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Int. J. Cancer: **112,** 14–25 (2004)

Publication of the International Union Against Cancer

FAST TRACK

GENE EXPRESSION PROFILES IN PRIMARY OVARIAN SEROUS PAPILLARY TUMORS AND NORMAL OVARIAN EPITHELIUM: IDENTIFICATION OF CANDIDATE MOLECULAR MARKERS FOR OVARIAN CANCER DIAGNOSIS AND THERAPY

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With the goal of identifying genes with a differential pattern of expression between ovarian serous papillary carcinomas (OSPCs) and normal ovarian (NOVA) epithelium and using this knowledge for the development of novel diagnostic and therapeutic markers for ovarian cancer, we used oligonucleotide microarrays with probe sets complementary to 12,533 genes to analyze the gene expression profiles of 10 primary OSPC cell lines, 2 established OSPC cell lines (UCI-101, UCI-107) and 5 primary NOVA epithelial cultures. Unsupervised analysis of gene expression data identified 129 and 170 genes that exhibited >5-fold upregulation and downregulation, respectively, in primary OSPC compared to NOVA. Genes overexpressed in established OSPC cell lines had little correlation with those overexpressed in primary OSPC, highlighting the divergence of gene expression that occurs as a result of long-term *in vitro* growth. Hierarchical clustering of the expression data readily distinguished normal tissue from primary OSPC. *Laminin, claudin 3, claudin 4, tumor-associated calcium signal transducers 1 and 2 (TROP-1/Ep-CAM, TROP-2), ladinin 1, S100A2, SERPIN2 (PAI-2), CD24, lipocalin 2, osteopontin, kallikrein 6 (protease M), kallikrein 10, matriptase (TADG-15)* and *stratifin* were among the most highly overexpressed genes in OSPC compared to NOVA. Downregulated genes in OSPC included *transforming growth factor-β receptor III, platelet-derived growth factor receptor α, SEMACAP3, ras homolog gene family member I (ARHI), thrombospondin 2* and *disabled-2/differentially expressed in ovarian carcinoma 2 (Dab2/DOC2).* Differential expression of some of these genes, including *claudin 3, claudin 4, TROP-1* and *CD24,* was validated by quantitative RT-PCR and flow cytometry on primary OSPC and NOVA. Immunohistochemical staining of formalin-fixed, paraffin-embedded tumor specimens from which primary OSPC cultures were derived further confirmed differential expression of *CD24* and *TROP-1/Ep-CAM* markers on OSPC *vs.* NOVA. These results, obtained with highly purified primary cultures of ovarian cancer, highlight important molecular features of OSPC and may provide a foundation for the development of new type-specific therapies against this disease.

Key words: *serous papillary ovarian cancer; gene expression profiling*

Ovarian carcinoma remains the cancer with the highest mortality rate among gynecologic malignancies, with 25,400 new cancer cases estimated in 2003 in the United States alone.[1] OSPC represents the most common histologic type of ovarian carcinoma.[2] Because of the insidious onset of the disease and the lack of reliable screening tests, two-thirds of patients have advanced disease when diagnosed; and although many patients with disseminated tumors respond initially to standard combinations of surgical and cytotoxic therapy, nearly 90% will develop recurrence and inevitably succumb to their disease.[2] Understanding the molecular basis of OSPC may significantly refine the diagnosis and management of these tumors and may eventually lead to the development of novel, more specific and more effective treatment modalities.

cDNA microarray technology has been used to identify genes involved in ovarian carcinogenesis.[3–11] Gene expression fingerprints representing large numbers of genes may allow precise and accurate grouping of human tumors and may identify patients who are unlikely to be cured by conventional therapy. Consistent with this view, evidence has been provided to support the notion that poor-prognosis B-cell lymphomas and biologically aggressive breast and ovarian carcinomas can be readily distinguished by gene expression profiles.[4,12,13] In addition, large-scale gene expression analysis has the potential to identify a number of differentially expressed genes in OSPC compared to NOVA epithelial cells and may therefore lay the groundwork for future studies of some of these markers for clinical utility in the diagnosis and treatment of OSPC.

Expression profiling studies involve the comparison of OSPC cells with their normal counterpart. However, selection of NOVA control cells may strongly influence the determination of differentially expressed genes in ovarian cancer expression profiling studies.[14] In this regard, because ovarian epithelial cells constitute only

Abbreviations: CPE, *Clostridium perfringens* enterotoxin; C_t, cycle threshold; Ep-CAM, epithelial cell adhesion molecule; FDR, false discovery rate; FIGO, International Federation of Gynecology and Obstetrics; MAb, monoclonal antibody; MFI, mean fluorescence intensity; NOVA, normal ovarian; OSPC, ovarian serous papillary carcinoma; PAI, plasminogen activator inhibitor; SAM, significance analysis of microarrays; TNF, tumor necrosis factor; WRS, Wilcoxon's rank sum.

Grant sponsor: Angelo Nocivelli and Camillo Golgi Foundation.

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Received 5 September 2003; Accepted after revision 20 April 2004

DOI 10.1002/ijc.20408
Published online 2 June 2004 in Wiley InterScience (www.interscience.wiley.com).

a small proportion of the total cells found in NOVA samples and because ovarian tumor tissue contains significant numbers of contaminant stromal cells as well as a variety of host-derived immune cells (*e.g.,* monocytes, dendritic cells, lymphocytes), we used oligonucleotide microarrays with probe sets complementary to 12,533 genes to analyze the gene expression profiles in primary OSPC cultures and NOVA epithelium cell lines. Short-term primary OSPC and NOVA cell cultures, minimizing the risk of selection bias inherent in any long-term *in vitro* growth, may provide an opportunity to study differential gene expression between relatively pure populations of tumor- and NOVA-derived epithelial cells.

We report that mRNA fingerprints readily distinguish OSPC from NOVA epithelial cells and identify a number of genes highly differentially expressed between nontransformed ovarian epithelia and OSPC. Several of the genes identified are already known to be overexpressed in ovarian cancer, validating our experimental approach as well as our criteria to determine the genes differentially expressed; but several represent novel findings. Of interest, many of the genes upregulated in ovarian cancer represent surface or secreted proteins, such as *laminin, claudin 3, claudin 4, tumor-associated calcium signal transducers 1 and 2 (TROP-1/Ep-CAM, TROP-2), ladinin 1, S100A2, SERPIN2 (PAI-2), CD24, lipocalin 2, osteopontin, kallikrein 6 (protease M), kallikrein 10, matriptase (TADG-15)* and *stratifin.* Quantitative real-time PCR was used to validate differences in gene expression between nontransformed ovarian epithelia and OSPC for some of these genes, including *TROP-1/Ep-CAM, CD24, claudin 3* and *claudin 4. TROP-1/Ep-CAM* and *CD24* gene expression was further validated through flow-cytometric analysis of primary OSPC and NOVA as well as by immunohistochemical analysis of archival OSPC and NOVA specimens.

MATERIALS AND METHODS

Establishment of OSPC and NOVA primary cell lines

A total of 15 primary cell lines (10 OSPC and 5 NOVA) were established after sterile processing of samples from surgical biopsies, as previously described for ovarian carcinoma specimens.[3,6,15] UCI-101 and UCI-107, 2 previously characterized OSPC cell lines,[16,17] were also included. All fresh tumor samples were obtained with appropriate consent according to institutional review board guidelines. Tumors were staged according to the FIGO operative staging system. Radical tumor debulking, including total abdominal hysterectomy and omentectomy, was performed in all ovarian carcinoma patients, while NOVA tissue was obtained from consenting donors of similar age undergoing surgery for benign pathology. No patient received chemotherapy before surgery. Patient characteristics are described in Table I. Briefly, normal tissue was obtained by scraping epithelial cells from the ovarian surface and placing them in RPMI-1640 medium (Sigma, St. Louis, MO) containing 10% FBS (Invitrogen, Grand Island, NY), 200 μ/ml penicillin and 200 μg/ml streptomycin. Epithelial explants were then allowed to attach and proliferate. Once the epithelial cells reached confluence, explants were trypsinized and subcultured for 3 or 4 passages before being collected for RNA extraction. Viable tumor tissue was mechanically minced in RPMI-1640 to portions no larger than 1–3 mm^3 and washed twice with RPMI-1640. Portions of minced tumor were then placed into 250 ml flasks containing 30 ml of enzyme solution [0.14% collagenase type I (Sigma) and 0.01% DNAse (Sigma, 2,000 KU/mg)] in RPMI-1640 and incubated in a magnetic stirring apparatus overnight at 4°C. Enzymatically dissociated tumor was then filtered through 150 μm nylon mesh to generate a single-cell suspension. The resultant cell suspension was then washed twice in RPMI-1640 plus 10% FBS. Primary cell lines were maintained in RPMI-1640 supplemented with 10% FBS, 200 μ/ml penicillin and 200 μg/ml streptomycin at 37°C, 5% CO_2 in tissue culture flasks (75–150 cm^2 Corning, Corning, NY). Tumor cells were collected for RNA extraction at 50–80% confluence after 2–12 passages *in vitro.* The epithelial nature and purity of OSPC and NOVA cultures were verified by immunohistochemical staining and flow-cytometric analysis with antibodies against cytokeratin, as previously described.[3,15] Only primary cultures with at least 90% viability and >99% epithelial cells were used for total RNA extraction.

