Patterns of Gene Expression in Different Histotypes of Epithelial Ovarian Cancer Correlate with Those in Normal Fallopian Tube, Endometrium, and Colon

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Abstract

Purpose: Epithelial ovarian cancers are thought to arise from flattened epithelial cells that cover the ovarian surface or that line inclusion cysts. During malignant transformation, different histotypes arise that resemble epithelial cells from normal fallopian tube, endometrium, and intestine. This study compares gene expression in serous, endometrioid, clear cell, and mucinous ovarian cancers with that in the normal tissues that they resemble.

Experimental Design: Expression of 63,000 probe sets was measured in 50 ovarian cancers, in 5 pools of normal ovarian epithelial brushings, and in mucosal scrapings from 4 normal fallopian tube, 5 endometrium, and 4 colon specimens. Using rank-sum analysis, genes whose expressions best differentiated the ovarian cancer histotypes and normal ovarian epithelium were used to determine whether a correlation based on gene expression existed between ovarian cancer histotypes and the normal tissues they resemble.

Results: When compared with normal ovarian epithelial brushings, alterations in serous tumors correlated with those in normal fallopian tube ($P = 0.0042$) but not in other normal tissues. Similarly, mucinous cancers correlated with those in normal colonic mucosa ($P = 0.0003$), and both endometrioid and clear cell histotypes correlated with changes in normal endometrium ($P = 0.0172$ and 0.0002, respectively). Mucinous cancers displayed the greatest number of alterations in gene expression when compared with normal ovarian epithelial cells.

Conclusion: Studies at a molecular level show distinct expression profiles of different histologies of ovarian cancer and support the long-held belief that histotypes of ovarian cancers come to resemble normal fallopian tube, endometrial, and colonic epithelium. Several potential molecular markers for mucinous ovarian cancers have been identified.

Most investigators believe that epithelial ovarian cancers develop from a single layer of cells that cover the ovary or that line inclusion cysts immediately beneath the ovarian surface (1). Despite their origin from flattened cells without distinctive features, ovarian cancers differentiate during malignant transformation into four major histotypes: serous, endometrioid, clear cell, and mucinous. Pathologists have pointed to a morphologic resemblance between these histotypes and the differentiation of normal mucosal cells in the gynecologic and intestinal tracts. Thus, serous carcinomas are thought to resemble fallopian tube epithelium, endometrioid carcinomas to normal endometrium, clear cell carcinomas to vaginal rests, and mucinous carcinomas to normal endocervical glands or intestinal mucosa. Molecular alterations that contribute to these morphologic changes have not been adequately studied.

Previous reports have shown that tumors of different histotypes can have distinct characteristics with regard to epidemiology, genetic abnormalities, expression of tumor markers, and response to chemotherapy (1–3). Parity, breastfeeding, and oral contraceptive use have been associated with decreased risk for the development of nonmucinous cancers (4–8) but do not affect risk for mucinous carcinomas. Conversely, cigarette smoking has been associated with increased risk of mucinous cancers but not serous carcinomas

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Table 1. Clinical characteristics of 50 epithelial ovarian cancer samples

	Stage	Grade	*n*
Serous	I	3	6
		4	3
	III	1	4
		3	10
Endometrioid	I	2	1
		3	1
	II	2	1
		3	2
	III	2	1
		3	3
Clear cell	I	3	2
	II	3	1
	III	3	4
	IV	3	2
Mucinous	I	1	2
		2	1
	II	2	1
	III	1	1
		2	1
		3	1
	IV	2	1
		3	1

Table 2. Number of genes and ESTs differentially expressed ≥2-fold compared to each individual histotype and with normal ovarian surface epithelial cells

Histotype	No. genes/ ESTs up-regulated	No. genes/ ESTs down-regulated	Total no. dysregulated genes/ESTs
Clear cell	24	1	25
Endometrioid	18	1	19
Serous	5	1	6
Mucinous	55	2	57

(9–11). Clear cell and endometrioid tumors have been associated with endometriosis (12–15). Genetic mutations in tumor suppressor genes and oncogenes vary between histotypes. Serous borderline tumors and low-grade serous ovarian cancers have high frequency of *K-ras* or *BRAF* mutations (16–19) but few p53 mutations (19–21). However, high-grade serous tumors commonly have p53 mutations (18–23) but rarely *BRAF* and *K-ras* mutations. Mucinous tumors have *K-ras* mutations (24–27) and endometrioid tumors have PTEN mutations (28). CA125 is expressed more frequently in serous cancers than in mucinous cancers (29). Clear cell cancers are thought to respond less frequently to chemotherapy (30–32).

Previous studies have been done to examine gene expression in different histotypes of ovarian cancer (33, 34). As in earlier reports, alterations in gene expression could distinguish different histotypes among the tumors analyzed in our study. Previous studies, however, had not compared gene expression in different histotypes with that in ovarian surface epithelium taken directly from patients. Using gene expression in normal ovarian surface epithelial cells as a standard, we have compared alterations in gene expression in different ovarian cancer histotypes with those in the mucosa of different normal organs. A significant correlation was observed between gene expression in normal colonic mucosa and in mucinous carcinomas, in fallopian tube mucosa and in serous carcinomas, and in endometrial mucosa and in

