EXHIBIT 5
(PART 2 OF 4)

Table 3. Primer pairs for RNA amplification.

Gene specific primer pair		SEQ ID NO.
ACTB	5'-ATGTGGATCAGCAAGCAGGA-3'	SEQ ID NO: 2
	5'-GGTGGCTTTTAGGATGGCAA-3'	SEQ ID NO: 3
HE4	5'-TTCGGCTTCACCCTAGTCTCA-3'	SEQ ID NO: 4
	5'-AGAGGGAATACAGAGTCCCGAA-3'	SEQ ID NO: 5
ZFP36	5'-ACCCTGATGAATATGCCAGCA-3'	SEQ ID NO: 6
	5'-GCTACTTGCTTTTGGAGGGTA-3'	SEQ ID NO: 7
RGS1	5'-GACTCTTATCCCAGGTTCCTCA-3'	SEQ ID NO: 8
	5'-TGACTCCCTGGTTTTAAGAGCA-3'	SEQ ID NO: 9
CD74	5'-CCAGTCCCCATGTGAGAGCA-3'	SEQ ID NO: 10
	5'-AGCTGATAACAAGCTTGGCTGA-3'	SEQ ID NO: 11
TOP2A	5'-TGTCCCTCCACGAGAAACAGA-3'	SEQ ID NO: 12
	5'-CGTACAGATTTTGCCCGAGGA-3'	SEQ ID NO: 13
HLA-DRB1	5'-GCGAGTTGAGCCTAAGGTGA-3'	SEQ ID NO: 14
	5'-TTGAAGATGAGGCGCTGTCA-3'	SEQ ID NO: 15

Amplified RT-PCR products were visualized on an agarose gel stained with ethidium bromide. The intensity of each band was an indicator of the quantity of DNA, as previously amplified by PCR. Thus, the intensity served as an indirect measure of the starting amount of the RNA amplified from the respective gene in each sample. Intensity was quantified using an ultraviolet light source and Alpha Imager software (Alpha Innotech Corp, San Leandro, CA). In addition to the above-mentioned tumor samples, sqRT-PCR evaluation of selected genes was also performed on the IOSE RNA for comparison.

Results

Global assessment of gene expression differences among tumor groups: Prior to investigating specific inter-group differences, the overall patterns of gene expression in the three tumor types (*BRCA*-1, *BRCA*-2, sporadic) were assessed. Multidimensional scaling (MDS), based on the expression levels of all 6,445 filtered genetic elements in the microarray, revealed that *BRCA1*- and *BRCA2*-linked tumors have distinct molecular profiles. In contrast, the sporadic samples showed a more heterogeneous distribution pattern, with many patterns clustering near the patterns of *BRCA1*-linked or *BRCA2*-linked samples (Figure 1A). The MDS results suggested that the *BRCA1*- and *BRCA2*-associated groups would be the most different and that gene expression patterns for each of the BRCA groups and the sporadic tumors would have fewer differences. In support of this hypothesis, only a few genes showed statistically significant ($P<0.0001$) differential expression between the sporadic tumors and the *BRCA1*- or *BRCA2*-linked tumors, whereas 110 genes were differentially expressed between *BRCA1*-linked and *BRCA2*-linked tumors (Figure 1B). In addition 34 EST sequences were differentially expressed between *BRCA1*- and *BRCA2*-linked tumors. The group of 144 total markers that were differentially expressed between *BRCA1*- and *BRCA2*-type tumor cells compared to normal ovarian epithelial cells is listed in Table 9 (see Addendum).

Differential gene expression among all three groups was also performed, which identified 60 genes and 3 EST sequences whose expression segregated *BRCA1*-linked, *BRCA2*-linked, and

sporadic tumors (modified F test, wherein P<0.0001). Fifty-one of these 63 genes and EST sequences were also among the statistically significant discriminators of *BRCA1* and *BRCA2* tumors, highlighting the distinct gene expression profiles of these two groups. In addition, the expression profile of the combined *BRCA1*- and *BRCA2*-linked group was remarkably similar to that of the sporadic tumors, as demonstrated by only three genes showing differential expression (P<0.0001) between these groups [*PSTPIP1* (SEQ ID NO: 538-540), *IDH2* (SEQ ID NO: 541-542), and *PCTK1* (SEQ ID NO: 527-528)]. These observations were in agreement with the multidimensional scaling analysis and demonstrated that, in terms of the overall pattern of gene expression, the *BRCA1*- and *BRCA2*-linked tumors are distinct from one another. Furthermore, the gene expression profiles of the sporadic tumors appear to share features of either *BRCA1*- or *BRCA2*-linked cancers, and these sporadic tumors are referred to herein as *BRCA1*-type or *BRCA2*-type sporadic ovarian tumors.

The group of 144 nucleic acid molecules listed in Table 9 was further investigated using hierarchical clustering (Figure 2A, B). As expected, the *BRCA*-associated tumors showed distinct and contrasting expression profiles (Figure 2A). Strikingly, the sporadic samples also segregated into two groups based on the expression patterns of the same 144 genes, exhibiting sporadic sample had a molecular profile similar to that of either the *BRCA1*- or the *BRCA2*-linked tumors. This observation was illustrated by hierarchical clustering of all samples, revealing distinct "*BRCA1*-type" and "*BRCA2*-type" clusters (Figure 2A). This clustering further demonstrates that sporadic tumors (which do not contain the *BRCA1* or *BRCA2* mutations) can often be classified as *BRCA1*-type or *BRCA2*-type. Classification of sporadic tumors into these subtypes may provide guidance in treating the patient. For example, a subject who has a *BRCA1*-type or *BRCA2*-type sporadic tumor may be treated similarly to a subject who has a *BRCA1*-linked or *BRCA2*-linked tumor. The identification of *BRCA1*- or *BRCA2*-type sporadic tumors also allows tumors (or subjects) to be selected for specific drug regimens that are particularly effective with the associated mutation type.

Color-coding is usually used to represent the relative transcript expression ratio, as measured by cDNA microarray analysis. Red customarily indicates the maximum point in gene expression, green the minimum, and levels closer to the mean approach black

To ensure that the *BRCA*-linked samples were not biasing the observed clustering patterns, the hierarchical architecture of gene expression in sporadic tumors was examined separately. Even in the absence of the *BRCA*-linked samples, two distinct cluster phenotypes were observed, each comprised of those sporadic samples that previously grouped with *BRCA1*- and *BRCA2*-linked tumors (Figure 2B). Tumor histology and patient age were also evaluated for possible confounding effects on the observed *BRCA1*-type and *BRCA2*-type clusters. Neither variable was found to influence clustering patterns (Figure 2A, 2B).

Genes differentially expressed between BRCA1- and BRCA2-linked ovarian carcinomas: The analysis of overall gene expression patterns established that the same genes whose expression differentiated *BRCA1* and *BRCA2*-linked tumors, also identified two major sub-populations of sporadic cancers (Figure 3). As such, these nucleic acids are believed to represent important mediators of common genetic pathways in ovarian cancer and/or carcinogenesis. Many of these

genes are involved in important cellular functions including signal transduction, RNA processing and translation, chemokine signaling and immune modification, and DNA repair. By way of example, the *BRCA1*-associated tumors were characterized by higher *AKT1* (SEQ ID NO: 504-506) and lower *PTEN* (SEQ ID NO: 507-509) relative expression. In addition *UBL1* (SEQ ID NO: 510-512) (also known as *SUMO*-1 and sentrin) was more highly expressed in *BRCA1*- associated tumors. This molecule interacts with RAD51 and RAD52 and has been proposed to have a regulatory role in homologous recombination (see Li *et al.*, *Nuc. Ac. Res*: 28: 1145-1153, 2000). The preferential expression of UBL1 (SEQ ID NO: 510-512) in the *BRCA1*-linked samples may prove to be relevant to possible differences in DNA repair actions of the BRCA tumor suppressor genes.

By way of example, the *BRCA2*-linked tumors showed higher relative expression of *WNT2* (SEQ ID NO: 513-514 and *SFRP4* (SEQ ID NO: 515-517), which are members of the wnt-β-catenin-TCF signaling pathway. Another notable observation is that both *BRCA1*- and *BRCA2*-linked tumors showed preferential expression of proto-oncogenes commonly altered in hematologic malignancies. *BRCA1* tumors showed higher expression levels of *RUNX1(SEQ ID NO: 518-520)/AML1*, while *BRCA2*-associated samples showed preferential expression of *TAL1 (SEQ ID NO: 521-523)/SCL*. Both of these oncogenes are transcription factors involved in proliferation, and their preferential expression in *BRCA1*- and *BRCA2*-linked tumors may indicate that the activation of such a "proliferation driver" is a necessary step in ovarian carcinogenesis.

Gene expression differences between BRCA-linked and sporadic tumors: Nine non-redundant genes showed significant differential expression between *BRCA1*-linked and sporadic tumors [*CD72* (SEQ ID NO: 805), *SLC25A11* (SEQ ID NO: 544), *LCN2* (SEQ ID NO: 545-547), *PSTPIP1* (SEQ ID NO: 538-540), *SIAHBP1* (SEQ ID NO: 543), *UBE1* (SEQ ID NO: 533), *WAS* (SEQ ID NO: 524-526), *IDH2* (SEQ ID NO: 541-542), *PCTK1* (SEQ ID NO: 527-528), P<0.0001, Figure 4A . A noteworthy observation was that three of these genes, *WAS (SEQ ID NO: 524-526)*, *PCTK1 (SEQ ID NO: 527-528)*, and *UBE1 (SEQ ID NO: 533)*, have all been mapped to the Xp11.23 and all were higher expressed in the *BRCA1*-linked tumors. This observation seemed unlikely to be explained by chance alone as only 35 of the total 6,445 filtered spots (0.5%) on the microarray represent genes mapped to Xp11. To further investigate this pattern, a larger group of 53 genes was considered for differential expression between *BRCA1*-linked and sporadic tumors under the less stringent significance level of P<0.001. Among this group three additional genes, *SMC1L1 (SEQ ID NO: 530)*, *ARAF1 (SEQ ID NO: 531-532)*, and *EBP (SEQ ID NO: 529)*, were discovered that also mapped to the Xp11.23 locus and also showed higher mean expression in *BRCA1*-associated samples (FIG 4D). Thus, six of fifty-three genes differentially expressed between *BRCA1*-linked and sporadic samples (P<.001) mapped to Xp11.23 and all showed higher mean expression in *BRCA1*-linked tumors. *In silico* analysis of the location of these genes revealed that they are all confined to a 5-Mb region of DNA in Xp11.23 (Ensemble database, Prous Science, Philadelphia, PA 19102, U.S.A.).

The comparison between *BRCA2*-linked and sporadic tumors revealed only two genes with differential expression among these groups at the significance level of *P*<0.0001 (Figure 3). The gene designated as *LOC51760 (SEQ ID NO: 534-535)* is also known as B/K (encoding the

brain/kidney protein) and is moderately homologous to the synaptotagmin family of vesicular transport molecules. The second differentially expressed gene encodes low-density lipoprotein-related protein-associated protein 1 (*LRPAP1*), also known as alpha-2-macroglobulin receptor-associated protein 1.

A further comparison consisted of investigating gene expression differences between the combined *BRCA*-linked group and the sporadic group, which revealed only three non-redundant, differentially expressed genes [*PSTPIP1* (SEQ ID NO: 538-540), *IDH2* (SEQ ID NO: 541-542), and *PCTK1* (SEQ ID NO: 527-528), Figure 4C. All three genes were among the group of genes that differentiated *BRCA1*-linked and sporadic samples. This finding is consistent with the observation that the RNA profiles of sporadic ovarian cancers share significant similarities with those of *BRCA1*-linked or *BRCA2*-linked tumors. It is believed that the similarities shown in the RNA profiles is a general characteristic that applies to gene and protein component profiles as well. The small number of differentially expressed genes obtained from the comparison of the combined *BRCA* group to the sporadic tumors is the result of the latter also consisting of *BRCA1*-type and *BRCA2*-type molecular classes.

Gene expression features distinguishing ovarian cancers from ovarian surface epithelial cells: Gene expression patterns common among all tumor types were investigated to identify genes that may be associated with the transformed state, *i.e.*, genes commonly expressed in ovarian tumors irrespective of their hereditary or sporadic nature. Gene expression in all sixty-one primary tumor samples was compared to immortalized ovarian surface epithelial (IOSE) cells used as the common reference. Using the selection criterion of two-fold or greater expression ratio relative to the IOSE reference in at least two-thirds of all tumors, a list of 201 non-redundant genes and ESTs was generated. The top twenty-five overexpressed (*IL8 (SEQ ID NO: 449-451), GRO1 (SEQ ID NO: 452-453), ALDH1A3 (SEQ ID NO: 454-456), MMP1 (SEQ ID NO: 457-459), OSF-2 (SEQ ID NO: 460-461), CDC25B (SEQ ID NO: 462-464), FLNA (SEQ ID NO: 465-467), TFP12 (SEQ ID NO: 468-469), FGF2 (SEQ ID NO: 470-472) , CD44 (SEQ ID NO: 473-475), DYT1 (SEQ ID NO: 476-477), UCHL1 (SEQ ID NO: 478), FGF2 (SEQ ID NO: 470-472), PLAU (SEQ ID NO: 479-480), LDHA (SEQ ID NO: 256), PTGS2 (SEQ ID NO: 481-483), PRNP (SEQ ID NO: 484-486), MT1X (SEQ ID NO: 487-488), UGB (SEQ ID NO: 489-490), PBEF (SEQ ID NO: 491-493), TXNRD1 (SEQ ID NO: 494-496), NT5 (SEQ ID NO: 497-499), PTGS2 (SEQ ID NO: 481-483), MT2A (SEQ ID NO: 500-502), ZNF220 (SEQ ID NO: 503)*) and twenty-five down-regulated (*FLJ22174 (SEQ ID NO: 30-31), DDR1 (SEQ ID NO: 74-76), SERPINF2 (SEQ ID NO: 18-19), HLA-DRB1 (SEQ ID NO: 87-88), IFITM2 (SEQ ID NOS: 55-57, 58-59), HGF (SEQ ID NO: 174-175), SORL1 (SEQ ID NO: 149-151), CP (SEQ ID NO: 83-84), HLA-DRA (SEQ ID NO: 94-96), BRF2 (SEQ ID NO: 190-192), ABCB1 (SEQ ID NO: 164-166), G1P3 (SEQ ID NO: 68-69), RGS1 (SEQ ID NO: 122-123), IFITM1 (SEQ ID NOS: 50-51, 52-54), FOS (SEQ ID NO: 133-135), PPP1R7 (179-180), HLA-DPA (SEQ ID NO: 97-99), HLA-DRB5 (SEQ ID NO: 85-86), TLR3 (SEQ ID NO: 199-201), ZFP36 (SEQ ID NOS: 167-168, 169-171, 172-173), SGK (SEQ ID NO: 176-178), HLA-DRB1 (SEQ ID NO: 87-88), HE4 (SEQ ID NO: 60), CD74 (SEQ ID NO: 89-91, CD24 (SEQ ID NO: 181-182))* named genes (by order of

magnitude) are presented in Figure 4A and 4B. This analysis revealed two potentially significant functional groups of genes to be overexpressed in ovarian cancers. The first group consisted of several of the genes that have all been previously shown to be interferon-inducible (*HLA-DRB1 (SEQ ID NO: 87-88)*, *HLA-DRB5 (SEQ ID NO: 85-86)*, *HLA-DRA (SEQ ID NO: 373-374)*, *HLA-DPA (SEQ ID NO: 97-99)*, *CD74 (SEQ ID NO: 89-91)*, *IFITM1 (SEQ ID NOS: 50-51, 52-54)*, and *IFITM2 (SEQ ID NOS: 55-57, 58-59)*, as indicated by italics in Figure 4A and 4B). The second group consisted of immediate-early response genes (*BRF2*, *ZFP36*, *SGK*, and *FOS*). In addition, several genes previously reported to be overexpressed in ovarian epithelial tumors were present in the list of genes overexpressed in tumors relative to the IOSE cells (Figure 4A and 4B). Elevated levels of *CLU*, *CD24*, and *MUC1* were also observed. These results identify additional potential markers of ovarian cancer. Table 9 lists the 144 nucleic acids that showed significantly elevated expression in ovarian cancer. These genes were selected based on consistency across all the pooled experiments and a significant difference in the average expression in the 40 independent samples, using a criteria of a tumor-to-ovarian surface epithelial cell line ratio of two or greater in at least 66% of all tumors.

