

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
16 December 2004 (16.12.2004)

PCT

(10) International Publication Number
WO 2004/108896 A2

(51) International Patent Classification⁷: **C12N**

(21) International Application Number:
PCT/US2004/017515

(22) International Filing Date: 1 June 2004 (01.06.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/475,446 3 June 2003 (03.06.2003) US

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(81) Designated States (*unless otherwise indicated, for every
kind of national protection available*): AE, AG, AL, AM,

AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN,
CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI,
GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE,
KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD,
MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG,
PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM,
TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM,
ZW.

(84) Designated States (*unless otherwise indicated, for every
kind of regional protection available*): ARIPO (BW, GH,
GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM,
ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI,
FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI,
SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ,
GW, ML, MR, NE, SN, TD, TG).

Published:

— *without international search report and to be republished
upon receipt of that report*

*For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.*

(54) Title: GENE EXPRESSION PROFILING OF UTERINE SEROUS PAPILLARY CARCINOMAS AND OVARIAN SEROUS PAPILLARY TUMORS

(57) Abstract: Oligonucleotide microarrays were used to profile and compare gene expression patterns between uterine serous papillary carcinoma and ovarian serous papillary carcinoma or normal endometrial epithelial cells. mRNA fingerprints readily distinguish the more biologically aggressive and chemotherapy resistant USPC from OSPC or NEC. *Plasminogen activator inhibitor* is the most highly up-regulated gene in OSPC relative to USPC, whereas the *c-erbB2* gene product (HER-2/neu) is strikingly overexpressed in USPC relative to OSPC and may therefore represent a novel diagnostic and therapeutic marker for this highly aggressive subset of endometrial tumors.



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**GENE EXPRESSION PROFILING OF UTERINE SEROUS PAPILLARY
CARCINOMAS AND OVARIAN SEROUS PAPILLARY TUMORS**

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BACKGROUND OF THE INVENTION

10 Field of the Invention

The present invention relates generally to the field of cancer research. More specifically, the present invention relates to gene expression profiling of uterine serous papillary carcinomas and ovarian serous papillary tumors.

15 Description of the Related Art

Ovarian serous papillary cancer (OSPC) represents the most common histological type of ovarian carcinoma and is the fourth leading cause of cancer-related death in women in the United States. Endometrial cancer is the most frequent cancer of the female genital tract with endometrioid (type 1) and serous papillary (type 2) being the most common cell types. Histologically indistinguishable to high grade serous ovarian carcinoma, uterine serous papillary cancer (USPC) has a propensity for early intra-abdominal, lymphatic and distant metastatic spread even at presentation and is characterized by a highly aggressive biological behavior. Unlike ovarian serous papillary cancer which is responsive to first line combined cisplatinum-based chemotherapy in 70% to 80% of the cases, uterine serous papillary cancer is a chemotherapy-resistant disease from outset, with responses to cytostatic agents in the order of 20% and of short duration.

Gene expression fingerprints representing large numbers of genes have the potential to allow precise and accurate grouping of tumors endowed with similar phenotype. Gene microarrays may identify cancers endowed with a more aggressive biologic behavior (i.e., rapidly metastatic tumors) that are unresponsive to standard adjuvant therapies and may thus allow improved prediction of response and clinical

outcome. Consistent with this view, in large B-cell lymphomas and breast carcinomas, gene expression profiles have been shown to identify patients who are unlikely to be cured by conventional therapy.

5 In ovarian carcinoma, cDNA microarray technology has recently been used to identify numerous genes differentially expressed in normal and tumor derived ovarian epithelial cells. Interestingly, several of the most up-regulated genes encode surface or secreted proteins, such as Kop, SLPI and claudin-3, making these products attractive candidate biomarkers. In contrast, very little is known about the possible genetic diversity between ovarian serous papillary cancer and uterine serous papillary cancer, two histologically similar serous carcinomas characterized by a dramatically different biological behavior and response to chemotherapy. Thus, the prior art is deficient in understanding the molecular basis of the differences between ovarian serous papillary cancer and uterine serous papillary cancer. The present invention fulfills this need in the art by providing gene expression profiling for these two types of cancer.

SUMMARY OF THE INVENTION

20 High grade ovarian serous papillary cancer (OSPC) and uterine serous papillary carcinoma (USPC) represent two malignancies that are histologically indistinguishable but are characterized by markedly different biological behavior and response to chemotherapy. Understanding the molecular basis of these differences may significantly refine differential diagnosis and management, and may lead to development of novel, more specific and more effective treatment modalities for ovarian serous papillary cancer and uterine serous papillary carcinoma.

25 In the present invention, an oligonucleotide microarray with probe sets complementary to >10,000 human genes was used to determine whether patterns of gene expression may differentiate ovarian serous papillary carcinoma from uterine serous papillary carcinoma.

30 Unsupervised analysis of gene expression in ovarian serous papillary cancer and uterine serous papillary carcinoma identified 116 genes that exhibited > 2-fold differences ($p < 0.05$) and that readily distinguished ovarian serous papillary

cancer from uterine serous papillary carcinoma. *Plasminogen activator inhibitor (PAI-2)* was the most highly overexpressed gene in ovarian serous papillary carcinoma when compared to uterine serous papillary carcinoma, while *c-erbB2* was the most strikingly overexpressed gene in uterine serous papillary carcinoma when compared to ovarian serous papillary carcinoma. Over-expression of the *c-erbB2* gene and its expression product (i.e., HER-2/neu receptor) was validated by quantitative real-time PCR as well as by flow cytometry on primary uterine serous papillary carcinoma and ovarian serous papillary carcinoma, respectively. Immunohistochemical staining of serous tumor samples from which primary ovarian serous papillary cancer and uterine serous papillary carcinoma cultures were derived further confirmed HER-2/neu as a novel molecular diagnostic and therapeutic marker for uterine serous papillary carcinoma.

In conclusion, gene expression fingerprints have the potential to predict the anatomical site of tumor origin and readily identify the biologically more aggressive uterine serous papillary carcinoma from ovarian serous papillary cancer. A therapeutic strategy targeting HER-2/neu may be beneficial in patients harboring chemotherapy-resistant uterine serous papillary carcinoma.

Moreover, unsupervised analysis of mRNA fingerprints readily distinguished uterine serous papillary carcinoma from normal endometrial epithelial cells and identified 139 and 390 genes that exhibited >5-fold up-regulation and down-regulation, respectively, in primary uterine serous papillary carcinoma compared to normal endometrial epithelial cells. Many of the genes up-regulated in uterine serous papillary carcinoma were found to represent oncogenes, adhesion molecules, and secreted proteins such as L1 cell adhesion molecule (L1CAM), claudin 3 and claudin-4, kallikrein 6 (protease M) and kallikrein 10, (NES1), interleukin-6, interleukin-18, urokinase plasminogen activator receptor (UPAR), and *c-erbB2*. Down-regulated genes in uterine serous papillary carcinoma included *transforming growth factor beta receptor III*, *platelet-derived growth factor receptor alpha*, *SEMACAP3*, *ras homolog gene family, member I (ARHI)*, and *differentially downregulated in ovarian carcinoma 1 (DOC1)*. High affinity epithelial receptor for *Clostridium perfringens* enterotoxin (CPE) claudin 4 was further validated through immunohistochemical analysis as a novel therapeutic marker for uterine serous papillary carcinoma. The results

presented herein, which were obtained with highly purified primary tumor cultures, highlight novel molecular features of uterine serous papillary carcinoma and provide foundation for the development of new type-specific therapies against this highly aggressive variant of endometrial cancer.

5 Other and further aspects, features, and advantages of the present invention will be apparent from the following description of the presently preferred embodiments of the invention. These embodiments are given for the purpose of disclosure.

10 BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows hierarchical clustering of 59 genes with differential expression between 6 ovarian serous papillary cancer and 5 uterine serous papillary carcinoma groups ($P < 0.05$) using a two-fold threshold. The cluster is color-coded
15 using red for up-regulation, green for down-regulation, and black for median expression. Agglomerative clustering of genes was illustrated with dendrograms. The symbol for each gene is followed by the Affymetrix clone number of the corresponding oligonucleotide spotted on the array. Expression ratios comparing the two groups to each other are listed.

20 **Figure 2** shows hierarchical clustering of 116 genes with differential expression between 5 ovarian serous papillary cancer and 5 uterine serous papillary carcinoma groups ($P < 0.05$) using a two-fold threshold. The cluster is color-coded using red for up-regulation, green for down-regulation, and black for median expression. Agglomerative clustering of genes was illustrated with dendrograms. The
25 symbol for each gene is followed by the Affymetrix clone number of the corresponding oligonucleotide spotted on the array. Expression ratios comparing each of the two groups to each other are listed.

Figure 3 shows quantitative real-time PCR and microarray expression analysis of *PAI-2* (*SERPINB2*) and *c-erbB2* (*ERBB2*). These two genes are
30 differentially expressed between ovarian serous papillary cancer and uterine serous papillary carcinoma.

Figure 4 shows FACS analysis of Herceptin™ staining of 3 primary

ovarian serous papillary cancer and 3 uterine serous papillary carcinoma cell lines. Data with Herceptin™ are shown in solid black while isotype control monoclonal antibody profiles are shown in white. HER-2/neu expression was significantly higher on uterine serous papillary carcinoma cell lines compared to ovarian serous papillary cancer cell lines ($p < 0.001$ by student t test).

Figure 5 shows immunohistochemical staining for HER-2/neu expression on 3 paraffin embedded ovarian serous papillary cancer (OSPC) and 3 uterine serous papillary carcinoma (USPC) specimens. OSPC1, OSPC2 and OSPC3 (right panel) showed negative or light (1+) staining for HER-2/neu. USPC1, USPC3, and USPC4 (left panel), showed heavy (3+) staining for HER-2/neu. Original magnification 400X.

Figure 6 shows unsupervised hierarchical clustering of fifteen primary uterine cell lines (i.e., 10 uterine serous papillary carcinoma and 5 normal endometrial epithelial cultures). The cluster is color-coded using red for up-regulation, green for down-regulation, and black for median expression. Agglomerative clustering of genes is illustrated with dendrograms.

Figure 7 shows quantitative real-time PCR and microarray expression analysis of *CDKN2A/p16*, *CDKN2A/p14ARF*, *L1CAM*, *claudin 3*, *claudin 4*, *GRB-7* and *c-erbB2* genes differentially expressed between uterine serous papillary carcinoma and normal endometrial epithelial cells.

Figure 8 shows representative immunohistochemical staining for claudin-4 of 2 paraffin-embedded uterine serous papillary carcinoma (USPC) and 1 normal endometrial epithelial cell (NEC) specimens. NEC 1 (upper panel) showed light membrane staining for claudin-4 while USPC 1 and USPC 3 showed heavy cytoplasmic and membranous staining for claudin-4 (middle and lower panel). Original magnification 400X.

DETAILED DESCRIPTION OF THE INVENTION

Advanced and/or metastatic serous papillary gynecologic tumors, regardless of their ovarian or uterine origin, are currently treated as a single disease. However, uterine serous papillary carcinoma is likely derived from metaplastic

Mullerian epithelium, whereas ovarian serous papillary cancer is likely derived from the ovarian surface epithelium. Dramatic difference in response to standard cisplatin-based chemotherapy is commonly reported for these histologically indistinguishable serous carcinomas. Hence, it is probable to have significant diversity in gene expression between these two types of tumors.

Ovarian Serous Papillary Carcinoma vs. Uterine Serous Papillary Carcinoma

All 5 uterine serous papillary cancer patients evaluated in this study either developed progressive disease during chemotherapy or recurred within six months from the end of treatment. In contrast, four out of five of the ovarian serous papillary cancer patients responded completely to standard adjuvant chemotherapy treatment. To study differential gene expression in highly enriched populations of epithelial tumor cells, short-term primary tumor cultures were used in the following study to minimize the risk of a selection bias inherent in any long term *in vitro* growth.

Hierarchical clustering of gene expression levels within the samples led to unambiguous separation of ovarian serous papillary carcinoma from uterine serous papillary carcinoma. Two hundred twenty seven genes were differentially expressed between ovarian serous papillary carcinoma and uterine serous papillary carcinoma, with 116 genes having at least two-fold differences between the two groups. Of interest, OSPC2, the only ovarian serous papillary cancer patient with mixed clear cell features included in the present analysis, clustered with uterine serous papillary carcinoma. These data are congruent with a recent report that clear cell ovarian tumors present a distinctive molecular signature from pure high grade ovarian serous papillary cancer. Thus, the following findings add to previous knowledge showing that clear cell tumors, a variant of ovarian cancer with a particularly unfavorable prognosis, express a molecular signature closer to that of the more aggressive uterine serous papillary carcinoma.

Lysophosphatidic Acid

Some of the differentially expressed genes may have diagnostic and therapeutic applications. For example, elevated serum levels of lysophosphatidic acid

(LPA) are found in more than 90% of ovarian cancer patients and the level of lysophosphatidic acid in plasma has been proposed as a potential biomarker for this disease. In addition, lysophosphatidic acid signaling may have a role in the progression of ovarian cancer cells through stimulation of cellular proliferation, enhanced cellular survival and suppression of apoptosis. It seems therefore likely that the higher lysophosphatidic acid receptor expression found in ovarian serous papillary cancer relative to uterine serous papillary carcinoma may represent a distinctive marker that plays a role in transduction of growth-promoting signals from high local concentrations of lysophosphatidic acid. Consistent with this view, phospholipase C, another gene that is differentially overexpressed in ovarian serous papillary cancer relative to uterine serous papillary carcinoma, has been previously reported to contribute to lysophosphatidic acid production in ovarian cancer cells.

Plasminogen Activator Inhibitor-2

Plasminogen activator inhibitor-2 (PAI-2), a protein capable of inhibiting invasion, may represent a molecular biomarker for several human tumors including ovarian carcinomas. Over-expression of plasminogen activator inhibitor-2 in epithelial ovarian cancer has been previously identified as a favorable prognostic factor. Indeed, high plasminogen activator inhibitor-2 expression in invasive ovarian tumors seems to be limited to a group of ovarian serous papillary cancer patients which experience a more prolonged disease-free and overall survival. High expression of plasminogen activator inhibitor-2 in ovarian serous papillary cancer relative to uterine serous papillary carcinoma may indicate a biologically less aggressive disease.

Glypican-1 And Syndecan-2

Membrane-associated heparan sulfate proteoglycans are thought to play important roles in many aspects of cell behavior, including cell-cell and cell-extracellular matrix adhesion and growth factor signaling. Two families of polypeptides appear to carry the majority of heparan sulfate on mammalian cells: glypicans, which are attached to the plasma membrane via glycosylphosphatidylinositol (GPI) anchors, and syndecans, which are transmembrane proteins. Convincing evidence has recently been provided that

glypican-1 can interact with FGF-2 and stimulate signaling of the FGF receptor. Importantly, whereas *glypican-1* and *FGF receptor 2* genes were expressed at higher levels in ovarian serous papillary cancer relative to uterine serous papillary carcinoma, *syndecan-2* gene expression was significantly higher in uterine serous papillary carcinoma as compared to ovarian serous papillary cancer. These data therefore support a major difference in the expression of heparan sulfate proteoglycans between these two tumor types. Furthermore, because bFGF is produced by ovarian serous papillary cancer cells and can bind to FGF receptor 2 expressed on these tumors, the combined overexpression of *glypicans* and *FGF receptor 2* genes found in ovarian serous papillary cancer cells may represent a common molecular abnormality with important functional consequences for the progression of ovarian serous papillary cancer.

Insulin Receptor

Over-expression of the insulin receptor by ovarian serous papillary cancer cells has been reported and is able to mediate a proliferative response in ovarian cancer cells. Results reported herein indicate that ovarian serous papillary cancer cells differentially overexpressed the insulin receptor gene relative to uterine serous papillary carcinoma. These results suggest a role for insulin receptor in the growth and regulation of ovarian serous papillary cancer cells, but not in uterine serous papillary carcinoma.

c-erbB2

Unlike ovarian serous papillary cancer, there have been few studies aimed at identifying molecular markers characteristic of uterine serous papillary carcinoma. Of particular interest, the *c-erbB2* gene was the most highly overexpressed gene in uterine serous papillary carcinoma with over 17-fold up-regulation relative to ovarian serous papillary cancer cells. Furthermore, *GRB7*, a gene tightly linked to *c-erbB2* and previously reported to be co-amplified and co-expressed with *c-erbB2* in several cancer types was also highly differentially expressed in uterine serous papillary carcinoma compared to ovarian serous papillary cancer cells. The striking overexpression of the *c-erbB2* gene product (HER-2/neu) on uterine serous papillary

carcinoma may therefore represent a distinctive molecular marker for these tumors and may also provide insights into the disproportionately poor prognosis of uterine serous papillary carcinoma relative to ovarian serous papillary cancer. Consistent with this view, previous studies have reported that amplification of this gene in a subset of ovarian cancer patients is associated with resistance to chemotherapeutic drugs and shorter survival. High overexpression of the *c-erbB2* gene strongly suggests that Herceptin™ monoclonal antibody, a humanized anti-HER-2/neu antibody that has shown great promise for treatment of metastatic breast cancers overexpressing HER-2/neu protein, may be a novel and potentially highly effective treatment option for uterine serous papillary carcinoma.