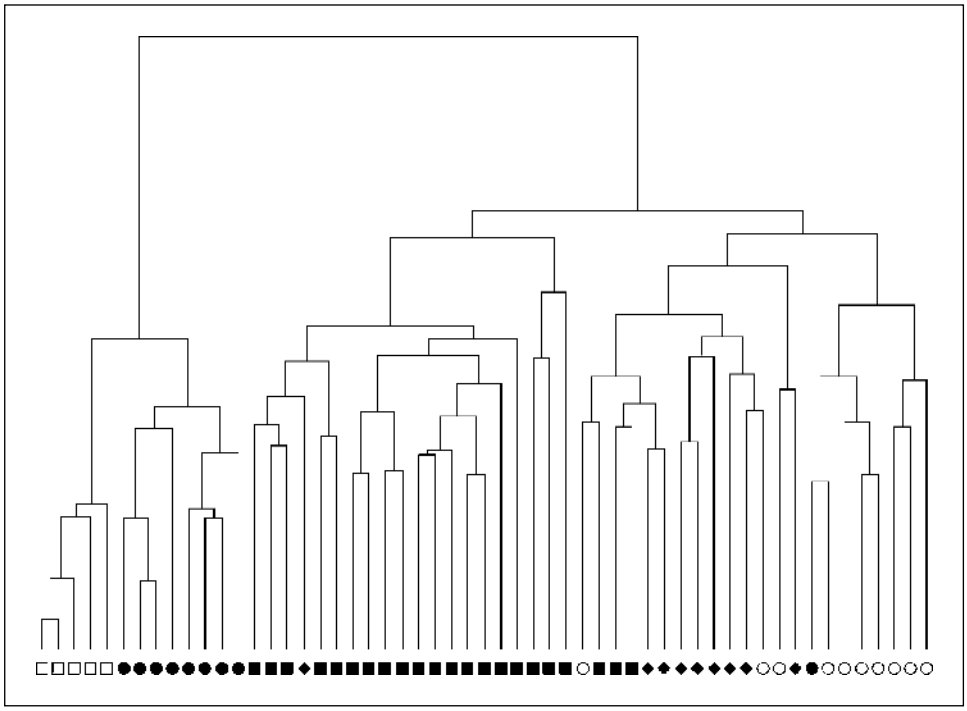

Fig. 1. Dendrogram of the top 490 differentially expressed genes distinguishing different histotypes and ovarian surface epithelium. □, ovarian surface epithelium; ■, serous cancers; ○, clear cell cancers; ●, mucinous cancers; ♦, endometrioid cancers.

Table 3.

A. Genes 2-fold differentially expressed in mucinous ovarian cancers compared to other histotypes and with normal ovarian surface epithelial cells

Symbol	Gene description	M vs CC	M vs E	M vs S	Av	M vs OSE	Colon vs OSE
Cell growth, differentiation, cell cycle progression, apoptosis, and hormonal regulation							
TM4SF3	*Transmembrane 4 superfamily member 3*	8.1	9.0	11.5	9.5	3.0	6.5
SOX2	*SRY (sex determining region Y) box 2*	8.3	5.9	6.7	7.0	7.1	−1.3
S100P	*S100 calcium-binding protein P*	4.5	7.3	8.9	6.9	10.7	2.6
G0S2	*Putative lymphocyte G_0/G_1 switch gene*	2.6	3.6	3.3	3.2	2.0	−3.6
MIG-6	*Gene 33/Mig-6*	2.4	2.8	3.1	2.8	2.1	−1.1
TM4SF4	*Transmembrane 4 superfamily member 4*	2.6	2.4	2.4	2.5	2.2	−1.0
CLU	*Clusterin*	−2.1	−3.2	−3.7	−3.0	−2.0	−5.9
EMX2	*Empty spiracles homologue 2 (Drosophila)*	−3.1	−4.6	−4.3	−4.0	−3.9	−7.3
Cytoskeletal structure, extracellular matrix formation, cell adhesion, and coagulation							
AGR2	*Anterior gradient 2 (Xenopus laevis) homologue*	7.5	7.4	18.1	11.0	15.3	14.2
CEACAM6	*Carcinoembryonic antigen-related cell adhesion molecule 6 (nonspecific cross-reacting antigen)*	8.0	7.1	8.4	7.8	8.2	6.0
LGALS4	*Lectin, galactoside-binding, soluble, 4 (galectin 4)*	7.3	6.5	6.8	6.9	6.4	36.5
MMP1	*Matrix metalloproteinase 1 (interstitial collagenase)*	7.6	4.0	7.7	6.4	10.0	1.2
CEACAM5	*Carcinoembryonic antigen – related cell adhesion molecule 5*	6.3	6.0	6.5	6.3	5.3	18.4
GAS	*Gastrin*	6.3	5.9	6.0	6.1	5.7	−1.2
BCMP11	*Breast cancer membrane protein 11*	4.8	4.7	7.1	5.5	3.4	6.4
CLDN18	*Claudin 18*	4.4	4.3	4.4	4.4	4.6	1.1
TUBB	*Tubulin, β polypeptide*	2.1	3.1	2.9	2.7	4.1	1.8
ANXA10	*Annexin A10*	2.6	2.5	2.6	2.6	2.2	−1.3
THBS2	*Thrombospondin 2*	2.2	2.3	2.6	2.4	3.0	−2.6
PLS1	*Plastin 1 (I isoform)*	2.4	2.3	2.3	2.3	2.5	5.1
EPLIN	*Epithelial protein lost in neoplasm β*	2.2	2.2	2.2	2.2	2.1	2.2
Metabolism, proteolysis, and proteinase inhibition							
CYP3A5	*Cytochrome P450, subfamily IIIA (niphedipine oxidase), polypeptide 5*	6.9	4.9	4.2	5.3	3.0	4.2
CTSE	*Cathepsin E*	5.0	5.0	5.0	5.0	4.6	1.4
SERPINE1	*Serine (or cysteine) proteinase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1*	3.1	5.5	4.9	4.5	3.5	−2.5
GCNT3	*Glucosaminyl (N-acetyl) transferase 3, mucin type*	4.9	2.6	5.4	4.3	6.4	12.2
LDLR	*Low-density lipoprotein receptor (familial hypercholesterolemia)*	2.4	2.9	2.4	2.6	3.7	2.4
SERPINB1	*Serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 1*	2.8	2.2	2.3	2.4	2.9	2.3
RNASE1	*RNase A family, 1 (pancreatic)*	2.4	2.5	2.1	2.3	3.1	1.6
Protein transport and binding							
REG4	*Regenerating islet-derived family, member 4*	8.1	8.4	8.4	8.3	8.8	1.5
SULT1C1	*Sulfotransferase family, cytosolic, 1C, member 1*	4.6	6.1	4.6	5.1	7.6	1.0
FABP1	*Fatty acid – binding protein 1, liver*	4.1	4.2	4.3	4.2	4.2	122.8
SDCBP2	*Syndecan-binding protein (syntenin) 2*	4.7	3.8	3.6	4.0	3.9	15.0
SLC28A2	*Solute carrier family 28 (sodium-coupled nucleoside transporter), member 2*	2.1	2.1	2.2	2.1	2.0	1.3
EFHD2	*EF hand domain containing 2*	2.0	2.2	2.0	2.1	2.2	1.7
Immune response and cellular defense							
TFF1	*Trefoil factor 1 (breast cancer, estrogen-inducible sequence expressed in)*	12.0	12.7	13.8	12.8	13.2	1.8
TFF3	*Trefoil factor 3 (intestinal)*	19.9	6.9	10.2	12.3	23.7	45.6
LILRB1	*Leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 1*	6.3	6.0	6.9	6.4	4.0	1.2

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Table 3. (Cont'd)

A. Genes 2-fold differentially expressed in mucinous ovarian cancers compared to other histotypes and with normal ovarian surface epithelial cells