EXAMPLE 2

Semiquantitative RT-PCR confirms and complements cDNA microarray data

This example describes how the results found in the previous example were confirmed using semiquantitative RT-PCR.

To validate the array data, semiquantitative RT-PCR (sqRT-PCR) analysis of several mRNAs was performed in a representative subset of tumors consisting of five *BRCA1*-linked, five *BRCA2*-linked, and five sporadic RNA samples. The tumor samples were randomly selected. The expression of *TOP2A (SEQ ID NO: 448)*, *RGS1 (SEQ ID NO: 398*, *CD74 (SEQ ID NOS: 89-91, 92-93)*, *HE4 (SEQ ID NO: 60)*, *HLA-DRB1 (SEQ ID NO: 87-88)*, and *ZFP36 (SEQ ID NO: 167-168, 169-171, 172-173)* were evaluated using sqRT-PCR, with β-actin as a normalizing control. Because data obtained from cDNA microarrays is in the form of relative expression ratios between tumors and the reference, RNA from IOSE cells and a histologically normal, postmenopausal ovarian RNA sample in the sqRT-PCR experiments was included for comparison. The results of these sqRT-PCR experiments were consistent with the cDNA microarray relative expression data for all six genes evaluated (Figure 5A and 5B). As anticipated from the microarray results (Figure 4), HE4 expression was consistently elevated in all fifteen tumor samples compared to IOSE reference cell-line and normal ovary (Figure 5A and 5B). Invariant chain genes, also known as *CD74* and *RGS1*, were overexpressed in the majority of tumors as indicated by microarray analysis (Figure 4). Both were also found to have increased expression in the majority of tumors as evaluated by sqRT-PCR (Figure 5A and 5B). The expression of *TOP2A* was found to be highest in the reference IOSE RNA. Furthermore, compared with the expression level in the normal postmenopausal ovary, twelve of the fifteen tested tumor samples showed elevated and variable *TOP2A* gene expression.

Several members of the immediate-early response cascade showed elevated expression in tumors as compared to IOSE cells in the microarray experiments (as indicated by the notation * in Figure 4A and 4B); however, some of these genes have previously been shown to have lower expression in ovarian cancer compared to normal ovary (see Welsh *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 98: 1176-1181, 2001, and Wang *et al.*, *Gene* 229: 101-108, 1999).

This discrepancy suggested that the elevated relative ratio observed in these experiments may be driven by low expression levels of these genes in IOSE cells grown in culture. In order to test this hypothesis, sqRT-PCR was used to compare *ZFP36* (an immediate-early gene also known as *G0S24* and *Tis11*) expression in tumors to that of normal ovary and IOSE cells. As suspected, normal ovary had one of the highest expression levels of *ZFP36*, followed by that of the majority of tumors (Figure 5A), while the lowest expression level was observed in the IOSE cells.

In addition to statistical analysis, multidimensional scaling (MDS) and hierarchical clustering techniques using a correlation metric and average linkage were used for evaluating overall gene expression (see Eisen *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 95: 14863-14868, 1998).

EXAMPLE 3

Identification of Additional Genes with Altered Expression in Ovarian Cancer

This example provides a description of how additional disclosed ovarian cancer-related nucleic acid molecules were identified. These ovarian cancer-related molecules show differences in expression in subjects having ovarian cancer compared to expression in normal ovarian surface epithelial cells.

Using a different microarrays and methods essentially similar to those described above in Example 1, thirty-one ovarian epithelial cancers were compared to two normal postmenopausal ovarian samples. 141 additional ovarian cancer-related nucleic acid molecules were identified and further characterized (Tables 6 and 7, Addendum).

Methods and Materials:

Methods and materials were similar to those described in Example 1, except that different microarrays were used. The nucleic acids constituted 7,600 features, and representing different (non-redundant) transcripts including multiple known named genes and ESTs. The cDNA microarrays were constructed by Dr. Eric Chuang (Division of Radiation Oncology) at the Advanced Technology Center (Gaithersburg, MD 20877). The genes represented on these arrays are composed of 7,600 cDNA clones and ESTs and are commercially available (Research Genetics, 2130 Memorial Parkway, Huntsvillle, AL 35801, U.S).

The nucleic acid molecule expression patterns of thirty-one ovarian epithelial cancers were compared to two normal postmenopausal ovarian samples. The tissues were analyzed once, as the

correlation coefficient from previously repeated array experiments was shown to be 0.92-0.95. Each tumor and normal sample was directly compared to a "reference RNA" consisting of a mix of nine different human cell lines (Stratagene, La Jolla, CA), allowing for indirect comparison of gene expression in tumors and normal ovarian samples.

Hierarchcal clustering was performed as described above and as set forth in Eisen *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 95: 14863-8, 1998.

Results

Systematically Altered Genes

Using these methods, two additional sets of nucleic acid molecules were identified that showed differential expression in subjects having ovarian cancer. Table 4 (see Addendum) provides a list of nucleic acid molecules that were found to be underexpressed in subjects having ovarian cancer, and their average gene log expression ratios. Table 5 (see Addendum) shows nucleic acid molecules that were found to be overexpressed in persons having ovarian cancer, and their average gene log expression ratios.

Genes underexpressed in ovarian tumors (see Table 4) may represent potential tumor suppressors. The induction of the expression of these genes through therapeutic means, for instance by induction through drug or gene therapy, may slow tumor growth and/or increase tumor cell death.

Among the 100 underexpressed genes were several oncogenes coding for proteins that are normally associated with the process of malignant transformation, 8ncluding *JUN* (SEQ ID NO: 137-138), *KIT (SEQ ID NO: 298-299)*, and *MAF (SEQ ID NO: 229-230)*. The lower expression of these genes in cancers compared to expression in non-cancerous subjects is unexpected and is believed to reflect novel effects unique to ovarian cancer. Additionally, *CDKN1C (SEQ ID NO: 249)*, *NBL1 (SEQ ID NO: 273)*, and *ING1L (SEQ ID NO: 322)* are recognized tumor suppressors, the downregulation of which may be involved in the process of tumor formation and/or progression. *TGF* beta cascade members *TGFBR3 (SEQ ID NO: 216-218)* and *EBAF (SEQ ID NO: 294)* (both shown herein to be underexpressed in ovarian cancer) present potential interest in light of the recent implication of the TGF beta pathway in normal and oral contraceptive-induced ovarian epithelial cell death and turnover (see Rodriguez *et al.*, J. *Natl. Can. Inst.* 94(1): 50-60, 2002). Thus, downregulation of these nucleic acids may lead to inappropriate growth and possible transformation.

Genes that were overexpressed in ovarian tumors (Table 5) compared to normal tissue are believed to represent suitable targets for therapy and/or diagnosis, prognosis and staging of ovarian cancer. The decrease of the expression of these genes through therapeutic means, for instance by drug or gene therapy, presents a potential method of inhibition of ovarian cancer.

Among the fifty-nine overexpressed genes were several genes coding for proteins that are believed to be particularly promising as gene targets, including the following: *SLPI* (Secretory leukocyte protease inhibitor) (SEQ ID NO: 340-341); *SPP1* (Secreted phosphoprotein 1) (SEQ ID NO: 342); *CKS1* (CDC28 protein kinase 1) (SEQ ID NO: 345-347); *ZWINT* (ZW10 interactor) (SEQ ID NO: 354); *BF* (B-factor, properdin) (SEQ ID NO: 343-344); *MMP7* (Matrix metalloproteinase 7)

(SEQ ID NO: 348-349); *FOLR1* (Folate receptor 1) (SEQ ID NO: 364-365); *KLK8* (Kallikrein 8) (SEQ ID NO: 368; *CRIP1* (Cysteine-rich protein 1) (SEQ ID NO: 375; *EYA2* (Eyes absent) (SEQ ID NO: 392-393); and *PAX8* (Paired box gene 8) (SEQ ID NO: 350-351).

SLPI is a particularly promising candidate as a potential ovarian cancer marker or detector. This protein has also been shown to be overexpressed in lung cancer (see Ameshima *et al.*, *Cancer* 89(7): 1448-1456, 2000) and is detectable in the saliva, enabling non-invasive testing (see Shugars *et al.*, *Gerontology*, 47(5): 246-253, 2001). *MMP7* over-expression has been described in primary and metastatic gastric cancers (see Mori *et al.*, *Surgery*, 131(1 Pt 2): S39-S47, 2002) as well as colorectal carcinomas (see Ougolkov *et al.*, *Gastroenterology*. 122(1): 60-71, 2002). MMP7 appears to be involved in new blood vessel formation, which is a prerequisite for tumor growth (see Nishizuka *et al.*, *Cancer Lett.* 173(2): 175-182, 2001). SPP1 (otherwise known as osteopondin) has also been associated with a number of malignancies (see Fedarko *et al.*, *Clin. Cancer Res.* 12: 4060-4066, 2001) including a recent report showing higher expression in ovarian cancer (see Mok *et al.*, *J. Natl. Cancer Inst.* 93(19) 1458-64, 2001). ZWINT is a newly discovered protein involved in kinetochore binding and centromere function (see Starr *et al.*, *J. Cell Sci.* 113(Pt 11): 1939-1950, 2000). Properdin is involved in immune function and encodes complement factor B, a component of the alternative pathway of complement activation. CRIP1 is believed to be involved in zinc transport. Kallikrein 8 (also *TADG14*) is normally expressed in neural tissue, but appears to be altered such that it is highly expressed in ovarian cancers (see Underwood *et al.*, *Cancer Res.* 59(17): 4435-4439, 1999). *EYA2*, named for its involvement in eye development, is an important developmental gene that is potentially important in ovarian cancer. *EYA2* is located on the 20q13 chromosomal locus, which is the most frequently amplified chromosome region in ovarian cancers (see Tanner *et al.*, *Clin. Cancer Res.* 5: 1833-1839, 2000). Other genes localized to the same 20q13 chromosomal region are *BMP7*, which is also involved in development, and *SLPI* (discussed above), as well as HE4 (identified in Example 1, above), all of which show higher expression in ovarian tumors. Thus, the upregulation of these nucleic acids may in part be due to amplification of 20q13 in the tumors studied.

PAX8 is involved in thyroid differentiation and normal function (see Pasca *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 97(24): 13144-13149, 2000). Furthermore, the folate receptor has been shown to be overexpressed in ovarian cancer (see Hough *et al.*, *Cancer Res.* 61(10): 3869-3876, 2001 and Bagnoli *et al.*, *Oncogene*, 19(41): 4754-4763, 2000). Finally, the specific pattern of caveolin (*CAV1*) under-expression and Folate receptor (*FOLR1*) over-expression disclosed herein (see Tables 4 & 5, Addendum) is consistent with the reciprocal regulation of the expression of genes in ovarian cancer (see Bagnoli *et al.*, *Oncogene*, 19(41): 4754-4763, 2000).

For each of the above specifically enumerated genes, a survey of the Serial Analysis of Gene Expression (SAGE) database (available through the UniGene search engine on the National Center for Biotechnology Information website) revealed that the expression of these genes is limited to a relatively small number of tissues, including ovarian cancers and some other tumors, for instance

pancreatic or breast. In addition, SLPI and SPP1 are secreted proteins that may be detectable as a diagnostic marker in the serum of a subject.

EXAMPLE 4

Classification of a Tumor into *BRCA1*-linked or *BRCA2*-linked tumor class.

This example describes how to classify a tumor into a *BRCA1*-like or *BRCA2*-like tumor type using compound covariate prediction analysis.

Class prediction can be performed using a Compound Covariate Predictor tool, available as part of the BRB Array Tools software provided for download on the National Cancer Institute Internet website. Detailed information about the Compound Covariate Predictor is provided by the Biometric Research Branch, National Cancer Institute and can be found in the following technical reports listed at that site" McShane *et al.*, "Methods for assessing reproducibility of clustering patterns observed in analyses of microarray data" and Radmacher *et al.*, "A paradigm for class prediction using gene expression profiles."

The compound covariate predictor tool creates a multivariate predictor for one of two classes for each sample using markers in the multivariate predictor that are univariately significant at the selected significance cutoff for a given set of data (see discussion above in ***Section V. D, "Compound Covariate Predictor Analysis."***). The statistical significance cutoff for a given set of data can be chosen based upon the level of confidence desired.

By way of example, the markers provided in Table 10 satisfy a cutoff of $P<0.0005$, and are therefore suitable for use with compound covariate predictor analysis. The multivariate predictor is a weighted linear combination of log-ratios for genes that are univariately significant. The weight consists of the univariate t-statistics for comparing the classes.

Using the compound covariate predictor and the markers provided in Table 10, a sample of ovarian tissue can be classified into a *BRCA1*-like or *BRCA2*-like tumor. Samples are prepared as described in Example 1, and logarithmic expression ratios obtained for each marker used in the compound covariate predictor analysis.

The markers provided in Table 10 were used to segregate *BRCA1*-linked and *BRCA1*-type sporadic tumor samples from *BRCA2*-linked and *BRCA2*-type sporadic samples, in a multivariate analysis. Based upon the information regarding these classes that was obtained using other approaches (such as hierarchical clustering, see Example 1), compound covariate predictor analysis classified the tumors with 92% accuracy (see Table 11).