RNA purification and microarray hybridization and analysis

Detailed protocols for RNA purification, cDNA synthesis, cRNA preparation and hybridization to the Affymetrix (Santa Clara, CA) Human U95Av2 GeneChip microarray were performed according to the manufacturer's protocols, as reported previously.[18]

Data processing

All data used were derived from Affymetrix 5.0 software. GeneChip 5.0 output files are given as a signal that represents the difference between the intensities of the sequence-specific perfect-match probe set and the mismatch probe set or as detection of present, marginal or absent signals as determined by the program's algorithm. Gene arrays were scaled to an average signal of 1,500 and then analyzed independently. Signal calls were transformed by log base 2, and each sample was normalized to give a mean of 0 and variance of 1.

Gene expression data analysis

Statistical analyses of the data were performed with the software package SPSS10.0 (SPSS, Chicago, IL) and the SAM method.[19] Genes were selected for analysis based on detection and fold change. In each comparison, genes having "present" detection calls in more than half of the samples in the overexpressed group were retained for statistical analysis if they showed a >2-fold change between groups. Retained genes were subjected to SAM to establish a FDR, then further filtered *via* the WRS test at $\alpha = 0.05$. The FDR obtained from the initial SAM was assumed to characterize genes found significant *via* WRS.

Gene Cluster/Treeview

The hierarchical clustering of the average-linkage method with the centered correlation metric was used.[20] The dendrogram was constructed with a subset of genes from 12,533 probe sets present on the microarray, whose expression levels varied the most among

TABLE I – CHARACTERISTICS OF THE PATIENTS

Patient	Age	Race	Grade	Stage	Presence of ascites	Chemotherapy regimen	Response to therapy
OSPC 1	42	White	G2/3	IVA	yes	TAX+CARB	Complete response
OSPC 2	67	White	G3	IIIB	yes	TAX+CARB	Complete response
OSPC 3	61	White	G3	IIIC	no	TAX+CARB	Partial response
OSPC 4	60	White	G3	IIIC	no	TAX+CARB	Complete response
OSPC 5	59	Afro-American	G2/3	IIIC	yes	TAX+CARB	Complete response
OSPC 6	72	White	G3	IVA	yes	TAX+CARB	Stable disease
OSPC 7	63	White	G3	IIIC	yes	TAX+CARB	Progressive disease
OSPC 8	74	Afro-American	G2/3	IIIC	no	TAX+CARB	Partial response
OSPC 9	68	White	G3	IIIB	yes	TAX+CARB	Complete response
OSPC 10	77	White	G2/3	IIIC	no	TAX+CARB	Complete response

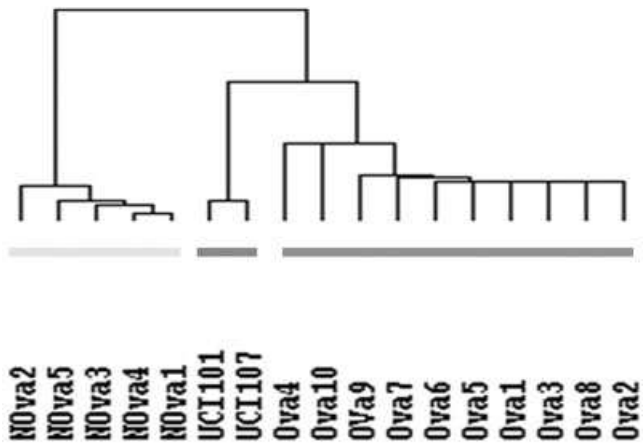

FIGURE 1 – Hierarchical clustering of 15 primary ovarian cell lines (10 OSPC and 5 NOVA) and 2 established OSPC cell lines (UCI-101 and UCI-107).

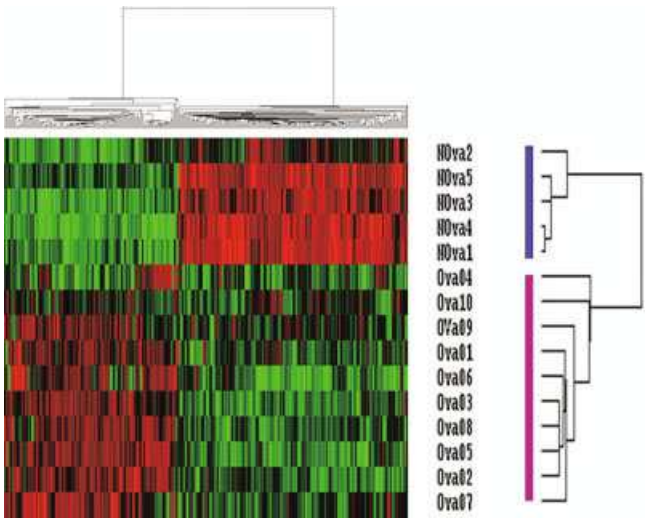

FIGURE 2 – Molecular profile of 10 primary OSPC and 5 NOVA cell lines. Hierarchical clustering of 299 genes with differential expression between 10 OSPC and 5 NOVA groups ($p < 0.05$) using a 5-fold threshold. The cluster is color-coded, using red for upregulation, green for downregulation and black for median expression. Agglomerative clustering of genes was illustrated with dendrograms.

the samples and were thus most informative. For the hierarchical clustering shown in Figures 1 and 2, only genes significantly expressed and whose average change in expression level was at least 5-fold were chosen. The expression value of each selected gene was renormalized to a mean of 0.

Quantitative real-time PCR

Quantitative real-time PCR was performed with an ABI Prism 7000 Sequence Analyzer using the manufacturer's recommended protocol (Applied Biosystems, Foster City, CA) to validate differential expression of selected genes in samples from all 15 primary cell lines. Each reaction was run in triplicate. The comparative C_t method was used to calculate amplification fold as specified by the manufacturer. Briefly, 5 μg of total RNA from each sample were reverse-transcribed using SuperScript II RNAse H Reverse Transcriptase (Invitrogen, Carlsbad, CA). Reverse-transcribed RNA samples (10 μl, from 500 μl of total volume) were amplified using the TaqMan Universal PCR Master Mix (Applied Biosystems) to produce PCR products specific for *TROP-1, CD24, claudin 3* and *claudin 4.* Primers specific for 18S ribosomal RNA and empirically determined ratios of 18S competimers (Applied Biosystems) were used to control for the amounts of cDNA generated from each sample. Primers for *TROP-1, claudin 3* and *claudin 4* were obtained from Applied Biosystems as assay on demand products. Assay IDs were Hs00158980_m1 (*TROP-1*), Hs00265816_s1 (*claudin 3*) and Hs00533616_s1 (*claudin 4*). *CD24* primer sequences were as follows: forward, 5′-cccaggtgttactgtaattcctcaa; reverse, 5′-gaacagcaatagctcaacaatgtaaac. Differences among OSPC and NOVA samples in the quantitative real-time PCR expression data were tested using the Kruskal-Wallis nonparametric test. Pearson product-moment correlations were used to estimate the degree of association between the microarray and quantitative real-time PCR data.

Flow cytometry

To validate microarray data on primary OSPC and NOVA cell lines at the protein level, TROP-1/EP-CAM and CD24 expression were evaluated by flow-cytometric analysis of a total of 13 primary cell lines (*i.e.,* 10 OSPC and 3 NOVA). Unconjugated anti-TROP-1/EP-CAM (IgG2a), anti-CD24 (IgG2a) and isotype control (mouse IgG2a) antibodies were obtained from BD Pharmingen (San Diego, CA). Goat antimurine FITC-labeled mouse Ig was purchased from Becton Dickinson (San Jose, CA). Analysis was conducted with a FACScan, utilizing Cell Quest software (Becton Dickinson).

