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(54) GRADING OF BREAST CANCER

(76) Inventors: **Mark G. Erlander**, Encinitas, CA (US); **Xiao-Jun Ma**, San Diego, CA (US); **Dennis C. Sgroi**, Winchester, MA (US)

Correspondence Address:
Kawai Lau
Morrison & Foerster LLP
Suite 500
3811 Valley Centre Drive
San Diego, CA 92130-2332 (US)

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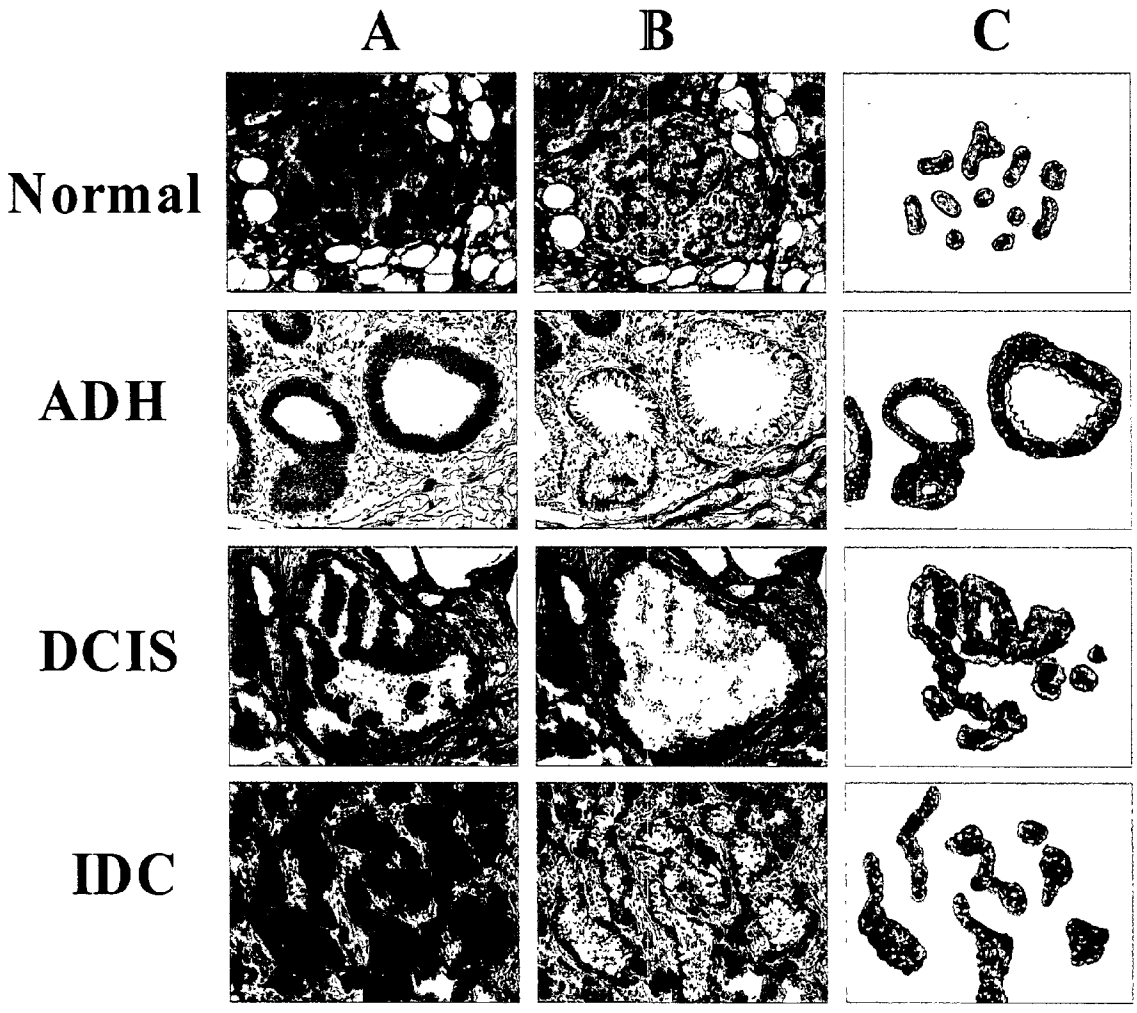
(57) **ABSTRACT**

Methods and compositions for the identification of breast cancer grade signatures are provided. The signature profiles are identified based upon multiple sampling of reference breast tissue samples from independent cases of breast cancer and provide a reliable set of molecular criteria for identification of cells as being in one or more particular stages and/or grades of breast cancer.