Using this method, an unknown tumor can be classified into one of any two groups provided that markers that are univariately significant at the selected significance cutoff for the desired groups are known. In addition, the gene expression data for the markers should be obtained using the same reference standard as the sample tumor.

Further analysis, such as a "leave-one-out" approach may be employed to check the veracity of the compound covariate predictor model. In this approach, each of the tumors is individually segregated, and the analysis completed using that tumor against the remaining samples. In this way, the strength of the data set is measured against each individual sample (tumor),

confirming that the data set is useful, independently of any individual sample. See Radmacher *et al.*, "A paradigm for class prediction using gene expression profiles," available on the Biometric Research Branch, National Cancer Institute Internet site.

EXAMPLE 5

Expression of Ovarian Cancer-related Polypeptides

This example describes how to express the ovarian cancer-related proteins disclosed herein using various techniques.

The disclosed ovarian cancer-related proteins (and fragments thereof) can be expressed by standard laboratory technique. After expression, the purified ovarian cancer-related protein or polypeptide may be used for instance for functional analyses, antibody production, diagnostics, prognostics, and patient therapy, *e.g.*, for prevention or treatment of ovarian cancer. Furthermore, the DNA sequences encoding the disclosed ovarian cancer-related proteins can be manipulated in studies to understand the expression of these genes and the function of their products. Mutant forms of human ovarian cancer-related proteins (and corresponding encoding sequences) may be isolated based upon information contained herein, and may be studied in order to detect alteration in expression patterns in terms of relative quantities, tissue specificity and functional properties of the encoded mutant ovarian cancer-related protein. Partial or full-length cDNA sequences that encode the subject protein may be ligated into bacterial expression vectors. Methods for expressing large amounts of protein from a cloned gene introduced into *Escherichia coli* (*E. coli*) or other prokaryotes may be utilized for the purification, localization, and functional analysis of proteins. For example, fusion proteins consisting of amino terminal peptides encoded by a portion of the *E. coli lacZ* or *trpE* gene linked to an ovarian cancer-related protein may be used to prepare polyclonal and monoclonal antibodies against these proteins. Thereafter, these antibodies may be used to purify proteins by immunoaffinity chromatography, in diagnostic assays to quantitate the levels of protein and to localize proteins in tissues and individual cells by immunofluorescence.

Intact native protein may also be produced in *E. coli* in large amounts for functional studies. Methods and plasmid vectors for producing fusion proteins and intact native proteins in bacteria are described in Sambrook *et al.* (In *Molecular Cloning: A Laboratory Manual*, Ch. 17, CSHL, New York, 1989). Such fusion proteins may be made in large amounts, are easy to purify, and can be used to elicit antibody response. Native proteins can be produced in bacteria by placing a strong, regulated promoter and an efficient ribosome-binding site upstream of the cloned gene. If low levels of protein are produced, additional steps may be taken to increase protein production; if high levels of protein are produced, purification is relatively easy. Suitable methods are presented in Sambrook *et al.* (In *Molecular Cloning: A Laboratory Manual*, CSHL, New York, 1989) and are well known in the art. Often, proteins expressed at high levels are found in insoluble inclusion bodies. Methods for extracting proteins from these aggregates are described by Sambrook *et al.* (In *Molecular Cloning: A*

Laboratory Manual, Ch. 17, CSHL, New York, 1989). Vector systems suitable for the expression of *lacZ* fusion genes include the pUR series of vectors (see Ruther and Muller-Hill, *EMBO J.* 2:1791, 1983), pEX1-3 (see Stanley and Luzio, *EMBO J.* 3:1429, 1984) and pMR100 (see Gray *et al., Proc. Natl. Acad. Sci. USA* 79:6598, 1982). Vectors suitable for the production of intact native proteins include pKC30 (see Shimatake and Rosenberg, *Nature* 292:128, 1981), pKK177-3 (see Amann and Brosius, *Gene* 40:183, 1985) and pET-3 (see Studiar and Moffatt, *J. Mol. Biol.* 189:113, 1986). Fusion proteins, for instance fusions that incorporate a portion of an ovarian cancer-related protein, may be isolated from protein gels, lyophilized, ground into a powder and used as an antigen. The DNA sequence can also be transferred from its existing context to other cloning vehicles, such as other plasmids, bacteriophages, cosmids, animal viruses and yeast artificial chromosomes (YACs) (see Burke *et al., Science* 236:806-812, 1987). These vectors may then be introduced into a variety of hosts including somatic cells, and simple or complex organisms, such as bacteria, fungi (see Timberlake and Marshall, *Science* 244:1313-1317, 1989), invertebrates, plants (see Gasser and Fraley, *Science* 244:1293, 1989), and animals (see Pursel *et al., Science* 244:1281-1288, 1989), which cell or organisms are rendered transgenic by the introduction of the heterologous ovarian cancer-related cDNA.

For expression in mammalian cells, the cDNA sequence may be ligated to heterologous promoters, such as the simian virus (SV) 40 promoter in the pSV2 vector (see Mulligan and Berg, *Proc. Natl. Acad. Sci. USA* 78:2072-2076, 1981), and introduced into cells, such as monkey COS-1 cells (see Gluzman, *Cell* 23:175-182, 1981), to achieve transient or long-term expression. The stable integration of the chimeric gene construct may be maintained in mammalian cells by biochemical selection, for example with neomycin (see Southern and Berg, *J. Mol. Appl. Genet.* 1: 327-341, 1982) or mycophenolic acid (see Mulligan and Berg, *Proc. Natl. Acad. Sci. USA* 78: 2072-2076, 1981).

DNA sequences can be manipulated with standard procedures such as restriction enzyme digestion, fill-in with DNA polymerase, deletion by exonuclease, extension by terminal deoxynucleotide transferase, ligation of synthetic or cloned DNA sequences, site-directed sequence-alteration via single-stranded bacteriophage intermediate or with the use of specific oligonucleotides in combination with PCR.

The cDNA sequence (or portions derived from it) or a mini gene (a cDNA with an intron and its own promoter) may be introduced into eukaryotic expression vectors by conventional techniques. These vectors are designed to permit the transcription of the cDNA in eukaryotic cells by providing regulatory sequences that initiate and enhance the transcription of the cDNA and ensure its proper splicing and polyadenylation. Vectors containing the promoter and enhancer regions of the SV40 or long terminal repeat (LTR) of the Rous Sarcoma virus and polyadenylation and splicing signal from SV40 are readily available (see Mulligan *et al., Proc. Natl. Acad. Sci. USA* 78:1078-2076, 1981; Gorman *et al., Proc. Natl. Acad. Sci USA* 78:6777-6781, 1982). The level of expression of the cDNA can be manipulated with this type of vector, either by using promoters that have different activities (for example, the baculovirus pAC373 can express cDNAs at high levels in *S. frugiperda* cells (see Summers and Smith, In *Genetically Altered Viruses and the Environment*,

Fields *et al.* (Eds.) 22:319-328, CSHL Press, Cold Spring Harbor, New York, 1985) or by using vectors that contain promoters amenable to modulation, for example, the glucocorticoid-responsive promoter from the mouse mammary tumor virus (see Lee *et al., Nature* 294:228, 1982). The expression of the cDNA can be monitored in the recipient cells 24 to 72 hours after introduction (transient expression).

In addition, some vectors contain selectable markers such as the *gpt* (see Mulligan and Berg, *Proc. Natl. Acad. Sci. USA* 78:2072-2076, 1981) or *neo* (see Southern and Berg, *J. Mol. Appl. Genet.* 1:327-341, 1982) bacterial genes. These selectable markers permit selection of transfected cells that exhibit stable, long-term expression of the vectors (and therefore the cDNA). The vectors can be maintained in the cells as episomal, freely replicating entities by using regulatory elements of viruses such as papilloma (see Sarver *et al., Mol. Cell Biol.* 1:486, 1981) or Epstein-Barr (see Sugden *et al., Mol. Cell Biol.* 5:410, 1985). Alternatively, one can also produce cell lines that have integrated the vector into genomic DNA. Both of these types of cell lines produce the gene product on a continuous basis. One can also produce cell lines that have amplified the number of copies of the vector (and therefore of the cDNA as well) to create cell lines that can produce high levels of the gene product (see Alt *et al., J. Biol. Chem.* 253:1357, 1978).

The transfer of DNA into eukaryotic, in particular human or other mammalian cells, is now a conventional technique. The vectors are introduced into the recipient cells as pure DNA (transfection) by, for example, precipitation with calcium phosphate (see Graham and vander Eb, *Virology* 52:466, 1973) or strontium phosphate (see Brash *et al., Mol. Cell Biol.* 7:2013, 1987), electroporation (see Neumann *et al., EMBO J* 1:841, 1982), lipofection (see Felgner *et al., Proc. Natl. Acad. Sci USA* 84:7413, 1987), DEAE dextran (see McCuthan *et al., J. Natl. Cancer Inst.* 41:351, 1968), microinjection (see Mueller *et al., Cell* 15:579, 1978), protoplast fusion (see Schafner, *Proc. Natl. Acad. Sci. USA* 77:2163-2167, 1980), or pellet guns (see Klein *et al., Nature* 327:70, 1987). Alternatively, the cDNA, or fragments thereof, can be introduced by infection with virus vectors. Systems are developed that use, for example, retroviruses (see Bernstein *et al., Gen. Engr'g* 7:235, 1985), adenoviruses (see Ahmad *et al., J. Virol.* 57:267, 1986), or Herpes virus (see Spaete *et al., Cell* 30:295, 1982). MB1 encoding sequences can also be delivered to target cells *in vitro* via non-infectious systems, for instance liposomes.

These eukaryotic expression systems can be used for studies of ovarian cancer-related nucleic acids (such as those listed in Table 1) and mutant forms of these molecules, as well as ovarian cancer-related proteins and mutant forms of these protein. Such uses include, for example, the identification of regulatory elements located in the 5' region of ovarian cancer-related genes on genomic clones that can be isolated from human genomic DNA libraries. The eukaryotic expression systems may also be used to study the function of the normal ovarian cancer-related proteins, specific portions of these proteins, or of naturally occurring or artificially produced mutant versions of ovarian cancer-related proteins.

Using the above techniques, the expression vectors containing ovarian cancer-related gene sequence or cDNA, or fragments or variants or mutants thereof, can be introduced into human cells,

mammalian cells from other species or non-mammalian cells as desired. The choice of cell is determined by the purpose of the treatment. For example, monkey COS cells (see Gluzman, *Cell* 23:175-182, 1981) that produce high levels of the SV40 T antigen and permit the replication of vectors containing the SV40 origin of replication may be used. Similarly, Chinese hamster ovary (CHO), mouse NIH 3T3 fibroblasts or human fibroblasts or lymphoblasts may be used.

The present disclosure thus encompasses recombinant vectors that comprise all or part of an ovarian cancer-related gene or cDNA sequence (*e.g.*, those listed in Table 1), for expression in a suitable host. In some embodiments, the ovarian cancer-related nucleic acid sequence is operatively linked in the vector to an expression control sequence to form a recombinant DNA molecule, so that the ovarian cancer-related polypeptide can be expressed. The expression control sequence may be selected from the group consisting of sequences that control the expression of genes of prokaryotic or eukaryotic cells and their viruses, and combinations thereof. The expression control sequence may be specifically selected from the group consisting of the *lac* system, the *trp* system, the *tac* system, the *trc* system, major operator and promoter regions of phage lambda, the control region of fd coat protein, the early and late promoters of SV40, promoters derived from polyoma, adenovirus, retrovirus, baculovirus and simian virus, the promoter for 3-phosphoglycerate kinase, the promoters of yeast acid phosphatase, the promoter of the yeast alpha-mating factors, and combinations thereof.

The host cell, which may be transfected with the vector of this disclosure, may be selected from the group consisting of *E. coli*, *Pseudomonas*, *Bacillus subtilis*, *B. stearothermophilus* or other bacilli; other bacteria; yeast; fungi; insect; mouse or other animal; or plant hosts; or human tissue cells.

It is appreciated that for mutant or variant ovarian cancer-related DNA sequences, similar systems are employed to express and produce the mutant product. In addition, fragments of an ovarian cancer-related protein can be expressed essentially as detailed above. Such fragments include individual ovarian cancer-related protein domains or sub-domains, as well as shorter fragments such as peptides. Ovarian cancer-related protein fragments (*e.g.*, those having therapeutic properties) may be expressed in this manner also.

EXAMPLE 6

Suppression of Ovarian Cancer-related Increased Gene Expression

This example describes how the ovarian cancer-related nucleic acids disclosed herein may be suppressed using various techniques.

A reduction of ovarian cancer-related protein expression in a transgenic cell may be obtained by introducing into cells an antisense construct based on an ovarian cancer-related protein encoding sequence, such as a cDNA or gene sequence or flanking regions thereof of any one of the proteins encoded by the nucleic acid molecules listed in Table 1, Table 9 or elsewhere herein. For antisense suppression, a nucleotide sequence encoding an ovarian cancer-related protein that is overexpressed