TROP-1 *and* CD24 *immunostaining of formalin-fixed tumor tissues*

To evaluate whether the differential *TROP-1* and *CD24* expression detected by flow cytometry in primary OSPC cell lines was comparable to the expression of *TROP-1* and *CD24* in uncultured OSPC from which the primary cell lines were derived, protein expression was evaluated by standard immunohistochemical staining on formalin-fixed tumor tissue from all surgical specimens (*i.e.,* 10 OSPC and 5 NOVA controls). Study blocks were selected after histopathologic review by a surgical pathologist. The most representative hematoxylin and eosin–stained block sections were used for each specimen. Briefly, immunohistochemical stains were performed on sections (4 μm thick) of formalin-fixed, paraffin-embedded tissue. After pretreatment with 10 mM citrate buffer (pH 6.0) using a steamer, sections were incubated with anti-Ep-CAM Ab-3 and anti-CD24 MAbs (Neo Markers, Fremont, CA) at 1:2,000 dilution. Slides were subsequently labeled with streptavidin–biotin (Dako, Glostrup, Denmark), stained with diaminobenzidine and counterstained with hematoxylin. The intensity of staining was graded as 0 (not greater than negative control), 1+ (light), 2+ (moderate) or 3+ (heavy).

RESULTS

Gene expression profiles distinguish OSPC from NOVA and identify differentially expressed genes

Flash-frozen biopsies from ovarian tumor tissue contain significant numbers of contaminant stromal cells as well as a variety of host-derived immune cells (*e.g.,* monocytes, dendritic cells, lymphocytes). In addition, because ovarian epithelial cells represent a small proportion of the total cells found in the normal ovary, it is difficult to collect primary material that is free of contaminating ovarian stromal cells in sufficient quantities to conduct comparative gene expression analyses. However, ovarian epithelial cells can be isolated and expanded in culture for about 15 passages,[3,6] while the majority of primary ovarian carcinomas can be expanded *in vitro* for several passages.[15] Thus, short-term primary OSPC and NOVA cell cultures, minimizing the risk of selection bias inherent in any long-term *in vitro* growth, may provide an opportunity to study differential gene expression between relatively pure populations of normal and tumor-derived epithelial cells. Accordingly,

comprehensive gene expression profiles of 10 primary OSPC and 5 primary NOVA cell lines were generated using high-density oligonucleotide arrays with 12,533 probe sets, which in total included some 10,000 genes. In addition, gene expression profiles derived from 2 established and previously characterized OSPC cell lines (UCI-101 and UCI-107) were analyzed. By combining the detection levels of genes significantly expressed in primary and established OSPC cultures, we found very little correlation between the 2 groups of OSPC. Indeed, as shown in Figure 1, UCI-101 and UCI-107 cells grouped together in the dendrogram, while all 10 primary OSPCs clustered tightly together in the rightmost columns separate from the 5 NOVA controls. Because of these results, we focused our analysis on the detection of differentially expressed genes between the 2 homogeneous groups of primary OSPC and NOVA cell lines. Figure 2 shows the cluster analysis on hybridization intensity values for each gene in all 15 primary cultures of NOVA and OSPC. Using the nonparametric WRS test ($p < 0.05$), which readily distinguished between the 2 groups of primary cultures, we found 1,546 genes differentially expressed between OSPC and NOVA. There were 365 genes showing a >5-fold change along with "present" detection calls in more than half the samples in the overexpressed group. Of these, 350 were significant by SAM, with a median FDR of 0.35% and a 90th percentile FDR of 0.59%. Of the 365 genes, 299 yielded $p < 0.05$ *via* WRS and 298 were among those found significant by SAM. Figure 2 describes the cluster analysis performed on hybridization intensity values for 298 gene segments whose average change in expression level was at least 5-fold and which were significant with both the WRS test and SAM. All 10 OSPCs were grouped together in the rightmost columns. Similarly, in the leftmost columns, all 5 NOVA lines clustered tightly together. The tight clustering of OSPC from NOVA lines was "driven" by 2 distinct profiles of gene expression. The first was represented by a group of 129 genes that were highly expressed in OSPC and underexpressed in NOVA (Table II). Many genes shown previously to be involved in ovarian carcinogenesis are present on these lists, providing some validity to our array analysis, while others are novel in ovarian carcinogenesis. Included in these group of genes are *laminin, claudin 3, claudin 4, tumor-associated calcium signal transducers 1 and 2 (TROP-1/Ep-CAM, TROP-2), ladinin 1, S100A2, SERPIN2 (PAI-2), CD24, lipocalin 2, osteopontin, kallikrein 6 (protease M), kallikrein 10, matriptase (TADG-15)* and *stratifin* (Table II). *TROP-1/Ep-CAM,* encoding for a transmembrane glycoprotein previously found to be overexpressed in various carcinoma types including colorectal and breast[21] and where antibody-directed therapy has resulted in improved survival,[22] was 39-fold differentially expressed in OSPC compared to NOVA (Table II). The second profile was represented by 170 genes that were highly expressed in NOVA and underexpressed in OSPC (Table III). Included in this group of genes are *transforming growth factor-β receptor III, platelet-derived growth factor receptor α, SEMACAP3, ras homolog gene family member I (ARHI), thrombospondin 2* and *disabled-2/differentially expressed in ovarian carcinoma 2 (Dab2/DOC2)* (Table III).

Validation of microarray data

We used quantitative real-time PCR assays to validate the microarray data. Four highly differentially expressed genes between OSPC and NOVA (*i.e., TROP-1, CD24, claudin-3* and *claudin-4*) were selected for quantitative real-time PCR analysis. A comparison of the microarray and quantitative real-time PCR data for these genes is shown in Figure 3. Expression differences between OSPC and NOVA for *TROP-1* ($p = 0.02$), *CD24* ($p = 0.004$), *claudin 3* ($p = 0.02$) and *claudin 4* ($p = 0.01$) were readily apparent (Table II, Fig. 3). Moreover, for all 4 genes tested, the quantitative real-time PCR data were highly correlated to the microarray data ($p < 0.001$; $r = 0.81, 0.90, 0.80$ and 0.85, respectively), as estimated from the samples (*i.e.,* 10 OSPC and 5 NOVA) included in both the quantitative real-time PCR and microarray experiments. Thus, quantitative real-time PCR data suggest that most array probe sets are likely to accurately measure the levels of the intended transcript within a complex mixture of transcripts.

TROP-1 *and* CD24 *expression by flow cytometry in primary OSPC and NOVA cell lines*

An important issue is whether differences in gene expression result in meaningful differences in protein expression. Because the *TROP-1/Ep-CAM* gene encodes the target for the anti-Ep-CAM antibody (17-1A) edrecolomab (Panorex), which has previously been shown to increase survival in patients harboring stage III colon cancer,[22] expression of Ep-CAM protein by FACS analysis was analyzed in 13 primary cell lines (10 OSPC and 3 NOVA). As positive controls, breast cancer cell lines (*i.e.,* BT-474 and SK-BR-3; ATCC, Rockville, MD) known to overexpress *TROP-1/Ep-CAM* were also studied. High *TROP-1/Ep-CAM* expression was found in all 10 primary OSPC cell lines tested (100% positive cells for all 10 OSPC), with MFI ranging 116–280 (Fig. 4). In contrast, primary NOVA cell lines were negative for *TROP-1/Ep-CAM* surface expression ($p < 0.001$) (Fig. 4). Similarly, *CD24* expression was found in all primary OSPC cell lines tested (100% positive cells for all 10 OSPC), with MFI ranging 26–55 (Fig. 4). In contrast, primary NOVA cell lines were negative for *CD24* surface expression ($p < 0.005$) (Fig. 4). These results show that high expression of the *TROP-1/Ep-CAM* and *CD24* gene products by OSPC correlates tightly with high protein expression by tumor cells. Breast cancer-positive controls expressed high levels of *TROP-1/Ep-CAM* (data not shown).

TROP-1/Ep-CAM *and* CD24 *expression by immunohistology on OSPC and NOVA tissue blocks*

To determine whether the high (OSPC) or low (NOVA) expression of the genes and Ep-CAM and CD24 proteins detected by microarray and flow cytometry, respectively, in primary cell lines was the result of selection of a subpopulation of cancer cells present in the original tumor or whether *in vitro* expansion conditions may have modified gene expression, we performed immunohistochemical analysis of TROP-1/Ep-CAM and CD24 protein expression on formalin-fixed tumor tissue from all uncultured primary surgical specimens of OSPC and NOVA. As shown in the left panel of Figure 5, heavy apical membranous staining for CD24 protein expression was noted in all OSPC specimens that also overexpressed the *CD24* gene and its gene product by microarray and flow cytometry, respectively. In contrast, negative or low (*i.e.,* score 0 or 1+) staining was found in all NOVA samples tested by immunohistochemistry. Similarly, as shown in the right panel of Figure 5, heavy membranous staining for TROP-1/Ep-CAM protein expression (*i.e.,* score 3+) was noted in all OSPC specimens that also overexpressed the *TROP-1/Ep-CAM* gene and its gene product by microarray and flow cytometry, respectively. In contrast, negative or low (*i.e.,* score 0 or 1+) staining was found in all NOVA samples tested by immunohistochemistry.

