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The molecular signature of endometriosis-associated endometrioid ovarian cancer differs significantly from endometriosis-independent endometrioid ovarian cancer

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Abstract

Objective—To determine whether endometriosis-associated endometrioid cancer (EAO) is a specific entity compared with endometrioid cancer not associated with endometriosis (OC).

Design—Case-control study.

Setting—University hospital research laboratory.

Patient(s)—Seven patients with endometriosis-associated ovarian cancer EAO and five patients each with OC, ovarian endometriosis, and benign ovaries.

Intervention(s)—Ovarian tissue samples were collected from surgical procedures.

Main Outcome Measure(s)—We hybridized cRNA samples to the Affymetrix HG-U133A microarray chip. Representative genes were validated by real time polymerase chain reaction.

Result(s)—We identified two main groups of genes: The first group contained the genes SICA2, CCL14, and TDGF1. These genes were equally regulated in endometriosis and EAO but not in OC and benign ovaries. The second group contained the genes StAR, SPINT1, Keratin 8, FoxM1B, FOLR1, CRABP1, and Claudin 7. They were equally regulated in EAO and OC but not in ovarian endometriosis and benign ovaries.

Conclusion(s)—That the first group is composed of the cytokines SICA2 and CCL14 and the growth factor TDGF1 indicates that the regulation of the autoimmune system and of inflammatory cytokines may be very important in the etiology of endometriosis and EAO. That the second

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group is composed of genes that play a central role in cell-cell interaction, differentiation, and cell proliferation indicates that they may be important in the development of ovarian cancer in women with endometriosis.

Keywords

Endometriosis; ovarian cancer; microarray analysis

Endometriosis is a very common disease which affects approximately 10% of all women of reproductive age (1). The cause of endometriosis is unclear. Retrograde menstruation, long believed to be the primary cause of endometriosis, occurs to some extent in most women. Therefore, there must be genetic and/or immunologic reasons that some women develop endometriosis and others do not. Approximately 10% of women with endometriosis have an affected first-degree relative, whereas the incidence in the control population is significantly lower (5%) (2).

Endometriosis is believed to be a precursor lesion for ovarian cancer, and to some degree it behaves similarly, because even without malignant transformation it invades and damages the tissue of neighboring organs. It is known that women with endometriosis have a higher prevalence of ovarian cancer (standardized incidence ratio [SIR] 2.48). In a large retrospective observational cohort study with 12,193 women, Brinton et al. (3) were able to show that the SIR for ovarian cancer is increased to 4.2 in women with a combination of endometriosis and primary infertility. Furthermore, 15%–40% of endometrioid ovarian carcinoma cases are associated with endometriosis (4–7). A recent genetic study was able to detect a loss of heterozygosity of the same alleles in coexisting ovarian carcinoma and endometriosis samples (8).

Ovarian cancer is the most lethal of all gynecologic cancers. The high mortality rate occurs mainly because the disease is asymptomatic in its initial stages and difficult to detect (9). If endometriosis is, in fact, a premalignant condition for some women, we need to find genetic markers that will identify this special group of patients, because they are more likely to suffer from ovarian cancer later in life. We studied gene expression in ovarian endometriosis, endometriosis-associated endometrioid cancer (EAOC), ovarian cancer (OC), and benign ovaries by microarray analysis and the confirmation of randomly selected genes by quantitative real time PCR. We report here that several genes were found to be either up- or down-regulated in EAOC and OC compared with endometriosis and benign ovaries, identifying molecular changes that may contribute to the transition of endometriosis to endometrioid ovarian cancer. Other genes were found to be either up- or down-regulated in endometriosis and EAOC compared with OC and benign ovaries.

MATERIALS AND METHODS

Samples

We obtained tissue from patients undergoing either an ovariectomy for treatment of endometriosis, EAOC, or OC or a prophylactic ovariectomy of benign ovaries after informed preoperative consent. The study was approved by the respective local Institutional Review

Board. Clinical data of the patients for microanalysis are listed in Table 1 and for real-time polymerase chain reaction (PCR) analysis (with additional samples) in Table 2. The diagnosis of endometriosis, EAO, OC, or benign ovaries was confirmed histologically.