in ovarian cancer, *e.g.* all or a portion of the small cell lung carcinoma cluster 4 antigen (*CD24*) (SEQ ID NO: 181-182), secretory leukocyte protease inhibitor antileukoproteinase (*SLPI*) (SEQ ID NO: 340-341), secreted phosphoprotein 1 (*SPP1*) (SEQ ID NO: 342), B-factor, properdin (*BF*) (SEQ ID NO: 343-344), "homolog of Cks1=p34Cdc28/Cdc2-associated protein" (*CKS1*) (SEQ ID NO: 345-347), matrix metalloproteinase 7 (*MMP7*) (SEQ ID NO: 348-349), paired box gene 8 (*PAX8*) (SEQ ID NO: 350-351), serine protease inhibitor, Kunitz type, 2 (*SPINT2*) (SEQ ID NO: 352-353), ZW10 interactor (*ZWINT*) (SEQ ID NO: 354), diacylglycerol kinase (*DGKH*) (SEQ ID NO: 355), high-mobility group (nonhistone chromosomal) protein isoforms I and Y (*HMGIY*) (SEQ ID NO: 356), Syndecan-4 - amphiglycan - ryudocan core protein (SDC4) (SEQ ID NO: 357-359), cyclin-dependent kinase inhibitor 2A (*CDKN2A*) (SEQ ID NO: 360), sodium channel, nonvoltage-gated 1 alpha (*SCNN1A*) (SEQ ID NO: 361-362), lactate dehydrogenase A (*LDHA*) (SEQ ID NO: 363), adult folate receptor (*FOLR1*) (SEQ ID NO: 364-365), Triosephosphate isomerase 1 (*TPI1*) (SEQ ID NO: 366-367), kallikrein 8 (neuropsin/ovasin) (*KLK8*) (SEQ ID NO: 368), CXC chemokine receptor 4- fusin-neuropeptide Y receptor-L3 (*CXCR4*) (SEQ ID NO: 200), kinesin-like 1 (*KNSL1*) (SEQ ID NO: 369-370), H2A histone family, member O (*H2AFO*) (SEQ ID NO: 371-372), major histocompatibility complex, class II, DR alpha, HLA-DRA, cysteine-rich protein 1 (intestinal) (*CRIP1*) (SEQ ID NO: 375), pyrophosphatase (inorganic), (*PP*) (SEQ ID NO: 376), EST 666391, glucose transporter (HepG2) (*SLC2A1*) (SEQ ID NO: 379-381), EST 897770, hepatoma-derived growth factor (*HDGF*) (SEQ ID NO: 383-385), argininosuccinate synthetase (*ASS*) (SEQ ID NO: 386), claudin 4 (*CLDN4*) (SEQ ID NO: 387-388), preferentially expressed antigen in melanoma (*PRAME*) (SEQ ID NO: 389), LAR = LCA-homologue (*PTPRF*) (SEQ ID NO: 390-391), eyes absent (Drosophila) homolog 2 (*EYA2*) (SEQ ID NO: 392-393), L-myc (*MYCL1*) (SEQ ID NO: 394-396), STAT1=IFN alpha/beta-responsive transcription factor ISGF3 beta subunits (p91/p84) (*STAT1*) (SEQ ID NO: 397-399), mitochondrial carrier homolog 2 (*MTCH2*) (SEQ ID NO: 400-401), 5-hydroxytryptamine (serotonin) receptor 3A (*HTR3A*) (SEQ ID NO: 402), cyclin E1 (*CCNE1*) (SEQ ID NO: 403-404), cadherin 6, type 2, K-cadherin (fetal kidney) (*CDH6*) (SEQ ID NO: 405), 5'-AMP-activated protein kinase - gamma-1 subunit (*PRKAG1*) (SEQ ID NO: 406-408), defensin beta 1 (*DEFB1*) (SEQ ID NO: 409), actin related protein 2/3 complex, subunit 1A (41 kD) (*ARPC1B*) (SEQ ID NO: 410-411), PKC iota=Protein kinase C, iota (*PRKCI*) (SEQ ID NO: 412-414), glyceraldehyde-3-phosphate dehydrogenase (*GAPD*) (SEQ ID NO: 415), complement component 2 (C2) (SEQ ID NO: 416-417), H2A histone family, member Y (*H2AFY*) (SEQ ID NO: 418-419), transmembrane 4 superfamily member 1 (*TM4SF1*) (SEQ ID NO: 420-421), glyceraldehyde-3-phosphate dehydrogenase (*GAPD*) (SEQ ID NO: 422-423), Interferon-inducible protein 1-8U (*IFITM3*) (SEQ ID NO: 424-426), glycine dehydrogenase (decarboxylating; glycine decarboxylase, glycine cleavage system protein P) (*GLDC*) (SEQ ID NO: 427-428), calumenin (*CALU*) (SEQ ID NO: 429-430), hemoglobin alpha 2 (*HBA2*) (SEQ ID NO: 431-432), S100 calcium-binding protein A11 (calgizzarin) (*S100A11*) (SEQ ID NO: 433), Lactate dehydrogenase A (LDHA) (SEQ ID NO: 434-436), ubiquitin-conjugating enzyme E2C (*UBE2C*) (SEQ ID NO: 437), E2F-3=pRB-binding transcription factor=KIAA0075 (*E2F3*) (SEQ ID NO: 438-440), E-cadherin (*CDH1*) (SEQ ID NO: 441-442), proteasome (prosome, macropain)

activator subunit 2 (PA28 beta) (*PSME2*) (SEQ ID NO: 443-444), OP-1=osteogenic protein in the TGF-beta family (*BMP7*) (SEQ ID NO: 445-447), and topoisomerase II (*TOP2A*) (SEQ ID NO: 448) cDNA or gene, is arranged in reverse orientation relative to the promoter sequence in the transformation vector. Other aspects of the vector may be chosen as for any other expression vector (*see, e.g.*, Example 4).

The introduced sequence need not be a full-length human ovarian cancer-related cDNA or gene, and need not be exactly homologous to the equivalent sequence found in the cell type to be transformed. Generally, however, where the introduced sequence is of shorter length, a higher degree of homology to the ovarian cancer-related sequence likely will be needed for effective antisense suppression. The introduced antisense sequence in the vector may be at least thirty nucleotides in length, and improved antisense suppression will typically be observed as the length of the antisense sequence increases. The length of the antisense sequence in the vector advantageously may be greater than 100 nucleotides.

Although the exact mechanism by which antisense RNA molecules interfere with gene expression has not been elucidated, it is believed that antisense RNA molecules bind to the endogenous mRNA molecules and thereby inhibit translation of the endogenous mRNA.

Suppression of endogenous ovarian cancer-related gene expression can also be achieved using ribozymes. Ribozymes are synthetic RNA molecules that possess highly specific endoribonuclease activity. The production and use of ribozymes are disclosed in U.S. Patent No. 4,987,071 to Cech and U.S. Patent No. 5,543,508 to Haselhoff. The inclusion of ribozyme sequences within antisense RNAs may be used to confer RNA cleaving activity on the antisense RNA, such that endogenous mRNA molecules that bind to the antisense RNA are cleaved, which in turn leads to an enhanced antisense inhibition of endogenous gene expression.

In addition, dominant negative mutant forms of the disclosed ovarian cancer-related sequences may be used to block endogenous activity of the corresponding gene products.

Suppression can also be achieved using small inhibitory RNA molecules (siRNAs) (see, for instance, Caplen *et al.*, *Proc. Natl. Acad. Sci.* 98(17): 9742-9747, 2001, and Elbashir *et al.*, *Nature* 411: 494-498, 2001). Thus, this disclosure also encompasses siRNAs that correspond to an ovarian cancer-related nucleic acid, which siRNA is capable of suppressing the expression or function of its cognate (target) ovarian cancer-related protein. Also encompassed are methods of suppressing the expression or activity of an ovarian cancer-related molecule using an siRNA.

Suppression of expression of an ovarian cancer-related gene can be used, for instance, to treat, reduce, or prevent cell proliferative and other disorders caused by over-expression or unregulated expression of the corresponding ovarian cancer-related gene. In particular, suppression of expression of sequences disclosed herein as being up-regulated in ovarian cancer can be used to treat, reduce, or prevent progression to a later stage of ovarian cancer.

EXAMPLE 7

Nucleic Acid-Based Analysis

This example describes how to use the ovarian cancer-related nucleic acids disclosed herein to detect and analyze neoplasms and mutations in ovarian cancer-related nucleic acids that may result in neoplasms.

The ovarian cancer-related nucleic acid molecules provided herein, and combinations of these molecules, can be used in methods of genetic testing for neoplasms (*e.g.*, ovarian or other cancers) or predisposition to neoplasms owing to altered expression of ovarian cancer-related nucleic acid molecules (*e.g.*, deletion, genomic amplification or mutation, or over- or under-expression in comparison to a control or baseline). For such procedures, a biological sample of the subject, which biological sample contains either DNA or RNA derived from the subject, is assayed for a mutated, amplified or deleted ovarian cancer-related nucleic acid molecule, or for over- or under-expression of an ovarian cancer-related nucleic acid molecule. Suitable biological samples include samples containing genomic DNA or RNA (including mRNA), obtained from body cells of a subject, such as those present in peripheral blood, urine, saliva, tissue biopsy, surgical specimen, amniocentesis samples and autopsy material.

The detection in the biological sample of a mutant ovarian cancer-related nucleic acid molecule, a mutant ovarian cancer-related RNA, an amplified or homozygously or heterozygously deleted ovarian cancer-related nucleic acid molecule, or over- or under-expression of an ovarian cancer-related nucleic acid molecule, may be performed by a number of methodologies, examples of which are provided.

A. Detection of Unknown Mutations:

Unknown mutations in ovarian cancer-related nucleic acid molecules can be identified through polymerase chain reaction amplification of reverse transcribed RNA (RT-PCR) or DNA isolated from breast or ovary or other tissue, followed by direct DNA sequence determination of the products; single-strand conformational polymorphism analysis (SSCP) (for instance, see Hongyo *et al., Nucleic Acids Res.* 21:3637-3642, 1993); chemical cleavage (including HOT cleavage) (Bateman *et al., Am. J. Med. Genet.* 45:233-240, 1993; reviewed in Ellis *et al., Hum. Mutat.* 11:345-353, 1998); denaturing gradient gel electrophoresis (DGGE); ligation amplification mismatch protection (LAMP); or enzymatic mutation scanning (Taylor and Deeble, *Genet. Anal.* 14:181-186, 1999), followed by direct sequencing of amplicons with putative sequence variations.

B. Detection of Known Mutations:

The detection of specific known DNA mutations in ovarian cancer-related nucleic acid molecules may be achieved by methods such as hybridization using allele specific oligonucleotides (ASOs) (see Wallace *et al., CSHL Symp. Quant. Biol.* 51:257-261, 1986), direct DNA sequencing

(see Church and Gilbert, *Proc. Natl. Acad. Sci. USA* 81:1991-1995, 1988), the use of restriction enzymes (see Flavell *et al., Cell* 15:25, 1978; Geever *et al., Proc. Natl. Acad. Sci. U.S.A.* 8(8): 5081-5085, 1981), discrimination on the basis of electrophoretic mobility in gels with denaturing reagent (see Myers and Maniatis, Cold Spring Harbor *Symp. Quant. Biol.* 51:275-284, 1986), RNase protection (see Myers *et al., Science* 230:1242, 1985), chemical cleavage (see Cotton *et al., Proc. Natl. Acad. Sci. USA* 85:4397-4401, 1985), and the ligase-mediated detection procedure (see Landegren *et al., Science* 241:1077-1080, 1988). Oligonucleotides specific to normal or mutant MB1 sequences can be chemically synthesized using commercially available machines. These oligonucleotides can then be labeled radioactively with isotopes (such as ^{32}P) or non-radioactively, with tags such as biotin (see Ward and Langer *et al., Proc. Natl. Acad. Sci. USA* 78:6633-6657, 1981), and hybridized to individual DNA samples immobilized on membranes or other solid supports by dot-blot or transfer from gels after electrophoresis. These specific sequences are visualized by methods such as autoradiography or fluorometric (see Landegren *et al., Science* 242:229-237, 1989) or colorimetric reactions (see Gebeyehu *et al., Nucleic Acids Res.* 15:4513-4534, 1987). Using an ASO specific for a normal allele, the absence of hybridization would indicate a mutation in the particular region of the gene, or deleted MB1 gene. In contrast, if an ASO specific for a mutant allele hybridizes to a clinical sample then that would indicate the presence of a mutation in the region defined by the ASO.

C. Detection of Genomic Amplification or Deletion

Gene dosage (copy number) can be important in neoplasms; it is therefore advantageous to determine the number of copies of ovarian cancer-related nucleic acids in biological samples of a subject, *e.g.*, serum or ovary samples. Probes generated from the disclosed encoding sequence of in ovarian cancer-related nucleic acid molecules can be used to investigate and measure genomic dosage of the corresponding ovarian cancer-related genomic sequence.

Appropriate techniques for measuring gene dosage are known in the art; see for instance, US Patent No. 5,569,753 ("Cancer Detection Probes") and Pinkel *et al.* (*Nat. Genet.* 20:207-211, 1998) ("High Resolution Analysis of DNA Copy Number Variation using Comparative Genomic Hybridization to Microarrays").

Determination of gene copy number in cells of a patient-derived sample using other techniques is known in the art. For example, amplification of an ovarian cancer-related nucleic acid sequence in cancer-derived cell lines as well as uncultured ovarian cancer or other cells can be carried out using bicolor FISH analysis. By way of example, interphase FISH analysis of breast cancer cell lines can be carried out as previously described (see Barlund *et al., Genes Chromo. Cancer* 20:372-376, 1997). The hybridizations can be evaluated using a Zeiss fluorescence microscope.

For tissue microarrays, the FISH can be performed as described in Kononen *et al.* (*Nat. Med.* 4:844-847, 1998). Briefly, consecutive sections of the array are deparaffinized, dehydrated in ethanol, denatured at 74° C for 5 minutes in 70% formamide/2 x SSC, and hybridized with test and reference probes. The specimens containing tight clusters of signals or >3-fold increase in the

number of test probe as compared to chromosome 17 centromere in at least 10% of the tumor cells may be considered as amplified. Microarrays can be constructed as described in WO 99/44063A2 and WO 99/44062A1.

C. Detection of mRNA Expression Levels

Altered expression of an ovarian cancer-related molecule also can be detected by measuring the cellular level of ovarian cancer-related nucleic acid molecule-specific mRNA. mRNA can be measured using techniques well known in the art, including for instance Northern analysis, RT-PCR and mRNA *in situ* hybridization. Details of mRNA analysis procedures can be found, for instance, in Example 1, Example 3, and Sambrook *et al.* (ed.), *Molecular Cloning: A Laboratory Manual*, 2nd ed., vol. 1-3, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.

The nucleic acid-based diagnostic methods of this disclosure are predictive of ovarian cancer. Cells of any tumors that demonstrate altered expression levels (*e.g.*, through genomic amplification, deletion, mutation, or other over- or under-expression) of nucleotide sequences that share homology with the ovarian cancer-related nucleic acids disclosed herein are aggressive tumor cells, and result in decreased survival, increased metastasis, increased rates of clinical, and overall worsened prognosis.

EXAMPLE 8

Production of Protein Specific Binding Agents

This example describes how to use the ovarian cancer-related molecules disclosed herein to produce binding agents useful in preventing ovarian cancer.

Monoclonal or polyclonal antibodies may be produced to any of the disclosed ovarian cancer-related proteins, or mutant forms of these proteins. Optimally, antibodies raised against these proteins, or peptides from within such proteins, would specifically detect the protein or peptide with which the antibodies are generated. That is, an antibody generated to the BMP7 protein or another specified protein (see Table 1) or a fragment thereof would recognize and bind that protein and would not substantially recognize or bind to other proteins found in human cells.

The determination that an antibody specifically detects a designated protein (*e.g.*, an ovarian cancer-related protein as disclosed herein) can be made by any one of a number of standard immunoassay methods; for instance, the Western blotting technique (see Sambrook *et al.*, In *Molecular Cloning: A Laboratory Manual*, CSHL, New York, 1989). To determine that a given antibody preparation (such as one produced in a mouse) specifically detects a designated protein by Western blotting, total cellular proteins are extracted from cells (for example, human ovary) and electrophoresed on a sodium dodecyl sulfate-polyacrylamide gel. The proteins are then transferred to a membrane (for example, nitrocellulose) by Western blotting, and the antibody preparation is incubated with the membrane. After washing the membrane to remove non-specifically bound

antibodies, the presence of specifically bound antibodies is detected by the use of an anti-mouse antibody conjugated to an enzyme such as alkaline phosphatase. Application of an alkaline phosphatase substrate 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium results in the production of a dense blue compound by immunolocalized alkaline phosphatase. Antibodies that specifically detect the designated protein will, by this technique, be shown to bind to the designated protein band (which will be localized at a given position on the gel determined by its molecular weight). Non-specific binding of the antibody to other proteins may occur and may be detectable as a weak signal on the Western blot. The non-specific nature of this binding will be recognized by one skilled in the art by the weak signal obtained on the Western blot relative to the strong primary signal arising from the specific antibody-protein binding.