DISCUSSION

Because of the lack of an effective ovarian cancer screening program and the common development of chemotherapy-resistant disease after an initial response to cytotoxic agents (*i.e.,* platinum-based regimen), ovarian cancer remains the most lethal among the gynecologic malignancies.[2] Thus, identification of novel ovarian tumor markers to be used for early detection of the disease as well as the development of effective therapy against chemotherapy-resistant/recurrent ovarian cancer remains a high priority.

High-throughput technologies for assaying gene expression, such as high-density oligonucleotide and cDNA microarrays, may offer the potential to identify clinically relevant genes highly differentially expressed between ovarian tumors and NOVA control epithelial cells.[3–11] Our investigation involved genomewide examination of differences in gene expression between primary OSPC and NOVA epithelial cells. We used short-term primary OSPC and NOVA cultures (to minimize the risk of selection bias inherent in any long-term *in vitro* growth) to study differential

TABLE II – UPREGULATED GENES EXPRESSED AT LEAST 5 FOLD HIGHER IN OSPC COMPARED WITH NOVA

Probe set	Gene symbol	Score(d)(SAM)	p of WRS	Ratio ova/nova	Probe set	Gene symbol	Score(d)(SAM)	p of WRS	Ratio ova/nova
35280_at	LAMC2	1.68927386	0.006	46.45	39016_r_at	KRT6E	0.973486831	0.008	7.91
35276_at	CLDN4	1.734410451	0.015	43.76	31610_at	MAP17	1.0156502	0.027	7.81
33904_at	CLDN3	1.650076713	0.02	40.24	2027_at	S100A2	0.941919001	0.008	7.76
575_s_at	TACSTD1	1.705816336	0.02	39.36	418_at	MKI67	0.826426448	0.011	7.46
32154_at	TFAP2A	1.667038647	0.002	33.31	1536_at	CDC6	1.08868941	0.017	7.37
39015_f_at	KRT6E	1.062629117	0.047	28.02	634_at	PRSS8	0.899891713	0.02	7.30
1713_s_at	CDKN2A	1.137682905	0.015	26.96	34342_s_at	SPP1	1.318723271	0.02	7.27
41376_i_at	UGT2B7	0.939735032	0.047	24.81	182_at	ITPR3	1.107167336	0.006	7.27
38551_at	L1CAM	1.151935363	0.008	24.66	32382_at	UPK1B	0.731294678	0.047	7.16
291_s_at	TACSTD2	1.249487388	0.047	24.46	863_g_at	SERPINB5	0.783530451	0.015	7.14
33282_at	LAD1	1.422481563	0.006	24.31	904_s_at	TOP2A	0.971648429	0.02	7.12
34213_at	KIBRA	1.533570321	0.002	23.06	40095_at	CA2	0.798857154	0.027	7.02
38489_at	HBP17	1.522882814	0.004	22.54	41294_at	KRT7	1.082553892	0.011	7.00
36869_at	PAX8	1.43906836	0.004	22.20	39951_at	PLS1	0.995091449	0.006	6.94
38482_at	CLDN7	1.307716566	0.027	20.01	38051_at	MAL	0.819842532	0.036	6.82
37909_at	LAMA3	1.121654521	0.027	19.24	40726_at	KIF11	0.803689697	0.036	6.78
34674_at	S100A1	1.219106334	0.008	19.01	1148_s_at	—	0.683569558	0.047	6.72
1620_at	CDH6	0.908193479	0.036	18.69	37920_at	PITX1	0.996497645	0.015	6.67
32821_at	LCN2	1.99990601	0.008	18.13	37117_at	ARHGAP8	1.129131077	0.002	6.65
522_s_at	FOLR3	1.113781518	0.02	17.90	38881_i_at	TRIM16	0.721698355	0.047	6.59
39660_at	DEFB1	0.837612681	0.036	17.34	34251_at	HOXB5	1.219463307	0.002	6.52
2011_s_at	BIK	1.594057668	0.006	17.23	41359_at	PKP3	1.047269618	0.004	6.50
41587_g_at	FGF18	0.965726983	0.02	17.10	40145_at	TOP2A	0.961173129	0.02	6.48
36929_at	LAMB3	1.115590892	0.047	16.76	37534_at	CXADR	0.888147605	0.006	6.32
35726_at	S100A2	1.036576352	0.004	15.05	40303_at	TFAP2C	0.948734146	0.004	6.30
1887_g_at	WNT7A	1.186990893	0.004	14.75	31805_at	FGFR3	0.969764101	0.011	6.28
35879_at	GAL	1.223278825	0.002	14.65	33245_at	MAPK13	0.877514586	0.011	6.27
266_s_at	CD24	1.756569076	0.004	14.45	885_g_at	ITGA3	0.702747685	0.036	6.19
1108_s_at	EPHA1	1.242309171	0.006	14.36	34693_at	STHM	0.872525584	0.008	6.15
37483_at	HDAC9	1.406744957	0.006	14.28	38555_at	DUSP10	0.880305317	0.008	6.12
31887_at	—	1.311220827	0.011	13.68	38418_at	CCND1	1.071102249	0.002	5.97
1788_s_at	DUSP4	1.22421987	0.003	13.65	33730_at	RAI3	0.813298748	0.011	5.90
32787_at	ERBB3	0.996784565	0.02	13.21	39109_at	TPX2	1.040973216	0.011	5.87
41660_at	CELSR1	1.634286803	0.004	13.11	36658_at	DHCR24	1.122129795	0.004	5.81
33483_at	NMU	1.100849065	0.004	13.04	35281_at	LAMC2	0.747766326	0.047	5.78
31792_at	ANXA3	0.896090153	0.011	12.90	38749_at	MGC29643	0.683275086	0.036	5.77
36838_at	KLK10	1.026306829	0.02	12.71	1083_s_at	MUC1	0.746980491	0.027	5.75
1585_at	ERBB3	1.102058608	0.011	12.51	40079_at	RAI3	0.709840659	0.02	5.73
1898_at	TRIM29	1.071987353	0.002	12.44	2047_s_at	JUP	0.815282235	0.011	5.62
37185_at	SERPINB2	0.815945986	0.027	12.26	32275_at	SLPI	0.940625784	0.02	5.61
406_at	ITGB4	1.296194559	0.006	11.66	2020_at	CCND1	0.926408163	0.002	5.51
1914_at	CCNA1	0.9363427778	0.011	11.21	33324_s_at	CDC2	1.026683994	0.008	5.47
977_s_at	CDH1	0.93637461	0.036	11.19	36863_at	HMMR	0.96343264	0.006	5.46
37603_at	IL1RN	1.103624942	0.015	11.14	1657_at	PTPRR	0.764510362	0.02	5.41
35977_at	DKK1	1.123240701	0.006	10.74	37985_at	LMNB1	0.895475347	0.008	5.36
36133_at	DSP	1.280269127	0.002	10.69	36497_at	C14orf78	0.942921564	0.008	5.33
36113_s_at	TNNT1	1.269558595	0.002	10.19	2021_s_at	CCNE1	0.893228297	0.006	5.33
1802_s_at	ERBB2	0.787465706	0.006	9.61	37890_at	CD47	0.775908217	0.015	5.33
2092_s_at	SPP1	1.34315986	0.02	9.53	40799_at	C16orf34	0.852774782	0.008	5.30
35699_at	BUB1B	1.026388835	0.006	9.49	35309_at	ST14	0.852534105	0.008	5.30
37554_at	KLK6	0.895036336	0.027	9.45	1599_at	CDKN3	0.925527261	0.02	5.29
38515_at	BMP7	0.945367	0.027	9.32	981_at	MCM4	1.058558782	0.006	5.28
34775_at	TSPAN-1	1.001195829	0.02	9.01	32715_at	VAMP8	0.938171642	0.006	5.28
37558_at	IMP-3	1.023799379	0.011	8.99	38631_at	TNFAIP2	0.72369235	0.015	5.26
38324_at	LISCH7	1.308000521	0.006	8.96	34715_at	FOXM1	1.31035831	0.008	5.24
39610_at	HOXB2	1.355268631	0.006	8.64	33448_at	SPINT1	0.924028022	0.015	5.21
572_at	TTK	1.122796615	0.006	8.53	419_at	MKI67	0.938133197	0.015	5.16
1970_s_at	FGFR2	1.022708001	0.02	8.30	1651_at	UBE2C	1.436239741	0.008	5.14
160025_at	TGFA	1.065272755	0.015	8.28	35769_at	GPR56	0.937347548	0.015	5.08
41812_s_at	NUP210	1.39287031	0.006	8.26	37310_at	PLAU	0.885110741	0.036	5.08
34282_at	NFE2L3	1.165273649	0.008	8.06	36761_at	ZFN339	0.937123503	0.011	5.05
2017_s_at	CCND1	1.114984456	0.002	8.04	37343_at	ITPR3	1.001079303	0.003	5.05
33323_r_at	SFN	1.202433185	0.008	8.01	40425_at	EFNA1	0.813414458	0.047	5.04
38766_at	SRCAP	1.131917941	0.008	7.99	1803_at	CDC2	0.732852195	0.027	5.00
41060_at	CCNE1	1.151246634	0.006	7.97					

gene expression in highly enriched populations of epithelial tumor cells. Only cancer cells derived from papillary serous histology tumors, which is the most common histologic type of ovarian cancer, were included, to limit the complexity of gene expression analysis.