RNA Preparation and Amplification

Total RNA was extracted from the samples with the RNeasyMini Kit from Qiagen (Hilden, Germany) following the manufacturer's instructions for total RNA isolation from tissue. RNA concentration was quantified by UV-spectroscopy and its quality checked by analysis on a LabChip (BioAnalyzer; Agilent Technologies, Santa Clara, CA). The cDNA was synthesized from 5 µg total RNA starting with the annealing to a T7-(dT)₂₄ Primer. Reverse transcription and second-strand synthesis were carried out using a complete cDNA reaction mix. Synthesis of biotin-labeled cRNA was performed using the BioArray High Yield RNA Transcription kit (Enzo Diagnostics, Farmingdale, NY). The amplified cRNA was purified with an affinity resin column and fragmented. The cRNA amount was determined by UV-spectroscopy and distribution of fragment sizes analyzed on a LabChip (BioAnalyzer).

Microarray

The fragmented cRNA, taken from each patient separately, was hybridized to the HG U133A/MG U74Av2 array (Affymetrix, Santa Clara, CA). Hybridization was carried out at 45°C in a hybridization oven for 16 hours. Washing and staining of the array was performed using the GeneChip fluidics station protocol EukGE-WS2. Probe arrays were then scanned twice at 3 µm resolution using the GeneChip System confocal scanner (Hewlett-Packard, Santa Barbara, CA). Data analysis was performed using Microarray Suite Version 5.0 software and Data Mining Tool version 2.0 software (Affymetrix).

Because the sample groups were of different sizes (five or seven samples per group), methods such as analysis of variance or significance analysis of microarrays for multiple correcting testing were not applicable. Therefore, classic filter technique of twofold or greater change of mean gene expression between groups was used in two substeps to identify genes of interest.

Validation of Gene Expression Data by Quantitative Real-Time RT-PCR

Total RNA from tissues was subjected to a reverse-transcription (RT) reaction using Superscript II Reverse Transcriptase (Invitrogen, Karlsruhe, Germany). Quantitative real-time PCR was performed in a Light Cycler System using the Platinum Sybr Green qPCR Supermix UDG kit as recommended by the manufacturer (Invitrogen). Primer sets are shown in Table 3. Quantification of the targets in the unknown samples was performed using a relative quantification method with external standard references. The target concentration is expressed relative to the concentration of a housekeeping gene, hypoxanthine guanine phosphoribosyltransferase 1 (HPRT-1). Among four different possible housekeeping genes (GAPDH, β-Actin, HPRT-1, and PGK-1), HPRT-1 showed the smallest differences between the samples of endometriosis, EAO, OC, and benign ovaries in the microarray experiments. After each run, melting curve analysis was performed to verify the specificity of the PCR reaction. Calculation of $2^{-\Delta\Delta CT}$ was performed to determine fold change of gene expression.

RESULTS

To identify possible differences in gene expression between EAO and OC not associated with endometriosis, we used total RNA microarray technology and validated randomly selected genes by real-time PCR.

There were no statistically significant differences in age, menopausal status (Tables 1 and 2), and histologic stage between the samples used for microarray analysis and real-time PCR in each sample group.

In addition, microarray analysis showed no difference in expression of the detected genes depending on menopausal status within one sample group.

For an overview, we listed the differentially expressed genes in two main groups: genes up- or down-regulated in endometriosis and EAO but not in OC and benign ovaries (Table 4); and genes that are up- or down-regulated in EAO and OC but not in ovarian endometriosis and benign ovaries (Table 5). Representative genes in each group were validated by quantitative real-time PCR, including 25 additional samples to enlarge the number of patients (genes printed in Tables 4 and 5 in boldface).