Substantially pure ovarian cancer-related protein or protein fragment (peptide) suitable for use as an immunogen may be isolated from transfected or transformed cells, as described above. Concentration of protein or peptide in the final preparation is adjusted, for example, by concentration on an Amicon filter device, to the level of a few micrograms per milliliter. Monoclonal or polyclonal antibody to the protein can then be prepared as follows:

A. Monoclonal Antibody Production by Hybridoma Fusion

Monoclonal antibody to epitopes of a designated protein (such as an ovarian cancer-related protein, including one encoded by a nucleic acid listed in Table 1) identified and isolated as described can be prepared from murine hybridomas according to the classical method of Kohler and Milstein (*Nature* 256:495-497, 1975) or derivative methods thereof. Briefly, a mouse is repetitively inoculated with a few micrograms of the selected protein over a period of a few weeks. The mouse is then sacrificed, and the antibody-producing cells of the spleen isolated. The spleen cells are fused by means of polyethylene glycol with mouse myeloma cells, and the excess un-fused cells destroyed by growth of the system on selective media comprising aminopterin (HAT media). The successfully fused cells are diluted and aliquots of the dilution placed in wells of a microtiter plate where growth of the culture is continued. Antibody-producing clones are identified by detection of antibody in the supernatant fluid of the wells by immunoassay procedures, such as ELISA, as originally described by Engvall (*Meth. Enzymol.* 70: 419-439, 1980), and derivative methods thereof. Selected positive clones can be expanded and their monoclonal antibody product harvested for use. Detailed procedures for monoclonal antibody production are described in Harlow and Lane (*Antibodies, A Laboratory Manual*, CSHL, New York, 1988).

B. Polyclonal Antibody Production by Immunization

Polyclonal antiserum containing antibodies to heterogeneous epitopes of a single protein can be prepared by immunizing suitable animals with the expressed protein, which can be unmodified or modified to enhance immunogenicity. Effective polyclonal antibody production is affected by many factors related both to the antigen and the host species. For example, small molecules tend to be less immunogenic than others and may require the use of carriers and adjuvant. Also, host animals vary

in response to site of inoculations and dose, with either inadequate or excessive doses of antigen resulting in low titer antisera. Small doses (ng level) of antigen administered at multiple intradermal sites appear to be most reliable. An effective immunization protocol for rabbits can be found in Vaitukaitis *et al.* (*J. Clin. Endocrinol. Metab.* 33: 988-991, 1971).

Booster injections can be given at regular intervals, and antiserum harvested when antibody titer thereof, as determined semi-quantitatively, for example, by double immunodiffusion in agar against known concentrations of the antigen, begins to fall. See, for example, Ouchterlony *et al.* (In *Handbook of Experimental Immunology*, Wier (ed.) Chapter 19. Blackwell, 1973). Plateau concentration of antibody is usually in the range of about 0.1 to 0.2 mg/ml of serum (about 12 μM). Affinity of the antisera for the antigen is determined by preparing competitive binding curves, as described, for example, by Fisher (*Manual of Clinical Immunology*, Ch. 42, 1980).

C. Antibodies Raised against Synthetic Peptides

A third approach to raising antibodies against the subject ovarian cancer-related proteins or peptides is to use one or more synthetic peptides synthesized on a commercially available peptide synthesizer based upon the predicted amino acid sequence of the desired ovarian cancer-related protein or peptide.

D. Antibodies Raised by Injection of Protein Encoding Sequence

Antibodies also may be raised against proteins and peptides related to ovarian cancer as described herein by subcutaneous injection of a DNA vector that expresses the desired ovarian cancer-related protein, or a fragment thereof, into laboratory animals, such as mice. Delivery of the recombinant vector into the animals may be achieved using a hand-held form of the Biolistic system (see Sanford *et al., Particulate Sci. Technol.* 5:27-37, 1987) as described by Tang *et al.* (*Nature* 356:152-154, 1992). Expression vectors suitable for this purpose may include those that express the ovarian cancer-related sequence under the transcriptional control of either the human β-actin promoter or the cytomegalovirus (CMV) promoter.

Antibody preparations prepared according to these protocols are useful in quantitative immunoassays that determine concentrations of antigen-bearing substances in biological samples; they also can be used semi-quantitatively or qualitatively to identify the presence of antigen in a biological sample; or for immunolocalization of the corresponding ovarian cancer-related protein.

For administration to human patients, antibodies, *e.g.*, ovarian cancer-related protein specific monoclonal antibodies (such as antibodies to the proteins encoded by the encoding sequences listed to in Table 1), can be humanized by methods known in the art. Antibodies with a desired binding specificity can be commercially humanized (Scotgene, Scotland, UK; Oxford Molecular, Palo Alto, CA). Alternatively, human antibodies can be produced. Methods for producing human antibodies are known in the art; see, for instance, Canevari *et al., Int. J. Biol. Markers* 8:147-150, 1993 and Green, *J. Immunol. Meth.* 231:11-23, 1999, for instance.

EXAMPLE 9

Protein-Based Analysis

This example describes how to use the ovarian cancer-related molecules disclosed herein to quantitate the level of one or more ovarian cancer-related proteins in a subject.

An alternative method of diagnosing, staging, detecting, or predicting ovarian cancer is to quantitate the level of one or more ovarian cancer-related proteins in a subject, for instance in the cells of the subject. This diagnostic tool is useful for detecting reduced or increased levels of ovarian cancer-related proteins. Localization and/or coordinated expression (temporally or spatially) of ovarian cancer-related proteins can also be examined using well known techniques. The determination of reduced or increased ovarian cancer-related protein levels, in comparison to such expression in a normal subject (*e.g.*, a subject not having ovarian cancer or not having a predisposition developing this condition, disease or disorder, would be an alternative or supplemental approach to the direct determination of ovarian cancer-related nucleic acid levels by the methods outlined above and equivalents. The availability of antibodies specific to specific ovarian cancer-related protein(s) will facilitate the detection and quantitation of cellular ovarian cancer-related protein(s) by one of a number of immunoassay methods which are well known in the art and are presented in Harlow and Lane (*Antibodies, A Laboratory Manual*, CSHL, New York, 1988). Methods of constructing such antibodies are discussed above, in Example 7.

Any standard immunoassay format (*e.g.*, ELISA, Western blot, or RIA assay) can be used to measure altered expression of ovarian cancer-related polypeptide or protein levels; comparison is to wild-type (normal) ovarian cancer-related protein levels, and a difference in ovarian cancer-related polypeptide levels is indicative of a biological condition resulting from altered expression of ovarian cancer-related polypeptides or proteins, such as neoplasia. Whether the key difference is an increase or a decrease is dependent on the specific ovarian cancer-related protein under examination, as discussed herein. Immunohistochemical techniques may also be utilized for ovarian cancer-related polypeptide or protein detection and quantification. For example, a tissue sample may be obtained from a subject, and a section stained for the presence of an ovarian cancer-related protein using the appropriate ovarian cancer-related protein specific binding agent and any standard detection system (*e.g.*, one which includes a secondary antibody conjugated to horseradish peroxidase). General guidance regarding such techniques can be found in, *e.g.*, Bancroft and Stevens (*Theory and Practice of Histological Techniques*, Churchill Livingstone, 1982) and Ausubel *et al.* (*Current Protocols in Molecular Biology*, John Wiley & Sons, New York, 1998).

For the purposes of quantitating an ovarian cancer-related protein, a biological sample of the subject, which sample includes cellular proteins, is required. Such a biological sample may be obtained from body cells, such as those present in peripheral blood, urine, saliva, tissue biopsy, amniocentesis samples, surgical specimens and autopsy material, particularly breast cells. Quantitation of an ovarian cancer-related protein can be achieved by immunoassay and the amount

compared to levels of the protein found in healthy cells. A significant difference (either increase or decrease) in the amount of ovarian cancer-related protein in the cells of a subject compared to the amount of the same ovarian cancer-related protein found in normal human cells is usually about a 10% or greater change, for instance 20%, 30%, 40%, 50% or greater difference. Substantial under- or over-expression of one or more ovarian cancer-related protein(s), may be indicative of neoplasia or a predilection to neoplasia or metastasis, and especially ovarian epithelial cancer.

The protein-based diagnostic methods as described herein are predictive of ovarian cancer. Cells of any tumors that demonstrate altered expression levels (*e.g.*, through genomic amplification, deletion, mutation, or other over- or under-expression) of nucleotide sequences that share homology with the ovarian cancer-related nucleic acids disclosed herein are aggressive tumor cells, and result in decreased survival, increased metastasis, increased rates of clinical recurrence, and overall worsened prognosis.

EXAMPLE 10

Gene Therapy

This example describes how to use the ovarian cancer-related molecules and analysis methods disclosed herein to effect gene therapy for the treatment of ovarian cancer.

Gene therapy approaches for combating neoplasia (particularly ovarian cancer) in subjects are made possible by the present disclosure.

Retroviruses have been considered a preferred vector for experiments in gene therapy, with a high efficiency of infection and stable integration and expression (see Orkin *et al., Prog. Med. Genet.* 7:130-142, 1988). A full-length ovarian cancer-related gene or cDNA can be cloned into a retroviral vector and driven from either its endogenous promoter or from the retroviral LTR (long terminal repeat). Other viral transfection systems may also be utilized for this type of approach, including adenovirus, adeno-associated virus (AAV) (see McLaughlin *et al., J. Virol.* 62:1963-1973, 1988), *Vaccinia* virus (Moss *et al., Annu. Rev. Immunol.* 5:305-324, 1987), Bovine Papilloma virus (Rasmussen *et al., Methods Enzymol.* 139:642-654, 1987) or members of the herpesvirus group such as Epstein-Barr virus (Margolskee *et al., Mol. Cell. Biol.* 8:2837-2847, 1988).

Developments in gene therapy techniques include the use of RNA-DNA hybrid oligonucleotides, as described by Cole-Strauss *et al.* (*Science* 273:1386-1389, 1996). This technique may allow for site-specific integration of cloned sequences, thereby permitting accurately targeted gene replacement.

In addition to delivery of ovarian cancer-related protein encoding sequences to cells using viral vectors, it is possible to use non-infectious methods of delivery. For instance, lipidic and liposome-mediated gene delivery has recently been used successfully for transfection with various genes (for reviews, see Templeton and Lasic, *Mol. Biotechnol.* 11:175-180, 1999; Lee and Huang, *Crit. Rev. Ther. Drug Carrier Syst.* 14:173-206; and Cooper, *Semin. Oncol.* 23:172-187, 1996). For

instance, cationic liposomes have been analyzed for their ability to transfect monocytic leukemia cells, and shown to be a viable alternative to using viral vectors (de Lima *et al.*, *Mol. Membr. Biol.* 16:103-109, 1999). Such cationic liposomes can also be targeted to specific cells through the inclusion of, for instance, monoclonal antibodies or other appropriate targeting ligands (see Kao *et al.*, *Cancer Gene Ther.* 3:250-256, 1996).

To reduce the level of ovarian cancer-related gene expression, gene therapy can be carried out using antisense or other suppressive constructs, the construction of which is discussed above (Example 4).

EXAMPLE 11

Kits

This example describes various kits for using the ovarian cancer-related molecules and analysis methods disclosed herein.

Kits are provided to determine the level (or relative level) of expression of one or more species of ovarian cancer-related nucleic acids (*e.g.*, mRNA) or one or more ovarian cancer-related protein (*i.e.*, kits containing nucleic acid probes or antibodies or other ovarian cancer-related protein specific binding agents). Kits are also provided that contain the necessary reagents for determining gene copy number (genomic amplification or deletion), such as probes or primers specific for an ovarian cancer-related nucleic acid sequence. These kits can each include instructions, for instance instructions that provide calibration curves or charts to compare with the determined (*e.g.*, experimentally measured) values.

A. Kits for Detection of Ovarian Cancer-related Genomic Amplification or Deletion

The nucleotide sequence of ovarian cancer-related nucleic acid molecules disclosed herein, and fragments thereof, can be supplied in the form of a kit for use in detection of ovarian cancer-related genomic amplification/deletion and/or diagnosis of progression to or predilection to progress to ovarian epithelial cancer. In such a kit, an appropriate amount of one or more oligonucleotide primer specific for an ovarian cancer-related-sequence is provided in one or more containers. The oligonucleotide primers may be provided suspended in an aqueous solution or as a freeze-dried or lyophilized powder, for instance. The container(s) in which the oligonucleotide(s) are supplied can be any conventional container that is capable of holding the supplied form, for instance, microfuge tubes, ampoules, or bottles. In some applications, pairs of primers may be provided in pre-measured single use amounts in individual, typically disposable, tubes, or equivalent containers. With such an arrangement, the sample to be tested for the presence of ovarian cancer-related genomic amplification/deletion can be added to the individual tubes and *in vitro* amplification carried out directly.

The amount of each oligonucleotide primer supplied in the kit can be any amount, depending for instance on the market to which the product is directed. For instance, if the kit is adapted for research or clinical use, the amount of each oligonucleotide primer provided likely would be an amount sufficient to prime several *in vitro* amplification reactions. Those of ordinary skill in the art know the amount of oligonucleotide primer that is appropriate for use in a single amplification reaction. General guidelines may for instance be found in Innis *et al.* (*PCR Protocols, A Guide to Methods and Applications*, Academic Press, Inc., San Diego, CA, 1990), Sambrook *et al.* (In *Molecular Cloning: A Laboratory Manual,* Cold Spring Harbor, New York, 1989), and Ausubel *et al.* (In *Current Protocols in Molecular Biology*, John Wiley & Sons, New York, 1998).

A kit may include more than two primers, in order to facilitate the *in vitro* amplification of ovarian cancer-related genomic sequences (or a protein of such a sequence), for instance an ovarian cancer-related nucleic acid listed in Table 1, or the 5' or 3' flanking region thereof.

In some embodiments, kits may also include the reagents necessary to carry out *in vitro* amplification reactions, including, for instance, DNA sample preparation reagents, appropriate buffers (*e.g.*, polymerase buffer), salts (*e.g.*, magnesium chloride), and deoxyribonucleotides (dNTPs). Written instructions may also be included.

Kits may in addition include either labeled or unlabeled oligonucleotide probes for use in detection of the *in vitro* amplified sequences. The appropriate sequences for such a probe will be any sequence that falls between the annealing sites of two provided oligonucleotide primers, such that the sequence the probe is complementary to is amplified during the *in vitro* amplification reaction (if it is present in the sample).