Collectively, the data presented herein show that ovarian serous papillary cancer and uterine serous papillary carcinoma, two diseases where further molecular characterization is needed to improve differential diagnosis and therapeutic strategies, can be readily discriminated by gene expression profiles. These findings suggest that global gene expression signatures can be an important adjunct to the morphology-based classification schemes for serous papillary tumors currently used.

Uterine Serous Papillary Carcinoma vs. Normal Endometrial Epithelium

Hierarchical clustering of gene expression levels led to unambiguous separation of uterine serous papillary carcinoma from normal endometrial epithelial cells (NEC), with 529 genes having at least five-fold differences between the two groups. The known function of some of these genes may provide insights to the molecular pathogenesis and the highly aggressive biologic behavior of uterine serous tumors while others may prove to be useful diagnostic and therapeutic markers against this disease.

Cyclin-Dependent Kinase Inhibitor 2A

The *cyclin-dependent kinase inhibitor 2A* (*CDKN2A*) gene was found to be the most highly differentially expressed gene in uterine serous papillary carcinoma with over 101-fold up-regulation relative to normal endometrial epithelial cells. Importantly, *CDKN2A* gene is a putative oncosuppressor gene encoding two unrelated cellular growth inhibitors in different reading frames. One is p16, which regulates

retinoblastoma protein (pRb)-dependent G1 arrest, and the second is p14ARF, which blocks MDM2-induced p53 degradation resulting in an increase in p53 levels that leads to cell cycle arrest. Although loss of p53 function is considered critical for the molecular pathogenesis of uterine serous papillary carcinoma, it is only recently that abnormality of the Rb pathway was suggested to define a subgroup of aggressive endometrial carcinomas with poor prognosis. Quantitative real-time PCR results investigating the expression of both *p16* and *p14ARF* in the uterine serous papillary carcinoma series described below showed extremely high levels of both transcripts, suggesting that the marked overexpression of the *CDKN2A* gene may be attributable to a negative feedback loop due to the loss of function of both pRb and p53 proteins. Consistent with this view, an inverse relationship between expression of p16 and p14ARF proteins and the presence of normal or functional Rb and p53 in human cancer cells has been previously demonstrated. Thus, the data presented below suggest for the first time that *CDKN2A* gene overexpression may represent a consistent genetic anomaly of uterine serous papillary carcinoma secondary to an auto-regulatory feedback loop due to a disruption of both the p16-CDK4/cyclin D1-pRb pathway (RB pathway) and the p14ARF-MDM2-p53 pathway (p53 pathway).

Lipocalin-2

Lipocalin-2 has not been previously linked to uterine cancer. *Lipocalin-2* represents an interesting marker because of several features. Lipocalins are extracellular carriers of lipophilic molecules such as retinoids, steroids, and fatty acid, all of which may play important roles in the regulation of epithelial cell growth. In addition, because lipocalin is a secreted protein, it may play a role in the regulation of cell proliferation and survival. Of interest, two recent publications on gene expression profiling of breast and pancreatic cancer have proposed *lipocalin-2* as a novel therapeutic and diagnostic marker for prevention and treatment of these diseases. The present invention indicates that *lipocalin-2* may be added to the known markers for uterine serous papillary carcinoma.

Claudin-3 And Claudin-4

Genes encoding tight junction proteins claudin-3 and claudin-4 were

consistently found as two of the most highly up-regulated genes in uterine serous papillary carcinoma with over 8 and 12-fold up-regulation, respectively. *Claudin-3* and *claudin-4* overexpression have not been previously linked to uterine serous papillary carcinoma. Although the exact function of claudin-3 and claudin-4 in uterine serous papillary carcinoma is still unclear, claudin-3 and claudin-4 have recently been shown to represent the epithelial receptors for *Clostridium perfringens* enterotoxin (CPE), and to be the only family members of the transmembrane tissue-specific claudin proteins capable of mediating CPE binding and cytolysis. Because CPE may trigger a multistep mechanism leading to efficient lysis of mammalian target cells overexpressing *claudin-3* and *claudin-4*, CPE-mediated therapy might be a novel, potentially highly effective strategy for the treatment of uterine serous papillary carcinoma refractory to chemotherapy as well as other human tumors overexpressing *claudin-3* and/or *claudin-4*. Consistent with this view, treatment-resistant prostate cancer and pancreatic cancer, two human malignancies characterized by an extremely poor prognosis, have already been shown to be potentially responsive *in vitro* as well as *in vivo* to CPE-mediated therapy. Protein expression data obtained by immunohistochemistry with anti-claudin-4 antibody on uncultured uterine serous papillary carcinoma blocks further support this view.

Kallikrein 6 And Kallikrein 10

The organization of kallikreins, a gene family now consisting of 15 genes which encode for trypsin-like or chymotrypsin-like serine proteases, has been recently elucidated. Serine proteases have been described to have well characterized roles in diverse cellular activities, including blood coagulation, wound healing, digestion, and immune responses, as well as tumor invasion and metastasis. Importantly, because of the secreted nature of some of these enzymes, *prostate-specific antigen (PSA)* and *kallikrein 2* have already found important clinical application as prostate cancer biomarkers. Of interest, *kallikrein 6* (also known as *zyme/protease M/neurosin*) and *kallikrein-10 (NES1)*, two serine proteases recently shown to be present at high levels in the circulation of a subset of ovarian cancer patients, were found to be highly differentially expressed in uterine serous papillary carcinoma when compared to normal endometrial epithelial cells. Both *kallikrein 6*

and *kallikrein 10* overexpression have been shown to correlate with intrinsic resistance to adjuvant chemotherapy and with poor prognosis in ovarian cancer patients. These data are thus consistent with the results presented herein showing high expression of *kallikreins 6* and *kallikrein 10* in uterine serous papillary carcinoma, a variant of endometrial carcinoma characterized by an aggressive biologic behavior and an inborn resistance to chemotherapy. Importantly, these results further emphasize the view that *kallikreins 6* and *kallikrein 10* have the potential to become novel cancer markers for early diagnosis and/or monitoring of uterine serous papillary carcinoma as well as possible immunotherapeutic targets of vaccination strategies against recurrent/refractory serous papillary gynecologic tumors.

c-erbB2

c-erbB2 gene was found to be one of the most highly differentially expressed genes in uterine serous papillary carcinoma with over 14-fold up-regulation compared with normal endometrial epithelial cells. Furthermore, the *growth factor receptor-bound protein 7 (GRB7)*, a gene tightly linked to *c-erbB2* and previously reported to be co-amplified and co-expressed with this gene in several cancer types, was also highly differentially expressed in uterine serous papillary carcinoma compared to normal endometrial epithelial cells. These data are in agreement with recent discovery of a striking overexpression of the *c-erbB2* gene as well as of its gene expression product HER2/neu on 60 to 80% of pure uterine serous papillary carcinoma. Therefore, HER2/neu overexpression may represent a distinctive molecular marker for this highly aggressive subset of endometrial tumors. Overexpression of the *c-erbB2* gene on uterine serous papillary carcinoma provides support for the notion that trastuzumab (Herceptin™, Genentech, San Francisco, CA), a humanized anti-HER-2/Neu antibody that is showing great promise for treatment of metastatic breast cancer patients overexpressing HER-2/Neu protein, may be a novel and potentially highly effective therapy against this aggressive variant of serous papillary carcinomas. Consistent with this view, uses of Herceptin™ in uterine serous papillary carcinoma patients have been reported with high sensitivity of uterine serous papillary carcinoma to the killing activity mediated by natural killer cells when triggered by anti-HER-2/Neu-specific antibody.

L1 Adhesion Molecule

L1 adhesion molecule (L1CAM), a 200-220 kD type I membrane glycoprotein of the immunoglobulin family, has been shown to play an important function in the development of nervous system by regulating cell adhesion and migration. Although initially characterized and most extensively studied in the nervous system, L1CAM has been recently reported to be expressed on a variety of human tumor cell lines such as neuroblastomas, melanomas, and lung carcinomas. Because overexpression of L1CAM by tumor cells may enhance cell migration on various extracellular membrane substrates, this molecule has been suggested to play a crucial role in the adhesion and migration events crucial for tumor spreading. Data presented herein indicate that *L1CAM* is one of the most highly differentially expressed genes in uterine serous papillary carcinoma with over 25-fold up-regulation relative to normal endometrial epithelial cells. These data, together with the results presented below, further support using *L1CAM* as a novel biomarker for predicting clinical outcome in uterine serous papillary carcinoma patients.

Urokinase Plasminogen Activator Receptor

Urokinase plasminogen activator receptor (UPAR) is a glycosyl-phosphatidylinositol-anchored glycoprotein whose role in promoting tumor cell invasion and metastases has been well established in a number of experimental studies. Consistent with this view, urokinase plasminogen activator receptor has been shown to regulate membrane-associated plasmin activity, facilitating cellular movement for tumor-cell invasion, chemotaxis, and cellular adhesion in a variety of human tumors. Furthermore, a direct correlation between *urokinase plasminogen activator receptor* expression and a higher invasive and metastatic potential in several human tumors has been previously reported. However, it is only recently that an abnormal *urokinase plasminogen activator receptor* expression in endometrial cancer has been positively correlated with the grade of disease, and in particular, with uterine serous papillary carcinoma phenotype. The present gene expression profiling results show that *urokinase plasminogen activator receptor* is one of the most highly differentially expressed genes in uterine serous papillary carcinoma with over 7-fold up-regulation relative to normal endometrial epithelial cells. Of interest, because urokinase

plasminogen activator receptor protein exists in two forms, as the glycosyl-phosphatidylinositol-anchored glycoprotein (50-60 kDa) present on cell surface, and as a soluble form of UPAR (sUPAR) produced after cleavage of urokinase plasminogen activator receptor by urokinase (35 kDa), measurement of urokinase plasminogen activator receptor levels by ELISA, in analogy to breast cancer, may have potential as prognostic marker to identify early recurrences in endometrial cancer patients associated with poor outcome. Finally, the recent demonstration of urokinase plasminogen activator receptor as a suitable cancer target for both therapeutic and diagnostic application by specific antibody directed against its ligand binding domain may provide a foundation for developing a new type-specific therapy against this highly aggressive disease.

Down-Regulated Genes

The present invention identifies a large number of down-regulated (at least 5-fold) genes in uterine serous papillary carcinoma versus normal endometrial epithelial cells. These genes include *transforming growth factor beta receptor III*, *platelet-derived growth factor receptor alpha*, *SEMACAP3*, *ras homolog gene family member I (ARHI)*, and *differentially downregulated in ovarian carcinoma 1 (DOC1)*. Some of these genes are tumor suppressor genes such as *SEMACAP3*, *ARHI*, and *DOC1*, whereas others encode for proteins important for tissue homeostasis or that have been previously implicated in apoptosis, proliferation, adhesion or tissue maintenance.

In conclusion, multiple uterine serous papillary carcinoma-restricted markers have been identified in the present invention. The identification of HER2/neu and *Clostridium perfringens* enterotoxin epithelial receptors as some of the most highly differentially expressed genes in uterine serous papillary carcinoma when compared to normal endometrial epithelial cells suggest that therapeutic strategies targeting HER2/neu by monoclonal antibodies or claudin 3 and claudin 4 by local and/or systemic administration of *Clostridium Perfringens* enterotoxin may represent novel potentially effective modalities for the treatment of patients harboring this highly aggressive and chemotherapy-resistant variant of endometrial cancer.

Thus, the present invention provides a method of detecting ovarian

serous papillary carcinoma. The method involves performing statistical analysis on the expression levels of a group of genes listed in Table 2. Included in this group of genes are *plasminogen activator inhibitor-2*, *fibroblast growth factor receptor-2*, *glypican 1*, *lysophosphatidic acid receptor*, *phospholipase C*, *glucose-6-phosphate dehydrogenase*, and *insulin receptor*. Over-expression of these genes would indicate that such individual has ovarian serous papillary carcinoma. Gene expression can be examined by a number of standard techniques in the art, e.g. DNA microarray and hierarchical cluster analysis. In general, gene expression can be examined at the protein or RNA level.

In another embodiment of the present invention, there is provided a method of detecting uterine serous papillary carcinoma based on overexpression of a group of genes listed in Tables 3 and 5. Included in these group of genes are *epidermal growth factor type 2 receptor*, *inhibin*, *multiple endocrine neoplasia*, *growth factor receptor-bound protein 7*, *BCL2*, *E-cadherin*, *syndecan*, *cyclin-dependent kinase inhibitor 2A*, *lipocalin-2*, *L1 cell adhesion molecule (L1CAM)*, *claudin 3*, *claudin-4*, *kallikrein 6 (protease M)*, *kallikrein 10*, *interleukin-6*, *interleukin-18*, and *urokinase plasminogen activator receptor (UPAR)*.

In another embodiment of the present invention, there is provided a method of detecting uterine serous papillary carcinoma based on down-regulation of a group of genes listed in Table 6. Included in this group of genes are *transforming growth factor beta receptor III*, *platelet-derived growth factor receptor alpha*, *SEMACAP3*, *ras homolog gene family member I (ARHI)*, and *differentially down-regulated in ovarian carcinoma 1 (DOC1)*.

In yet another embodiment of the present invention, there is provided a method of treating uterine serous papillary carcinoma by inhibiting the expression or function of *epidermal growth factor type 2 receptor (c-erbB2)*. In general, inhibition of gene expression can be obtained using anti-HER2/neu antibody or anti-sense oligonucleotide according to standard protocols generally available in the art.

The following examples are given for the purpose of illustrating various embodiments of the invention and are not meant to limit the present invention in any fashion. One skilled in the art will appreciate readily that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as

well as those objects, ends and advantages inherent herein. Changes therein and other uses which are encompassed within the spirit of the invention as defined by the scope of the claims will occur to those skilled in the art.

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EXAMPLE 1**Establishment of Ovarian Serous Papillary Cancer And Uterine Serous Papillary Carcinoma Primary Cell Lines**

Eleven primary serous papillary cell lines (6 ovarian serous papillary carcinoma and 5 uterine serous papillary carcinoma) were established after sterile processing of the tumor samples from surgical biopsies. Tumors were staged according to the F.I.G.O. operative staging system. Total abdominal hysterectomy and regional lymph node sampling for invasive uterine serous papillary carcinoma were performed in all cases. Radical tumor debulking, including a total abdominal hysterectomy and omentectomy, was performed in all ovarian carcinoma patients. No patient received chemotherapy before surgical therapy. The patient characteristics are described in Table 1. The epithelial nature and the purity of uterine serous papillary carcinoma and ovarian serous papillary carcinoma cultures was verified by immunohistochemical staining and flow cytometric analysis with antibodies against cytokeratin as described (Ismail et al., 2000; Satin et al., 2000, 2002). Only primary cultures which had at least 90% viability and contained >99% tumor cells were used for total RNA extraction.

TABLE 1
Characteristics of Patients Disclosed In Example 1

Patient	Age	Race	Stage	Chemotherapy regimen
USPC 1	66	Afro-American	IV B	TAX+CARB
USPC 2	77	White	III C	TAX+CARB
USPC 3	61	Afro-American	III C	TAX+CARB
USPC 4	62	Afro-American	III C	TAX+CARB
USPC 5	63	Afro-American	III C	TAX+CARB
OSPC 1	42	White	III C	TAX+CIS
OSPC 2	43	White	III C	TAX+CARB
OSPC 3	34	White	III C	TAX+CARB
OSPC 4	51	White	III C	TAX+CARB
OSPC 5	59	Afro-American	III B	TAX+CARB
OSPC 6	52	White	III C	TAX+CARB

5 USPC, Uterine Serous Papillary Carcinoma; OSPC, Ovarian Serous Papillary Carcinoma.

EXAMPLE 2

Microarray Hybridization and Analysis

10 RNA purification, cDNA synthesis, cRNA preparation, and hybridization to the Affymetrix Human U95Av2 GeneChip microarray were performed according to the manufacturer's protocols and as reported (Zhan et al., 2002).

15 All data used in the analyses 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 a detection of present, marginal, or absent signals as

determined by the GeneChip 5.0 algorithm. Gene arrays were scaled to an average signal of 1500 and then analyzed independently. Signal calls were transformed by the log base 2 and each sample was normalized to give a mean of 0 and variance of 1.

Statistical analyses of the data were performed with the software package SPSS10.0 (SPSS, Chicago, IL). The first test applied was the detection. In each comparison, genes having "present" detection calls in more than half of the samples in the overexpressed gene group were retained. To compare gene expression levels, the nonparametric Wilcoxon rank sum (WRS) test ($p < 0.05$) was applied to the normalized signal call. By combining the detection and WRS data, differentially expressed genes were identified between ovarian serous papillary carcinoma and uterine serous papillary carcinoma.

The hierarchical clustering of average-linkage method with the centered correlation metric was used (Eisen et al., 1998). The dendrogram was constructed with a subset of genes from 12,588 probe sets present on the microarray, whose expression levels vary the most among the 11 samples, and thus most informative. For the hierarchical clustering shown in Figure 1 and Figure 2, only genes significantly expressed and whose average change in expression level was at least two-fold were chosen. The expression value of each selected gene was re-normalized to have a mean of zero.