Symbol	Gene description	M vs CC	M vs E	M vs S	Av	M vs OSE	Colon vs OSE
Cell growth, differentiation, cell cycle progression, apoptosis, and hormonal regulation							
IL6	*Interleukin-6 (IFN, β2)*	2.4	7.0	4.7	4.7	6.2	−2.0
RNF128	*Ring finger 128*	3.4	2.8	6.1	4.1	3.9	4.8
IL8	*Interleukin-8*	3.6	4.5	3.3	3.8	6.6	−1.4
FCGBP	*Fc fragment of IgG-binding protein*	4.1	3.4	3.5	3.7	3.2	9.4
CXCL12	*Stromal cell – derived factor 1*	3.3	3.7	3.0	3.3	2.5	1.0
TFF2	*Trefoil factor 2 (spasmolytic protein 1)*	2.9	3.1	3.3	3.1	2.9	−1.2
MRC1	*Mannose receptor, C-type 1*	2.5	3.1	3.2	2.9	2.4	1.5
Unknown function							
PLAC8	*Placenta-specific 8*	3.5	4.2	4.0	3.9	3.2	10.8
MS4A7	*Membrane-spanning 4 domains, subfamily A, member 7*	2.2	2.8	2.8	2.6	3.0	1.1

B. Genes 2-fold differentially expressed in endometrioid ovarian cancers compared to other histotypes and with normal ovarian surface epithelial cells (genes with negative fold changes are down-regulated)

Symbol	Gene description	E vs CC	E vs S	E vs M	Av	E vs OSE	Endometrium vs OSE
Cell growth, differentiation, cell cycle progression, apoptosis, and hormonal regulation							
FGF9	*Fibroblast growth factor 9 (glia-activating factor)*	5.9	4.3	5.5	5.2	3.2	−1.2
DKK4	*Dickkopf homologue 4 (X. laevis)*	3.6	3.6	3.1	3.4	3.3	1.3
APCDD1	*Adenomatosis polyposis coli down-regulated 1*	3.0	3.1	3.6	3.2	2.8	2.6
FZD10	*Frizzled (Drosophila) homologue 10*	2.4	2.7	2.6	2.6	5.0	1.3
IHH	*Indian hedgehog homologue*	2.6	2.5	2.1	2.4	2.3	1.1
Cytoskeletal structure, extracellular matrix formation, cell adhesion, and coagulation							
SFN	*Stratifin*	2.2	2.7	2.0	2.3	4.3	1.9
SCGB1D2	*Secretoglobin, family 1D, member 2*	2.0	2.0	2.6	2.2	2.4	10.4
Metabolism, proteolysis, and proteinase inhibition							
SERPINA5	*Serine (or cysteine) proteinase inhibitor, clade A (α-1 antiproteinase, antitrypsin), member 5*	2.5	4.2	4.4	3.7	5.5	5.2
SERPINA1	*Serine (or cysteine) proteinase inhibitor, clade A (α-1 antiproteinase, antitrypsin), member 1*	3.4	4.0	3.6	3.7	8.4	1.1
GAD1	*Glutamate decarboxylase 1 (brain, 67 kDa)*	2.6	2.5	2.6	2.6	2.3	1.0
SERPINA3	*Serine (or cysteine) proteinase inhibitor, clade A (α-1 antiproteinase, antitrypsin), member 3*	2.0	2.3	2.2	2.2	2.0	1.0
Protein transport and binding							
MT4	*Metallothionein IV*	3.1	3.4	3.4	3.3	2.8	−1.2
MT1G	*Metallothionein 1G*	4.0	2.2	3.5	3.2	9.1	11.5
Immune response and cellular defense							
C4BP4	*Complement component 4 binding protein, α*	3.3	4.4	4.2	4.0	2.9	1.4
CXCL14	*Small inducible cytokine subfamily B (Cys-X-Cys), member 14 (BRAK)*	2.4	3.5	3.0	3.0	17.0	23.9

C. Genes 2-fold differentially expressed in clear cell ovarian cancers compared to other histotypes and with normal ovarian surface epithelial cells (genes with negative fold changes are down-regulated)

Symbol	Gene Description	CC vs E	CC vs S	CC vs M	Av	CC vs OSE	Endometrium vs OSE
Cell growth, differentiation, cell cycle progression, apoptosis, and hormonal regulation							
BCAT1	*Branched chain aminotransferase 1, cytosolic*	4.7	3.1	5.6	4.5	5.0	1.1
CALB1	*Calbindin 1 (28 kDa)*	3.5	4.5	4.7	4.2	4.7	−1.1
LEFTB	*Left-right determination, factor B*	4.4	2.7	3.0	3.4	4.1	1.2
LGR7	*Leucine-rich repeat-containing G protein – coupled receptor 7*	2.5	3.1	3.5	3.0	3.1	6.3

(*Continued on the following page*)

Table 3. (Cont'd)

C. Genes 2-fold differentially expressed in clear cell ovarian cancers compared to other histotypes and with normal ovarian surface epithelial cells (genes with negative fold changes are down-regulated)

Symbol	Gene Description	CC vs E	CC vs S	CC vs M	Av	CC vs OSE	Endometrium vs OSE
Cytoskeletal structure, extracellular matrix formation, cell adhesion, and coagulation							
PROM1	*Prominin-like 1 (mouse)*	2.5	3.0	2.9	2.8	10.4	2.8
Metabolism, proteolysis, and proteinase inhibition							
GALNT11	*UDP-N-acetyl-α-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 11*	2.9	2.6	3.8	3.1	2.8	1.0
GPX3	*Glutathione peroxidase 3 (plasma)*	2.6	2.4	2.6	2.5	2.4	2.5
GDA	*Guanine deaminase*	2.1	3.2	2.2	2.5	2.9	2.1
Protein transport and binding							
ABP1	*Amiloride-binding protein 1 [amine oxidase (copper-containing)]*	9.6	7.4	4.1	7.0	14.1	1.4
RBP4	*Retinol-binding protein 4, plasma*	6.2	6.7	3.0	5.3	6.4	2.6
GLRX	*Glutaredoxin (thioltransferase)*	4.8	4.7	3.0	4.2	5.4	1.9
FXYD2	*FXYD domain-containing ion transport regulator 2*	2.8	2.6	2.9	2.8	2.8	1.0
SLC7A2	*Solute carrier family 7 (cationic amino acid transporter, y+ system), member 2*	2.6	2.0	2.1	2.2	2.6	2.4
SLC40A1	*Solute carrier family 40 (iron-regulated transporter), member 1*	−2.0	−2.6	−2.6	−2.4	−4.9	−1.5
Unknown function							
PHF8	*PHD finger protein 8*	2.3	2.2	2.3	2.2	2.4	1.6

D. Genes 2-fold differentially expressed in serous ovarian cancers compared to other histotypes and with normal ovarian surface epithelial cells (genes with negative fold changes are down-regulated)

