
0.0028	SCGB2A1	NM_002407	Secretoglobin, family 2A, member 1
0.00277	GSTA1	S49975	Glutathione S-transferase A1
0.00272	FLJ35773	NM_152599	Hypothetical protein FLJ35773
0.00253	NNMT	BC000234	Nicotinamide N-methyltransferase
0.00252	SAA1	NM_000331	Serum amyloid A1
0.00233	TYMS	BC013919	Thymidylate synthetase
0.00233	UGT2B7	BC030974	UDP glycosyltransferase 2, polypeptide B7
0.00226	KIAA1505	AK056542	KIAA1505 protein
0.00226	SAA2	NM_030754	Serum amyloid A2
0.00202	FLJ30296	NM_173495	Hypothetical protein FLJ30296
0.00195	GPR27	AB040799	G protein-coupled receptor 27
0.00192	LAMA3	L34155	Laminin, α 3
0.00188	NR4A2	BC009288	Nuclear receptor subfamily 4, group A, member 2
0.00188	TMEM14B	BC013913	Transmembrane protein 14B
0.0018	MGC17330	BC011049	HGFL gene
0.0018	PAPLN	NM_173462	Papilin, proteoglycan-like sulfated glycoprotein

Supplementary Table I. Continued.

P-value	Common	Genbank	Description
0.00168	HEC	BC035617	Highly expressed in cancer
0.00165	F13A1	BC027963	Coagulation factor XIII, A1 polypeptide
0.00165	SCGB1D1	NM_006552	Secretoglobin, family 1D, member 1
0.00155	DF	NM_001928	D component of complement (adipsin)
0.00152		BC035876	cDNA FLJ43911 fis, clone TESTI4010928
0.0015	SELE	M30640	Selectin E
0.00146	NELL2	D83018	NEL-like 2
0.0014	EBI2	BC020752	Epstein-Barr virus induced gene 2
0.00138	DKFZp761P1010	AL353940	Hypothetical protein DKFZp761P1010
0.00135	SSBP1	AF277319	Single-stranded DNA binding protein 1
0.00125	NR4A3	NM_173200	Nuclear receptor subfamily 4, group A, member 3
0.00125	PIK3R3	BC021622	Phosphoinositide-3-kinase, polypeptide 3
0.00106	NR4A1	BC016147	Nuclear receptor 4, group A, member 1
0.00104		BC035925	cDNA clone IMAGE:6150603
0.00101		AK056887	cDNA FLJ32325, clone PROST2003922.
0.000969	HIST1H2BG	NM_003518	Histone 1, H2bg
0.000961	SOCS3	AB004904	Suppressor of cytokine signaling 3
0.000939	GSTP1	BC010915	Glutathione S-transferase pi
0.000935	FLJ12960	NM_024638	Hypothetical protein FLJ12960
0.000935	OSR1	NM_145260	Odd-skipped related 1
0.000917	SOX17	AK025905	SRY (sex determining region Y)-box 17
0.000888	BIRC5	U75285	Survivin
0.000888	HIST1H1EH1F4	M60748	Histone H1 (H1F4) gene
0.000832	HIST1H4C	AY128656	Histone H4 gene
0.000826	HIST1H2BG	NM_003522	Histone 1, H2bg
0.000826	HIST1H4L	NM_003546	Histone 1, H4l
0.000776	CXCL2	NM_002089	Chemokine (C-X-C motif) ligand 2
0.000696	ACAT2	NM_005891	Acetyl-Coenzyme A acetyltransferase 2
0.000692	DOCK5	AK024569	Dedicator of cytokinesis 5
0.000668	E2IG5	NM_014367	Growth and transformation-dependent protein
0.000668	HIST1H1C; H1.2;	X57129	Histone H1, H1.2
0.000668	HSPE1	BC023518	Heat shock 10-kDa protein 1
0.000668	MYCBP	BC008686	c-myc binding protein
0.000668	PLEK	BC018549	Pleckstrin
0.000668	RPGRIP1	NM_020366	Retinitis pigmentosa GTPase interacting protein 1
0.000648	LAMB3	D37766	Laminin, ß 3
0.000626	BEX1	NM_018476	Brain expressed, X-linked 1
0.000626	EYA2	NM_172112	Eyes absent homolog 2 (Drosophila)
0.000626	SLC6A7	AK096607	Solute carrier family 6, member 7
0.000619	C7orf30	BC012331	Chromosome 7 open reading frame 30
0.000603	CDW52	BC000644	CDW52 antigen (CAMPATH-1 antigen)
0.000569	EYA2	NM_172113	Eyes absent homolog 2 (Drosophila)
0.000556	CDCA1	AK093348	Cell division cycle associated 1
0.000556	MGC13057	BC005083	Hypothetical protein MGC13057
0.000547	HBB	BC007075	Hemoglobin, ß
0.000525	CCL2	M24545	Chemokine (C-C motif) ligand 2
0.000525	CDCA7	NM_031942	Cell division cycle associated 7
0.000525	DJ79P11.1	BC015522	X-linked protein
0.000525	HIST1H2BJ	NM_021058	Histone 1, H2bj
0.000525	PRSS2	BC030260	Protease, serine, 2 (trypsin 2)
0.000525	SGNE1	NM_003020	Secretory granule, neuroendocrine protein 1
0.000525	SYCP2	BC040566	Synaptonemal complex protein 2