EXAMPLE 3

Gene Expression Profiles Distinguish Ovarian Serous Papillary Carcinoma from Uterine Serous Papillary Carcinoma

Flash frozen biopsies from ovarian and uterine tumor tissue are known to contain significant numbers of contaminant stromal cells as well as a variety of host derived immune cells (e.g., monocytes, dendritic cells, lymphocytes). Short term primary tumor cell cultures minimize the risk of a selection bias inherent in any long term *in vitro* growth and provide an opportunity to study differential gene expression between relatively pure populations of tumor cells. Thus, comprehensive gene expression profiles of 6 primary ovarian serous papillary carcinoma and 5 primary uterine serous papillary carcinoma cell lines were generated using high-density oligonucleotide arrays with 12,588 probe sets, which in total interrogated some 10,000

genes. One hundred sixty five genes were differentially expressed between ovarian serous papillary carcinoma and uterine serous papillary carcinoma (WRS test, $p < 0.05$).

Figure 1 shows the cluster analysis performed on hybridization intensity values for 59 gene segments whose average difference in expression level was at least two-fold. Two major branches on the dendrogram were identified. All 5 uterine serous papillary carcinoma were grouped together in the leftmost columns. Similarly, in the rightmost columns all 5 pure ovarian serous papillary carcinoma were found to cluster tightly together. Of interest, OSPC2, a serous papillary tumor with mixed clear cell features (i.e., a biologically aggressive variant of ovarian cancer characterized by a poor prognosis) clustered on a sub-branch with uterine serous papillary carcinoma (Figure 1).

Figure 2 shows the cluster analysis on hybridization intensity values for each gene in ten primary cultures of ovarian serous papillary carcinoma and uterine serous papillary carcinoma. By combining the detection levels of genes significantly expressed in ovarian serous papillary carcinoma and uterine serous papillary carcinoma, 227 genes were differentially identified between the two cancer types using the nonparametric WRS test ($p < 0.05$). The dendrogram shown in Figure 2 depicts a marked separation in the expression profiles of the two groups of serous papillary tumors, with 116 gene segments whose average change in expression level was at least two-fold.

The tight clustering of pure ovarian serous papillary carcinoma from uterine serous papillary carcinoma was "driven" by two distinct profiles of gene expression. The first was represented by a group of 40 genes that were highly expressed in ovarian serous papillary carcinoma and underexpressed in uterine serous papillary carcinoma (Table 2). Many genes shown previously to be involved in ovarian carcinogenesis are present on these lists, providing a degree of validity to our array analysis. Included in this group of genes are *plasminogen activator inhibitor-2* (PAI-2), *fibroblast growth factor receptor-2* (FGFR2), *glypican 1* (GPC1), *lysophosphatidic acid receptor* (EDG2), *phospholipase C* (PLCL2), *glucose-6-phosphate dehydrogenase* (G6PD), and *insulin receptor* (IGF1) (Table 2).

The second profile was represented by 76 genes that were highly

expressed in uterine serous papillary carcinoma and underexpressed in ovarian serous papillary carcinoma (Table 3). Included in this group of genes are *epidermal growth factor type 2 receptor (c-erbB2)*, *inhibin (INHBB)*, *multiple endocrine neoplasia I (MEN1)*, *growth factor receptor-bound protein 7 (GRB7)*, *BCL2*, *E-cadherin (CDH1)*, and *syndecan (SDC2)* (Table 3). Importantly, *c-erbB2* gene was the most highly differentially expressed gene in uterine serous papillary carcinoma when compared to ovarian serous papillary carcinoma (Table 3, Figure 2). OSPC2, the only serous tumor with mixed clear cell histology evaluated in the series, was also found to highly overexpress *c-erbB2*.

TABLE 2

Upregulated Genes Expressed At Least 2 Fold Higher In Ovarian Serous Papillary Carcinoma Compared With Uterine Serous Papillary Carcinoma

Probe Set Name	Gene Symbol	Map Location	p of WRS	Ratio Ov/Ut
37185_at	SERPINB2	18q21.3	0.00902344	21.2101742
40478_at	DJ971N18.2	20p12	0.0162936	7.391447995
38837_at	DJ971N18.2	20p12	0.047201768	6.933671714
34439_at	AIM2	1q22	0.00902344	6.689727463
36073_at	NDN	15q11.2-q12	0.028280124	6.460327167
859_at	CYP1B1	2p21	0.047201768	4.642443935
40387_at	EDG2	9q32	0.047201768	4.612620508
1669_at	WNT5A	3p21-p14	0.028280124	4.35214472
1363_at	FGFR2	10q26	0.0162936	3.958060853
1143_s_at			0.028280124	3.948020982
37816_at	C5	9q32-q34	0.0162936	3.945622621
40071_at	CYP1B1	2p21	0.047201768	3.826875845
38294_at	HOXD4	2q31-q37	0.028280124	3.804399853
33162_at	INSR	19p13.3-p13.2	0.047201768	3.772
34853_at	FLRT2	14q24-q32	0.047201768	3.471204819

Probe Set Name	Gene Symbol	Map Location	p of WRS	Ratio Ov/Ut
40395_at	PLXNA2	1q32.1	0.028280124	3.371729137
39805_at	ABCB6	2q36	0.047201768	3.369062784
41796_at	PLCL2	3p24.3	0.00902344	3.280007364
1403_s_at	SCYA5	17q11.2-q12	0.047201768	3.158368265
33929_at	GPC1	2q35-q37	0.028280124	3.15594993
39566_at	CHRNA7	15q14	0.047201768	3.14079953
34354_at	FGFR2	10q26	0.047201768	2.928346342
444_g_at	HOXD4	2q31-q37	0.047201768	2.892672123
38042_at	G6PD	Xq28	0.047201768	2.813117012
36077_at	RABL4	22q13.1	0.028280124	2.720984156
36453_at	KIAA0711	8p23.3	0.047201768	2.688792044
32668_at	SSBP2	5q14.1	0.047201768	2.663148439
32610_at	RIL	5q31.1	0.047201768	2.55031145
514_at	CBLB	3q13.12	0.028280124	2.511893491
40112_at	IDH3B	20p13	0.028280124	2.294973901
38271_at	HDAC4	2q37.2	0.028280124	2.245891142
1325_at	MADH1	4q28	0.047201768	2.228503651
32381_at	RORB	9q22	0.028280124	2.205852674
32800_at	RXRA	9q34.3	0.047201768	2.168594631
36312_at	SERPINB8	18q21.3	0.047201768	2.110497544
40142_at	DDX24	14q32	0.0162936	2.109997452
33227_at	IL10RB	21q22.11	0.047201768	2.082986437
32529_at	CKAP4	12q23.3	0.047201768	2.04858844
37280_at	MADH1	4q28	0.028280124	2.044781456
39709_at	SEPW1	19q13.3	0.028280124	2.017195806

TABLE 3

Upregulated Genes Expressed At Least 2 Fold Higher In Uterine Serous Papillary Carcinoma
Compared With Ovarian Serous Papillary Carcinoma

Probe Set Name	Gene Symbol	Map Location	p of WRS	Ratio Ut/Ov
1802_s_at	ERBB2	17q11.2-q12	0.028280124	17.39166248
39470_at			0.00902344	14.13960749
41470_at	PROML1	4p15.33	0.00902344	11.00274366
32521_at	SFRP1	8p12-p11.1	0.047201768	10.49619245
33218_at	ERBB2	17q11.2-q12	0.0162936	9.009761458
41354_at	STC1	8p21-p11.2	0.0162936	7.780569927
41700_at	F2R	5q13	0.028280124	7.299013748
38207_at	MEN1	11q13	0.028280124	6.578419265
36254_at	TAC1	7q21-q22	0.047201768	6.292979547
38268_at	SLC1A1	9p24	0.0162936	5.506571087
33576_at	KIAA0918	13q31.1	0.0162936	5.478319783
37883_i_at	AF038169	2q22.1	0.0162936	5.06566416
35704_at	HRASLS3	11q13.1	0.028280124	4.596441783
38267_at	SLC1A1	9p24	0.028280124	4.488128886
41376_i_at	UGT2B7	4q13	0.047201768	4.418941048
828_at	PTGER2	14q22	0.028280124	4.338041431
39506_at			0.028280124	4.313685637
1680_at	GRB7	17q12	0.047201768	4.262623744
38545_at	INHBB	2cen-q13	0.028280124	4.198823428
40679_at	SLC6A12	12p13	0.047201768	3.956969879
35912_at	MUC4	3q29	0.028280124	3.94095027
39966_at	CSPG5	3p21.3	0.047201768	3.918103678
32027_at	PDZK1	1q21	0.047201768	3.91484375

Probe Set Name	Gene Symbol	Map Location	p of WRS	Ratio Ut/Ov
31732_at	RLN2	9p24.1	0.0162936	3.913095715
36202_at	PKIA	8q21.11	0.047201768	3.89984472
37978_at	QPRT	16q13	0.0162936	3.845374532
994_at	PTPRM	18p11.2	0.047201768	3.812843137
37208_at	PSPHL	7q11.2	0.028280124	3.654717567
37884_f_at	AF038169	2q22.1	0.028280124	3.593346825
995_g_at	PTPRM	18p11.2	0.028280124	3.555706062
35985_at	AKAP2	9q31-q33	0.028280124	3.319448607
32963_s_at	RAGD	6q15-q16	0.00902344	3.280777993
33358_at	KIAA1157	12q13.13	0.0162936	3.250881457
311_s_at			0.0162936	3.138465417
35674_at	PADI2	1p35.2-p35.1	0.047201768	3.100307522
2021_s_at	CCNE1	19q12	0.028280124	3.081090355
32893_s_at	GGT2	22q11.23	0.047201768	3.055014721
36869_at	PAX8	2q12-q14	0.047201768	3.050015496
36508_at	GPC4	Xq26.1	0.0162936	2.887073572
39901_at	MYO7A	11q13.5	0.028280124	2.885983264
35148_at	TJP3	19p13.3	0.028280124	2.879832572
31892_at	PTPRM	18p11.2	0.047201768	2.844557651
36990_at	UCHL1	4p14	0.0162936	2.833524684
37209_g_at	PSPHL	7q11.2	0.047201768	2.780479031
38168_at	INPP4B	4q31.1	0.00902344	2.645321215
36943_r_at	PLAGL1	6q24-q25	0.0162936	2.57527834
37258_at	TMEFF1	9q31	0.047201768	2.55946924
36985_at	IDI1	10p15.3	0.047201768	2.538587569
39075_at	NEU1	6p21.3	0.0162936	2.521110072
40488_at	DMD	Xp21.2	0.00902344	2.507697552

Probe Set Name	Gene Symbol	Map Location	p of WRS	Ratio Ut/Ov
39332_at	TUBB	6p21.3	0.047201768	2.504487188
39757_at	SDC2	8q22-q23	0.047201768	2.452025072
933_f_at	ZNF91	19p13.1-p12	0.028280124	2.445525292
37210_at	INA	10q25.1	0.047201768	2.387532735
1860_at	TP53BP2	1q42.1	0.0162936	2.356857655
37869_at	ELKS	12p13.3	0.028280124	2.356300578
33878_at	FLJ13612	2q36.1	0.0162936	2.319659881
35143_at	DKFZP566A1 524		0.047201768	2.312331476
38997_at	SLC25A1	22q11.21	0.00902344	2.304275318
40077_at	ACO1	9p22-p13	0.028280124	2.297124855
36261_at	LOC51760	16p13.13	0.028280124	2.252602915
39436_at	BNIP3L	8p21	0.047201768	2.236567978
977_s_at	CDH1	16q22.1	0.00902344	2.212331718
36175_s_at	HIVEP2	6q23-q24	0.047201768	2.206300362
41269_r_at	API5	11p12-q12	0.0162936	2.189353711
1837_at			0.047201768	2.180124558
1818_at			0.047201768	2.177494716
366_s_at	NEK2	1q32.2-q41	0.047201768	2.157771457
40900_at			0.028280124	2.151464435
40194_at			0.028280124	2.133081444
41172_at	ARSDR1	14q23.3	0.0162936	2.113388456
37999_at	CPO	3q12	0.028280124	2.100322069
35978_at	PRRG1	Xp21.1	0.028280124	2.05552932
121_at	PAX8	2q12-q14	0.028280124	2.028946437
41715_at	PIK3C2B	1q32	0.00902344	2.024856688
41644_at	KIAA0790	6q24.3	0.047201768	2.004743183

EXAMPLE 4

Quantitative Real Time PCR Analysis of *PAI-2* And *c-erbB2* Expression

5 Quantitative real time PCR assays were used to validate the microarray data. The two most highly differentially expressed genes between uterine serous papillary carcinoma and ovarian serous papillary carcinoma (i.e., *PAI-2* and *c-erbB2*) were selected for the analysis.

 Quantitative real time PCR was performed with an ABI Prism 7000
10 Sequence Analyzer using the manufacturer's recommended protocol (Applied Biosystems, Foster City, CA). Each reaction was run in triplicate. The comparative threshold cycle (C_T) method was used for the calculation of amplification fold as specified by the manufacturer. Briefly, five mg of total RNA from each sample was reverse transcribed using SuperScript II Rnase H Reverse Transcriptase (Invitrogen,
15 Carlsbad, CA). Ten ml of reverse transcribed RNA samples (from 500 ul of total volume) were amplified by using the TaqMan™ Universal PCR Master Mix (Applied Biosystems) to produce PCR products specific for *PAI-2* and *c-erbB2*. Primers specific for 18s ribosomal RNA and empirically determined ratios of 18s competitors (Applied Biosystems) were used to control for the amounts of cDNA generated from
20 each sample. Differences among uterine serous papillary carcinoma and ovarian serous papillary carcinoma 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.

25 A comparison of the microarray and quantitative real time PCR data for the two genes is shown in Figure 3. Expression differences between tumor types for *PAI-2* ($p = 0.009$) and *c-erbB2* ($p = 0.02$) were readily apparent (Table 2 and 3). Moreover, for both genes tested, the quantitative real time PCR data were highly correlated to the microarray data ($p < 0.001$, $r = 0.91$ and 0.71 , respectively) as
30 estimated from 6 samples (i.e., 3 uterine serous papillary carcinoma and 3 ovarian serous papillary carcinoma). The quantitative real time PCR data mirror the microarray data both qualitatively and quantitatively, suggesting that most array probe

sets are likely to accurately measure the levels of the intended transcript within a complex mixture of transcripts.

EXAMPLE 5

5 Flow Cytometry Analysis of HER-2/neu Expression

To validate microarray data on primary uterine serous papillary carcinoma and ovarian serous papillary carcinoma cell lines at the protein level, HER-2/neu receptor expression was evaluated by flow cytometry on six primary serous papillary cell lines (3 uterine serous papillary carcinoma and 3 ovarian serous papillary carcinoma). The HER-2/neu MAb Herceptin™ (Genentech, San Francisco, CA) was used as the primary antibody. FITC-conjugated goat anti-human F(ab)² immunoglobulin was used as a secondary reagent (BioSource International, Camarillo, CA). Analysis was conducted with a FACScan, utilizing Cell Quest software (Becton Dickinson).

As positive and negative controls, breast cancer cell lines known to overexpress HER-2/neu (BT-474 and SK-BR-3, American Type Culture Collection), and Epstein-Barr virus-transformed lymphoblastoid cell lines (LCL) established from the same patients were also studied. High HER-2/neu receptor expression was found on all three primary uterine serous papillary carcinoma cell lines tested (100% positive cells for all three cell lines), with mean fluorescence intensity (MFI) ranging from 94 to 140 (Figure 4). In contrast, primary ovarian serous papillary carcinoma cell lines were found to express significantly lower levels of HER-2/neu (average MFI was ten-fold lower) than the uterine serous papillary carcinoma cells ($p < 0.001$) (Figure 4). These results show that high expression of the *c-erbB2* gene product by uterine serous papillary carcinoma correlates tightly with high protein expression by the tumor cells. Autologous LCL were consistently negative for HER-2/neu expression, while breast cancer cell lines expressed high levels of HER-2/neu.

EXAMPLE 6

30 Immunohistochemical Analysis of HER2/neu Expression

Formalin fixed tumor tissue blocks from six primary surgical specimens were tested for HER-2/neu expression by immunohistochemical staining. The

intensity of staining was graded as 0 (staining not greater than negative control), 1+ (light staining), 2+ (moderate staining) or 3+ (heavy staining).

Heavy staining for HER-2/neu protein expression (i.e., score 3+) was noted in all three uterine serous papillary carcinoma specimens that also overexpressed the *c-erbB2* gene product detected by microarray and flow cytometry (Figure 5). In contrast, negative or low (i.e., score 0 or 1+) staining was found in all 3 representative ovarian serous papillary carcinoma samples (Figure 5).

EXAMPLE 7

10 Establishing Primary Cell Lines of Uterine Serous Papillary Carcinoma And Normal Endometrial Epithelial Cells

A total of fifteen primary cell lines (i.e., 10 uterine serous papillary carcinoma cell lines and 5 normal endometrial epithelial cell lines) were established after sterile processing of samples from surgical biopsies collected from 1997 to 2003.