Symbol	Gene description	S vs E	S vs CC	S vs M	Av	S vs OSE	Fallopian tube vs OSE
Cell growth, differentiation, cell cycle progression, apoptosis, and hormonal regulation							
ID1	*Inhibitor of DNA binding 1, dominant-negative helix-loop-helix protein*	−2.8	−2.5	−2.7	−2.7	−2.4	−1.7
Cytoskeletal structure, extracellular matrix formation, cell adhesion, and coagulation							
CHI3L1	*Chitinase 3–like 1 (cartilage glycoprotein-39)*	2.1	3.8	2.7	2.9	3.8	1.5
Metabolism, proteolysis, and proteinase inhibition							
AMY2B	*Amylase, α2B; pancreatic*	3.4	2.6	3.5	3.2	2.7	4.5

NOTE: Genes with negative fold changes are down-regulated. M, mucinous; S, serous; CC, clear cell; E, endometrioid; OSE, ovarian surface epithelium.

endometrioid and clear cell carcinomas. In contrast to previous reports, we found the largest number of differentially expressed genes in mucinous cancers. Alterations in gene expression might provide useful markers for studies of differentiation among the histotypes of epithelial ovarian cancer.

Materials and Methods

Tissues. Fifty flash-frozen primary epithelial ovarian cancers of different histologies, stages, and grades were collected: 14 serous from University of Texas M.D. Anderson Cancer Center; 9 endometrioid, 9 clear cell, and 9 mucinous from Duke University; and 9 serous from the Mayo Clinic (Table 1). All tumors were classified according to grade and stage using standard International Federation of Gynecology and Obstetrics criteria. Five pools of normal ovarian epithelial brushings from 42 different individuals were obtained from Northwestern University (Evanston, IL). Brushings contained at least 95% epithelial cells. Patients ranged in age from 43 to 79 years, with a median age of 55 years; two-thirds were postmenopausal and one-third were premenopausal. Of the postmenopausal donors, ~60% were receiving hormone replacement therapy. The normal cells were collected using a cytobrush and were immediately suspended and frozen in RLT buffer (Qiagen, Valencia, CA). Four colon, four fallopian tube scrapings, and five secretory endometria were collected from University of Texas M.D. Anderson Cancer Center. Institutional review board approval had been obtained at each participating institution before the initiation of this study.

RNA purification and gene expression analysis. Total RNA was extracted from all ovarian cancers and normal ovarian epithelial scrapings using the RNeasy kit (Qiagen) according to the manufacturer's protocol. The Affymetrix Human Genome U95 set of arrays (Affymetrix, Santa Clara, CA) was used for this expression analysis. This series contains ~63,000 probe sets. The biotinylated cRNA preparation, hybridization, and scanning of microarrays were done according to the manufacturer's protocols. Data were collected using GeneChip software (Affymetrix), whereas expression values were determined using dChip perfect match–only model (35). Gene expression data can be found at http://www.mdanderson.org/departments/expther/bastovcalab/.

Data analysis. Samples were contrasted by histotype, grade, and stage to see which stratifications produced the most pervasive differences. Kruskal-Wallis contrasts were done both with and without inclusion of the normal ovary pools. To assess the relative strengths of the histotype separations, we first identified the genes showing the greatest ability to differentiate five subtypes using Kruskal-Wallis tests. Setting a univariate P cutoff of 0.0005, we identified 490 genes from 63,000 probe sets, suggesting a false discovery rate slightly less than 5%. Hierarchical clustering using rank correlation with complete linkage was then applied to the matrix of the ranks of these genes across samples. We began with the hypothesis that many of the differences between histotypes might be due to the tumors arising from different types of local normal mucosae, such as mucinous tumors arising from colonic tissue. If this hypothesis holds, then we might expect that several of the differences between histotypes might parallel the differences between the associated normal tissue types. To test this hypothesis, we first arrayed a panel of 18 normal epithelial samples for comparison: 4 colon, 4 fallopian tube scrapings, 5 endometria, and the 5 ovarian surface epithelial pools described above. Next, by contrasting histotypes, we assembled the lists of genes we would use for comparing the normal mucosae and checking for parallelism. There is one list per histotype. A gene made it onto the mucinous list (for example) by having an average expression level in mucinous tumors that was (*a*) >2-fold greater (less) than the average expression level in each of the four other groups (three histotypes and ovarian surface epithelium) and (*b*) >100 units greater (less) than the average expression level in each of the four other groups. The expression levels of each gene on the list were then ranked across the 18 normal samples. If gene expression is elevated in the target histotype, a 1 indicates the lowest expression in the normal samples and 18 the highest; if reduced, a 1 indicates the highest expression and 18 the lowest. For a gene more strongly expressed in the mucinous histotype, if this difference is due in part to (*a*) origin in colonic cells and (*b*) higher levels of expression in colon than in other normal mucosae, then we would expect the highest ranks for this gene to be observed in the colon samples. The ranks are then summed over (*a*) all samples in the tissue group (e.g., colon) and (*b*) all genes in the list, and a "large" value of the sum indicates that genes chosen for elevation in a histotype show a parallel elevation in the corresponding normal mucosae, suggesting an association. Significance was assessed by randomly permuting the group labels among samples and summing multiple times; the observed distribution of a given type of sums (e.g., "mucinous") is our null distribution.

Reverse transcription-PCR. Semiquantitative reverse transcription-PCR (RT-PCR) was done to confirm gene expression using the DNA 500 LabChip and Agilent 2100 Bioanalyzer (Agilent Technologies, Germantown, MD). Gene expression was evaluated in four new pools of normal ovarian surface epithelial scrapings and five ovarian cancers from each histotype. Due to limited material, two new samples for both mucinous and serous cancers were combined with three ovarian cancers included in the microarray analysis. For clear cell cancers, one new sample was combined with four ovarian cancers included in the microarray analysis. All five endometrioid samples were used previously in the microarray analysis. cDNA was synthesized using 1 μg total RNA, oligo(dT_{16}), and Superscript II reverse transcriptase (Invitrogen, Carlsbad, CA). Synthesis was done according to the manufacturer's instructions. Gene expression was calculated as a percent relative to glyceraldehyde-3-phosphate dehydrogenase expression in the corresponding samples.

To further quantitate gene expression of mucinous specific markers, real-time quantitative RT-PCR was done using the ABI PRISM 7000 (Applied Biosystems, Foster City, CA). The same samples used for semiquantitative RT-PCR were used for the real-time quantitative RT-PCR. In each reaction, 1 μL target cDNA was amplified in a 50 μL reaction using SYBR Green PCR Master Mix (Applied Biosystems). The comparative threshold cycle (C_T) method was used to calculate relative quantitation between ovarian surface epithelium and ovarian cancers. Fold differences relative to normal ovarian surface epithelium were determined by $2^{-\Delta\Delta CT}$.