The first group of genes is equally regulated in endometriosis and EAO but not in OC and benign ovaries, as validated by quantitative real-time PCR.

The highest expression of the cytokine SICA2 (monocyte chemotactic protein 1 [MCP-1]) is found in EAO compared with endometriosis (1,689 times higher; $P=.00051$), OC (425,854 times higher; $P=.00017$), and benign ovary (216,169 times higher; $P=.003$). There is no statistical difference in the very low expression of SICA2 in OC and benign ovary ($P=.84$). Quantitative real-time PCR shows a trend for higher expression of SICA2 in endometriosis compared with OC ($P=.07$) and benign ovary ($P=.12$).

Cytokine CCL14 expression is 89 times higher in endometriosis and 87 times higher in EAO when compared to OC ($P=.0021$) and benign ovary ($P=.038$). There is no statistically significant difference in the elevated expression of CCL14 in endometriosis and in EAO ($P=.98$). CCL14 expression is equally low in OC and benign ovary ($P=.98$).

Conversely, the lowest expression of the growth factor TDGF1 is observed in endometriosis and EAO compared to benign ovaries (22 times lower; $P=.00039$). There is no statistically significant difference in the expression of TDGF1 between endometriosis (11 times higher; $P=.02$) and EAO (7 times higher; $P=.59$) compared with OC.

The expression of small inducible cytokine subfamily A, member 14 (CCL14), small inducible cytokine A2 (SICA2/MCP-1), and TDGF1 was validated by quantitative real-time PCR with a total of 47 samples.

The second group is comprised of genes that are up- or down-regulated in EAO and OC but not in endometriosis and benign ovaries, as validated by quantitative real time PCR.

We investigated the expression of SPINT1 and found it to be more highly expressed in EAO and OC ($P=.92$) compared with endometriosis (11 times higher; $P=.01$) or benign ovary (8 times higher; $P=.02$).

In the same group, the expression of the intermediary filament Keratin 8 is higher in EAO and OC ($P=.63$) compared with endometriosis (18 times higher; $P=.001$) or benign ovary (6 times higher; $P=.004$).

We found the expression of the transcription factor FoxM1B to be higher in EAO and OC ($P=.91$) compared with ovarian endometriosis (27 times higher; $P=.001$) or benign ovary (18 times higher; $P=.03$).

Additionally, the expression of FOLR1 is higher in EAO and OC ($P=.91$) compared with ovarian endometriosis (20 times higher; $P=.0005$) or benign ovary (159 times higher; $P=1.56E-5$). The expression of FOLR1 is elevated in ovarian endometriosis compared with benign ovary (7 times higher; $P=.01$) as well.

The expression of another gene, CRABP1, is higher in EAO and OC ($P=.62$) compared with endometriosis (107 times higher; $P=.004$) or benign ovary (158 times higher; $P=.005$).

Finally, the expression of Claudin7 is elevated in OC compared with EAO (3 times higher; $P=.0001$), ovarian endometriosis (40 times higher; $p=1.22E-7$), or benign ovary (45 times higher; $P=.01$). The second highest expression of Claudin7 is found in EAO compared with endometriosis (12 times higher; $P=.0001$) or benign ovary (13 times higher; $P=.04$).

StAR, however, is down-regulated in OC (196 times lower; $P=.0008$) and EAO (40 times lower; $P=.01$) compared with benign ovaries and endometriosis ($P=.73$). There is no statistically significant difference in the low expression of StAR in EAO and OC ($P=.15$).

DISCUSSION

We determined that SICA2, CCL14, and TDGF1 are equally regulated in endometriosis and EAO compared with OC and benign ovaries. In this group of genes, cytokines in particular were up-regulated in ovarian endometriosis and EAO. Cytokines are important mediators of intercellular communication within the immune system. The peritoneal fluid of women with endometriosis shows increased levels of many cytokines (10, 11), supporting the theory that endometriosis is a chronic inflammatory process with an altered immune answer.