We found that hierarchical clustering of samples and gene expression levels within samples led to the unambiguous separation of OSPC from NOVA. Of interest, the expression patterns detected in primary OSPC cells were consistently different from those seen in established OSPC cell lines (*i.e.,* UCI-101 and UCI-107). These data, thus, further highlight the divergence of gene expression that occurs as a result of long-term *in vitro* growth. Furthermore, these data emphasize that, although established ovarian cancer cell lines provide a relatively simple model to examine gene expression, primary OSPC and NOVA cultures

TABLE III – UPREGULATED GENES EXPRESSED AT LEAST 5 FOLD HIGHER IN NOVA COMPARED WITH OSPC

Probe set	Gene symbol	Score(d)(SAM)	p of WRS	Ratio ova/nova	Probe set	Gene symbol	Score(d)(SAM)	p of WRS	Ratio ova/nova
39701_at	PEG3	1.991111245	0.006	113.32	36867_at	—	1.273166453	0.008	8.21
32582_at	MYH11	1.921434447	0.002	67.31	38653_at	PMP22	1.422063697	0.002	8.19
39673_i_at	ECM2	1.740409609	0.011	53.54	38875_r_at	GREB1	1.026886865	0.015	8.10
37394_at	C7	1.597329897	0.02	50.45	35366_at	NID	1.483421362	0.002	8.10
37247_at	TCF21	2.261979734	0.002	39.29	34417_at	FLJ36166	0.783978445	0.047	7.98
1897_at	TGFBR3	1.648143277	0.003	38.12	37221_at	PRKAR2B	0.927090765	0.036	7.91
36627_at	SPARCL1	1.610346382	0.008	37.84	39031_at	COX7A1	1.564725491	0.004	7.89
37015_at	ALDH1A1	1.886579474	0.002	35.18	39757_at	SDC2	1.288106392	0.002	7.80
38469_at	TM4SF3	1.620821878	0.003	34.43	36629_at	DSIPI	0.981563882	0.008	7.79
35717_at	ABCA8	1.709820793	0.008	33.92	35390_at	ABCA6	1.026714913	0.036	7.79
32664_at	RNASE4	1.720857082	0.003	32.94	39629_at	PLA2G5	1.405181995	0.002	7.70
40775_at	ITM2A	1.393751125	0.006	31.35	40961_at	SMARCA2	0.996692724	0.015	7.68
38519_at	NR1H4	1.431579641	0.004	27.02	719_g_at	PRSS11	1.399043078	0.002	7.65
37017_at	PLA2G2A	1.263990266	0.011	26.68	40856_at	SERPINF1	1.077533093	0.008	7.55
36681_at	APOD	1.44030134	0.008	26.04	37008_r_at	SERPINA3	1.134224016	0.002	7.53
34193_at	CHL1	1.738491852	0.006	25.97	33834_at	CXCL12	1.060878451	0.002	7.51
34363_at	SEPP1	1.490374268	0.015	25.93	31880_at	D8S2298E	1.177864913	0.002	7.45
1501_at	IGF1	1.116943817	0.027	25.87	37628_at	MAOB	1.194963489	0.004	7.43
33240_at	SEMACAP3	1.818843975	0.003	25.54	34853_at	FLRT2	1.250330254	0.027	7.41
36939_at	GPM6A	0.924236354	0.047	25.47	38887_r_at	ARHI	1.169953614	0.015	7.32
614_at	PLA2G2A	1.391395227	0.003	23.15	38220_at	DPYD	1.024334451	0.02	7.26
37407_s_at	MYH11	1.72766007	0.002	22.73	1327_s_at	MAP3K5	0.891703475	0.02	7.23
39325_at	EBAF	1.248164036	0.02	22.49	1380_at	FGF7	1.096254206	0.004	7.14
767_at	—	1.688001805	0.002	21.90	37573_at	ANGPTL2	1.052539345	0.002	7.08
37595_at	—	1.582101386	0.004	20.94	718_at	PRSS11	1.381205346	0.002	6.99
1290_g_at	GSTM5	1.383630361	0.003	20.84	36712_at	—	1.15195149	0.005	6.88
34388_at	COL14A1	1.400078214	0.015	20.39	1709_g_at	MAPK10	1.160327795	0.002	6.85
607_s_at	VWF	1.314435559	0.002	19.05	39123_s_at	TRPC1	1.060327922	0.015	6.79
37599_at	AOX1	1.669903577	0.003	17.61	38627_at	HLF	0.911787462	0.036	6.79
41504_s_at	MAF	1.463988429	0.008	16.40	32076_at	DSCR1L1	1.127515982	0.002	6.77
41412_at	PIPPIN	1.799353403	0.002	16.08	36669_at	FOSB	1.023057503	0.011	6.65
279_at	NR4A1	1.194733065	0.008	15.42	38194_s_at	IGKC	1.239936045	0.015	6.64
38427_at	COL15A1	1.570514035	0.002	15.38	39545_at	CDKN1C	1.040717569	0.004	6.62
41405_at	SFRP4	1.478603828	0.002	14.44	36993_at	PDGFRB	1.384657766	0.004	6.60
39066_at	MFAP4	1.91469237	0.004	14.26	35837_at	SCRG1	1.023840456	0.036	6.48
1731_at	PDGFRA	1.791307012	0.003	13.91	1507_s_at	EDNRA	1.23933124	0.004	6.48
36595_s_at	GATM	1.382271028	0.004	13.86	40488_at	DMD	1.291791538	0.002	6.42
34343_at	STAR	2.080476608	0.003	13.67	38364_at	—	1.030881108	0.004	6.35
36917_at	LAMA2	1.359731285	0.006	13.51	41424_at	PON3	0.946224951	0.036	6.32
38430_at	FABP4	1.054221974	0.02	13.05	32109_at	FXYD1	1.005577422	0.004	6.19
36596_r_at	GATM	1.22177547	0.008	12.67	1182_at	PLCL1	1.097390316	0.002	6.17
35898_at	WISP2	1.276226302	0.004	12.55	31897_at	DOC1	1.533672652	0.003	6.13
36606_at	CPE	1.608244463	0.003	12.30	37208_at	PSPHL	1.007759699	0.015	6.08
32057_at	LRRC17	1.345223643	0.011	12.22	36396_at	—	1.009684807	0.015	6.07
33431_at	FMOD	1.516795166	0.003	12.17	41505_r_at	MAF	1.116101319	0.006	6.06
34985_at	CILP	0.905018335	0.02	11.53	37765_at	LMOD1	1.127716375	0.003	6.00
755_at	ITPR1	1.433938835	0.002	11.06	37398_at	PECAM1	0.970664041	0.008	5.98
1466_s_at	FGF7	1.184028604	0.027	11.00	41013_at	FLJ31737	1.036561659	0.003	5.98
36727_at	—	0.98132702	0.036	10.96	39279_at	BMP6	1.106724571	0.002	5.93
1103_at	RNASE4	1.456068199	0.002	10.88	1527_s_at	CG018	0.804755548	0.047	5.91
32666_at	CXCL12	1.342426238	0.006	10.72	39038_at	FBLN5	1.279283798	0.004	5.89
914_g_at	ERG	1.264721284	0.002	10.54	32542_at	FHL1	1.134214637	0.002	5.88
40698_at	CLECSF2	1.325237675	0.002	10.46	38508_s_at	TNXB	0.878513741	0.011	5.74
36873_at	VLDLR	1.344197327	0.004	10.45	32696_at	PBX3	0.888011703	0.027	5.69
1090_f_at	—	0.914708216	0.027	10.34	41796_at	PLCL2	0.857601993	0.02	5.68
36042_at	NTRK2	0.950553444	0.02	10.32	34473_at	TLR5	0.871815246	0.027	5.67
36311_at	PDE1A	1.356950738	0.004	10.21	661_at	GAS1	1.267909476	0.004	5.66
41685_at	NY-REN-7	0.8848466	0.036	10.08	41449_at	SGCE	1.050056933	0.004	5.65
32847_at	MYLK	1.545610138	0.002	10.00	35740_at	EMILIN1	1.366368794	0.011	5.58
35358_at	TENC1	1.539140855	0.003	9.97	37908_at	GNG11	0.989043327	0.004	5.43
32249_at	HFL1	1.257702238	0.02	9.86	37406_at	MAPRE2	1.265872665	0.002	5.41
36695_at	na	1.452847153	0.003	9.82	33802_at	HMOX1	1.034088234	0.015	5.41
1987_at	PDGFRA	1.50655467	0.002	9.76	39106_at	APOA1	1.266005754	0.008	5.40
37446_at	GASP	1.219014593	0.004	9.76	1771_s_at	PDGFRB	1.336082701	0.006	5.39
35752_s_at	PROS1	1.211272096	0.008	9.66	39409_at	C1R	1.05784087	0.011	5.39
36533_at	PTGIS	1.882348646	0.004	9.62	32535_at	FBN1	1.422415283	0.006	5.35
38886_i_at	ARHI	1.127672988	0.02	9.59	37710_at	MEF2C	0.98149558	0.011	5.35
36733_at	FLJ32389	1.420588897	0.011	9.57	37958_at	TM4SF10	1.293658009	0.003	5.35
38717_at	DKFZP586A0522	1.158933663	0.015	9.50	33756_at	AOC3	0.829203515	0.02	5.29
32551_at	EFEMP1	1.385495033	0.004	9.38	36569_at	TNA	0.926096917	0.006	5.25
1968_g_at	PDGFRA	1.364848071	0.003	9.31	39771_at	RHOBTB1	1.048906896	0.008	5.20
33910_at	PTPRD	1.129963902	0.008	9.20	39852_at	SPG20	0.82401517	0.027	5.20
32778_at	ITPR1	1.370809534	0.002	9.08	35168_f_at	COL16A1	1.509830282	0.011	5.18
280_g_at	NR4A1	1.074894321	0.006	8.79	33244_at	CHN2	0.92878389	0.015	5.18
35389_s_at	ABCA6	1.209294071	0.011	8.79	35681_r_at	ZFHX1B	1.170745794	0.006	5.14
32889_at	RPIB9	1.145333813	0.003	8.74	2087_s_at	CDH11	1.656534188	0.008	5.12
37248_at	CPZ	1.238797022	0.002	8.69	40496_at	C1S	0.973175912	0.011	5.10
39674_r_at	ECM2	0.874009817	0.027	8.67	41137_at	PPP1R12B	1.12885067	0.008	5.07
33911_at	PTPRD	1.099609918	0.02	8.66	39698_at	HOP	0.797252583	0.011	5.05
35234_at	RECK	1.407865518	0.008	8.58	38211_at	ZNF288	0.926263264	0.015	5.04
32119_at	—	1.153957574	0.011	8.57	41839_at	GAS1	1.127093791	0.006	5.03
35998_at	LOC284244	1.104281231	0.008	8.54	39979_at	F10	0.890787173	0.002	5.02
37279_at	GEM	1.012760866	0.008	8.31	1135_at	GPRK5	1.150554994	0.002	5.01
35702_at	HSD11B1	1.164189513	0.004	8.28	479_at	DAB2	1.255638531	0.006	5.01
32126_at	FGF7	1.336918337	0.008	8.22					