Results

Gene expression array analysis distinguishes ovarian cancer histotypes. Microarray analysis was done to compare gene expression in 50 ovarian cancer specimens, including all four histotypes to gene expression in 5 pools of normal ovarian surface epithelial cells. Data were analyzed to determine whether changes in gene expression correlated with different histotypes, grade, or stage. In broad outline, differences were most pervasive when samples were stratified by histotype, followed by grade, and finally followed by stage. When normal samples were included in the mix, the numbers of probe sets showing significant differences ($P < 0.0005$ without adjustment for multiple testing) were 490 for histotype, 292 for grade, and 94 for stage. Focusing solely on the tumors, the numbers were 190 for histotype, 87 for grade, and 53 for stage. We would expect ~30 by chance alone. We briefly investigated the expression patterns by filtering the genes for tumor expression values exhibiting both a minimal level of variation (coefficient of variation > 0.5) and a minimal level of expression ($\mu > 100$) and clustering. The normals clearly split off from everything else, as did most of the mucinous samples. Restricting attention to a partially supervised list produced by a Kruskal-Wallis filter, we would expect to see some separation by histotype, but the internal grouping structure is still open. Here, we saw a major division grouping normals with mucinous and splitting the pair off from the rest. A dendrogram (Fig. 1) was constructed by calculating the Kruskal-Wallis values for 63,000 probe sets and then creating a cluster based on the top 490 differentially expressed probe sets ($P < 0.0005$). The dendrogram shows a clear separation between individual histotypes and normal ovarian surface epithelial cells. Overall, 50 of the 55 samples were correctly categorized. Expression profiles were separated into two main divisions. Normal ovarian surface epithelial cells and mucinous ovarian cancers clustered together in one branch, whereas the other histotypes clustered in a second branch. Within the second branch, the majority of serous ovarian cancers could be separated from endometrioid and clear cell cancers that clustered farthest from normal ovarian surface epithelial cells. We examined the stage and grade of the five misclassified samples to determine whether these factors contributed to the misclassification. Similar samples with the same histotype, stage, and grade segregated properly; therefore, misclassification does not seem to be a consequence of stage or grade.

Mucinous carcinomas exhibit a greater number of dysregulated genes than other histotypes. Previously reported studies that had identified differences in gene expression among ovarian cancer histotypes had not compared expression in cancer with that in normal ovarian epithelial brushings (33, 34). Comparing expression between histotypes and normal ovarian surface epithelial cells might identify genes that are related to ovarian oncogenesis. In examining the array data, more than half of the genes that distinguished histotypes were eliminated from further consideration, as their expression did not differ from that in normal ovarian surface epithelial cells. For those genes whose expression distinguished histotypes and differed from normal ovarian surface epithelial cells, marked differences were observed among histotypes in the number of up-regulated and down-regulated genes observed (Table 2). Mucinous ovarian

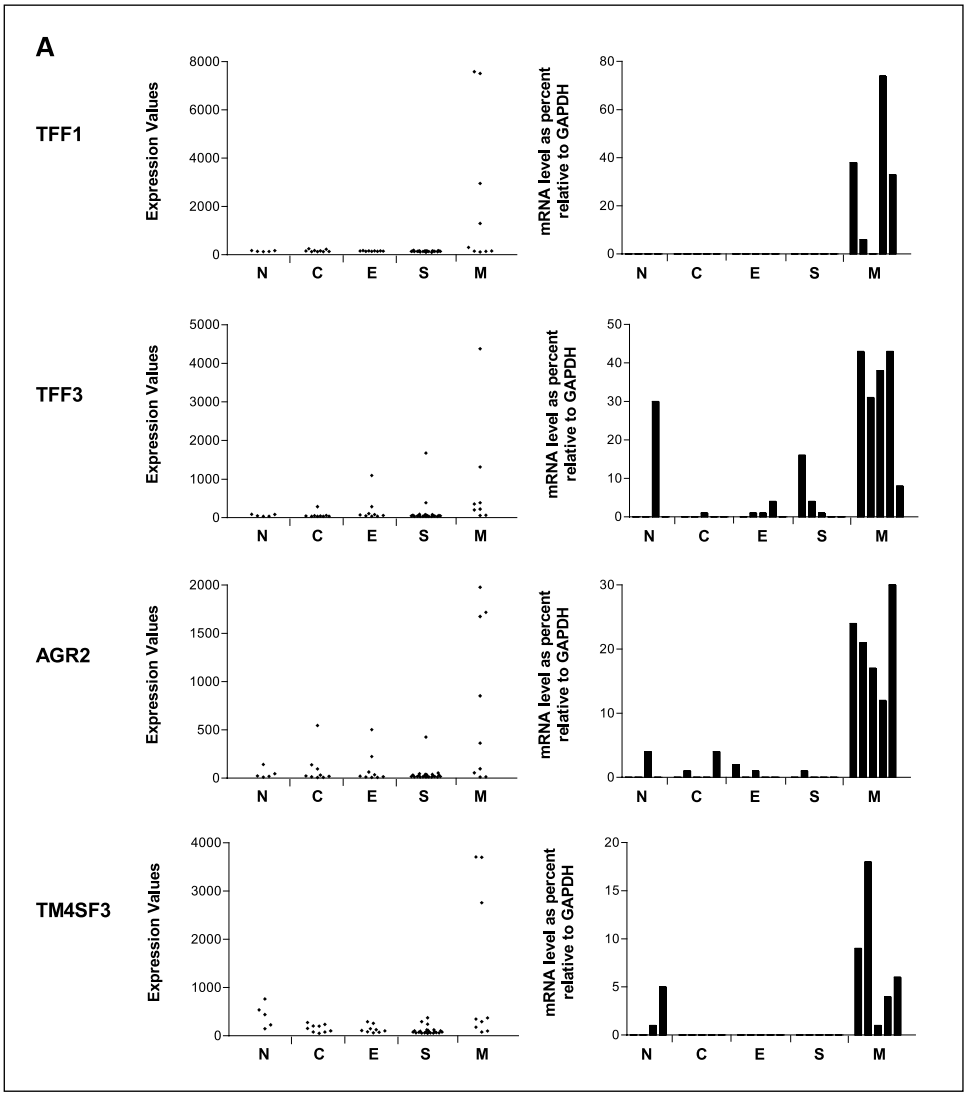
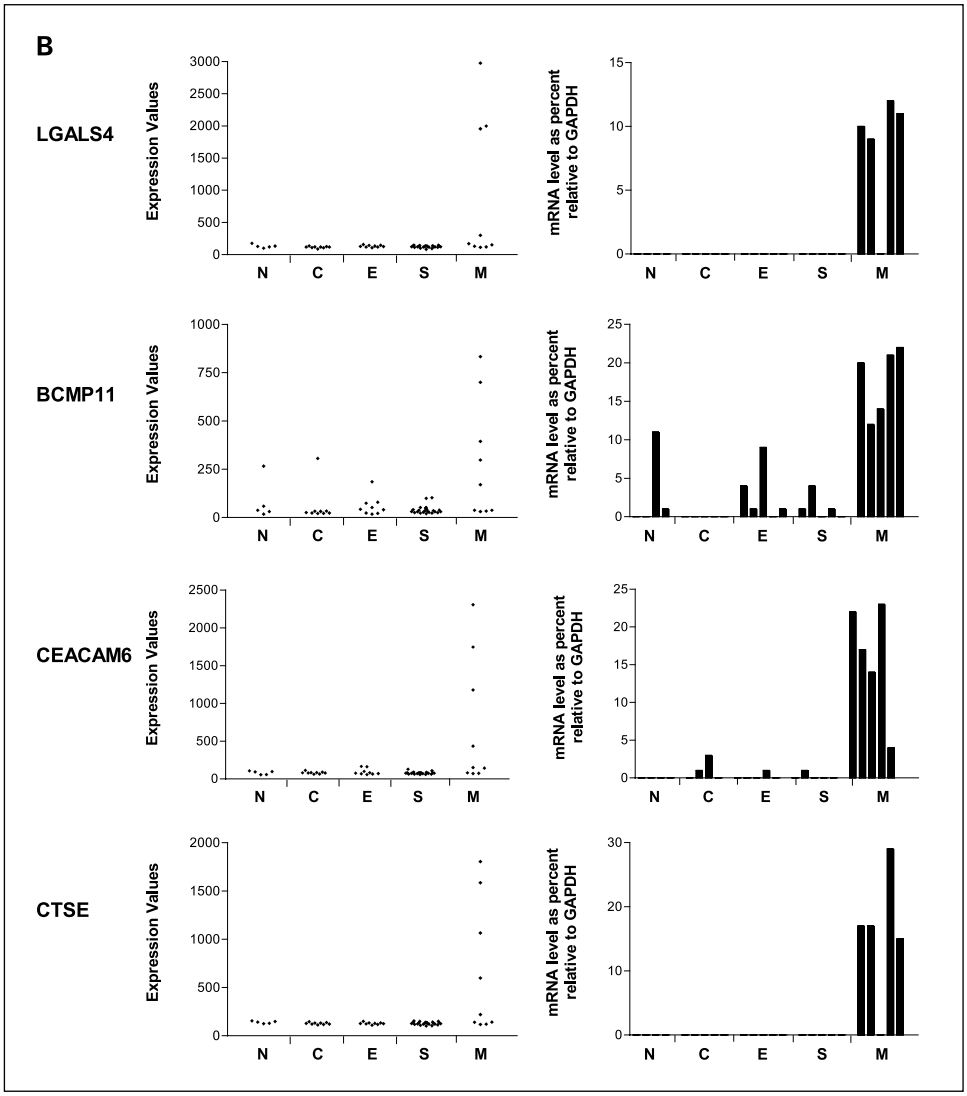