Small inducible cytokine subfamily A, member 14 (CCL14), and SICA2/MCP-1 are members of the small inducible gene family and play a role in the recruitment of monocytes to sites of injury and infection. They are secreted by several cell types, such as endothelial cells, fibroblasts (12), and leukocytes (13). Their concentration is high in the peritoneal fluid of women with endometriosis and correlates with the severity of the disease (14). SICA2 and CCL14 seem to be specific markers for endometriosis and ovarian cancer associated with endometriosis. Although the direct or indirect effects of chemokines on tumor invasion are poorly evaluated, it is known that chemokines affect tumor development by attracting immuno-competent cells with pro- or antitumoral activities (15).

Teratocarcinoma-derived growth factor (TDGF1, Cripto-1) was down-regulated in endometriosis and EAOB versus OC. Interestingly, the central portion of this protein is structurally similar to human transforming growth factor alpha and epidermal growth factor, both proteins involved in the pathogenesis of ovarian cancer. Strizzi et al. (16) reviewed the role of TDGF1 during embryogenesis, cell migration, invasion, and angiogenesis and concluded that these activities may relate to cellular transformation and oncogenesis. TDGF1 is expressed in 47% of ovarian cancer samples (17).

The gene StAR is down-regulated and the genes SPINT1, Keratin 8, FoxM1B, FOLR1, CRABP1, and Claudin 7 are up-regulated in EAOB and OC but not in ovarian endometriosis or benign ovaries.

We identified the steroidogenic acute regulatory protein (StAR) in the group of proteins down-regulated in EAOB and OC compared with ovarian endometriosis and benign ovary. StAR mediates the rapid increase in pregnenolone synthesis stimulated by tropic hormones and is mainly expressed in the adrenal cortex. Transcript variants of StAR were detected in ovary and testis (18). A very recent study was able to demonstrate the expression of a few steroidogenic genes in endometriosis. StAR and aromatase were among the most important genes identified (19). Abd-Elaziz et al. (20) studied the expression of StAR in ovarian cancer and found a significant inverse relationship between FIGO stage and the residual size of the tumor.

Serine protease inhibitor 1 (SPINT1) was up-regulated in EAOB and OC compared with ovarian endometriosis and benign ovary. Hepatocyte growth factor (HGF) stimulates tumor cell-cell interactions, matrix adhesions, migration, and angiogenesis. SPINT1 inhibits the generation of biologically active HGF (21). Although there are currently no publications that demonstrate an association between SPINT1 and ovarian cancer or endometriosis, there are several reports concerning the role of SPINT1 in cancer cells. Parr et al. examined the expression of SPINT1 in human cancerous and normal breast tissue (22). The breast cancer specimen expressed a significantly higher level of SPINT1 compared with the normal background tissues.

We identified Keratin 8 in the group of genes highly up-regulated in EAOB and OC. Intermediary filaments, such as keratin 8, are involved in cell motility and cancer progression. Stimpfl et al. (23) studied the expression of keratin 8 in seven human ovarian cancer cell lines. Keratin 8 was detectable in all of the cell lines and was strongly expressed in four of them. As yet, there is no relevant literature about keratin 8 and endometriosis or ovarian cancer in vivo. A correlation between the expression of keratin 8 and the prognosis of certain human cancer seems to exist (24).

Forkhead Box M1B transcription factor (FoxM1B) was found to be highly up-regulated in EAOB and OC but not in ovarian endometriosis or benign ovary. The transcription factor FoxM1B regulates expression of cell cycle genes essential for progression into DNA replication and mitosis. Although there is, as yet, no literature on the expression of FoxM1b in ovarian cancer or endometriosis, FoxM1b has been found to be expressed in a variety of distinct human cancers. Indeed, Kim et al. (25) studied the role of FoxM1B in human

nonsmall cell lung cancers. Transient transfection experiments with lung adenocarcinoma cells showed that depletion of FoxM1B levels by short interfering RNA caused diminished DNA replication and mitosis and reduced growth of cell colonies.