15 Tumors were staged according to the F.I.G.O. operative staging system. A total abdominal hysterectomy with bilateral salpingo oophorectomy and bilateral pelvic lymphadenectomy was performed in all uterine carcinoma patients while normal endometrial tissue was obtained from consenting donors undergoing surgery for benign pathology. No patient received chemotherapy or radiation before surgery. Patient
20 characteristics are described in Table 4. The cell lines were established as previously described for uterine serous papillary carcinoma specimens (Santin et al., 2002) and normal endometrial epithelial cell cultures (Bongso et al., 1988; Meresman et al., 2003). Briefly, normal tissue was obtained from healthy endometria mechanically minced and enzymatically dissociated with 0.14% collagenase Type I (Sigma, St.
25 Louis, MO) in RPMI 1640 as described previously by Bongso et al. (1988) with minor modifications. After 1-2 hrs incubation with enzymes on a magnetic stirring apparatus at 37°C in an atmosphere of 5% CO₂, the resulting suspension was collected by centrifugation at 100 g for 5-10 minutes and washed twice with RPMI 1640 medium (Sigma Chemical Co., St. Louis, MO) containing 10% fetal bovine serum
30 (FBS, Invitrogen, Grand Island, NY). The final pellet was then placed in fresh RPMI 1640 medium containing 10% fetal bovine serum (FBS, Invitrogen), 200 U/ml penicillin, and 200 µg/ml streptomycin in tissue culture flasks or Petri dishes

(Invitrogen). The epithelial explants were then allowed to attach and proliferate. Explants were trypsinized and subcultured for 1 to 2 passages before being collected for RNA extraction.

5 Tumor tissue was mechanically minced in RPMI 1640 to portions no larger than 1-3 mm³ and washed twice with RPMI 1640. The portions of minced tumor were then placed into 250 ml flasks containing 30 ml of enzyme solution [0.14% collagenase Type I (Sigma, St. Louis, MO) and 0.01% DNase (Sigma, 2000 KU/mg)] in RPMI 1640, and either incubated on a magnetic stirring apparatus for 1-2 hrs at 37°C in an atmosphere of 5% CO₂ or overnight at 4°C. Enzymatically
10 dissociated tumor was then filtered through 150 mm 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 U/ml penicillin, 200 µg/ml streptomycin at 37°C, 5% CO₂. Some of the primary uterine serous papillary carcinoma cell lines had been cultured *in vitro*
15 over 2-4 weeks, whereas others had been cultured over a much longer period of time. The epithelial nature and the purity of the cell cultures were verified by immunohistochemical staining and flow cytometric analysis with antibodies against cytokeratin and vimentin as previously described (Santin et al., 2002; Meresman et al., 2003). Only primary cultures which had at least 90% viability and contained >99%
20 epithelial cells were used for total RNA extraction.

TABLE 4
Characteristics of Patients Disclosed In Example 7

Patient	Age	Race	Stage
USPC 1	65	White	IV B
USPC 2	75	Afro-American	III C
USPC 3	75	Afro-American	IV A
USPC 4	59	White	IV A
USPC 5	59	White	III C
USPC 6	62	Afro-American	IV B
USPC 7	63	Afro-American	III C
USPC 8	61	Afro-American	III C
USPC 9	78	White	III C
USPC 10	64	Afro-American	IV A

5

EXAMPLE 8

Gene Expression Profiles Distinguish Uterine Serous Papillary Carcinoma From Normal Endometrial Epithelial Cells

10 Tumor tissue flash frozen biopsies are known to 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, uterine serous papillary carcinoma represents rare tumors which may present in either pure forms, or admixed with endometrioid or clear cell tumor cells. To minimize the risk of contamination of

15 uterine serous papillary carcinoma RNA with that of normal cells or tumor cells with different histology (i.e., endometrioid or clear cells), as well as to reduce the complexity of gene expression data analysis, RNA was extracted from short term primary tumor cell cultures collected only from uterine serous papillary carcinoma with single type differentiation. Pure uterine serous papillary carcinoma and normal

20 endometrial epithelial cell cultures, minimizing the risk of a selection bias inherent in

any long term *in vitro* growth, provide an opportunity to study differential gene expression between highly enriched populations of normal and tumor-derived epithelial cells.

Accordingly, comprehensive gene expression profiles of 10 primary
5 uterine serous papillary carcinoma cell lines and 5 primary normal endometrial
epithelial cell lines were generated using high-density oligonucleotide arrays with
12,533 probe sets, which in total interrogated some 10,000 genes. Statistical analyses
of the data were performed with the software packages SPSS10.0 (SPSS, Chicago, IL)
and the significance analysis of microarrays (SAM) method (Tusher et al., 2001).
10 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 gene group were retained for statistical analysis if they showed >5-
fold change between groups. Retained genes were subjected to SAM to establish a
false discovery rate, then further filtered via the Wilcoxon rank sum (WRS) test at
15 $\alpha=0.05$. The false discovery rate obtained from the initial SAM analysis was
assumed to characterize genes found significant via WRS.

Hierarchical clustering of average-linkage method with the centered
correlation metric was used (Eisen et al., 1998). For the unsupervised hierarchical
clustering shown in Figure 6, a total of 7,328 probe sets were scanned across the two
20 groups of primary cultures. The 7,328 probe sets were derived from 12,588 by
filtering out all control genes, all genes with absent detections, and genes not fulfilling
the test of standard deviation greater than 0.5 (0.5 being the log base 2 of the signal).

As shown in Figure 6, all 10 uterine serous papillary carcinoma cell
lines were found to group together in the rightmost columns of the dendrogram.
25 Similarly, in the leftmost columns all 5 normal endometrial epithelial cell lines were
found to cluster tightly together. After filter out most "absent" genes, the SAM and
the nonparametric WRS test ($p < 0.05$) were performed to identify genes differentially
expressed between uterine serous papillary carcinoma and normal endometrial
epithelial cells. A total of 2,829 probe sets were found differentially expressed
30 between the two groups with $p < 0.05$ by WRS and with a median false discovery rate
of 0.35% and a 90th percentile false discovery rate of 0.59% by SAM.

Of the 2,829 aforementioned probe sets, there were 529 probe sets

showing >5-fold change. As shown in Table 5, a group of 139 probe sets were found highly expressed in uterine serous papillary carcinoma and underexpressed in normal endometrial epithelial cells. Included in these group of genes are *CDKN2A/p16/p14ARF*, *L1 cell adhesion molecule (L1CAM)*, *claudin 3 and claudin-4*,
 5 *kallikrein 6 (protease M) and kallikrein 10, (SCCE)*, *interleukin-6*, *interleukin-18* and *plasminogen activator receptor (PLAUR)*. Importantly, *c-erbB2*, which is highly expressed in uterine serous papillary carcinoma when compared to ovarian serous papillary tumors, was 14-fold more expressed in uterine serous papillary carcinoma when compared to normal endometrial epithelial cells (Table 5).

10 The second profile was represented by 390 genes that were highly expressed in normal endometrial epithelial cells and underexpressed in uterine serous papillary carcinoma (Table 6). Included in this group of genes are *transforming growth factor beta receptor III*, *platelet-derived growth factor receptor alpha*, *SEMACAP3*, *ras homolog gene family, member I (ARHI)*, and differentially
 15 *downregulated in ovarian carcinoma 1 (DOC1)*.

TABLE 5

Upregulated Genes Expressed At Least 5-Fold Higher In Uterine Serous Papillary
 20 Carcinoma Compared With Normal Endometrial Epithelial Cells

Probe Set	Gene Symbol	SAM Score(d)	p of WRS	Ratio USPC/NEC
1713_s_at	CDKN2A	10.59223007	0.0027	101.9077377
36288_at	KRTHB1	4.573430656	0.0027	77.3983986
33272_at	SAA1	3.777977393	0.0063	45.74937337
41294_at	KRT7	7.173346265	0.0027	41.46788873
32154_at	TFAP2A	7.636996321	0.0027	32.47929396
31610_at	MAP17	3.621151787	0.0093	30.28302802
408_at	---	4.070053148	0.0063	30.14111158
32821_at	LCN2	5.126089463	0.0027	27.69975608
35174_i_at	EEF1A2	2.839620426	0.0278	26.80482891

Probe Set	Gene Symbol	SAM Score(d)	p of WRS	Ratio USPC/NEC
38551_at	L1CAM	3.115032534	0.0196	25.60938089
38249_at	VGLL1	5.273984976	0.0027	24.69495091
35879_at	GAL	5.593811144	0.0027	23.48953559
36838_at	KLK10	3.455062978	0.0136	23.17518549
38299_at	IL6	3.62957424	0.0041	19.05873079
38051_at	MAL	4.877642645	0.0041	17.51555106
41469_at	PI3	2.853526521	0.0063	16.90464558
40412_at	PTTG1	5.218191198	0.0027	16.61222352
1886_at	WNT7A	3.544426758	0.0196	16.11519778
33128_s_at	CST6	4.221666931	0.0136	15.97856318
38414_at	CDC20	7.317470579	0.0027	15.64601435
34012_at	KRTHA4	2.410988057	0.0278	15.37247475
37554_at	KLK6	3.784630357	0.0093	15.23781352
1802_s_at	ERBB2	2.566389361	0.0136	14.52012028
41060_at	CCNE1	6.092165808	0.0027	14.16647068
36837_at	KIF2C	6.129605781	0.0027	14.1328483
34213_at	KIBRA	5.300586641	0.0027	13.27228177
1651_at	UBE2C	5.554093545	0.0027	12.87617243
35276_at	CLDN4	6.381184288	0.0027	12.74825421
36990_at	UCHL1	4.623383279	0.0027	12.30505908
35977_at	DKK1	4.494993915	0.0041	12.25382636
36113_s_at	TNNT1	4.071523595	0.0027	11.93824813
2011_s_at	BIK	3.451043397	0.0063	11.66959681
543_g_at	CRABP1	3.193471228	0.0093	11.55494382
34852_g_at	STK6	6.224691811	0.0027	11.51812047
33483_at	NMU	4.093975777	0.0027	11.42057993
39109_at	TPX2	6.161639109	0.0027	11.29208457
37018_at	HIST1H1C	2.26997194	0.0278	10.74270622

Probe Set	Gene Symbol	SAM Score(d)	p of WRS	Ratio USPC/NEC
1165_at	IL18	3.220966429	0.0041	10.65596528
36477_at	TNNI3	2.867426116	0.0136	10.61101382
572_at	TTK	3.720282658	0.0093	9.723902052
31542_at	FLG	2.622102112	0.0196	9.600831601
35937_at	MICB	4.238382451	0.0093	9.460109582
36155_at	SPOCK2	2.277735266	0.0278	9.216570003
32186_at	SLC7A5	4.148798845	0.0063	9.121679665
35766_at	KRT18	5.933225457	0.0027	9.01220054
35822_at	BF	3.266560726	0.0063	8.952514469
35714_at	PDXK	6.549900892	0.0027	8.898191704
1369_s_at	---	2.679010624	0.0196	8.773380878
40079_at	RAI3	4.515766371	0.0063	8.626843209
37168_at	LAMP3	2.837727959	0.0136	8.616807346
39704_s_at	HMGA1	5.322233414	0.0027	8.597894471
1887_g_at	WNT7A	3.003097491	0.0196	8.491813649
36929_at	LAMB3	5.769944566	0.0027	8.354149098
527_at	CENPA	6.125858747	0.0027	8.32992789
41081_at	BUB1	4.882654417	0.0027	8.213759056
885_g_at	ITGA3	4.447267172	0.0027	8.20660555
2021_s_at	CCNE1	4.399072926	0.0041	8.199388463
33904_at	CLDN3	3.296023945	0.0136	8.020010794
33730_at	RAI3	4.648262631	0.0041	7.923899439
34736_at	CCNB1	5.077963775	0.0063	7.896644626
757_at	ANXA2	3.514460359	0.0063	7.870466864
910_at	TK1	3.933693732	0.0093	7.869091533
34851_at	STK6	4.491412407	0.0041	7.764803777
34703_f_at	---	2.275488598	0.0278	7.710260816
34715_at	FOXMI	5.318031066	0.0027	7.659023602

Probe Set	Gene Symbol	SAM Score(d)	p of WRS	Ratio USPC/NEC
38971_r_at	TNIP1	6.799881197	0.0027	7.595036872
32263_at	CCNB2	4.245537907	0.0063	7.578513543
1680_at	GRB7	4.013375211	0.0027	7.471384928
38247_at	F2RL1	3.185259514	0.0093	7.432476326
160025_at	TGFA	6.08814344	0.0027	7.355344272
1945_at	CCNB1	5.297806506	0.0041	7.291039832
31792_at	ANXA3	4.872657477	0.0041	7.266892828
182_at	ITPR3	5.431705752	0.0027	7.172450367
1117_at	CDA	2.936875649	0.0093	7.114518646
902_at	EPHB2	5.186069433	0.0027	7.065363569
634_at	PRSS8	5.21560703	0.0041	7.001894703
41169_at	PLAUR	3.982498409	0.0063	7.00139089
33203_s_at	FOXD1	3.4642857	0.0093	6.989749222
40095_at	CA2	4.285159359	0.0027	6.946396937
38940_at	AD024	5.064744169	0.0041	6.928406028
34348_at	SPINT2	6.262957935	0.0027	6.877224695
33933_at	WFDC2	3.343526736	0.0136	6.820073691
35281_at	LAMC2	3.346662529	0.0093	6.7580474
349_g_at	KIFC1	5.275031682	0.0041	6.700913018
33218_at	ERBB2	2.710053625	0.0027	6.615105998
38881_i_at	TRIM16	3.000641338	0.0196	6.506893575
1536_at	CDC6	4.666139295	0.0041	6.463305623
38482_at	CLDN7	4.930843791	0.0041	6.409117877
40697_at	CCNA2	3.396480338	0.0093	6.40768505
41688_at	TM4SF11	4.390330663	0.0027	6.366861533
38158_at	ESPL1	6.007466409	0.0027	6.225688779
38474_at	CBS	3.379648389	0.0093	6.212078913
36483_at	GALNT3	3.889728637	0.0041	6.181109111

Probe Set	Gene Symbol	SAM Score(d)	p of WRS	Ratio USPC/NEC
35372_r_at	IL8	2.359705895	0.0278	6.133149591
41585_at	KIAA0746	4.436299723	0.0027	6.092207586
36832_at	B3GNT3	5.456967667	0.0027	5.941291793
1107_s_at	G1P2	3.937533177	0.0063	5.923287019
35207_at	SCNN1A	3.076038486	0.0136	5.920739634
36863_at	HMMR	2.830001586	0.0196	5.905038013
38631_at	TNFAIP2	4.924314508	0.0027	5.897745642
36813_at	TRIP13	5.665655915	0.0027	5.870351247
41048_at	PMAIP1	3.489974054	0.0062	5.853172336
2084_s_at	ETV4	3.742551143	0.0093	5.798002338
33245_at	MAPK13	3.774897977	0.0136	5.766618762
37347_at	CKS1B	5.542650247	0.0027	5.762817533
34282_at	NFE2L3	2.668167751	0.0136	5.734907375
330_s_at	---	4.026422371	0.0041	5.726752495
41732_at	na	6.920337146	0.0027	5.706487141
1516_g_at	---	6.725730866	0.0027	5.63870137
904_s_at	TOP2A	3.418887485	0.0063	5.634251452
36041_at	EXO1	4.970840916	0.0027	5.59235892
33143_s_at	SLC16A3	4.007293245	0.0063	5.56591457
37228_at	PLK	4.500601808	0.0041	5.564532365
1854_at	MYBL2	4.116712652	0.0063	5.54317592
40407_at	KPNA2	4.188947411	0.0041	5.51635645
33282_at	LAD1	3.904051584	0.0063	5.509367036
40145_at	TOP2A	3.30652637	0.0093	5.48127065
1100_at	IRAK1	5.530078337	0.0027	5.470162749
37883_i_at	AF038169	3.159630542	0.0027	5.460495655
37343_at	ITPR3	5.257721251	0.0027	5.449013729
31598_s_at	GALE	4.646763029	0.0027	5.442955253

Probe Set	Gene Symbol	SAM Score(d)	p of WRS	Ratio USPC/NEC
889_at	ITGB8	2.743766192	0.0093	5.370592815
37558_at	IMP-3	3.122846843	0.0093	5.364127468
32715_at	VAMP8	5.685902454	0.0027	5.352873419
36312_at	SERPINB8	3.611288676	0.0027	5.327343554
37210_at	INA	3.550708512	0.0063	5.307526088
35699_at	BUB1B	3.664553007	0.0196	5.279075308
32787_at	ERBB3	2.657607539	0.0041	5.247404657
32275_at	SLPI	3.726091901	0.0041	5.221163981
893_at	E2-EPF	3.774672918	0.0063	5.196412396
41583_at	FEN1	5.481105111	0.0027	5.196005796
41781_at	PPFIA1	4.113488223	0.0027	5.194931774
40726_at	KIF11	2.94101083	0.0093	5.1806793
41400_at	TK1	4.245983179	0.0093	5.167172588
41409_at	C1orf38	3.109232321	0.0063	5.100239097
40425_at	EFNA1	2.738432716	0.0196	5.067718102
32081_at	CIT	6.162032917	0.0027	5.043567722
1108_s_at	EPHA1	4.863995126	0.0027	5.040980858
33338_at	STAT1	3.274771895	0.0063	5.029498048

TABLE 6

Upregulated Genes Expressed At Least 5-Fold Higher In Normal Endometrial
Epithelial Cells Compared With Uterine Serous Papillary Carcinoma