Supplementary Table I. Continued.

P-value	Common	Genbank	Description
0.000525	ZNF183	BC000266	Zinc finger protein 183 (RING finger, C3HC4 type)
0.000525			Retired, was Hemoglobin α 1
0.000521	AHCY	BC011606	S-adenosylhomocysteine hydrolase
0.000484	VIP	AY101765	α-2 macroglobulin family protein VIP
0.000468	HIST1H2BD	NM_021063	Histone 1, H2bd
0.000468	SCEL	NM_003843	Sciellin
0.000464	FOLR1	M28099	Folate receptor 1 (adult)
0.000464	LDHB	BC015122	Lactate dehydrogenase B
0.000413	HBA2	BC032122	Hemoglobin, α 2
0.0004	PURB	AY039216	Purine-rich element binding protein B
0.000369	FOSB	NM_006732	FBJ murine osteosarcoma viral oncogene homolog B
0.000333	HIST1H4B	NM_003544	Histone 1, H4b
0.000331	BM039	NM_018455	Bone marrow protein BM039
0.000316	HINT1	BC007090	Histidine triad nucleotide binding protein 1
0.000316	XLKD1	AF118108	Extracellular link domain containing 1
0.000316	ZFP36	M92843	Zinc finger protein 36, C3H type, homolog (mouse)
0.000302	ASRGL1	BC021295	Asparaginase like 1
0.000302	MELK	D79997	Maternal embryonic leucine zipper kinase
0.000302	MRPS17	BC047445	Mitochondrial ribosomal protein S17
0.000291	CKS2	BC006458	CDC28 protein kinase regulatory subunit 2
0.000291	F11R	NM_144504	F11 receptor
0.000266	EGR2	NM_000399	Early growth response 2 (Krox-20 homolog, *Drosophila*)
0.000266	HIST1H4I	NM_003495	Histone 1, H4i
0.000266	SNRPF	BC002505	Small nuclear ribonucleoprotein polypeptide F
0.000244	Siva	U82938	Human CD27BP (Siva) mRNA, complete cds.
0.000177	TACSTD1	AK026585	Tumor-associated calcium signal transducer 1
0.000177	WAC	BC004258	WW domain-containing, with a coiled-coil region
0.000119	FLJ90586	NM_153345	Hypothetical protein FLJ90586
0.000115	HBG1; HBGA	BC020719	Hemoglobin, γ A
0.000115	POU5F1	Z11898	POU domain, class 5, transcription factor 1
0.000115	RFC5	BC001866	Replication factor C (activator 1) 5
0.00011	HIST1H2BC;	BC009612	Histone 1, H2bc
9,14E-05	ASNS	BC014621	Asparagine synthetase
9,14E-05	BUB1	AF046078	BUB1 budding (yeast)
9,14E-05	PGRMC1	Y12711	Progesterone receptor membrane component 1
9,14E-05	TLE4	AB033087	Transducin-like enhancer of split 4 [E(sp1) homolog, *Drosophila*]
8,47E-05	AD024	NM_020675	AD024 protein
8,47E-05	GPR49	AF062006	G protein-coupled receptor 49
8,47E-05	H2AV	BC000098	Histone H2A.F/Z variant
3,31E-05	FLJ14627	AK094682	Hypothetical protein FLJ14627
1,28E-05	HIST1H3E; H3.1;	AF531278	histone H3
1,28E-05	PTGDS	AK075333	Prostaglandin D2 synthase 21-kDa (brain)
1,28E-05	RAB38	BC015808	RAB38, member RAS oncogene family
1,28E-05		BC042036	Clone IMAGE:5314816
1,54E-06		NM_152562	Hypothetical protein FLJ25804