Fig. 2. Gene expression determined by microarray analysis and semiquantitative RT-PCR for genes up-regulated in mucinous ovarian cancers (*TFF1, TFF3, AGR2, TM4SF3, CEACAM6, LGALS4, BCMP11*, and *CTSE*). N, ovarian surface epithelium; C, clear cell; E, endometrioid; S, serous; M, mucinous.

cancers had the largest number of dysregulated genes (n = 57), which is more than twice as many dysregulated genes as the other histotypes. Clear cell and endometrioid ovarian cancers had similar numbers of dysregulated genes (n = 25 and 19, respectively). Serous cancers had only 6 dysregulated genes. In addition, genes that marked mucinous cancers exhibited a greater average fold change (12.8-fold) than the other three histotypes (range, 3.2- to 7.0-fold; Table 3A-D). Differentially expressed sequence tags (EST) were not included in Table 3. Across all histotypes, 102 differentially expressed genes were up-regulated and 5 were down-regulated 2-fold (Table 2). When gene expression in all 50 ovarian cancers was compared with that in 5 pools of normal ovarian surface epithelial cells without regard to histotype, 271 genes were 2-fold up-regulated and 200 were 2-fold down-regulated. Consequently, genes that distinguished histotypes from each other and from normal ovarian surface epithelial cells were largely up-regulated rather than down-regulated.

Reverse transcription-PCR analysis confirms up-regulation of genes identified in mucinous ovarian cancers but not in other histotypes. Array analysis has proven to be a reliable method for identifying differentially expressed genes in normal and cancer tissues, but differences in expression cannot always be confirmed with other techniques. We used semiquantitative RT-PCR to confirm gene expression for genes up-regulated at least 5-fold in mucinous cancers versus each individual histotype and at least 3-fold versus normal ovarian surface epithelial cells. Eight genes were evaluated: *TFF1*, *TFF3*, *AGR2*, *TM4SF3*, *LGALS4*, *BCMP11*, *CEACAM6*, and *CTSE*. Figure 2 shows gene expression data from microarray analysis and from semiquantitative RT-PCR for each up-regulated gene. All genes were up-regulated on RT-PCR in at least three or more of the five mucinous samples relative to the other histotypes. *TFF3*, *TM4SF3*, and *BCMP11* seem to be less specific markers for mucinous cancers on RT-PCR analysis, with up-regulation of each gene in one of five samples of normal ovarian surface epithelium. *BCMP11* was also up-regulated in two endometrioid samples, but all five mucinous samples showed greater up-regulation than was observed in the endometrioid ovarian cancers. Consequently, the most specific markers for mucinous ovarian cancers were *TFF1*, *AGR2*, *LGALS4*, *CEACAM6*, and *CTSE*. Further quantitation of *AGR2* and *LGALS4* showed a significant fold difference between mucinous tumors and normal ovarian surface epithelium. *AGR2* expression in all five mucinous samples ranged from



190- to 1,115-fold greater than normal ovarian surface epithelium, whereas expression in ovarian cancers ranged from 0 to 21-fold. Similarly, *LGALS4* expression in four of five mucinous samples ranged from 178- to 633-fold greater than normal ovarian surface epithelium. *LGALS4* expression in 19 ovarian cancer samples ranged from 1- to 11-fold greater than normal ovarian surface epithelium and 1 serous sample had a fold difference of 128.