Another protein that is up-regulated in EAOc and OC but not in endometriosis or normal ovarian tissue is folate receptor 1 (FOLR1). FOLR1 has a high affinity for folic acid and for several reduced folic acid derivatives. It mediates delivery of 5-methyltetrahydrofolate to the interior of cells. As early as 1991, FOLR1 was described as a marker for ovarian cancer (26). Hibbs et al. (27) found FOLR1 among the genes specifically up-regulated in ovarian carcinoma tissue compared with more than 350 other tissue samples.

We detected the up-regulation of retinoic acid-binding protein type 1 (CRABP1) in EAOc and OC compared with ovarian endometriosis and benign ovary. Cellular retinoic acid binding is assumed to play an important role in retinoic acid-mediated differentiation and proliferation processes. A very early study investigated the concentration of CRABP in carcinomas of the ovary compared with normal ovary and found highly elevated levels in ovarian carcinomas (28). Hibbs et al. (27) studied gene expression in ovarian carcinomas, ovarian carcinoma metastases of the omentum, and in 50 normal ovaries by microarray analysis. CRABP1 was one of several genes found to be up-regulated in ovarian carcinomas.

In the group of genes found to be up-regulated in EAOc and especially in OC, but not in benign ovaries or endometriosis, we detected Claudin 7. Claudins are involved in the formation of tight junctions between epithelial cells. Tight junctions restrict lateral diffusion of lipids and membrane proteins and thereby define the border between the apical and basolateral compartments of epithelial cells (29). Although Kominsky et al. (30) found that expression of claudin 7 was decreased in cancer such as invasive ductal carcinomas of the breast compared with normal breast epithelium, overexpression of other claudins, such as claudin 4, has been demonstrated in several types of cancer, including ovarian cancer (31, 32).

The results of the present study emphasize the usefulness of microarray analysis in elucidating the genetic profiles of ovarian endometriosis and ovarian cancer. We identified two main groups of genes.

The first group was composed of the cytokines SICA2 and CCL14 and the growth factor TDGF1. These genes were equally regulated in endometriosis and EAOc but not in OC or benign ovaries (Table 4). It seems likely that the regulation of the autoimmune system and of inflammatory cytokines is very important in the etiology of endometriosis and EAOc.

The second group was composed of a diversity of genes: StAR, SPINT1, Keratin 8, FoxM1B, FOLR1, CRABP1, and Claudin 7 (Table 5). They all play a central role in cell-cell interaction, differentiation, and cell proliferation and are equally regulated in EAOc and OC but not in ovarian endometriosis or benign ovaries. Therefore, these genes may be viewed as potential markers that could be used to distinguish malignant from benign ovarian tissue and to predict the development of ovarian cancer in women with endometriosis.

Further studies are necessary to show if it will be possible to identify those endometriosis patients at risk for ovarian cancer who would benefit from prophylactic treatment.

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TABLE 1

Clinical data of patients for microarray analysis.

Diagnosis	Age, y	n	Menopausal status
Endometriosis	36.4 ± 7.4	5	5 premenopausal (all ovarian endometriosis cysts)
EAO	58.8 ± 13.2	7	1 premenopausal, (FIGO IA) 6 postmenopausal (2× FIGO IB, 1× IC, 1× IIIB, 2× IIIC)
OC	57.8 ± 13.0	5	2 premenopausal, (FIGO IC, FIGO IIC) 3 postmenopausal (all FIGO IIIC)
Benign ovary	48.3 ± 22.7	5	2 premenopausal, (benign ovarian cysts) 3 postmenopausal (benign ovarian cysts)

Note: EAO = endometriosis-associated ovarian cancer; OC = ovarian cancer.

Banz. Gene profile of ovarian endometriosis. Fertil Steril 2010.

TABLE 2

Clinical data of patients for real-time polymerase chain reaction analysis.