Probe Set	Gene Symbol	SAM Score(d)	p of WRS	Ratio NEC/ USPC
774_g_at	MYH11	17.6111674	0.0027	1014.968759
773_at	MYH11	14.26541863	0.0027	968.0223497
32582_at	MYH11	11.27680376	0.0027	212.2458648
36681_at	APOD	11.7139616	0.0027	137.3140116
1501_at	IGF1	8.3207956	0.0027	128.0651104
39325_at	EBAF	8.177456434	0.0027	123.064609
767_at	---	12.70747752	0.0027	119.5409103
40398_s_at	MEOX2	10.18172072	0.0027	114.9489897
40776_at	DES	9.737597758	0.0027	109.416397
1197_at	ACTG2	5.86136146	0.0027	96.65715109
36627_at	SPARCL1	7.724402007	0.0027	93.89302842
37407_s_at	MYH11	11.89270454	0.0027	88.70362054
39673_i_at	ECM2	8.458527972	0.0027	82.52353674
39701_at	PEG3	7.644643483	0.0027	73.87997223
39066_at	MFAP4	9.382764201	0.0027	68.84723295
38737_at	IGF1	6.340769807	0.0027	65.13666715
35730_at	ADH1B	9.758743372	0.0027	64.94146631
41124_r_at	ENPP2	7.006678874	0.0027	53.43118221
36749_at	CPA3	9.095730461	0.0027	53.07347991
39616_at	PTGER3	10.18818646	0.0027	52.65326101
33440_at	TCF8	7.220247327	0.0027	49.54168001
32666_at	CXCL12	7.167916527	0.0027	49.2359637
38734_at	PLN	9.182449981	0.0027	47.86730799
34203_at	CNN1	5.0508523	0.0027	47.0209722
37576_at	PCP4	12.46833717	0.0027	45.45542357

Probe Set	Gene Symbol	SAM Score(d)	p of WRS	Ratio NEC/ USPC
36834_at	MOXD1	9.736431311	0.0027	44.20917891
37247_at	TCF21	9.809724062	0.0027	42.40051895
37701_at	RGS2	7.407533049	0.0027	42.29038464
779_at	COL4A6	5.840226002	0.0027	41.96325597
37394_at	C7	9.834950038	0.0027	40.72326307
36533_at	PTGIS	6.097204484	0.0027	39.10052985
32689_s_at	---	6.178363757	0.0027	36.96897988
32905_s_at	TPSB2	6.87294347	0.0027	36.85974711
33240_at	SEMACAP3	4.814091817	0.0027	36.54583796
1466_s_at	FGF7	8.628399247	0.0027	35.60413415
32686_at	PTGER3	7.396922485	0.0027	35.31357002
39690_at	ALP	6.951347359	0.0027	33.76045029
38427_at	COL15A1	7.196374501	0.0027	32.85391153
39939_at	COL4A6	6.846432716	0.0027	32.53815505
37630_at	NRLN1	5.895556339	0.0027	31.03030255
35717_at	ABCA8	7.587173679	0.0027	30.63819827
1709_g_at	MAPK10	6.522200835	0.0027	30.54331363
35679_s_at	DPP6	5.899443575	0.0027	30.33959319
35740_at	EMILIN1	7.445413041	0.0027	29.45261895
41123_s_at	ENPP2	6.597107224	0.0027	29.29614059
755_at	ITPR1	8.210929741	0.0027	29.02124207
32847_at	MYLK	5.388215885	0.0027	28.22518538
38001_at	CACNA1C	5.739826433	0.0027	27.31347302
37279_at	GEM	9.925280559	0.0027	26.56613192
36396_at	---	5.90098377	0.0027	26.32457357
41137_at	PPP1R12B	12.043235	0.0027	25.97089935
41405_at	SFRP4	10.07707264	0.0027	23.90433923
40775_at	ITM2A	6.15225431	0.0027	23.83083993

Probe Set	Gene Symbol	SAM Score(d)	p of WRS	Ratio NEC/ USPC
38059_g_at	DPT	6.507962247	0.0027	23.76376781
41504_s_at	MAF	5.331090667	0.0027	23.65323618
1596_g_at	TEK	4.54150328	0.0027	23.37422615
914_g_at	ERG	6.104980572	0.0027	22.63829292
34283_at	---	5.390052481	0.0041	22.19917455
34388_at	COL14A1	9.452205029	0.0027	21.48165459
38994_at	SOCS2	6.600175855	0.0027	21.47663968
36065_at	LDB2	5.790219755	0.0027	21.19321308
40230_at	FRZB	5.962169722	0.0027	21.00467856
33790_at	CCL15	9.01652793	0.0027	20.5510028
33890_at	RGS5	7.347159852	0.0027	20.2203337
36513_at	MAGP2	4.385435702	0.0041	20.10734871
32526_at	JAM3	6.12916782	0.0027	19.89641568
32687_s_at	PTGER3	5.902450533	0.0027	19.7194642
35638_at	CBFA2T1	10.47022487	0.0027	19.57233853
34637_f_at	ADH1A	6.291154915	0.0027	19.20847669
34675_at	SBLF	5.400378777	0.0027	19.04463285
38351_at	---	5.899004809	0.0026	18.69131208
1182_at	PLCL1	4.047061946	0.0027	18.50958764
39681_at	ZNF145	6.266146694	0.0027	18.41823634
1708_at	MAPK10	6.336420306	0.0027	18.31250756
37765_at	LMOD1	7.585915773	0.0027	18.11624265
1678_g_at	IGFBP5	4.237261246	0.0041	17.88349387
35358_at	TENC1	9.24287493	0.0027	17.68277053
33442_at	KIAA0367	6.829577702	0.0027	17.58195512
37249_at	PDE8B	5.03457122	0.0027	17.25176904
33834_at	CXCL12	7.735640221	0.0027	17.10893345
35324_at	SLIT3	5.157473623	0.0027	17.08441711

Probe Set	Gene Symbol	SAM Score(d)	p of WRS	Ratio NEC/ USPC
37015_at	ALDH1A1	4.941494764	0.0027	16.83181709
39266_at	---	6.23654664	0.0027	16.80733058
33462_at	GPR105	4.907484265	0.0027	16.49634139
32488_at	COL3A1	3.927988681	0.0027	16.44993439
483_g_at	CDH13	3.754937593	0.0027	16.43857351
38026_at	FBLN1	5.350151734	0.0027	16.06497318
1909_at	BCL2	7.355936992	0.0027	16.01160367
36245_at	HTR2B	4.697764773	0.0027	15.77119379
32057_at	LRRC17	5.469946898	0.0027	15.6891271
39544_at	DMN	5.361074255	0.0027	15.66465434
37112_at	C6orf32	4.719846975	0.0027	15.65975413
36733_at	FLJ32389	4.441898885	0.0027	15.64273348
1319_at	DDR2	5.400093727	0.0027	15.5817123
38057_at	DPT	8.478521377	0.0027	15.51358362
40358_at	GLI3	6.649732557	0.0027	15.11249492
38627_at	HLF	4.946851692	0.0027	14.89249894
1731_at	PDGFRA	7.049550835	0.0027	14.84422948
31897_at	DOC1	4.66382704	0.0027	14.80084961
2073_s_at	CDH13	5.076264455	0.0027	14.79118507
38577_at	---	7.283309694	0.0027	14.78670234
38004_at	CSPG4	6.071627359	0.0027	14.40637488
32109_at	FXYP1	7.333234712	0.0027	14.33463902
34853_at	FLRT2	9.833656085	0.0027	14.33080432
38298_at	KCNMB1	7.045889128	0.0027	14.25816751
41245_at	GDF10	6.642690314	0.0027	14.21467179
38322_at	GAGEC1	6.215440519	0.0027	14.10508119
1198_at	EDNRB	4.898747758	0.0027	14.08196951
41505_r_at	MAF	4.528150651	0.0027	14.0059518

Probe Set	Gene Symbol	SAM Score(d)	p of WRS	Ratio NEC/ USPC
36976_at	CDH11	3.691860596	0.0027	14.00329993
32688_at	PTGER3	4.997630756	0.0027	13.68791213
1575_at	ABCB1	6.210355138	0.0027	13.64196645
32778_at	ITPR1	4.9135006	0.0027	13.60359986
40737_at	KCNMA1	5.642312954	0.0027	13.60011778
36569_at	TNA	11.51239349	0.0027	13.14186685
1897_at	TGFBR3	4.922146191	0.0041	12.99722509
2087_s_at	CDH11	4.34470878	0.0027	12.96131894
34820_at	PTN	4.413004145	0.0041	12.94626537
743_at	NAP1L3	7.858619208	0.0027	12.91061134
1507_s_at	EDNRA	4.31923776	0.0041	12.88466839
38995_at	CLDN5	12.15582555	0.0027	12.86636376
1147_at	---	7.083718066	0.0027	12.69931721
1954_at	KDR	6.076599432	0.0027	12.6174344
38786_at	---	5.676030466	0.0027	12.61170471
40757_at	GZMA	4.574516084	0.0041	12.56281091
36695_at	na	5.77638459	0.0027	12.53905775
32052_at	HBB	5.107109957	0.0063	12.52262761
37446_at	GASP	8.226558005	0.0027	12.39360585
38038_at	LUM	4.46220463	0.0027	12.34757673
32889_at	RPIB9	6.336650756	0.0027	12.34540513
35234_at	RECK	4.47558162	0.0027	12.33793353
661_at	GAS1	4.647834984	0.0027	12.32556384
41195_at	LPP	6.291656851	0.0027	12.26636801
32664_at	RNASE4	6.39454963	0.0027	11.89680804
39038_at	FBLN5	4.324883809	0.0027	11.89440528
40693_at	KCNB1	4.95203129	0.0041	11.84773944
38052_at	F13A1	5.346464847	0.0027	11.69181717

Probe Set	Gene Symbol	SAM Score(d)	p of WRS	Ratio NEC/ USPC
35220_at	ENPEP	4.200434333	0.0027	11.66308093
37512_at	RODH	5.112208975	0.0027	11.65031202
103_at	THBS4	5.113726559	0.0027	11.6063075
36156_at	AQP1	6.926726938	0.0027	11.6016663
39593_at	FGL2	5.175765324	0.0027	11.38223584
33248_at	HOXA11	6.555467127	0.0027	11.34828098
41420_at	IGFBP5	4.165576966	0.0041	11.32365037
35644_at	HEPH	6.666296008	0.0027	11.26324425
39646_at	CACNB2	4.718873234	0.0027	11.25017414
35333_r_at	DVS27	4.403332874	0.0027	11.23759398
33182_at	---	4.990249409	0.0027	11.20258783
34303_at	FLJ90798	4.908581714	0.0027	11.10458088
33756_at	AOC3	5.185884024	0.0027	11.09776026
37710_at	MEF2C	7.484609789	0.0027	11.03832245
35680_r_at	DPP6	3.78344404	0.0027	10.88151987
40126_at	---	4.62026524	0.0027	10.87424764
31831_at	SMTN	5.711671156	0.0027	10.84917408
234_s_at	PTN	5.660081325	0.0041	10.803161
36939_at	GPM6A	4.980509075	0.0027	10.76644676
41158_at	PLP1	4.878943567	0.0027	10.76462278
41839_at	GAS1	4.40075134	0.0027	10.75375673
1186_at	GDF10	4.165439023	0.0063	10.74677086
35404_at	TACR2	4.416070077	0.0063	10.67366905
160023_at	WNT2	5.423667844	0.0027	10.67077677
34561_at	MS4A2	6.379719275	0.0027	10.6336763
36280_at	GZMK	4.739332721	0.0027	10.5607572
35668_at	RAMP1	3.430294187	0.0027	10.4886967
32521_at	SFRP1	4.488454671	0.0027	10.43051698

Probe Set	Gene Symbol	SAM Score(d)	p of WRS	Ratio NEC/ USPC
1975_s_at	IGF1	7.381010481	0.0027	10.34307322
37671_at	LAMA4	5.094401298	0.0027	10.34084543
40013_at	CLIC2	5.388103426	0.0027	10.2736905
32782_r_at	BPAG1	4.596569018	0.0027	10.2197061
36918_at	GUCY1A3	4.344175119	0.0027	10.18606373
32239_at	MATN2	6.196807922	0.0027	10.14895891
36503_at	CCL21	6.812268911	0.0027	10.09354903
38508_s_at	TNXB	8.826474643	0.0027	10.0794719
35146_at	TGFB1I1	5.5617525	0.0027	10.0680837
38653_at	PMP22	6.084746373	0.0027	10.04665889
342_at	ENPP1	5.008283422	0.0041	10.0434301
32826_at	ENTPD1	6.422625578	0.0027	10.01186286
40318_at	DNCI1	3.347919999	0.0063	10.00454416
32781_f_at	BPAG1	3.895818151	0.0027	9.99877625
40202_at	BTEB1	5.752005719	0.0027	9.966240854
40856_at	SERPINF1	6.638116781	0.0027	9.959239422
40560_at	TBX2	6.427628478	0.0027	9.957108857
33355_at	PBX1	6.203118598	0.0027	9.938502929
39945_at	FAP	3.442260355	0.0041	9.899321818
41549_s_at	AP1S2	6.153046901	0.0027	9.800866773
34760_at	DCL-1	4.968676715	0.0027	9.737016618
34990_at	SETBP1	4.786102171	0.0027	9.73531319
35459_at	RGS13	4.318971444	0.0063	9.73086055
37716_at	MOX2	6.058611716	0.0027	9.730316399
40301_at	RE2	4.30887411	0.0027	9.580304305
35742_at	BC008967	4.439963322	0.0027	9.57255333
39674_r_at	ECM2	6.458114792	0.0027	9.556122049
36993_at	PDGFRB	4.280198113	0.0027	9.498903399

Probe Set	Gene Symbol	SAM Score(d)	p of WRS	Ratio NEC/ USPC
37919_at	SLC21A2	5.026176297	0.0041	9.412820802
38837_at	DJ971N18.2	6.539256063	0.0027	9.396781505
36442_g_at	KCNMA1	5.555835457	0.0027	9.394855646
38177_at	RAMP2	5.549671233	0.0027	9.393838874
36894_at	CBX7	8.966175904	0.0027	9.37016198
40646_at	CX3CR1	5.239286889	0.0027	9.327761215
32143_at	OSR2	5.813502921	0.0027	9.305772959
35366_at	NID	3.807200874	0.0027	9.274271385
32667_at	COL4A5	4.185428011	0.0027	9.271586005
32780_at	BPAG1	5.55884169	0.0027	9.258012771
36042_at	NTRK2	5.561146153	0.0027	9.250870262
33295_at	FY	7.084139876	0.0027	9.106257274
38228_g_at	MITF	6.102946294	0.0027	9.105582903
36073_at	NDN	3.559985604	0.0027	9.063290854
39750_at	---	8.467774719	0.0027	9.019572879
39771_at	RHOBTB1	4.574178485	0.0027	8.990229715
607_s_at	VWF	5.356332342	0.0027	8.958228604
1577_at	AR	3.825402089	0.0041	8.912555487
33303_at	SSPN	4.096449745	0.0027	8.868579212
38028_at	DAT1	4.626935347	0.0027	8.86276571
37983_at	AGTR1	6.183093963	0.0027	8.827871738
40046_r_at	C18orf1	5.605179701	0.0027	8.779676657
1767_s_at	TGFB3	7.525905116	0.0027	8.765570787
39714_at	SH3BGRL	4.93580005	0.0027	8.76401131
36606_at	CPE	4.727031789	0.0027	8.748823414
38120_at	PKD2	6.187924359	0.0027	8.723317835
36867_at	---	5.502645605	0.0027	8.681195794
38113_at	SYNE1	4.961267087	0.0027	8.667220225

Probe Set	Gene Symbol	SAM Score(d)	p of WRS	Ratio NEC/ USPC
33733_at	ABCG2	5.755109102	0.0027	8.636931554
35681_r_at	ZFHX1B	3.362927284	0.0027	8.619823363
39317_at	---	4.725601909	0.0027	8.587430684
1500_at	WT1	4.331555572	0.0041	8.574447585
40017_at	DKFZP586 H2123	3.36169877	0.0027	8.559319409
1625_at	---	5.121462166	0.0027	8.490942275
129_g_at	CTSK	4.3842531	0.0027	8.419453396
41784_at	DKFZp564B 0769	4.61026234	0.0027	8.419008886
41290_at	---	3.544096357	0.0093	8.400345338
1591_s_at	IGF2	3.561710606	0.0041	8.324420931
36021_at	LEF1	4.051827392	0.0027	8.238834587
1771_s_at	PDGFRB	3.650542935	0.0027	8.225807768
38466_at	CTSK	3.787589001	0.0041	8.197720493
1380_at	FGF7	5.524953025	0.0027	8.121593377
41013_at	FLJ31737	6.592336358	0.0027	8.107620424
859_at	CYP1B1	3.751490096	0.0063	8.082690646
31902_at	DIO2	4.316229908	0.0027	8.06806751
34995_at	CALCRL	5.70953518	0.0027	8.052189461
32126_at	FGF7	4.234501237	0.0027	8.042021298
37398_at	PECAM1	4.329117802	0.0027	8.016895704
38042_at	G6PD	6.987010631	0.0027	8.016883235
32779_s_at	ITPR1	6.416481452	0.0027	7.974320137
32542_at	FHL1	4.425372331	0.0027	7.941951703
39031_at	COX7A1	3.414853092	0.0027	7.901991161
37863_at	EGR2	3.653408914	0.0027	7.862848151
40475_at	CAPN6	4.123958511	0.0027	7.840245281