RT-PCR analysis was also done for expression of the serous candidate gene *AMY2B*, the clear cell candidate gene *RBP4*, and the endometrioid candidate gene *FGF9* (Fig. 3). Although the highest levels of *AMY2B* were observed in serous ovarian cancers, expression was also detected in other histotypes and in normal epithelial cells. *RBP4* was strongly expressed by two of five clear cell cancers but was also detected at lower levels in mucinous cancers. *FGF9* was expressed across all histotypes and by each of five normal ovarian epithelial specimens. Thus, specific markers were not found for the nonmucinous histotypes.

Histotype-related changes in gene expression correlate with gene expression in normal tissues. Pathologists have pointed to the resemblance of the mucinous histotype to normal colonic epithelium, the serous histotype to fallopian tube, and the endometrioid histotype to endometrium. To determine whether similarities in morphology might reflect similarities in gene expression, we did expression array analysis of mucosal scrapings from four fallopian tubes, four colonic segments, and five endometria. Gene expression of histotypes and normal tissue were compared with that in normal ovarian epithelial cells as outlined in Materials and Methods. Correlation was sought between genes dysregulated in individual histotypes and in the corresponding normal tissues (Table 4). Statistically significant correlations were observed between gene expression in mucinous ovarian cancers and in normal colonic epithelium ($P = 0.0003$) as well as in serous ovarian cancers and normal fallopian tube epithelium ($P = 0.0013$). Interestingly, gene expression in both endometrioid and clear cell ovarian cancers correlated with that in normal endometrium ($P = 0.0002$ and 0.0172, respectively).

Discussion

Our data confirm the ability of gene expression profiles to distinguish epithelial ovarian cancers from normal ovarian

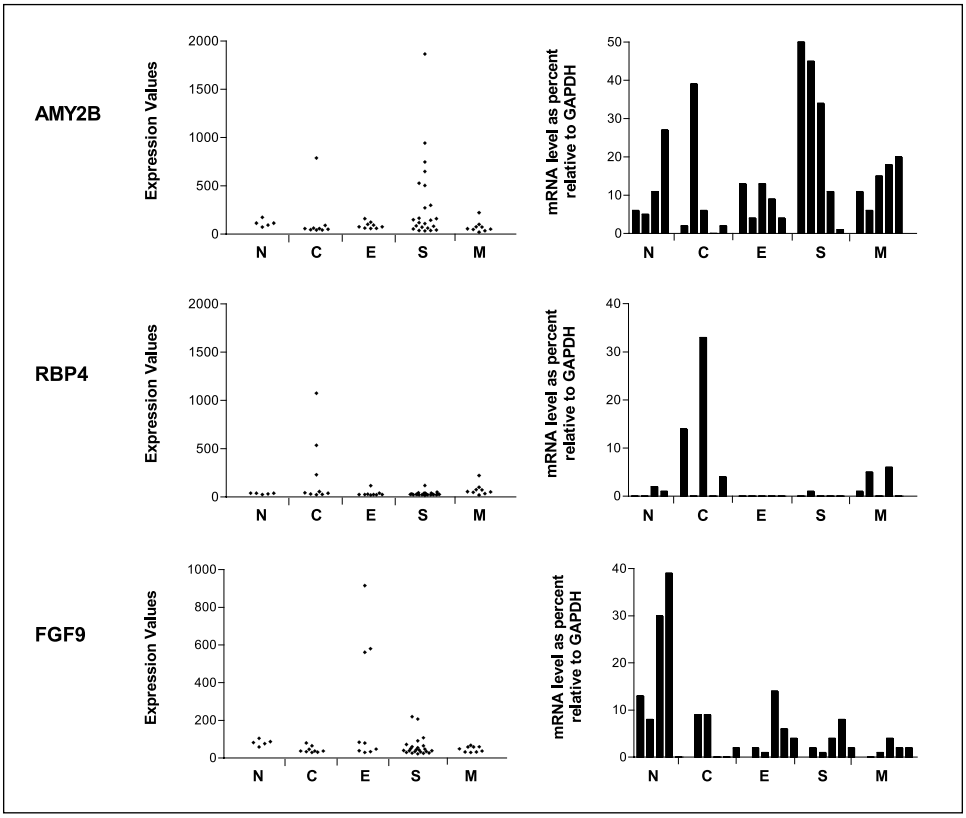


Fig. 3. Gene expression determined by microarray analysis and semiquantitative RT-PCR for serous, clear cell, and endometrioid specific genes (*AMY2B*, *RBP4*, and *FGF9*).

epithelial cells and to distinguish four different histotypes. Overall, a dendrogram (Fig. 1) correctly sorted 50 of 55 specimens (93%). Schwartz et al. had also found that differences in gene expression correlated with differences in morphology (33, 34) but had not compared gene expression in cancers with that in normal ovarian epithelial cells. In their initial study, the pattern of gene expression in endometrioid ovarian cancers overlapped with those of all other histotypes. In our analysis, apparent misclassification was balanced between the four histotypes. Misclassification did not seem to be associated with stage or grade.

In the earlier study, clear cell ovarian cancers exhibited the greatest number of changes in gene expression, whereas our data suggest that mucinous ovarian cancers have the most distinctive transcriptional profiles. Schwartz et al. found 158 up-regulated genes that distinguished clear cell carcinomas, whereas we identified only 31 up-regulated genes and ESTs that correlated with this histotype. Of these 31 genes, 24 were up-regulated >2-fold in normal ovarian surface epithelium. Differences in the number of genes identified may be due to the relatively small number of clear cell cancers studied in both reports and the use of different generations of Affymetrix arrays in the two studies and partly to our exclusion of genes that were not dysregulated when compared with normal ovarian epithelial cells. Clear cell candidate genes (*GPX3*, *GLRX*, *FXYD2*, and *RBP4*) were the only genes shown to be up-regulated in our data as well as in the Schwartz et al. data.

In our current study, mucinous cancers exhibited the most distinctive expression signature with the greatest number of dysregulated genes relative to all other histotypes. This may relate to the fact that our mucinous tumors were predominantly low-grade, well-differentiated carcinomas, whereas other histotypes were predominantly high grade. Only 2 of 9 mucinous carcinomas were grade 3, whereas 19 of 23 serous, 6 of 9 endometrioid, and all 9 clear cell carcinomas were high grade. Differences in results with clear cell cancers cannot, however, be related simply to grade in that all specimens of this histotype were high grade in both studies. Interestingly, our dendrogram grouped mucinous and normal ovarian surface epithelial cells in the same branch, suggesting that despite distinctive changes in differentiation the well-differentiated mucinous carcinomas still most closely resembled normal epithelial cells in their overall gene profile.