Diagnosis	Age, y	n	Menopausal status
Endometriosis	34.8 ± 6.3	14	14 premenopausal (all ovarian endometriosis cysts)
EAO	58.5 ± 12.6	8	1 premenopausal, (FIGO IA) 7 postmenopausal (2× FIGO IB, 2× IC, 1× IIIB, 2× IIIC)
OC	65.6 ± 12.1	16	2 premenopausal, (FIGO IC, FIGO IIC) 14 postmenopausal (1× FIGO IC, 1× IIIB, 1× IIIC, 8× IIIC, 3× IV)
Benign ovary	50.8 ± 16.3	9	4 premenopausal, (all benign ovarian cysts) 5 postmenopausal (all benign ovarian cysts)

Note: Abbreviations as in Table 1.

Banz. Gene profile of ovarian endometriosis. Fertil Steril 2010.

TABLE 3

Primer sequences for quantitative real-time polymerase chain reaction.

Primer sequence	5'-3' Forward	Reverse
Small inducible cytokine A2 (SICA2)	taa ctt cac caa tag gaa ga	atg gaa tcc tga acc cac tt
Small inducible cytokine subfamily A, member 14 (SIC14)	gga tta tgg att act atg ag	ctt cat gtc ctt gat ata gt
Teratocarcinoma-derived growth factor (TDGF1)	aca gaa cct gct gcc tga at	aca cag gga aca ctt ctt gg
Steroidogenic acute regulatory protein (StAR)	tgg agc gca tgg aag caa t	gcc agc tcg tga gta atg a
Serine protease inhibitor 1 (Spint1)	ctg aca gtg act agc tca ga	tag tac cag cgt ggg aaa ga
Keratin 8	agc tac atc aac aac ctt a	ctc tgt acg ctt att gat ct
Forkhead Box M1B transcription factor (FoxM1B)	gtc tcc ttc tgg acc att ca	ttc caa gtg ctc ggg caa tt
Folate receptor 1 (FOLR1)	gcc tgc aaa cgg cat ttc at	tgc tca cag tcc tct ttg ca
Retinoic acid-binding protein type 1 (CRABP1)	cgc acc act gag atc aac tt	gtg cag tgg atc ttg ttc tc
Claudin 7	aga cga caa agt gaa gaa gg	agt ctg tga caa tct gat gg

Banz. *Gene profile of ovarian endometriosis. Fertil Steril* 2010.

TABLE 4

Genes identified by microarray analysis that are up- or down-regulated in endometriosis and endometriosis-associated ovarian cancer (EAOC) compared with ovarian cancer and benign ovaries.

Probe set ID Up-regulated in EAOC and endometriosis	Gene symbol	Fold		Sequence derived from
209710_at	GATA2	1,67	AL563460	GATA-binding protein 2
202540_s_at	HMGCR	1,67	NM_000859.1	3-Hydroxy-3-methylglutaryl-coenzyme A reductase
201941_at	CPD	1,68	AA897514	Carboxypeptidase D
202458_at		1,87	NM_007173.1	Protease, serine, 23
221477_s_at	BNIP3L	2,03	AL132665.1	BCL2/adenovirus E1B 19kD interacting protein 3-like
210980_s_at	ASAH	2,03	U47674.1	N-Acylsphingosine amidohydrolase (acid ceramidase)
202422_s_at	FACL4	2,12	NM_022977.1	Fatty-acid-coenzyme A ligase, long-chain 4
201656_at	ITGA6	3,01	NM_000210.1	Integrin, alpha 6
213975_s_at		3,25	AV711904	Hypothetical protein FLJ23356
216598_s_at	SICA2, MCP1	4,70	S69738	Small inducible cytokine A2 (monocyte chemotactic protein 1)
214594_x_at	ATP8B1	4,80	BG252666	ATPase, Class I, type 8B, member 1
202948_at	IL1R1	5,63	NM_000877.1	Interleukin 1 receptor, type I
205392_s_at	CCL14, CC-1, CC-3, NCC2, SY14, HCC-1, HCC-3, NCC-2, SCYL2	5,85	NM_004166	Small inducible cytokine subfamily A (Cys-Cys), member 14
Down-regulated:				
206286_s_at	TDGF1, CR, CRIPTO	3,74	NM_003212	Teratocarcinoma-derived growth factor 1

Note: Genes confirmed by quantitative real-time polymerase chain reaction are shown in boldface.