It may also be advantageous to provided in the kit one or more control sequences for use in the *in vitro* amplification reactions. The design of appropriate positive control sequences is well known to one of ordinary skill in the appropriate art.

B. Kits for Detection of mRNA Expression

Kits similar to those disclosed above for the detection of ovarian cancer-related genomic amplification/deletion can be used to detect ovarian cancer-related mRNA expression levels (including over- or under-expression, in comparison to the expression level in a control sample). Such kits include an appropriate amount of one or more of the oligonucleotide primers for use in, for instance, reverse transcription PCR reactions, similarly to those provided above, with art-obvious modifications for use with RNA.

In some embodiments, kits for detection of ovarian cancer-related mRNA expression may also include reagents necessary to carry out RT-PCR or other *in vitro* amplification reactions, including, for instance, RNA sample preparation reagents (including *e.g.*, an RNAse inhibitor), appropriate buffers (*e.g.*, polymerase buffer), salts (*e.g.*, magnesium chloride), and deoxyribonucleotides (dNTPs). Written instructions may also be included.

Kits may in addition include either labeled or unlabeled oligonucleotide probes for use in detection of an *in vitro* amplified target sequence. The appropriate sequences for such a probe will be

any sequence that falls between the annealing sites of the two provided oligonucleotide primers, such that the sequence the probe is complementary to is amplified during the PCR reaction.

It may also be advantageous to provided in the kit one or more control sequences for use in the *in vitro* amplification reactions. The design of appropriate positive control sequences is well known to one of ordinary skill in the appropriate art.

Alternatively, kits may be provided with the necessary reagents to carry out quantitative or semi-quantitative Northern analysis of ovarian cancer-related mRNA. Such kits include, for instance, at least one ovarian cancer-related sequence-specific oligonucleotide for use as a probe. This oligonucleotide may be labeled in any conventional way, including with a selected radioactive isotope, enzyme substrate, co-factor, ligand, chemiluminescent or fluorescent agent, hapten, or enzyme.

C. Kits For Detection of Ovarian Cancer-linked Protein or Peptide Expression

Kits for the detection of ovarian cancer-linked protein expression, for instance altered (over or under) expression of a protein encoded for by a nucleic acid molecule listed in Table 1 or elsewhere, are also encompassed herein. Such kits may include for example at least one target (ovarian cancer-linked) protein (*e.g.*, all or a portion of the small cell lung carcinoma cluster 4 antigen (*CD24*) (SEQ ID NO: 181-182), secretory leukocyte protease inhibitor antileukoproteinase (*SLPI*) (SEQ ID NO: 340-341), secreted phosphoprotein 1 (*SPP1*) (SEQ ID NO: 342), B-factor, properdin (*BF*) (SEQ ID NO: 343-344), "homolog of Cks1=p34Cdc28/Cdc2-associated protein" (*CKS1*) (SEQ ID NO: 345-347), matrix metalloproteinase 7 (*MMP7*) (SEQ ID NO: 348-349), paired box gene 8 (*PAX8*) (SEQ ID NO: 350-351), serine protease inhibitor, Kunitz type, 2 (*SPINT2*) (SEQ ID NO: 352-353), ZW10 interactor (*ZWINT*) (SEQ ID NO: 354), diacylglycerol kinase (*DGKH*) (SEQ ID NO: 355), high-mobility group (nonhistone chromosomal) protein isoforms I and Y (*HMGIY*) (SEQ ID NO: 356), Syndecan-4 - amphiglycan - ryudocan core protein (*SDC4*) (SEQ ID NO: 357-359), cyclin-dependent kinase inhibitor 2A (*CDKN2A*) (SEQ ID NO: 360), sodium channel, nonvoltage-gated 1 alpha (*SCNN1A*) (SEQ ID NO: 361-362), lactate dehydrogenase A (*LDHA*) (SEQ ID NO: 363), adult folate receptor (*FOLR1*) (SEQ ID NO: 364-365), Triosephosphate isomerase 1 (*TPI1*) (SEQ ID NO: 366-367), kallikrein 8 (neuropsin/ovasin) (*KLK8*) (SEQ ID NO: 368), CXC chemokine receptor 4- fusin-neuropeptide Y receptor-L3 (*CXCR4*) (SEQ ID NO: 200), kinesin-like 1 (*KNSL1*) (SEQ ID NO: 369-370), H2A histone family, member O (*H2AFO*) (SEQ ID NO: 371-372), major histocompatibility complex, class II, DR alpha, HLA-DRA, cysteine-rich protein 1 (intestinal) (*CRIP1*) (SEQ ID NO: 375), pyrophosphatase (inorganic), (*PP*) (SEQ ID NO: 376), EST (SEQ ID NO: 377-378) 666391, glucose transporter (HepG2), (*SLC2A1*) (SEQ ID NO: 379-381), EST (SEQ ID NO: 377-378) 897770, hepatoma-derived growth factor (*HDGF*) (SEQ ID NO: 383-385), argininosuccinate synthetase (*ASS*) (SEQ ID NO: 386), claudin 4 (*CLDN4*) (SEQ ID NO: 387-388), preferentially expressed antigen in melanoma (*PRAME*) (SEQ ID NO: 389), LAR = LCA-homologue (*PTPRF*) (SEQ ID NO: 390-391), eyes absent (Drosophila) homolog 2 (*EYA2*) (SEQ ID NO: 392-

393), L-myc (*MYCL1*) (SEQ ID NO: 394-396), STAT1=IFN alpha/beta-responsive transcription factor ISGF3 beta subunits (p91/p84) (*STAT1*) (SEQ ID NO: 397-399), mitochondrial carrier homolog 2 (*MTCH2*) (SEQ ID NO: 400-401), 5-hydroxytryptamine (serotonin) receptor 3A (*HTR3A*) (SEQ ID NO: 402), cyclin E1 (*CCNE1*) (SEQ ID NO: 403-404), cadherin 6, type 2, K-cadherin (fetal kidney) (*CDH6*) (SEQ ID NO: 405), 5'-AMP-activated protein kinase - gamma-1 subunit (*PRKAG1*) (SEQ ID NO: 406-408), defensin beta 1 (*DEFB1*) (SEQ ID NO: 409), actin related protein 2/3 complex, subunit 1A (41 kD) (*ARPC1B*) (SEQ ID NO: 410-411), PKC iota=Protein kinase C, iota (*PRKCI*) (SEQ ID NO: 412-414), glyceraldehyde-3-phosphate dehydrogenase (*GAPD*) (SEQ ID NO: 415), complement component 2 (*C2*) (SEQ ID NO: 416-417), H2A histone family, member Y (*H2AFY*) (SEQ ID NO: 418-419), transmembrane 4 superfamily member 1 (*TM4SF1*) (SEQ ID NO: 420-421), glyceraldehyde-3-phosphate dehydrogenase (*GAPD*) (SEQ ID NO: 422-423), Interferon-inducible protein 1-8U (*IFITM3*) (SEQ ID NO: 424-426), glycine dehydrogenase (decarboxylating; glycine decarboxylase, glycine cleavage system protein P) (*GLDC*) (SEQ ID NO: 427-428), calumenin (*CALU*) (SEQ ID NO: 429-430), hemoglobin alpha 2 (*HBA2*) (SEQ ID NO: 431-432), S100 calcium-binding protein A11 (calgizzarin) (*S100A11*) (SEQ ID NO: 433), Lactate dehydrogenase A (*LDHA*) (SEQ ID NO: 434-436), ubiquitin-conjugating enzyme E2C (UBE2C) (SEQ ID NO: 437), E2F-3=pRB-binding transcription factor=KIAA0075 (*E2F3*) (SEQ ID NO: 438-440), E-cadherin (*CDH1*) (SEQ ID NO: 441-442), proteasome (prosome, macropain) activator subunit 2 (PA28 beta) (*PSME2*) (SEQ ID NO: 443-444), OP-1=osteogenic protein in the TGF-beta family (*BMP7*) (SEQ ID NO: 445-447), or topoisomerase II (*TOP2A*) (SEQ ID NO: 448) specific binding agent (*e.g.*, a polyclonal or monoclonal antibody or antibody fragment), and may include at least one control. The ovarian cancer-linked protein specific binding agent and control may be contained in separate containers. The kits may also include a means for detecting ovarian cancer-related protein:agent complexes, for instance the agent may be detectably labeled. If the detectable agent is not labeled, it may be detected by second antibodies or protein A, for example, either of both of which also may be provided in some kits in one or more separate containers. Such techniques are well known.

Additional components in some kits include instructions for carrying out the assay. Instructions will allow the tester to determine whether ovarian cancer-linked expression levels are elevated or reduced in comparison to a control sample. Reaction vessels and auxiliary reagents such as chromogens, buffers, enzymes, etc. also may be included in the kits.

EXAMPLE 12

Identification of Therapeutic Compounds

This example describes how to use the ovarian cancer-related molecules disclosed herein to identify compounds for potential therapeutic use in treating, reducing, or preventing ovarian cancer or development or progression of ovarian cancer.

The ovarian cancer-related molecules disclosed herein, and more particularly the linkage of these molecules to cancer, can be used to identify compounds that are useful in treating, reducing, or preventing ovarian cancer or development or progression of ovarian cancer. These molecules can be used alone or in combination, for instance in sets of two or more that are linked to cancer or cancer progression.

By way of example, a test compound is applied to a cell, for instance a test cell, and at least one ovarian cancer-related molecule level and/or activity in the cell is measured and compared to the equivalent measurement from a test cell (or from the same cell prior to application of the test compound). If application of the compound alters the level and/or activity of an ovarian cancer-related molecule (for instance by increasing or decreasing that level), then that compound is selected as a likely candidate for further characterization. In particular examples, a test agent that opposes or inhibits an ovarian cancer-related change is selected for further study, for example by exposing the agent to an ovarian epithelial cancer cell *in vitro*, to determine whether *in vitro* growth is inhibited. Such identified compounds may be useful in treating, reducing, or preventing ovarian cancer or development or progression of ovarian cancer. In particular embodiments, the compound isolated will inhibit or inactivate an ovarian cancer-related molecule, for instance those represented by the nucleic acids listed in Table 1.

Methods for identifying such compounds optionally can include the generation of an ovarian cancer-related gene expression profile, as described herein. Control gene expression profiles useful for comparison in such methods may be constructed from normal ovarian tissue, including primary ovarian cancer tissue.

EXAMPLE 12

Gene Expression Profiles (Fingerprints)

This example describes how to use the ovarian cancer-related nucleic acids and analysis methods disclosed herein to generate and use gene expression profiles, or "fingerprints."

With the provision herein of methods for determining molecules that are linked to ovarian cancer, and the provision of a large collection of such ovarian cancer-linked molecules (as represented for instance by those listed in Table 1), gene expression profiles that provide information on the ovarian cancer-state of a subject are now enabled.

Ovarian cancer-related expression profiles comprise the distinct and identifiable pattern of expression (or level) of sets of ovarian cancer-related genes, for instance a pattern of high and low expression of a defined set of genes, or molecules that can be correlated to such genes, such as mRNA levels or protein levels or activities. Useful sets of molecules for constructing nucleic acid expression profiles include at least one that is represented by the following genes and ESTs: *BCKDHB* (SEQ ID NO: 16-17), *ZNF33A* (SEQ ID NO: 20-22), EST 192198 (SEQ ID NO: 25), EST 128738 (SEQ ID NO: 26-27), EST 429211 (28-29), *FLJ22174* (SEQ ID NO: 30-31), EST 415562 (SEQ ID NO: 32-33), EST 296488 (SEQ ID NO: 34-35), EST 120124 (SEQ ID NO: 36-37), EST 132142 (SEQ ID NO: 38-39), EST 50635 (SEQ ID NO: 40), *POR* (SEQ ID NO: 41-43), EST 73702 (SEQ ID NO: 46-47), EST 2218314 (SEQ ID NO: 48), EST 2261113 (SEQ ID NO: 49), *IFITM1* (SEQ ID NO: 50-54), *IFITM2* (SEQ ID NO: 55-59), *KIAA0203* (SEQ ID NO: 61-62), *G1P3* (SEQ ID NO: 68-69), *BST2* (SEQ ID NO: 70-72), EST 1384797 (SEQ ID NO: 196) , *TLR3* (SEQ ID NO: 199-201), *SPON1* (SEQ ID NO: 160-161), *HSRNASEB* (SEQ ID NO: 162-163), EST 294506 (SEQ ID NO: 146-148), *SORL1* (SEQ ID NO: 149-151), *SIAT1* (SEQ ID NO: 73), *PL1* (SEQ ID NO: 77), EST 108422 (SEQ ID NO: 78-79), *CEBPG* (SEQ ID NO: 80), *HLA-DPA* (SEQ ID NO: 97-99), *H2AFL* (SEQ ID NO: 107-109), *IGKC* (SEQ ID NO: 112-116), *SCYB10* (SEQ ID NO: 120-121), *RGS1* (SEQ ID NO: 122-126), *LSR68* (SEQ ID NO: 168), *SGK* (SEQ ID NO: 176-178), and *ZFP36* (SEQ ID NO: 167-173). These genes and ESTs, which have not previously been correlated with cancer, present potentially useful novel markers for cancer, and in particular, ovarian cancer.

A second example set of molecules that could be used in a profile would include at least one that is represented by (or correlated to) the genes and ESTs represented by the SEQ ID NOs in Table 9. These nucleic acids, which are disclosed herein to be differentially expressed in ovarian cancer (see Figure 2), are suitable for markers to diagnose, prognose, and monitor ovarian cancer in a subject. In addition, these genes and ESTs are potentially useful as markers for classifying tumors into types, for instance into *BRCA1*-type or *BRCA2*-type tumors, using the methods disclosed herein.

A third example set of molecules that could be used in a profile would include at least one that is represented by (or correlated to) the genes and ESTs represented by SEQ ID NOs: 417, 284, 285, 281, 283, 278, 273, 282, 274. These represent markers disclosed herein that were found to be differentially expressed between *BRCA1*-Linked and sporadic tumors in a comparison to reference Immortalized Ovarian Epithelial Cells (IOSE). These markers are useful for classifying tumors into *BRCA1*-linked and sporadic types, and present potential targets for treatment of ovarian cancer.

A fourth example set of molecules that could be used in a profile would include at least one that is represented by (or correlated to) the genes and ESTs represented by SEQ ID NOs: 279-280, which, as disclosed herein, are markers that were found to be differentially expressed between *BRCA2*-Linked and sporadic tumors in a comparison to reference Immortalized Ovarian Epithelial Cells. These markers are useful for classifying tumors into *BRCA2*-linked and sporadic types, and present potential targets for treatment of ovarian cancer.