Probe Set	Gene Symbol	SAM Score(d)	p of WRS	Ratio NEC/ USPC
36882_at	HOXD9	4.651878446	0.0027	7.823908297
38174_at	PSD	6.229841181	0.0027	7.816130898
33431_at	FMOD	4.128438324	0.0027	7.815908163
40841_at	TACC1	6.85110732	0.0027	7.795011222
33817_at	HNRPA3	6.302017315	0.0027	7.787750832
40913_at	ATP2B4	3.846256488	0.0027	7.776240242
1396_at	---	3.120644286	0.0063	7.748987482
38288_at	SNAI2	4.162425417	0.0041	7.728278549
38312_at	LOC169611	5.745797039	0.0027	7.703588116
38650_at	---	2.889673044	0.0027	7.644382939
37598_at	RASSF2	3.481277121	0.0093	7.576353353
40698_at	CLECSF2	4.080958193	0.0027	7.516722321
36917_at	LAMA2	4.758295806	0.0027	7.50983691
38875_r_at	GREB1	3.932392202	0.0041	7.503944317
128_at	CTSK	3.912325952	0.0027	7.490867771
39063_at	ACTC	3.50984153	0.0136	7.462854816
36311_at	PDE1A	4.408892423	0.0027	7.400144084
38181_at	MMP11	3.440836069	0.0136	7.215260166
414_at	HOXD10	4.48610318	0.0027	7.165970315
37560_at	FLJ00133	4.618261212	0.0027	7.113754721
41388_at	MEIS2	5.209442647	0.0027	7.102590023
40716_at	---	5.682826784	0.0027	7.087402622
41837_at	---	3.749728537	0.0063	7.086919982
33239_at	MGC33887	3.781769332	0.0027	7.064070455
35678_at	PCDH7	4.599768253	0.0027	7.055958628
41287_s_at	CALM1	4.9159385	0.0027	6.980202617
32119_at	---	4.11125462	0.0027	6.969578438
36908_at	MRC1	3.916253996	0.0027	6.960776505

Probe Set	Gene Symbol	SAM Score(d)	p of WRS	Ratio NEC/ USPC
38636_at	ISLR	6.658140679	0.0027	6.952656256
40071_at	CYP1B1	3.542253289	0.0063	6.931496169
36149_at	DPYSL3	4.256411985	0.0041	6.92915584
37205_at	FBXL7	3.820866964	0.0027	6.919005089
268_at	---	3.904361484	0.0027	6.915726506
32728_at	AMPH	4.439623421	0.0027	6.837387527
36577_at	PLEKHC1	4.82253418	0.0027	6.829191126
38315_at	ALDH1A2	5.390933518	0.0027	6.759589253
41536_at	ID4	3.893377567	0.0027	6.742578708
38420_at	COL5A2	3.546028887	0.0041	6.703606937
39647_s_at	CACNB2	6.114983417	0.0027	6.669834349
36505_at	CASQ2	4.034975973	0.0027	6.608279434
32570_at	HPGD	4.182995735	0.0027	6.596383607
31790_at	STARD13	4.986919151	0.0041	6.580417745
40767_at	TFPI	5.047863935	0.0027	6.564786304
1535_at	CHES1	4.241079307	0.0027	6.553244809
32182_at	STK38L	4.706516629	0.0041	6.543532522
40191_s_at	KIAA0582	4.468954313	0.0027	6.537990728
34377_at	ATP1A2	4.345740288	0.0027	6.442188895
36534_at	KIAA1735	5.605691943	0.0027	6.434650624
34257_at	AIP1	5.597880058	0.0027	6.413861576
38317_at	TCEAL1	5.544448535	0.0027	6.400529452
38669_at	SLK	4.4731488	0.0027	6.395945769
33910_at	PTPRD	3.451476556	0.0063	6.326797395
38407_r_at	PTGDS	3.508676618	0.0041	6.2537194
36521_at	DZIP1	3.333044376	0.0027	6.229375084
35622_at	SLI	3.925359668	0.0063	6.225537025
40328_at	TWIST1	3.703806272	0.0063	6.213773777

Probe Set	Gene Symbol	SAM Score(d)	p of WRS	Ratio NEC/ USPC
41448_at	HOXA10	5.498866772	0.0027	6.207688396
36096_at	FLJ13110	5.000819145	0.0027	6.193515832
38408_at	TM4SF2	3.772993241	0.0063	6.181162174
41412_at	PIPPIN	4.595014514	0.0041	6.179362052
35645_at	SNX1	6.206010457	0.0027	6.139850996
1601_s_at	---	3.506288374	0.0093	6.131115111
40488_at	DMD	4.488235032	0.0027	6.086194006
482_at	CDH13	3.85482903	0.0027	6.078949974
31855_at	SRPX	4.594388673	0.0027	6.061823385
41796_at	PLCL2	4.149979666	0.0041	6.060598274
719_g_at	PRSS11	3.302739151	0.0027	6.021056622
696_at	---	3.545443995	0.0041	6.01634733
34216_at	KLF7	3.766915537	0.0063	5.957634877
35692_at	RIS1	3.353524837	0.0027	5.920347327
36258_at	PRKG1	3.430746034	0.0027	5.886138767
39069_at	AEBP1	2.937772018	0.0027	5.878905586
40839_at	UBL3	4.622359971	0.0041	5.876076773
31982_at	SORBS1	3.386365547	0.0041	5.845348994
34235_at	GPR116	4.838344706	0.0041	5.836924195
39397_at	NR2F2	6.424176938	0.0027	5.818493808
40075_at	SYT1	4.767353132	0.0041	5.776146594
37543_at	ARHGEF6	6.674769949	0.0027	5.771235708
33387_at	GAS7	3.527723529	0.0041	5.758434115
34015_at	PTGFR	3.205361762	0.0136	5.742603773
39950_at	ASM3A	5.096868564	0.0027	5.741120565
36095_at	CLIPR-59	3.524586182	0.0027	5.736208783
34797_at	PPAP2A	6.402521028	0.0027	5.705386452
40763_at	MEIS1	3.790421308	0.0027	5.6975795

Probe Set	Gene Symbol	SAM Score(d)	p of WRS	Ratio NEC/ USPC
35948_at	ITGA9	4.89260679	0.0041	5.693603423
34023_at	FCER1A	3.259096027	0.0041	5.685370893
37051_at	KCNN3	4.327976299	0.0027	5.681982346
37628_at	MAOB	3.961145375	0.0093	5.659562947
41478_at	---	4.097311966	0.0027	5.656302505
1467_at	EPS8	4.546724195	0.0041	5.647160627
32527_at	APM2	3.04326955	0.0136	5.646392299
40576_f_at	HNRPDL	5.544877803	0.0027	5.641607337
38748_at	ADARB1	4.239414077	0.0027	5.630091556
32307_s_at	COL1A2	3.077946747	0.0027	5.610938857
37604_at	HNMT	4.685376668	0.0027	5.60567914
41739_s_at	CALD1	3.835849971	0.0027	5.566506265
31830_s_at	SMTN	5.187606432	0.0027	5.536338862
1958_at	FIGF	3.87790423	0.0027	5.530479685
37842_at	HIC	5.230871247	0.0027	5.520045776
37600_at	ECM1	3.271948767	0.0027	5.51149006
40448_at	ZFP36	5.469746743	0.0027	5.505331184
36061_at	SEMA5A	5.299762537	0.0027	5.499436404
34192_at	COH1	4.663835693	0.0027	5.498908277
659_g_at	THBS2	3.250443928	0.0093	5.488143801
32851_at	CUGBP2	4.172100946	0.0027	5.480623377
34802_at	COL6A2	3.774964096	0.0027	5.472446424
481_at	SNRK	2.494696971	0.0196	5.44491364
41378_at	SGCD	3.588550024	0.0041	5.435392672
32740_at	Rab11-FIP2	3.54373784	0.0041	5.429822613
41028_at	RYR3	4.132282077	0.0027	5.384932561
39123_s_at	TRPC1	3.623665783	0.0027	5.327776817
37841_at	BCHE	4.581864247	0.0027	5.32221997

Probe Set	Gene Symbol	SAM Score(d)	p of WRS	Ratio NEC/ USPC
2094_s_at	FOS	3.072963845	0.0136	5.314613874
37366_at	LIM	6.021012841	0.0027	5.314441359
160029_at	PRKCB1	4.108606566	0.0063	5.294770685
615_s_at	PTHLH	3.120886497	0.0136	5.278419862
718_at	PRSS11	3.543714365	0.0027	5.257009628
38227_at	MITF	5.771493332	0.0027	5.251250656
40199_at	MSX1	3.266467021	0.0027	5.232971855
1529_at	13CDNA73	4.977414815	0.0027	5.214598483
32371_at	KIAA0527	3.324238194	0.0027	5.208457414
38957_at	DCAMKL1	3.228540429	0.0093	5.205512163
40746_at	GRIA2	2.97000806	0.0027	5.181047723
33658_at	ZNF124	3.0703345	0.0027	5.158102761
41859_at	UST	4.126895577	0.0093	5.151465368
38126_at	BGN	4.006564606	0.0027	5.14050562
38220_at	DPYD	2.983416919	0.0063	5.139610847
38294_at	HOXD4	4.823268726	0.0041	5.138269153
37225_at	KANK	5.494612189	0.0027	5.134298593
36089_at	INPP5F	5.042825581	0.0027	5.133241496
31823_at	CUTL1	5.021790489	0.0041	5.11768676
36448_at	---	3.890991953	0.0027	5.110789001
34417_at	FLJ36166	5.812546119	0.0027	5.106099227
31687_f_at	HBB	3.813508725	0.0136	5.092799127
38717_at	DKFZP586A0522	2.964163964	0.0063	5.085371203
1970_s_at	FGFR2	2.814367366	0.0136	5.080491239
32769_at	WDFY3	4.794833664	0.0027	5.064409438
41031_at	OMD	3.179437346	0.0093	5.049649007
38430_at	FABP4	3.825907049	0.0041	5.045289037

Probe Set	Gene Symbol	SAM Score(d)	p of WRS	Ratio NEC/ USPC
735_s_at	---	4.840928699	0.0027	5.035949376
36707_s_at	CDKL5	4.404644205	0.0041	5.032600633
32313_at	TPM2	3.296609047	0.0041	5.010974485
40570_at	FOXO1A	4.524987938	0.0027	5.007343169

EXAMPLE 9

Validation of Microarray Data

Quantitative real-time PCR assays as described above were used to validate the microarray data. Seven highly differentially expressed genes between uterine serous papillary carcinoma and normal endometrial epithelial cells (i.e., *CDKN2A/p16*, *CDKN2A/p14ARF*, *L1CAM*, *claudin 3*, *claudin 4*, *GRB-7* and *c-erbB2*) were selected for the analysis.

Primers for *L1CAM*, *claudin-3*, and *claudin-4* were obtained from Applied Biosystems as assay on demand products. Assays ID were Hs00170849_m1 (*L1CAM*), Hs00265816_s1 (*claudin-3*), and Hs00533616_s1 (*claudin-4*). *GRB7* primers sequences were: forward, 5'-TCTACGGGATGACCACTGA-3' (SEQ ID NO.1); reverse, 5'-CGAAGCCCCTTGTGTCCA-3' (SEQ ID NO.2). *c-erbB2* primers sequences were: forward, 5'-GTATACATTTCGGCGCCAGCT-3' (SEQ ID NO.3); reverse, 5'-GCAGACGAGGGTGCAGGA-3' (SEQ ID NO.4). *CDKN2A/p16* primers sequences were: forward, 5'-CCCAAACGCACCGAATAGTTAC-3' (SEQ ID NO.5); reverse, 5'-ATTCCAATTCCCCTGCAAAC-3' (SEQ ID NO.6). *CDKN2A/p14ARF* primers sequences were: forward, 5'-TGATGCTACTGAGGAGCCAGC-3' (SEQ ID NO.7); reverse, 5'-AGGGCCTTTCCTACCTGGTC-3' (SEQ ID NO.8). Amplification was carried out by using 1 unit of polymerase in a final volume of 20 µl containing 2.5 mM MgCl₂. TaqGold was activated by incubation at 96°C for 12 min, and the reactions were cycled 26-30 times at 95°C for 1 min, 55°C for 1 min, and 72°C for 1 min, followed by a final extension at 72°C for 10 min. PCR products were visualized on 2% agarose

gels stained with ethidium bromide, and images were captured by an Ultraviolet Products Image Analysis System. Differences between the tumor and normal cells 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.

As shown in Figure 7, expression differences between uterine serous papillary carcinoma and normal endometrial epithelial cells for *CDKN2A/p16* ($p = 0.002$), *CDKN2A/p14ARF* ($p = 0.002$), *L1CAM* ($p = 0.01$), *claudin 3* ($p = 0.01$), *claudin-4* ($p = 0.002$), *GRB-7* ($p = 0.002$) and *c-erbB2* ($p = 0.01$) were readily apparent. Moreover, for all seven genes tested, the quantitative real-time PCR data were highly correlated to the microarray data ($p < 0.001$) ($r = 0.81, 0.80, 0.75, 0.69, 0.82, 0.71$ and 0.65 , respectively). Thus, the 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.

EXAMPLE 10

Immunohistochemical Analysis of Claudin-4 Expression.

An important issue is whether differences in gene expression result in meaningful differences in protein expression. A second crucial issue is whether gene expression in short term *in vitro* culture of primary tumor and normal cells may be comparable to uncultured cells from which the primary cell lines were derived. Claudin-4 protein expression was thus evaluated by immunohistochemical staining on formalin-fixed tumor tissue from which primary cultures were obtained. In addition, to further confirm transcriptional profiling results of uterine serous papillary carcinoma, claudin-4 marker was also evaluated by immunohistochemistry in a second independent set of 10 uterine serous papillary carcinoma clinical tissue samples obtained from patients harboring advanced stage disease (i.e., stage III and IV).

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 4 mm-thick sections of formalin-fixed, paraffin embedded tissue. After pretreatment with 10 mM citrate buffer at pH 6.0 using a steamer, the samples were incubated with

mouse anti-claudin-4 antibodies (Zymed Laboratories Inc. San Francisco, CA) at 1 : 2000 dilution. Antigen-bound primary antibodies were detected using standard avidin-biotin immunoperoxidase complex (Dako Corp., Carpinteria, CA). Cases with less than 10% staining in tumor cells were considered negative for claudin expression, whereas positive cases were classified as follows regarding the intensity of claudin-4 protein expression: +, medium to weak staining; and ++, medium to intense staining. Subcellular localization (membrane or cytoplasm) was also noted.

Figure 8 shows immunohistochemical staining on formalin-fixed tumor tissue from which primary cultures were obtained. Heavy cytoplasmic and membranous staining for claudin-4 protein expression were noted in all uterine serous papillary carcinoma specimens that overexpressed the claudin-4 gene. In contrast, only low membranous staining for claudin-4 protein was found in normal endometrial epithelial tissue samples.

To confirm and validate the immunohistochemical result data in an independent series of tumor and normal tissues, formalin-fixed tumor tissue blocks from another 13 surgical specimens (i.e., 10 uterine serous papillary carcinoma obtained from patients harboring advanced stage disease and 3 age-matched normal endometrial epithelial cells samples) were tested for claudin-4 expression. Again, in agreement with microarray data, moderate to heavy cytoplasmic and membranous staining for the claudin-4 receptor was found in 100% of the uterine serous papillary carcinoma tested (i.e., 90% score ++ and 10% score +) while NEC showed light membranous staining in only 40% of the samples ($p = 0.02$, uterine serous papillary carcinoma vs normal endometrial epithelial cells by student t test).

The following references were cited herein:

- Bongso et al., *Human Reproduction* 3:705-13 (1988).
- Eisen et al., *Proc Natl Aca Sci USA* 95:14863-68 (1998).
- Ismail et al., *Cancer Res.* 60:6744-6749 (2000).
- Meresman et al., *Fertility & Sterility* 80 Suppl 2:702-7 (2003).
- Santin et al., *Am. J. Obstet. Gynecol.* 183: 601-609 (2000).
- Santin et al., *Brit. J. Cancer* 86:151-157 (2002).
- Tusher et al., *Proc Natl Acad Sci USA* 98:5116-5121 (2001).
- Zhan et al., *Blood* 99:1745-57 (2002).

WHAT IS CLAIMED IS:

1. A method of detecting ovarian serous papillary carcinoma, comprising the steps of:

5 examining gene expression levels of a group of genes comprising SERPINB2, DJ971N18.2, DJ971N18.2, AIM2, NDN, CYP1B1, EDG2, WNT5A, FGFR2, C5, CYP1B1, HOXD4, INSR, FLRT2, PLXNA2, ABCB6, PLCL2, SCYA5, GPC1, CHRNA7, HOXD4, G6PD, RABL4, KIAA0711, SSBP2, RIL, CBLB, IDH3B, HDAC4, MADH1, RORB, RXRA, SERPINB8, DDX24, IL10RB, CKAP4,
10 MADH1, and SEPW1; and

performing statistical analysis on the expression levels of said genes as compared to those in a normal individual, wherein overexpression of said genes indicates that said individual has ovarian serous papillary carcinoma.

15 2. The method of claim 1, wherein said gene expression is examined by DNA microarray.

3. The method of claim 1, wherein said gene expression is examined at the protein level or RNA level.

20 4. The method of claim 1, wherein said statistical analysis is hierarchical cluster analysis.