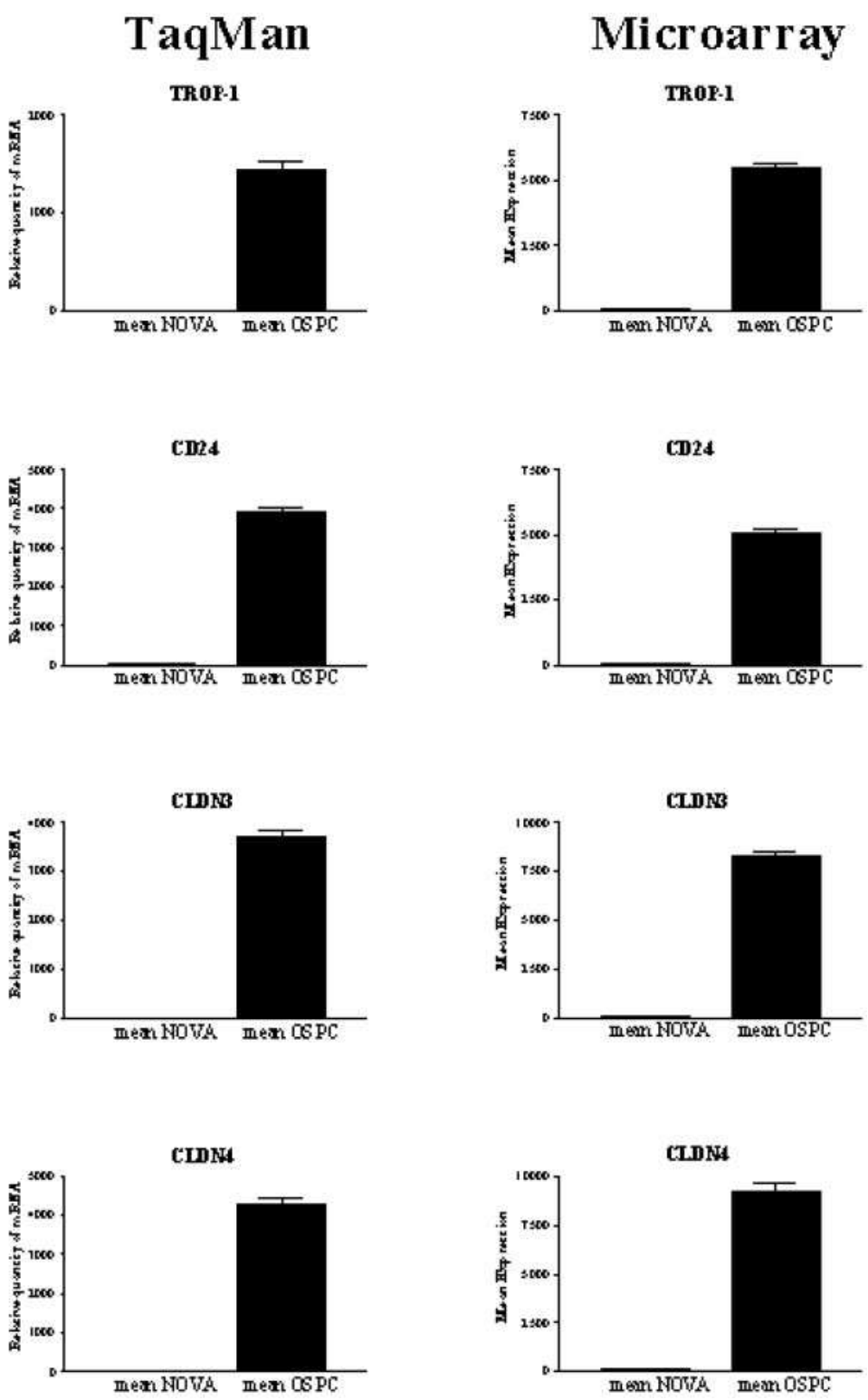

FIGURE 3 – Quantitative real-time PCR and microarray expression analyses of *TROP-1, CD24, claudin 3* and *claudin 4* genes differentially expressed between OSPC and NOVA.

represent a better model system of normal and cancerous ovarian tissues in comparative gene expression analysis. Because of these results, we focused our analysis on the detection of differentially expressed genes between the 2 homogeneous groups of primary OSPC and NOVA.