A fifth example set of molecules that could be used in a profile would include at least one that is represented by (or correlated to) the genes and ESTs represented by SEQ ID NOs: 281, 282

and 274, which, as disclosed herein, are markers that were found to be differentially expressed between combined *BRCA*-Linked and sporadic tumors in a comparison to reference Immortalized Ovarian Epithelial Cells. These markers are useful for classifying tumors into BRCA-linked and sporadic types, and present potential targets for treatment of ovarian cancer.

A sixth example set of molecules that could be used in a profile would include at least one that is represented by (or correlated to) the genes and ESTs represented by the SEQ ID NOs set forth in Table 10, which, as disclosed herein, are markers that can be used to segregate *BRCA1*-linked from *BRCA2*-linked tumor types using compound covariate prediction analysis. These markers are useful for classifying tumors into one of two types of tumors, which provides information helpful to a clinician in choosing a course of treatment for the patient based on the type of tumor into which the sample is classified.

A seventh example set of molecules that could be used in a profile would include at least one that is represented by (or correlated to) the genes and ESTs represented by SEQ ID NO: 16-201, 565-567, and 803-804. These genes and ESTs were found, as disclosed herein, to be differentially expressed in a comparison of *BRCA1*-linked and *BRCA2*-linked to sporadic tumors. Hence, these genes and ESTs present potentially useful markers for classifying tumors into types, using the methods disclosed herein. Furthermore, they represent potential targets for pharmaceutical treatment of tumors of each respective tumor type.

A eighth example set of molecules that could be used in a profile would include at least one that is represented by (or correlated to) the genes and ESTs represented by SEQ ID NOs: 124-126, 319, 429-430, 504-523, 533-535, 544, and 548-799. As disclosed herein, these nucleic acids were found to be overexpressed in a comparison of *BRCA1*-linked, *BRCA2*-linked and sporadic tumor samples. Hence, these genes and ESTs present potentially useful markers for classifying tumors into types, using the methods disclosed herein. Furthermore, they represent potential targets for pharmaceutical treatment of tumors of each respective tumor type.

A ninth example set of molecules that could be used in a profile would include at least one that is represented by (or correlated to) the genes and ESTs represented by SEQ ID NOs: 202-339. As disclosed herein, these nucleic acids were found to be overexpressed in ovarian cancer in a comparison of ovarian epithelial cancer to normal postmenopausal ovarian tissue. Hence, these genes and ESTs present potentially useful markers diagnosis, prognosis, and monitoring of ovarian cancer. In addition, they represent potential targets for pharmaceutical treatment of ovarian tumors.

A tenth example set of molecules that could be used in a profile would include at least one that is represented by (or correlated to) the genes and ESTs represented by SEQ ID NOs:97 and 340-448. As disclosed herein, these nucleic acids were found to be underexpressed in ovarian cancer in a comparison of ovarian epithelial cancer to normal postmenopausal ovarian tissue. Hence, these genes and ESTs present potentially useful markers diagnosis, prognosis, and monitoring of ovarian cancer. In addition, they represent potential targets for pharmaceutical treatment of ovarian tumors.

In other examples of ovarian cancer-related gene expression profiles, such profiles may be further broken down by the manner of molecules included in the profile. Thus, certain examples of

profiles may include a specific class of ovarian cancer markers, such as those molecules involved in cell cycle control.

Particular profiles may be specific for a particular stage of normal tissue (*e.g.*, ovarian tissue) growth or disease progression (*e.g.*, progression of ovarian cancer). Thus, gene expression profiles can be established for a pre-ovarian cancer tissue (*i.e.*, normal ovarian tissue), and a primary ovarian cancer tissue. Each of these profiles includes information on the expression level of at least one, but usually two or more, genes that are linked to ovarian cancer (*e.g.*, ovarian cancer-related genes). Such information can include relative as well as absolute expression levels of specific genes. Likewise, the value measured may be the relative or absolute level of protein expression, which can be correlated with a "gene expression level." Results from the gene expression profiles of an individual subject are often viewed in the context of a test sample compared to a baseline or control sample fingerprint.

The levels of molecules that make up a gene expression profile can be measured in any of various known ways, which may be specific for the type of molecule being measured. Thus, nucleic acid levels (such as direct gene expression levels, such as the level of mRNA expression) can be measured using specific nucleic acid hybridization reactions. Protein levels may be measured using standard protein assays, using immunologic-based assays (such as ELISAs and related techniques), or using activity assays, for instance. Examples for measuring nucleic acid and protein levels are provided herein; other methods are well known to those of ordinary skill in the art.

Examples of ovarian cancer-related gene expression profiles can be in array format, such as a nucleotide (*e.g.*, polynucleotide) or protein array or microarray. The use of arrays to determine the presence and/or level of a collection of biological macromolecules is now well known (see, for example, methods described in published PCT application number WO9948916, describing hypoxia-related gene expression arrays). In array-based measurement methods, an array may be contacted with polynucleotides (in the case of a nucleic acid-based array) or polypeptides (in the case of a protein-based array) from a sample from a subject. The amount and/or position of binding of the subject's polynucleotides or polypeptides then can be determined, for instance to produce a gene expression profile for that subject. Such gene expression profile can be compared to another gene expression profile, for instance a control gene expression profile from a subject having a known gynecological or ovary-related condition. Optionally, the subject's gene expression profile can be correlated with one or more appropriate treatments, which may be correlated with a control (or set of control) expression profiles for stages of ovarian cancer, for instance.

This disclosure provides the identification of ovarian cancer-related molecules that exhibit alterations in expression during development of ovarian cancer, and expression fingerprints (profiles) specific for ovarian cancers. It further provides methods of using these identified nucleic acid molecules, and proteins encoded thereby, and expression fingerprints or profiles, for instance to predict and/or diagnose ovarian cancer, and to elect treatments for instance based on likely response. These identified ovarian cancer-related molecules also can serve as therapeutic targets, and can be

used in methods for identifying, developing and testing therapeutic compounds. It will be apparent that the precise details of the methods described may be varied or modified without departing from the spirit of the described invention. We claim all such modifications and variations that fall within the scope and spirit of the claims below.

CLAIMS

We claim:

1. A method of classifying an ovarian tumor as a BRCA-1-like or BRCA-2-like or non-BRCA-like tumor, comprising:

determining a pattern of expression in the ovarian tumor of a plurality of markers listed in Table 1, wherein the pattern of expression in the ovarian tumor is determined relative to a standard ovarian tissue;

comparing a similarity of the pattern of expression of the plurality of markers in the ovarian tumor to a pattern of expression of the plurality of markers in a comparison tissue of a known BRCA-1-like or BRCA-2-like or non-BRCA-like tumor, wherein the pattern of expression in the comparison tissue is determined relative to the standard ovarian tissue;

wherein a similarity of the pattern of expression in the ovarian tumor to a pattern of expression of the comparison tissue of the known BRCA-1-like tumor classifies the ovarian tumor as a BRCA-1-like tumor, a similarity of the pattern of expression in the ovarian tumor to a pattern of expression of the known BRCA-2-like tumor classifies the ovarian tumor as a BRCA-2-like tumor, and a similarity of the pattern of expression in the ovarian tumor to a pattern of expression of the known non-BRCA-like tumor classifies the ovarian tumor as a non-BRCA-like tumor.

2. The method of claim 1, wherein the method comprises determining a pattern of over-expression or under-expression of the plurality of markers in the ovarian tumor to over-expression or under-expression of the plurality of markers of the comparison tissue.

3. The method of claim 2, wherein the method comprises determining a pattern of both over-expression and under-expression of the plurality of markers in the ovarian tumor to over-expression or under-expression of the plurality of markers in the comparison tissue.

4. The method of claim 1 wherein the comparison tissue is from a known BRCA-1-like tumor, and the method comprises determining whether the ovarian tumor is a BRCA-1-like tumor by comparing the pattern of expression in the ovarian tumor to the pattern of expression in the comparison tissue.

5. The method of claim 1 wherein the comparison tissue is from a subject known to have a mutation in BRCA-1 and the method comprises determining whether the ovarian tumor is a BRCA-1-like tumor by comparing the pattern of expression in the ovarian tumor to the pattern of expression in the comparison tissue.

6. The method of claim 1 wherein the comparison tissue is from a subject known to have a mutation in BRCA-2 and the method comprises determining whether the ovarian tumor is a BRCA-2-like tumor by comparing the pattern of expression in the ovarian tumor to the pattern of expression in the comparison tissue.

7. The method of claim 1 wherein the comparison tissue is from a known BRCA-2-like tumor, and the method comprises determining whether the ovarian tumor is BRCA-2-like by comparing the pattern of expression in the ovarian tumor to the pattern of expression in the comparison tissue.

8. The method of claim 7, wherein classifying the ovarian tumor comprises determining whether a tumor that does not contain a BRCA-1 or BRCA-2 mutation is BRCA-1-like or BRCA-2-like.

9. The method of claim 1 wherein the comparison tissue is from a known non-BRCA-like tumor, and the method comprises determining whether the ovarian tumor is non-BRCA-like by comparing the pattern of expression in the ovarian tumor to the pattern of expression in the comparison tissue.

10. The method of claim 1, wherein the standard ovarian tissue is tissue from an immortalized ovarian cell, ovarian tissue from a subject not having ovarian cancer, a subject not predisposed to developing ovarian cancer, or ovarian tissue from a subject from whom the ovarian tumor was obtained at an earlier point in time.

11. The method of claim 1, wherein the patterns of expression are patterns of logarithmic expression ratios.

12. The method of claim 1, wherein the patterns of expression are multidimensional scaling patterns.

13. The method of claim 12 wherein the multi-dimensional scaling patterns are visually compared to determine similarities.

14. The method of claim 1, wherein the patterns of expression are hierarchical clustering patterns.

15. The method of claim 14, wherein standard normal deviation values of the logarithmic expression ratios are assigned relative color intensities that assist in the visual comparison.

16. The method of claim 15, wherein the hierarchical clustering patters are visually compared to determine similarities.

17. The method of claim 11 comprising comparing the logarithmic expression ratios of the plurality markers using compound covariate predictor analysis.

18. The method of claim 11, wherein the method comprises differentiating a *BRCA1*-like ovarian tumor from a sporadic ovarian tumor by comparing relative logarithmic expression ratios of at least one marker shown in Table 6.

19. The method of claim 18, wherein differentiating a *BRCA1*-linked ovarian tumor from a sporadic ovarian tumor comprises comparing the relative logarithmic expression ratios of *CD72* (SEQ ID NO: 805), *SLC25A11* (SEQ ID NO: 544), *LCN2* (SEQ ID NO: 545-547), PSTPIP1 (SEQ ID NO: 538-540), SIAHBP1 (SEQ ID NO: 543), *UBE1* (SEQ ID NO: 533), *WAS* (SEQ ID NO: 524-526), *IDH2* (SEQ ID NO: 541-542), or *PCTK1* (SEQ ID NO: 527-528) in the ovarian tumor and comparison tissue.

20. The method of claim 11, wherein the method comprises differentiating a *BRCA2*-like ovarian tumor from a non-BRCA-like ovarian tumor by comparing relative logarithmic expression ratios of at least one marker shown in Table 7.

21. The method of claim 20, wherein the method comprises comparing the relative logarithmic expression ratios of *LOC51760* (SEQ ID NO: 279) or *LRPAP1* (SEQ ID NO: 280) to differentiate a *BRCA2*-like ovarian tumor from a non-BRCA like ovarian tumor

22. The method of claim 21, wherein the method comprises differentiating a non-BRCA-like tumor from a BRCA-1-like or BRCA-2-like ovarian tumor by comparing relative logarithmic expression ratios of at least one marker shown in Table 8.

23. The method of claim 22, wherein the method comprises comparing relative logarithmic expression ratios of *PSTPIP1* (SEQ ID NO: 281), *IDH2* (SEQ ID NO: 282), or *PCTK1* (SEQ ID NO: 274) to differentiate a combined *BRCA1*- and *BRCA2*-linked ovarian tumor from a sporadic ovarian tumor.

24. The method of claim 11, wherein the method comprises differentiating a *BRCA1*-like ovarian tumor from a *BRCA2*-like ovarian tumor by comparing relative logarithmic expression ratios of at least one marker shown in Table 10.

25. The method of claim 1, wherein the method further comprises selecting a treatment strategy based on classifying the ovarian tumor as *BRCA1*-like, *BRCA2*- like or non-*BRCA*-like.

26. The method of claim 25, wherein the treatment strategy comprises selecting a more aggressive treatment regimen for a *BRCA1*-like or *BRCA2*-like tumor.

27. The method of claim 26, wherein the treatment is chemotherapy, radiotherapy, or surgical removal of the affected tissue and/or surrounding area.

28. The method of claim 25, further comprising treating the subject with the selected treatment.

29. The method of claim 11, wherein comparing the patterns of logarithmic expression ratios comprises comparing the logarithmic expression ratios to patterns of logarithmic expression ratios in a database of patterns associated with *BRCA1*-like, *BRCA2*-like or non-BRCA-like ovarian tumors.

30. The method of claim 11, wherein comparing patterns of logarithmic expression ratios of the plurality of markers comprises obtaining the pattern of expression of the plurality of markers on an array.

31. The method of claim 1, wherein the pattern of expression of the plurality of markers comprises over-expression of one or more markers compared to the standard.

32. The method of claim 29, wherein the one or more markers that is overexpressed is listed in Table 5.

33. The method of claim 32, wherein determining the pattern of expression comprises providing nucleic acid sequences of the markers, and performing nucleic acid hybridization of specific oligonucleotide probes to the nucleic acid sequences.

34. The method of claim 33, wherein the sequence of the oligonucleotide probe is selected to bind specifically to a nucleic acid molecule listed in Table 1.

35. The method of claim 34, further comprising amplifying the one or more markers prior to performing nucleic acid hybridization.

36. The method of claim 33, further comprising quantitating hybridization to detect a level of differential expression.

37. The method of claim 33, wherein providing sequences of the markers comprises providing the nucleic acid sequences on an array carrying the plurality of markers.

38. The method of claim 37, wherein the array is a cDNA microarray.

39. The method of claim 33, wherein providing the nucleic acid sequences of the markers comprises providing at least 50 of the markers listed in Table 1.

40. The method of claim 33, wherein providing the nucleic acid sequences of the markers comprises providing at least 100 of the markers listed in Table 1.

41. The method of claim 33, wherein providing the nucleic acid sequences of the markers comprises providing at least 200 of the markers listed in Table 1.

42. A method of diagnosing or prognosing development or progression of ovarian cancer in a subject comprising detecting under-expression of one or more markers in Table 4 relative to a standard.

43. The method of claim 42, wherein the standard is immortalized ovarian epithelial cells, ovarian tissue from a subject not having cancer or a subject not predisposed to developing cancer, or ovarian tissue from the subject at an earlier point in time.