Table 4. *P*s for the rank-sum analysis to test the correlation between individual histotypes and normal tissues

	Normal ovary	Fallopian tube	Colon	Endometrium
Clear cell	0.9623	0.7791	0.6775	**0.0002**
Endometrioid	0.4915	0.5928	0.9748	**0.0172**
Serous	0.0743	**0.0042**	0.9993	0.8504
Mucinous	0.6905	0.4863	**0.0003**	0.9860

NOTE: Bold numbers identify significant correlation at the *P*s noted.

Of the dysregulated genes that marked different histotypes, 102 of 107 (95%) were up-regulated. By contrast, when gene expression by cancers of all histotypes was compared with gene expression in normal ovarian epithelial cells, 271 of 471 (58%) of the dysregulated genes were up-regulated. Thus, cellular differentiation, reflected in morphologic change, may relate to up-regulation of genes rather than to a loss of gene expression. In the mucinous and endometrioid histotypes, we identified clusters of genes that mapped to the same chromosomal bands. Of the genes up-regulated in mucinous cancers, *TFF1*, *TFF2*, and *TFF3* map to 21q22.3; *IL6* and members of the anterior gradient homologue family, *AGR2* and *BCMP11*, to 7p21; *LILRB1*, *CEACAM5*, *CEACAM6*, *LGALS4*, and *FCGBP* to 19q13; *CYP3A5* and *SERPINE1* to 7q21; and *TUBB* and *SERPINB1* to 6p25. Clusters of genes up-regulated in endometrioid cancers included *MTIV* and *MT1G* located at 16q31 and *SERPINA1*, *SERPINA3*, *SERPINA5*, and *IHH* at 14q32. Coordinate regulation might be due to chromosomal amplification or to transcriptional activation. The location of genes that were up-regulated in each histotype was compared with the location of amplicons on comparative genome hybridization analysis. Of the 79 genes that were differentially expressed across histotypes, 11 were found on amplicons with increased copy number on high-resolution BAC arrays.[9] Of the genes that marked different histotypes and that appeared to cluster, only *SERPINE1* was associated with increased copy number, but *CYP3A5* that localized to the same chromosomal band was not. Increased copy number can be found in the absence of peaks on comparative genome hybridization, but these data are most consistent with the importance of coordinate transcriptional regulation of genes, both individually and in clusters within each histotype.

Genes that distinguished different histotypes belonged to a broad range of functional groups (Table 3). Differentially expressed genes were found in several different functional categories across all four histotypes, including (*a*) cell growth, differentiation, cell cycle regulation, and apoptosis; (*b*) cytoskeletal structure, cell adhesion, extracellular matrix formation, and coagulation factors; and (*c*) metabolism, proteolysis, and proteinase inhibition. Distinctive transport and binding proteins were found in mucinous, endometrioid, and clear cell cancers. Consistent with a previous report (32), several genes that marked endometrioid differentiation were associated with the β-catenin signaling pathway, including *FGF9*, *SERPINA1*, *SERPINA3*, *SERPINA5*, *IHH*, and *SFN*. In addition, *APCDD1* and *FZD10* were also up-regulated in endometrioid cancers.

Histotype-related changes in gene expression correlated with gene expression in normal tissues when normal ovarian surface epithelial cells were used as a standard for comparison. Pathologists have pointed to the resemblance of the mucinous histotype to normal colonic epithelium, the serous histotype to fallopian tube, and the endometrioid histotype to endometrium. This report shows for the first time that genes expressed in different ovarian cancer histotypes are also concordantly expressed in the normal tissues that they resemble histologically.

According to our data, several genes that were distinctively expressed in mucinous carcinomas were also up-regulated in normal colon. Previous studies have detected expression of these genes in normal colonic epithelial cells. *CEACAM5* and *CEACAM6* are localized to the apical glycocalyx of normal colonic epithelium and may play a role in innate immunity (36). *CEACAM6* expression has correlated with apoptosis in normal colonic epithelial cells, and both *CEACAM5* and *CEACAM6* are absent from highly proliferating cells at the base of colonic crypts (37). *LGALS4*, confined to the epithelial cells of the embryonic and adult gastrointestinal tract, is expressed at about equal levels in the colon and small intestine but much less in the stomach (38). *TFF1*, *TFF2*, and *TFF3* are important for protection and healing of the human gastrointestinal tract (39). *FABP1* is overexpressed in inflammatory bowel disease (40).

Our results indicate endometrioid candidate genes, such as *FGF9*, *SFN*, *MT1G*, and *IHH*, were also expressed in endometrium. Previous reports state that *FGF9* is expressed in normal ovarian epithelial cells and is also expressed at high levels in normal uterine endometrium, especially during the late proliferative phase (41). *SFN* and *MT1G* are up-regulated in endometrium during secretory phase (42, 43). Studies with mice suggest that *IHH* is regulated by progesterone and that *IHH* signaling may play a role in the preparation of the uterus for implantation of the embryo (44). The correlation between gene expression in normal endometrium with that in both clear cell and endometrioid ovarian cancers is unexpected, but previous studies have suggested that both clear cell and endometrioid ovarian cancers can arise from endometriosis (45–47).

Two genes that marked serous ovarian cancers were up-regulated in normal fallopian tube: *AMY2B* and *CHI3L1*. Amylase proteins are overexpressed in a variety of cancers and have been studied as serum markers for ovarian cancer (48). Amylase 1 has been found in the salivary gland and in lung cancers (49). Amylase 2 is found in pancreas, normal lung tissue, and epithelial cells from the thyroid, trachea, and gynecologic tract. Amylase 2 is overexpressed in serous ovarian cancers and in fallopian tube. Consequently, amylase 2 seems to be a differentiation antigen associated with both normal and transformed tissues. *CHI3L1* (*YKL40*) has recently been proposed as a marker for early detection and prognosis in ovarian cancer, presumably related to its association with the most prevalent serous carcinomas (50).

In some cases, genes that were up-regulated in different histotypes were not up-regulated in normal tissues but up-regulated in cancers that developed from those organs. *GAS* is expressed in normal gastric mucosa but not in normal colon. *GAS* levels, however, have been increased in colon cancer (51). Other genes that were up-regulated in both ovarian and colon cancer included *MMP1*, *SERPINE1*, *REG4*, *TFF1*, *IL6*, and *IL8*. *MMP1* has been implicated in invasion and metastasis (52), *IL6* is an autocrine/paracrine growth factor (53), and *IL8* is an angiogenic factor (54). Consequently, these genes are likely to participate in malignant transformation.

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[9] J. Gray, personal communication.

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Clinical Cancer Research

Patterns of Gene Expression in Different Histotypes of Epithelial Ovarian Cancer Correlate with Those in Normal Fallopian Tube, Endometrium, and Colon

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