We detected 298 genes differentially expressed between OSPC and NOVA whose average change in expression level between the 2 groups was at least 5-fold and which were significant with both the WRS test and SAM. The known function of some of these genes may provide insights in the biology of serous ovarian tumors, while others may prove to be useful diagnostic and therapeutic markers against OSPC. For example, the *laminin γ2* gene was the most highly differentially expressed gene in OSPC, with over 46-fold upregulation relative to NOVA. Migration of ovarian epithelial cells is considered essential for cell dissemination and invasion of the submesothelial extracellular matrix commonly seen

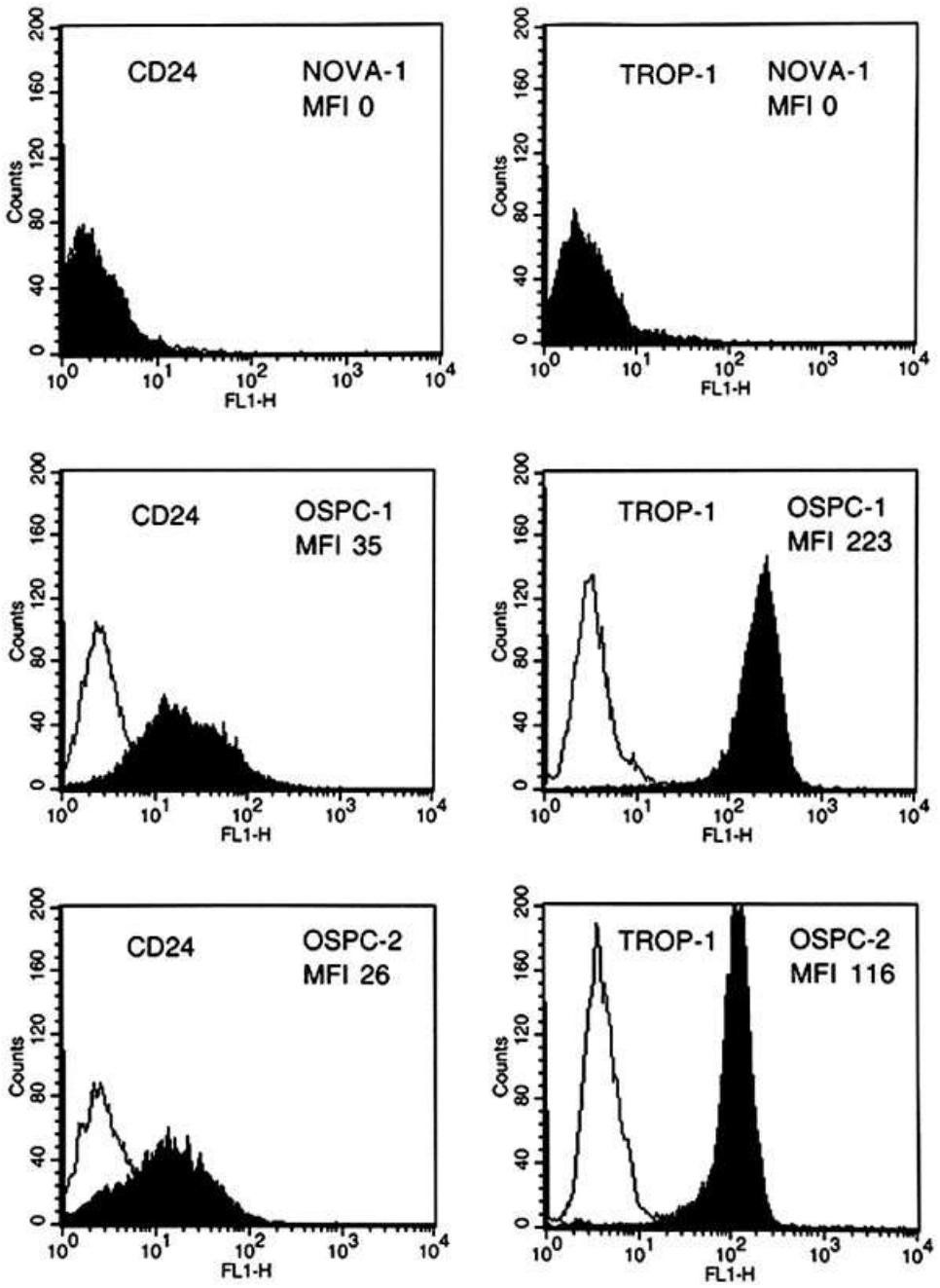

FIGURE 4 – Representative FACS analysis of *CD24* staining (left panel) and *TROP-1/Ep-CAM* staining (right panel) of 2 primary OSPC cell lines and 1 NOVA cell line. Data on *CD24* and *TROP-1/Ep-CAM* are shown in solid black, while isotype control MAb profiles are shown in white. Expression of both *CD24* and *TROP-1/Ep-CAM* was significantly higher in OSPC compared to NOVA cell lines ($p < 0.001$ by Student's *t*-test).

in ovarian cancer. Consistent with this view, the *laminin γ2* isoform has been previously suggested to play an important role in the adhesion, migration and scattering of ovarian carcinoma cells.[23–25] Thus, it is likely that the high laminin expression found in ovarian tumor cells may be a marker correlated with the invasive potential of OSPC. Consistent with this view, increased cell surface expression of laminin has been reported in highly metastatic tumors cells compared to cells of low metastatic potential.[26] Previous work has also shown that attachment and metastasis of tumor cells can be inhibited by incubation with antilaminin antibodies[27] or synthetic laminin peptides,[28] underscoring the novel potential for the treatment of chemotherapy-resistant ovarian cancer.

TROP-1/Ep-CAM (also called 17-1A, ESA, EGP40) is a 40 kDa epithelial transmembrane glycoprotein overexpressed in several normal epithelia and in various carcinomas, including colorectal and breast cancers.[21] In most adult epithelial tissues, enhanced expression of Ep-CAM is closely associated with either benign or malignant proliferation. Among mammals, Ep-CAM is an evolutionarily highly conserved molecule,[29] suggesting an important biologic function in epithelial cells and tissue. In this regard, Ep-CAM is known to function as an intercellular adhesion molecule and could have a role in tumor metastasis.[30] Because a randomized phase II trial with MAb CO17-1A in colorectal carcinoma patients has demonstrated a significant decrease in recurrence and mortality of MAb-treated patients *vs.* controls,[22] TROP-1/Ep-CAM antigen has attracted substantial attention as a target for immunotherapy in human carcinomas. In our work, TROP-1/Ep-CAM was 39-fold differentially expressed in OSPC compared to NOVA. These data support the notion that anti-Ep-CAM antibody therapy may be a novel and potentially effective treatment option for OSPC patients with residual/resistant disease after sur-