25 5. A method of detecting uterine serous papillary carcinoma, comprising the steps of:

examining gene expression levels of a group of genes comprising ERBB2, PROML1, SFRP1, STC1, F2R, MEN1, TAC1, SLC1A1, KIAA0918, AF038169, HRASLS3, UGT2B7, PTGER2, GRB7, INHBB, SLC6A12, MUC4, CSPG5, PDZK1, RLN2, PKIA, QPRT, PTPRM, PSPHL, PTPRM, AKAP2,
30 RAGD, KIAA1157, PADI2, CCNE1, GGT2, PAX8, GPC4, MYO7A, TJP3, UCHL1, INPP4B, PLAGL1, TMEFF1, IDI1, NEU1, DMD, TUBB, SDC2, ZNF91, INA, TP53BP2, ELKS, FLJ13612, DKFZP566A1524, SLC25A1, ACO1,

LOC51760, BNIP3L, CDH1, HIVEP2, API5, NEK2, ARSDR1, CPO, PRRG1,
PAX8, PIK3C2B, KIAA0790, CDKN2A, KRTHB1, SAA1, KRT7, TFAP2A,
MAP17, LCN2, EEF1A2, L1CAM, VGLL1, GAL, KLK10, IL6, MAL, PI3,
PTTG1, WNT7A, CST6, CDC20, KRTHA4, KLK6, CCNE1, KIF2C, KIBRA,
5 UBE2C, CLDN4, DKK1, TNNT1, BIK, CRABP1, STK6, NMU, TPX2,
HIST1H1C, IL18, TNNT3, TTK, FLG, MICB, SPOCK2, SLC7A5, KRT18, BF,
PDXK, RAI3, LAMP3, HMGA1, WNT7A, LAMB3, CENPA, BUB1, ITGA3,
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10 AD024, SPINT2, WFDC2, LAMC2, KIFC1, TRIM16, CDC6, CLDN7, CCNA2,
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HMMR, TNFAIP2, TRIP13, PMAIP1, ETV4, MAPK13, CKS1B, NFE2L3,
TOP2A, EXO1, SLC16A3, PLK, MYBL2, KPNA2, LAD1, TOP2A, IRAK1,
GALE, ITGB8, IMP-3, VAMP8, SERPINB8, INA, BUB1B, ERBB3, SLPI, E2-EPF,
15 FEN1, PPFIA1, KIF11, TK1, C1orf38, EFNA1, CIT, EPHA1, and STAT1; and

performing statistical analysis on the expression levels of said genes as
compared to those in normal individual, wherein overexpression of said genes indicates
that said individual has uterine serous papillary carcinoma.

20 6. The method of claim 5, wherein said gene expression is examined by
DNA microarray.

7. The method of claim 5, wherein said gene expression is examined at the
protein or RNA level.

25 8. The method of claim 5, wherein said statistical analysis is hierarchical
cluster analysis.

9. A method of treating uterine serous papillary carcinoma, comprising
30 the steps of inhibiting the expression or function of epidermal growth factor type 2
receptor gene.

10. The method of claim 9, wherein said inhibition is at the protein level, transcription level or translation level.

11. A method of detecting uterine serous papillary carcinoma, comprising
5 the steps of:

examining gene expression levels of a group of genes comprising
MYH11, APOD, IGF1, EBAF, MEOX2, DES, ACTG2, SPARCL1, ECM2, PEG3,
MFAP4, IGF1, ADH1B, ENPP2, CPA3, PTGER3, TCF8, CXCL12, PLN, CNN1,
PCP4, MOXD1, TCF21, RGS2, COL4A6, C7, PTGIS, TPSB2, SEMACAP3, FGF7,
10 ALP, COL15A1, COL4A6, NRLN1, ABCA8, MAPK10, DPP6, EMILIN1, ENPP2,
ITPR1, MYLK, CACNA1C, GEM, PPP1R12B, SFRP4, ITM2A, DPT, MAF, TEK,
ERG, COL14A1, SOCS2, LDB2, FRZB, CCL15, RGS5, MAGP2, JAM3,
CBFA2T1, ADH1A, SBLF, PLCL1, ZNF145, MAPK10, LMOD1, IGFBP5,
TENC1, KIAA0367, PDE8B, SLIT3, ALDH1A1, GPR105, COL3A1, CDH13,
15 FBLN1, BCL2, HTR2B, LRRC17, DMN, C6orf32, FLJ32389, DDR2, DPT, GLI3,
HLF, PDGFRA, DOC1, CDH13, CSPG4, FXYD1, FLRT2, KCNMB1, GDF10,
GAGEC1, EDNRB, MAF, CDH11, ABCB1, ITPR1, KCNMA1, TNA, TGFBR3,
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MGC33887, PCDH7, CALM1, MRC1, ISLR, CYP1B1, DPYSL3, FBXL7, AMPH, PLEKHC1, ALDH1A2, ID4, COL5A2, CACNB2, CASQ2, HPGD, STARD13, TFPI, CHES1, STK38L, KIAA0582, ATP1A2, KIAA1735, AIP1, TCEAL1, SLK, PTPRD, PTGDS, DZIP1, SLI, TWIST1, HOXA10, FLJ13110, TM4SF2, PIPPIN, SNX1, DMD, CDH13, SRPX, PLCL2, PRSS11, KLF7, RIS1, PRKG1, AEBP1, UBL3, SORBS1, GPR116, NR2F2, SYT1, ARHGEF6, GAS7, PTGFR, ASM3A, CLIPR-59, PPAP2A, MEIS1, ITGA9, FCER1A, KCNN3, MAOB, EPS8, APM2, HNRPDL, ADARB1, COL1A2, HNMT, CALD1, SMTN, FIGF, HIC, ECM1, ZFP36, SEMA5A, COH1, THBS2, CUGBP2, COL6A2, SNRK, SGCD, Rab11, FIP2, RYR3, TRPC1, BCHE, FOS, LIM, PRKCB1, PTHLH, PRSS11, MITF, MSX1, 13CDNA73, KIAA0527, DCAMKL1, GRIA2, ZNF124, UST, BGN, DPYD, HOXD4, KANK, INPP5F, CUTL1, FLJ36166, HBB, DKFZP586A0522, FGFR2, WDFY3, OMD, FABP4, CDKL5, TPM2, and FOXO1A; and

performing statistical analysis on the expression levels of said genes as compared to those in normal individual, wherein down-regulation of said genes indicates that said individual has uterine serous papillary carcinoma.