44. The method of claim 42 wherein the one or more markers comprise a nucleic acid encoded by SEQ ID NOs: 449-503.

45. A method of diagnosing or prognosing development or progression of ovarian cancer in a subject comprising detecting over-expression of one or more markers in Table 5 relative to a standard.

46. The method of claim 45, wherein the standard is immortalized ovarian epithelial cells, ovarian tissue from a subject not having cancer or a subject not predisposed to developing cancer, or ovarian tissue from the subject at an earlier point in time.

47. The method of claim 45 wherein the one or more markers comprise a nucleic acid encoded by SEQ ID NOs: 18-19, 30-31, 50-51, 52-54, 55-57, 58-59, 60, 68-69, 74-76, 85-86, 87-88, 89-91, 92-93, 94-95, 97-99, 122-123, 133-135, 149-151, 164-166, 167-168, 169-170, 174-175, 176-178, 179-180, 181-182, 190-192, or 199-201.

48. A method of screening for an agent for treating or inhibiting ovarian cancer in a subject, comprising exposing a tumor cell to a therapeutically effective amount of a pharmaceutical compound that restores wild-type expression of at least one *BRCA1*-like or *BRCA2*-like marker listed in Table 1.

49. The method of claim 48 wherein the agent corrects under-expression or over-expression of a marker listed in Table 1.

50. A method of monitoring a response to therapy for an ovarian tumor, comprising monitoring expression of the markers in the subject following administration of the therapy.

51. A method of diagnosing or prognosing development or progression of ovarian cancer in a subject comprising detecting differential expression of a gene that maps to Chromosome Xp11.2.

52. A kit for classifying one or more ovarian tumors as sporadic, *BRCA1*-like or *BRCA2*-like tumors, comprising components for measuring expression levels of markers in the one or more ovarian tumor samples and for comparing the expression levels of the markers to the markers in Table 10.

53. The kit of claim 52, wherein the expression levels of a plurality of markers from each tumor are measured.

54. The kit of claim 52, comprising an array carrying a plurality of markers.

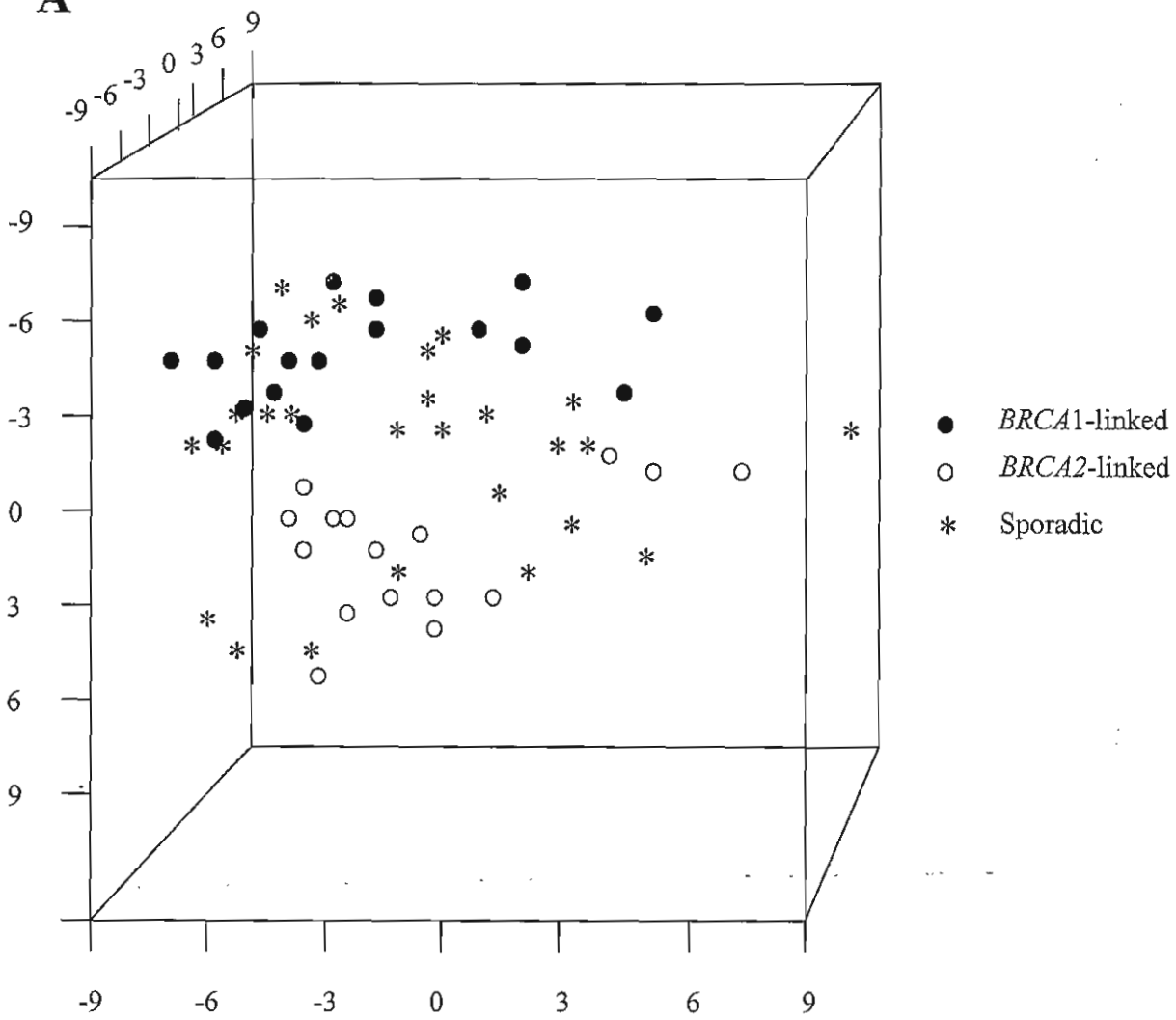
55. The kit of claim 52, comprising a binding molecule that selectively binds to a marker in the one or more tumor samples, and wherein the marker is listed in Table 10.

56. The kit of claim 52, wherein the expression levels measured are of a non-BRCA-like, *BRCA1*-like or *BRCA2*-like tumor protein, and the binding molecule is an antibody or antibody fragment that selectively binds the tumor protein.

57. The kit of claim 52, wherein the expression levels measured are of a BRCA-like, *BRCA1*-like or *BRCA2*-like nucleic acid marker, and the binding molecule is an oligonucleotide capable of hybridizing to the nucleic acid molecule marker.

Figure 1

A



B

Number of genes differentiating tumor types (P<0.0001)	
*BRCA*1-linked vs. Sporadic	9
BRCA2-linked vs. Sporadic	2
BRCA1-linked vs. BRCA2-linked vs. Sporadic	60
BRCA1-linked vs. BRCA2-linked	110
BRCA1 & *BRCA2*-linked vs. Sporadic	3

Figure 2A

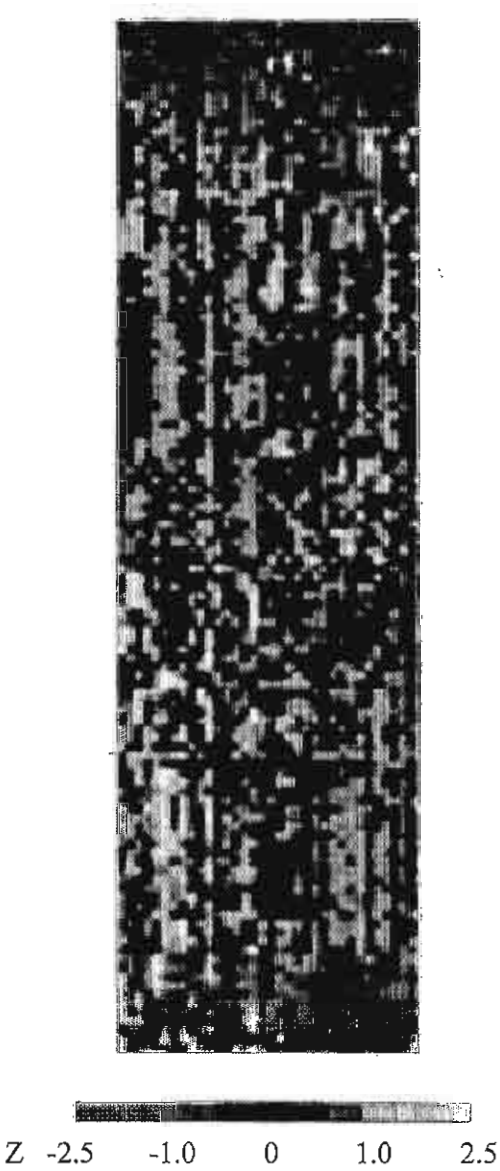
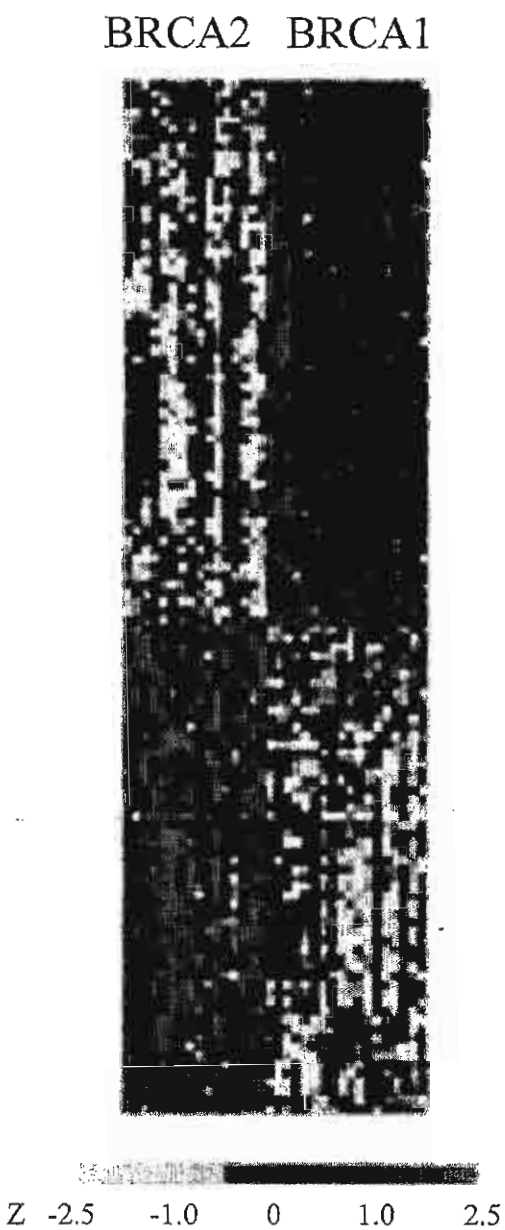


Figure 2A'



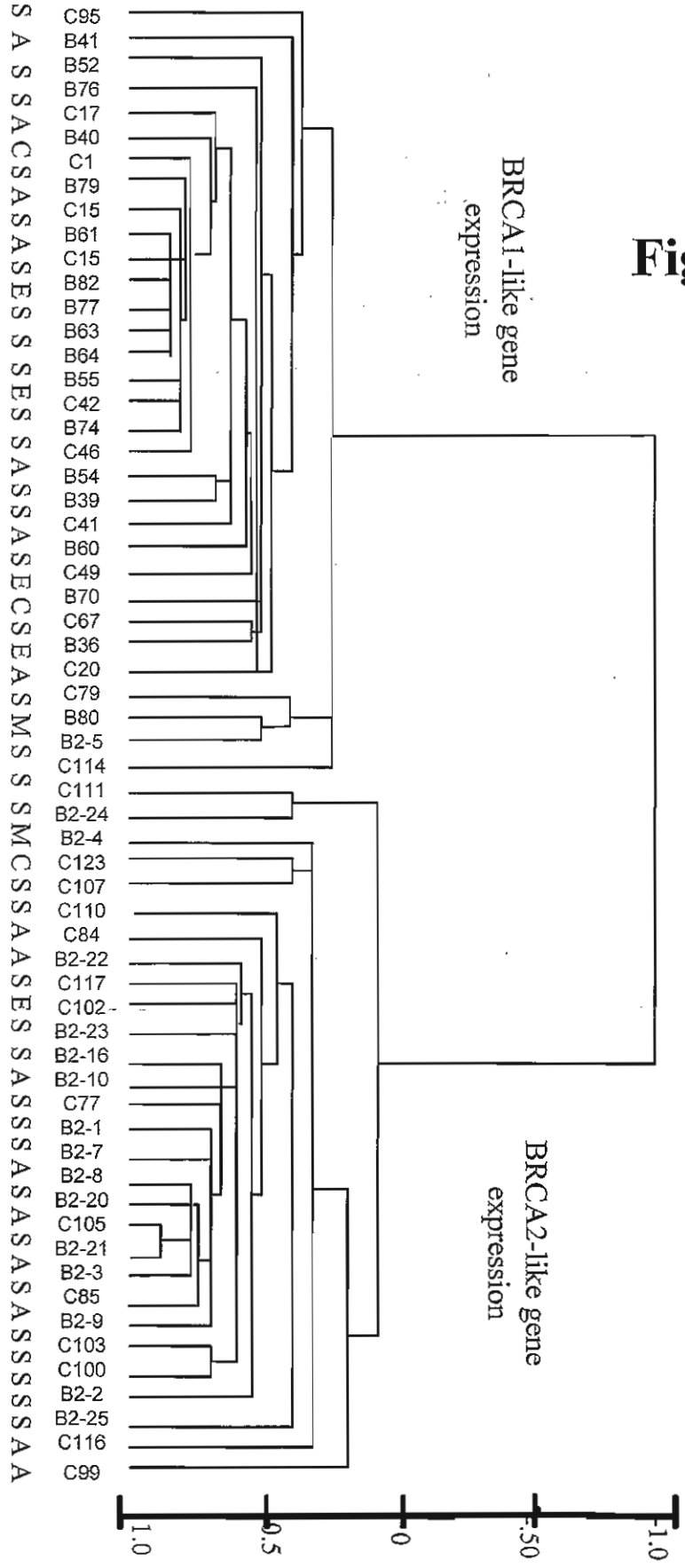

BRCA1-like gene expression
BRCA2-like gene expression
C95
B41
B52
B76
C17
B40
C1
B79
C15
B61
C15
B82
B77
B63
B64
B55
C42
B74
C46
B54
B39
C41
B60
C49
B70
C67
B36
C20
C79
B80
B2-5
C114
C111
B2-24
B2-4
C123
C107
C110
C84
B2-22
C117
C102
B2-23
B2-16
B2-10
C77
B2-1
B2-7
B2-8
B2-20
C105
B2-21
B2-3
C85
B2-9
C103
C100
B2-2
B2-25
C116
C99
1.0
0.5
0
.50
1.0
S A S S A C S A S A S E S S E S S A S S A S E C S E A S M S S S M C S S A A S E S S A S S S A S A S A S A S S S S S A A
Median Age = 59.5
Median Age = 63

Figure 2B