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TABLE 5

Genes identified by microarray analysis that are up- or down-regulated in EAOC and OC compared with endometriosis and benign ovaries.

Probe set ID Up-regulated in EAOC and OC	Gene symbol	Fold		Sequence derived from
205350_at	CRABP1	3,34	NM_004378	Cellular retinoic acid-binding protein 1
202580_x_at	FOXM1	3,64	NM_021953.1	Forkhead box M1
203954_x_at	CLDN3	7,11	NM_001306.1	Claudin 3
200660_at	S100A11	7,19	NM_005620.1	S100 calcium-binding protein A11 (calgizzarin)
201690_s_at	TPD52	8,16	BE974098	Tumor protein D52
201005_at	CD9	8,22	NM_001769.1	CD9 antigen (p24)
202826_at	SPINT1	8,26	NM_003710.1	Serine protease inhibitor, Kunitz type 1
204124_at	SLC34A2	8,69	AF146796.1	Solute carrier family 34 (sodium phosphate), member 2
202790_at	CLDN7	9,29	NM_001307.1	Claudin 7
33323_r_at	SFN	9,51	X57348	Stratifin
207847_s_at	MUC1	11,7	NM_002456.1	Mucin 1, transmembrane
209772_s_at	CD24	11,88	X69397.1	CD24 antigen (small cell lung carcinoma cluster 4 antigen)
200606_at	DSP	12,15	NM_004415.1	Desmoplakin (DPI, DPII)
204437_s_at	FOLR1	12,76	NM_016725	Folate receptor 1 (adult)
201131_s_at		12,93	NM_004360.1	Cadherin 1, type 1, E-cadherin (epithelial)
209875_s_at	SPP1	14,04	M83248.1	Secreted phosphoprotein 1 (osteopontin, bone sialoprotein I, early T-lymphocyte activation 1)
203917_at	CXADR	14,11	NM_001338.1	Coxsackie virus and adenovirus receptor
213693_s_at		15,56	AI610869	tp21e08.x1 NCL CGAP_Gas4 Homo sapiens cDNA clone IMAGE: 2188454 3' similar to gb:J05582 MUCIN 1 PRECURSOR (HUMAN);, mRNA sequence.
201596_x_at	KRT18	15,65	NM_000224.1	Keratin 18
205979_at	MGB2	15,84	NM_002407.1	Mammaglobin 2
208998_at	UCP2	17,21	U94592.1	Uncoupling protein 2 (mitochondrial, proton carrier)
209008_x_at	KRT8	17,94	U76549.1	Keratin 8
203892_at	WFDC2	18,39	NM_006103.1	WAP four-disulfide core domain 2
208650_s_at	CD24	23,6	BG327863	CD24 antigen (small cell lung carcinoma cluster 4 antigen)
202286_s_at	TACSTD2	27,06	J04152	Tumor-associated calcium signal transducer 2
266_s_at	CD24	30,62	L33930	CD24 antigen (small cell lung carcinoma cluster 4 antigen)
208651_x_at	CD24	30,66	M58664.1	CD24 antigen (small cell lung carcinoma cluster 4 antigen)
201839_s_at	TACSTD1	37,78	NM_002354.1	Tumor-associated calcium signal transducer 1
209771_x_at	CD24	62,98	AA761181	CD24 antigen (small cell lung carcinoma cluster 4 antigen)
Down-regulated				
204548_at	STAR	3,26	NM_000349	Steroidogenic acute regulatory protein

Note: Genes confirmed by quantitative real-time polymerase chain reaction are shown in boldface.

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