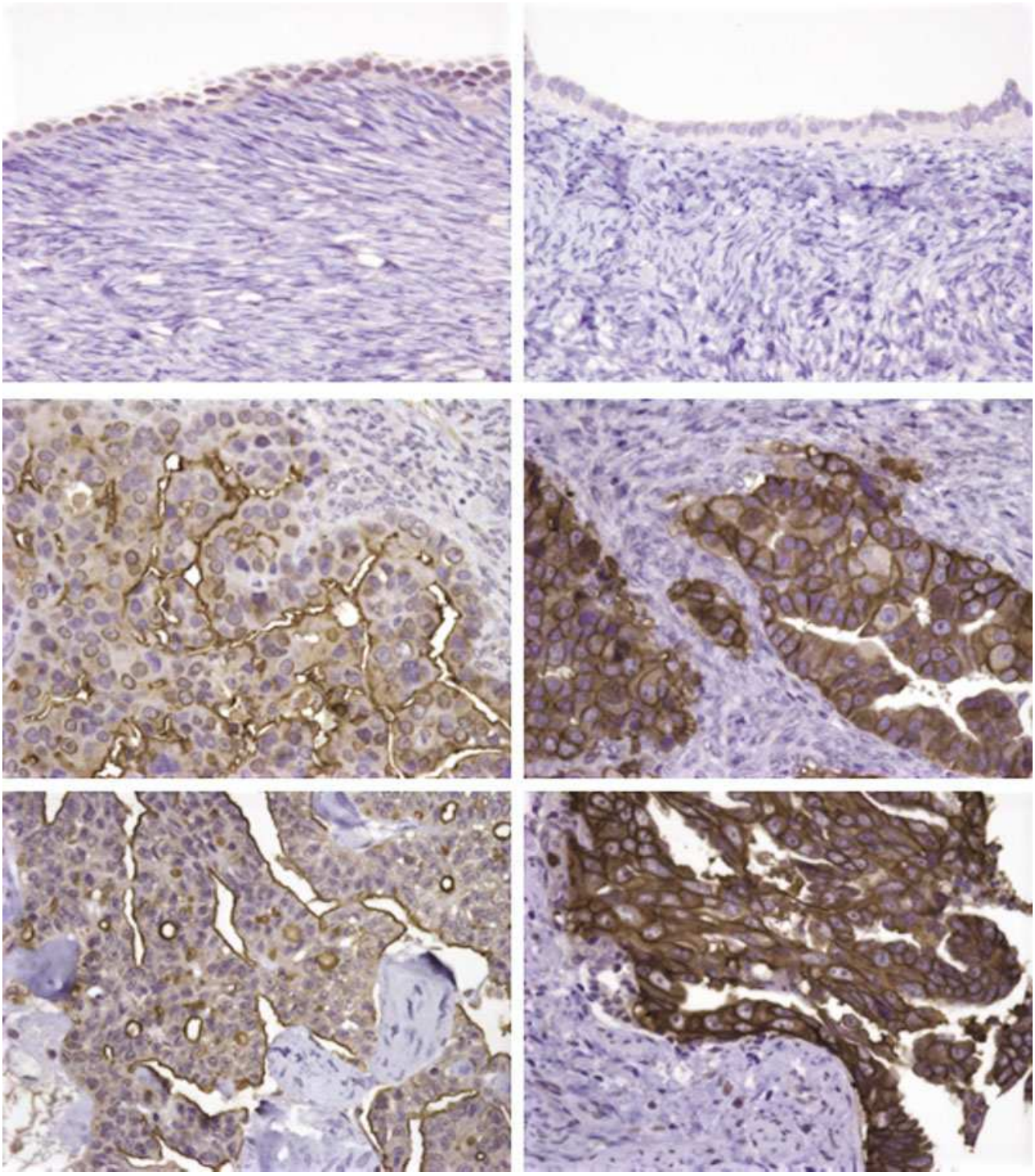

FIGURE 5 – Representative immunohistochemical staining for *CD24* (left panel) and *Trop-1/Ep-CAM* (right panel) markers of 2 paraffin-embedded OSPC specimens and 1 NOVA specimen. NOVA 1 (upper panel, right and left) showed negative or light (1+) staining for both *CD24* and *Trop-1/Ep-CAM,* while OSPC 1 and OSPC 3 showed heavy apical membranous staining for *CD24* (lower and middle panel, left) and strong membranous staining for *TROP-1/Ep-CAM* (right). Original magnification ×400.

gical and cytotoxic therapy. Protein expression data obtained by flow cytometry on primary OPSC cell lines and by immunohistochemistry on uncultured OSPC blocks support this view.

Claudin 3 and claudin 4, 2 members of claudin family of tight junction proteins, were 2 of the top 5 differentially expressed genes in OSPC. These results are consistent with a previous report on gene expression in ovarian cancer.[6] Although the function of claudin proteins in ovarian cancer remains unclear, they likely represent a transmembrane receptor.[31] Of interest, claudin 3 and claudin 4 are homologous to CPE-R, the low- and high-affinity intestinal epithelial receptors for CPE, respectively, and are sufficient to mediate CPE binding and trigger subsequent toxin-medi-

ated cytolysis.[32] These known functions of claudin 3 and claudin 4 combined with their extremely high level of expression in OSPC have suggested the use of CPE as a novel therapeutic strategy to treat chemotherapy-resistant disease in ovarian cancer patients. Supporting this view, the functional cytotoxicity of CPE in metastatic, androgen-independent prostate cancer overexpressing claudin 3 has been reported.[33]

PAI-2, a gene whose expression has been linked to cell invasion in several human malignancies[34,35] as well as to protection from TNF-α-mediated apoptosis,[36] was 12-fold differentially expressed in OSPC compared to NOVA. Consistent with our findings, previous studies have shown that elevated levels of *PAI-2* are detectable in the ascites of ovarian cancer patients and that high *PAI-2* levels are independently predictive of poor disease-free survival.[37,38] In some of these studies, a 7-fold increase in *PAI-2* content was found in the omentum of ovarian cancer patients compared to primary disease, suggesting that metastatic tumors may overexpress *PAI-2.*[38] Other studies, however, have identified *PAI-2* production as a favorable prognostic factor in epithelial ovarian cancer.[39] Indeed, high *PAI-2* expression in invasive ovarian tumors was limited to a group of OSPC patients who experienced more prolonged disease-free and overall survival.[39] The reasons for these differences are not clear but, as previously suggested,[40] may be related at least in part to the actions of macrophage colony stimulating factor-1, a cytokine which stimulates the release of *PAI-2* by ovarian cancer cells.

CD24 is a small, heavily glycosylated glycosylphosphatidylinositol-linked cell surface protein, which is expressed in hematologic malignancies as well as in a large variety of solid tumors.[41–46] However, it is only recently that CD24 overexpression has been reported at the RNA level in ovarian cancer.[4] Consistent with this report, we found the *CD24* gene to be 14-fold differentially expressed in OSPC compared to NOVA. Because CD24 is a ligand of P-selectin, an adhesion receptor on activated endothelial cells and platelets, its expression may contribute to the metastatic capacities of CD24-expressing ovarian tumor cells.[47–49] CD24 expression has been reported to be an independent prognostic marker for ovarian cancer patient survival.[50] These data combined with our findings further suggest that this marker may delineate aggressive ovarian cancer disease and may be targeted for therapeutic and/or diagnostic purposes.

Among the genes we have identified, to the best of our knowledge, lipocalin 2 has not been previously linked to ovarian cancer. Lipocalin 2 represents a particularly interesting marker because of several features. Lipocalins are extracellular carriers of lipophilic molecules, such as retinoids, steroids and fatty acids, all of which may play important roles in the regulation of epithelial cell growth.[51,52] In addition, because lipocalin is a secreted protein, it may play a role in the regulation of cell proliferation and survival.[51,52] Of interest, 2 publications, on gene expression profiling of breast and pancreatic cancers, have proposed lipocalin 2 as a novel therapeutic and diagnostic marker for prevention and treatment of these diseases.[53,54] On the basis of our findings, lipocalin 2 may be added to the known markers for ovarian cancer.

Osteopontin (SPP1) is an acidic, calcium-binding glycophosphoprotein that has been linked to tumorigenesis in several experimental animal models and human patient studies.[55–57] Because of its integrin-binding, arginine-glycine-aspartate domain and adhesive properties, osteopontin has been reported to play a crucial role in the metastatic process of several human tumors.[55,58] However, it is only recently that the upregulated expression of osteopontin in ovarian cancer has been identified.[59] Because of the secreted nature of this protein, osteopontin has been proposed as a novel biomarker for the early recognition of ovarian cancer.[59] In our study, the *SPP1* gene was 10-fold differentially expressed in OSPC compared to NOVA. Taken together, these data confirm a high expression of osteopontin in OSPC and further suggest that future research assessing its clinical usefulness in ovarian cancer would be worthwhile.

The organization of kallikreins, a gene family consisting of 15 genes that encode for trypsin-like or chymotrypsin-like serine proteases, has been elucidated.[60] Serine proteases have well-characterized roles in diverse cellular activities, including blood coagulation, wound healing, digestion and immune responses, as well as tumor invasion and metastasis (reviewed by Diamandis and Yousef[60]). Because of the secreted nature of some of these enzymes, prostate-specific antigen and kallikrein 2 have already found important clinical application as prostate cancer biomarkers.[60] Of interest, kallikrein 6 (also known as zyme/protease M/neurosin), kallikrein 10 and matriptase (TADG-15/MT-SP1) were highly differentially expressed in OSPC compared to NOVA. These data confirm previous results from our group as well as others showing high expression of several kallikrein genes and proteins in ovarian neoplasms.[60–64] Moreover, these results, obtained by high-throughput technologies for assaying gene expression, further emphasize the view that some members of the kallikrein family have the potential to become novel cancer markers for early diagnosis of ovarian cancer[64] as well as targets for novel therapies against recurrent/refractory ovarian disease.[65] Other highly ranked genes in OSPC included *stratifin, desmoplakin, S100A2, cytokeratin 6, cytokeratin 7* and *MUC-1.*

A large number of downregulated (at least 5-fold) genes in OSPC *vs.* NOVA, such as *transforming growth factor beta receptor III, platelet-derived growth factor receptor α, SEMACAP3, ras homolog gene family member I (ARHI), thrombospondin 2* and *disabled-2/differentially expressed in ovarian carcinoma 2 (Dab2/DOC2)* (Table III), were identified in our analysis. Some encode for widely held tumor-suppressor genes, such as *SEMACAP3, ARHI* and *Dab2/DOC2*,[66] and others for proteins important for ovarian tissue homeostasis or previously implicated in apoptosis, proliferation, adhesion or tissue maintenance. Because of space limitations, we will not comment further on the cluster of genes that showed downregulation of the transcripts in invasive tumors.

In conclusion, several OSPC-restricted markers have been identified through our analysis. Some of these genes have been previously reported to be highly expressed in ovarian cancer, while others have not been linked with this disease. Finally, identification of *TROP-1/Ep-CAM* and *CPE* epithelial receptors as some of the most highly differentially expressed genes in OSPC compared to NOVA suggests that novel therapeutic strategies targeting *TROP-1/Ep-CAM* by MAbs[22] and/or *claudin 3* and *claudin 4* by local and/or systemic administration of *CPE*[33] may represent novel therapeutic modalities in patients harboring OSPC refractory to standard treatment.

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