12. The method of claim 11, wherein said gene expression is examined by DNA microarray.

13. The method of claim 11, wherein said gene expression is examined at the protein or RNA level.

14. The method of claim 11, wherein said statistical analysis is hierarchical cluster analysis.

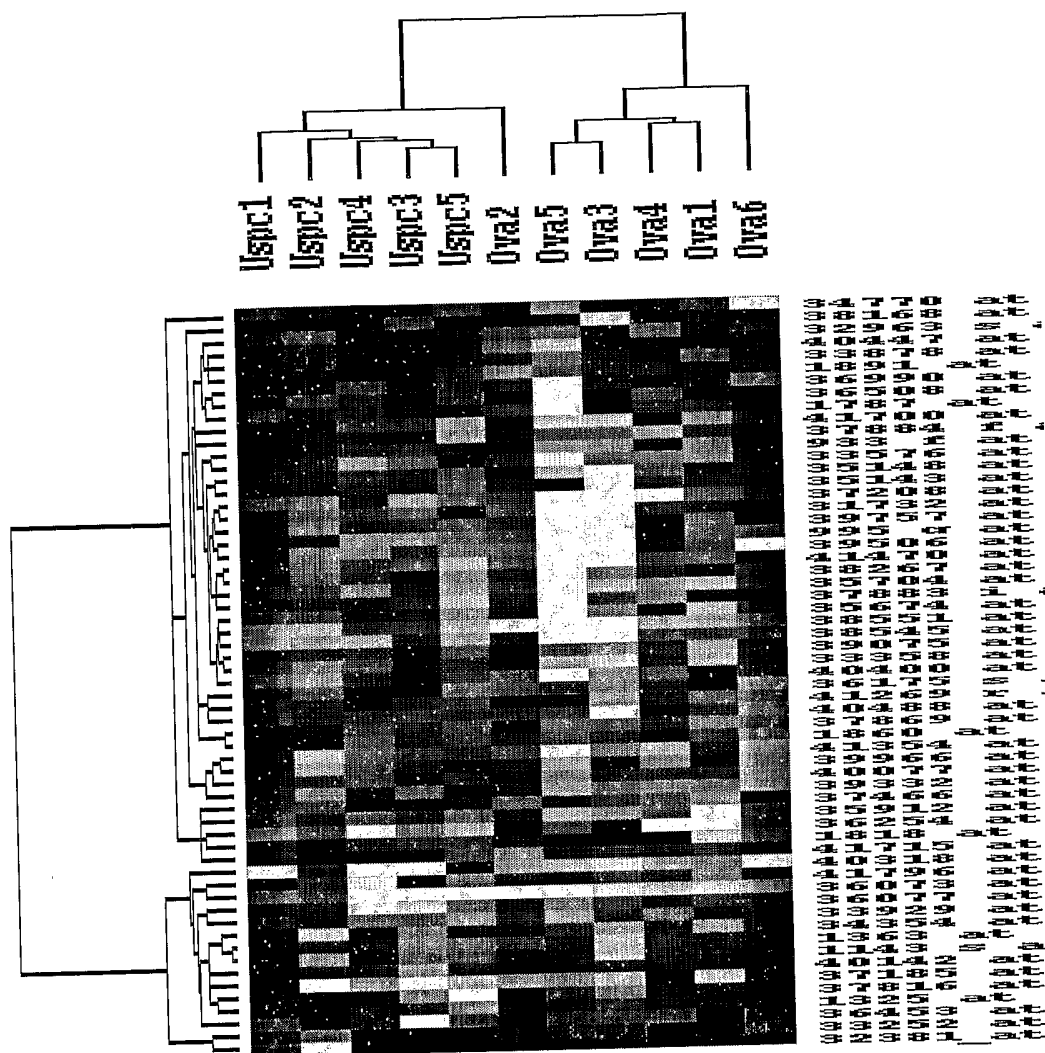


Fig. 1

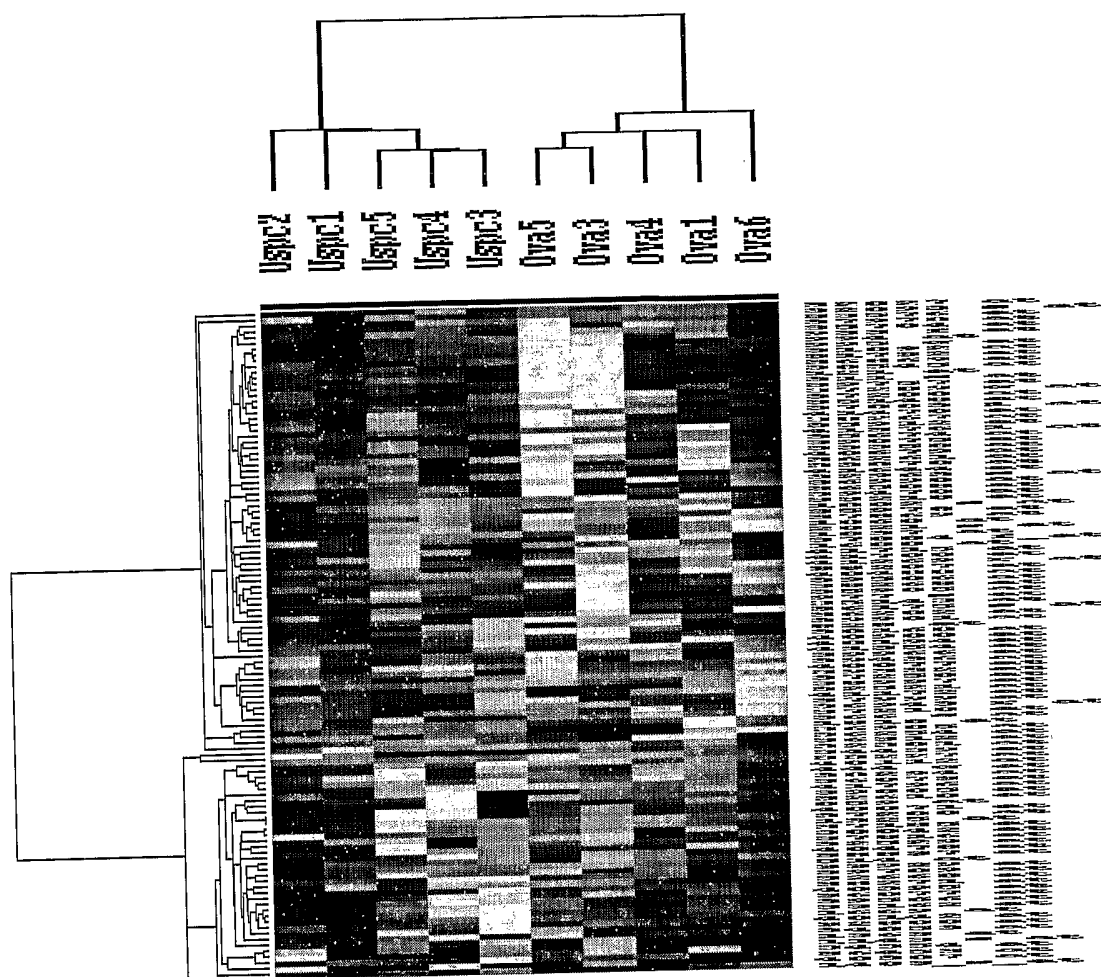


Fig. 2

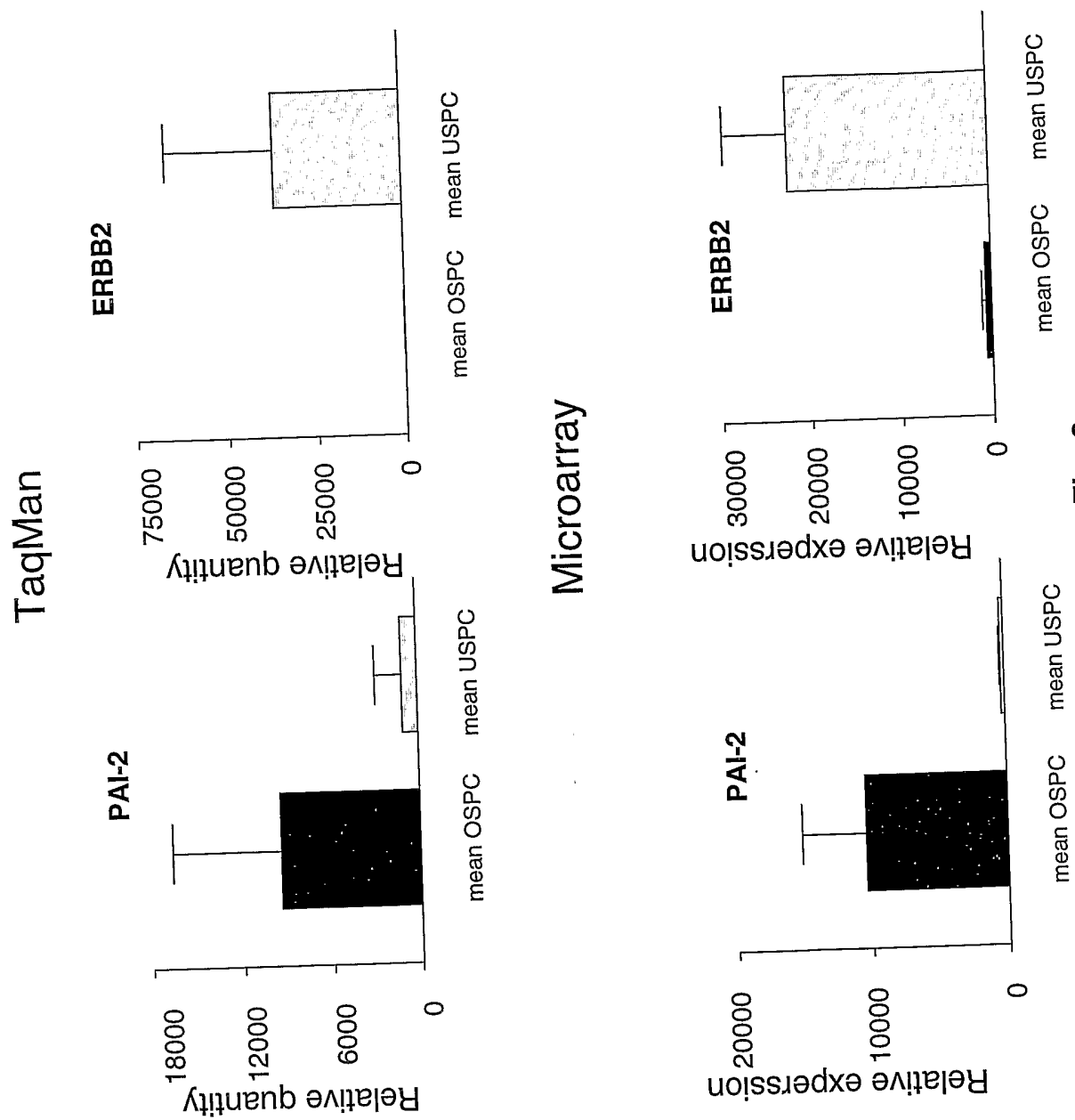


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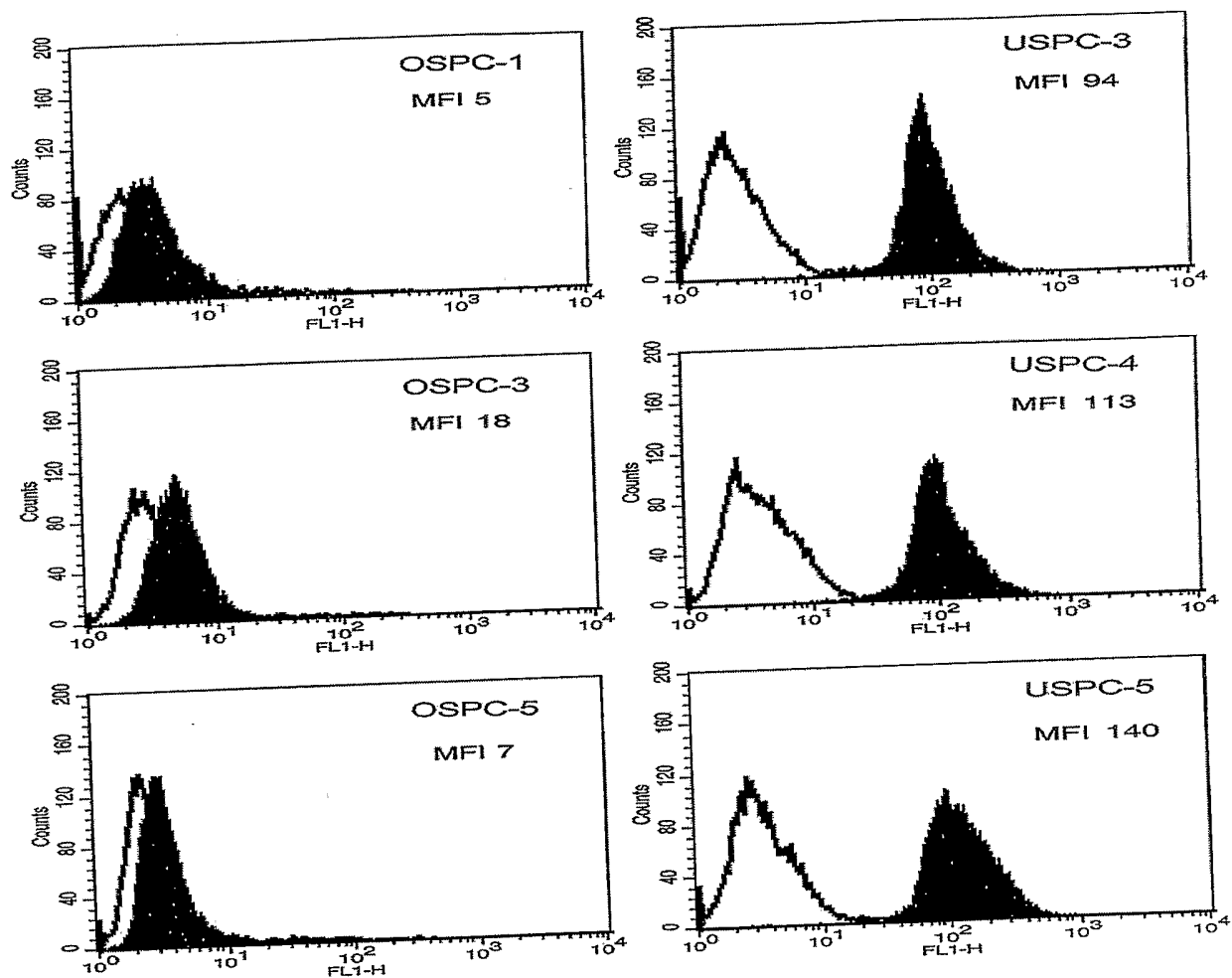


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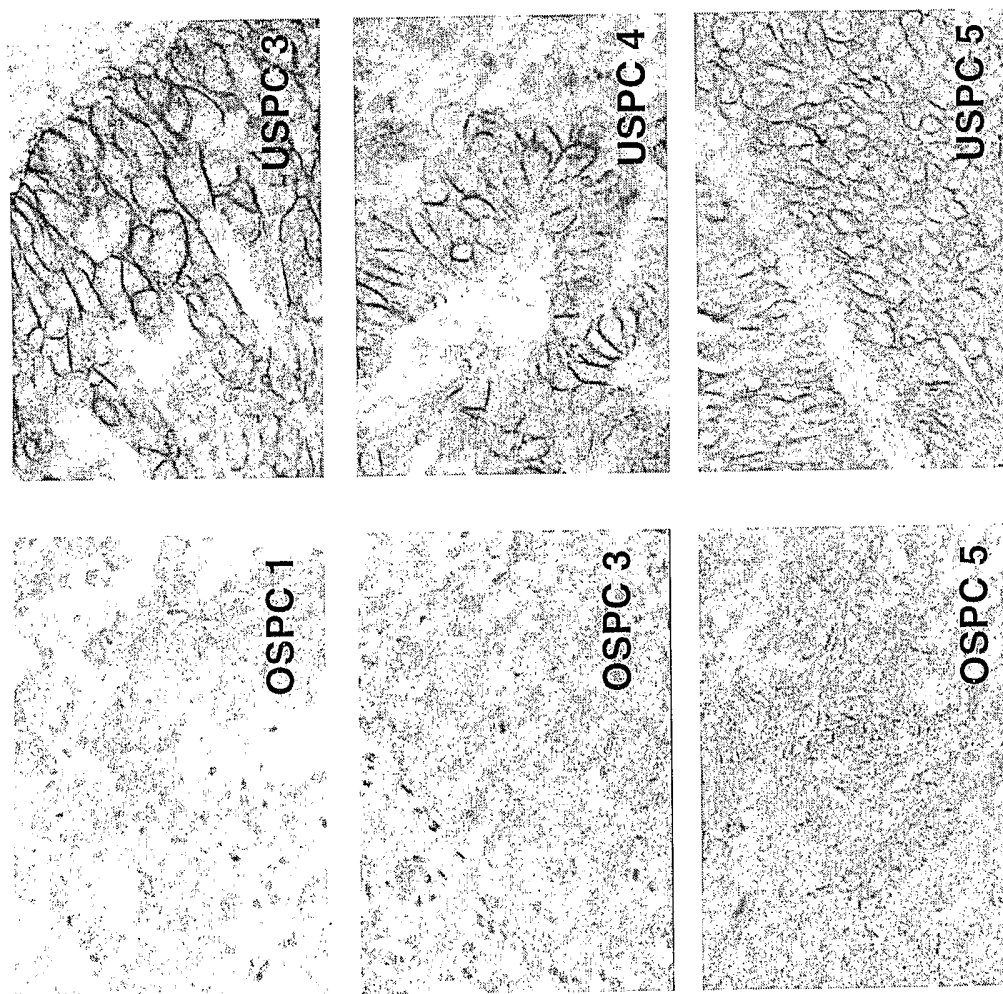


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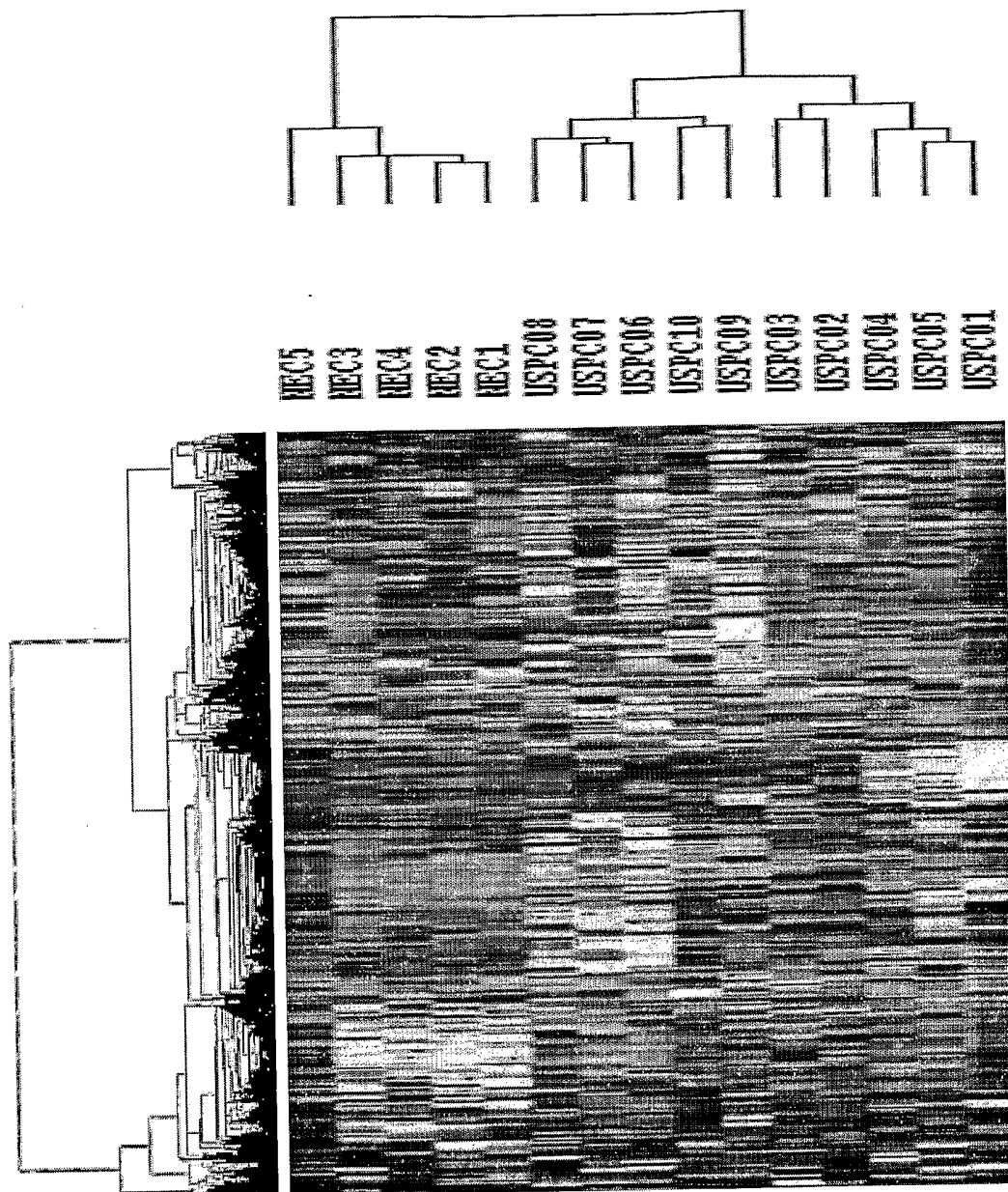


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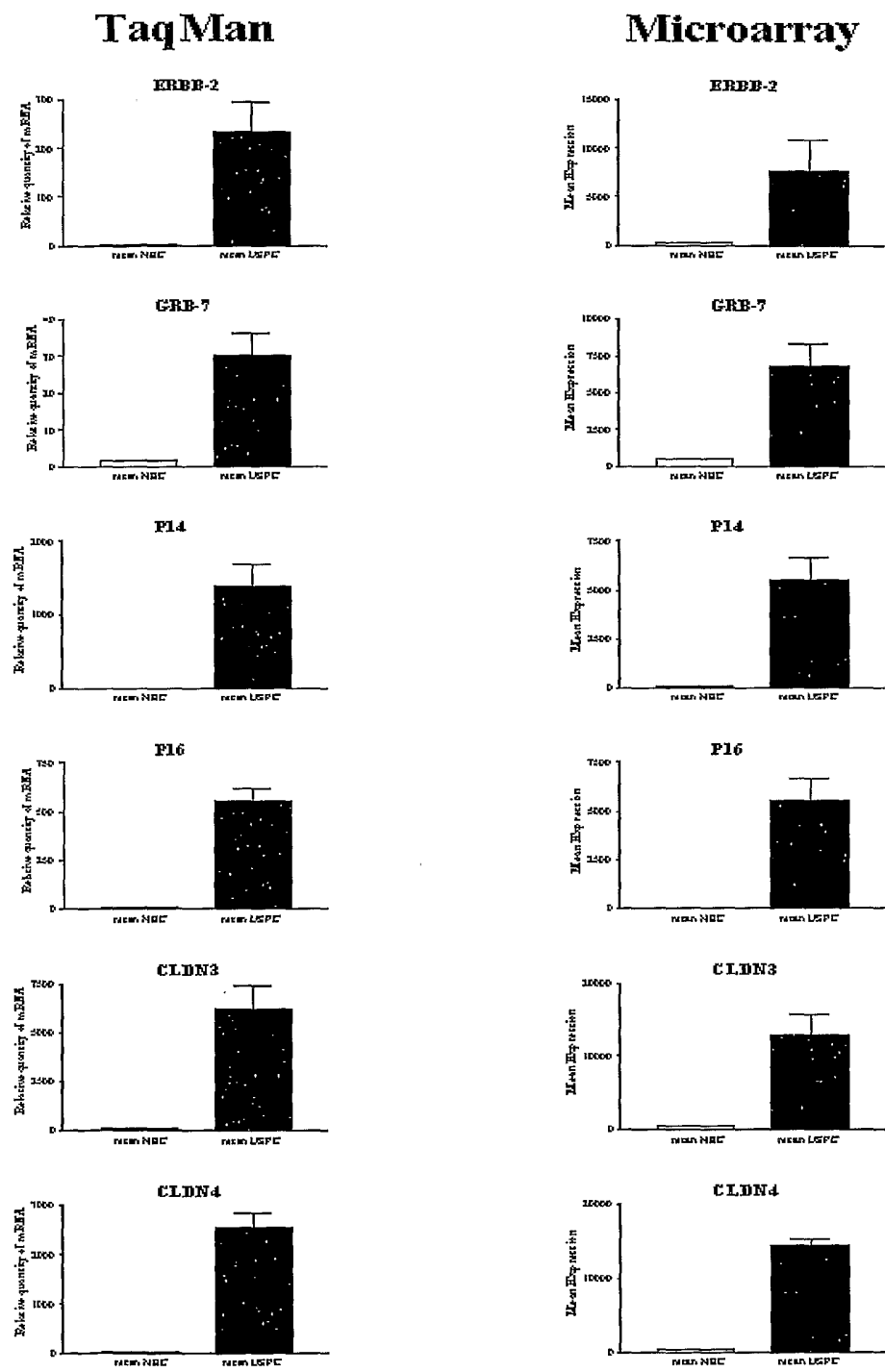


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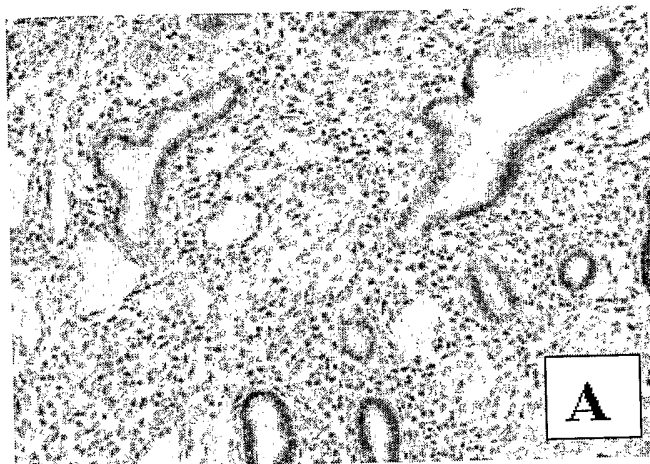


Fig. 8A

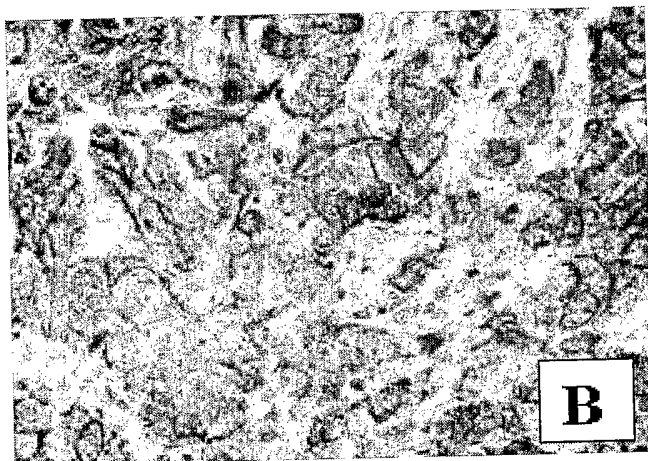


Fig. 8B

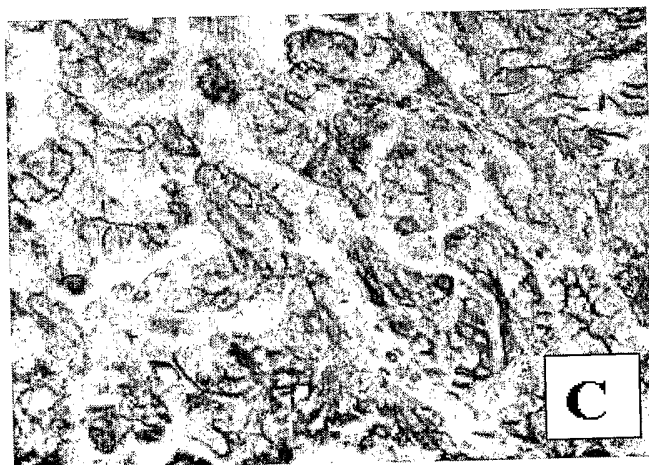


Fig. 8C

SEQUENCE LISTING

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