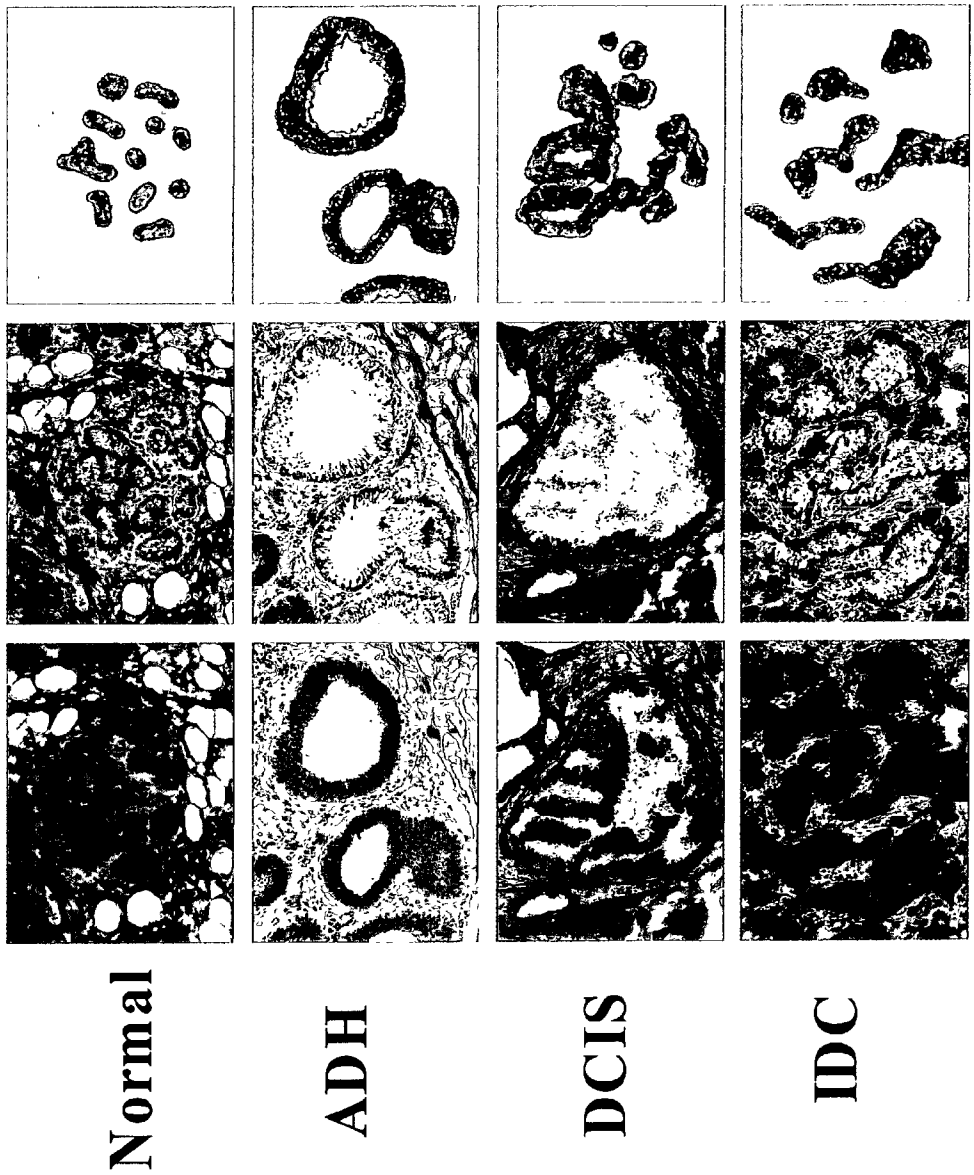


Fig. 1

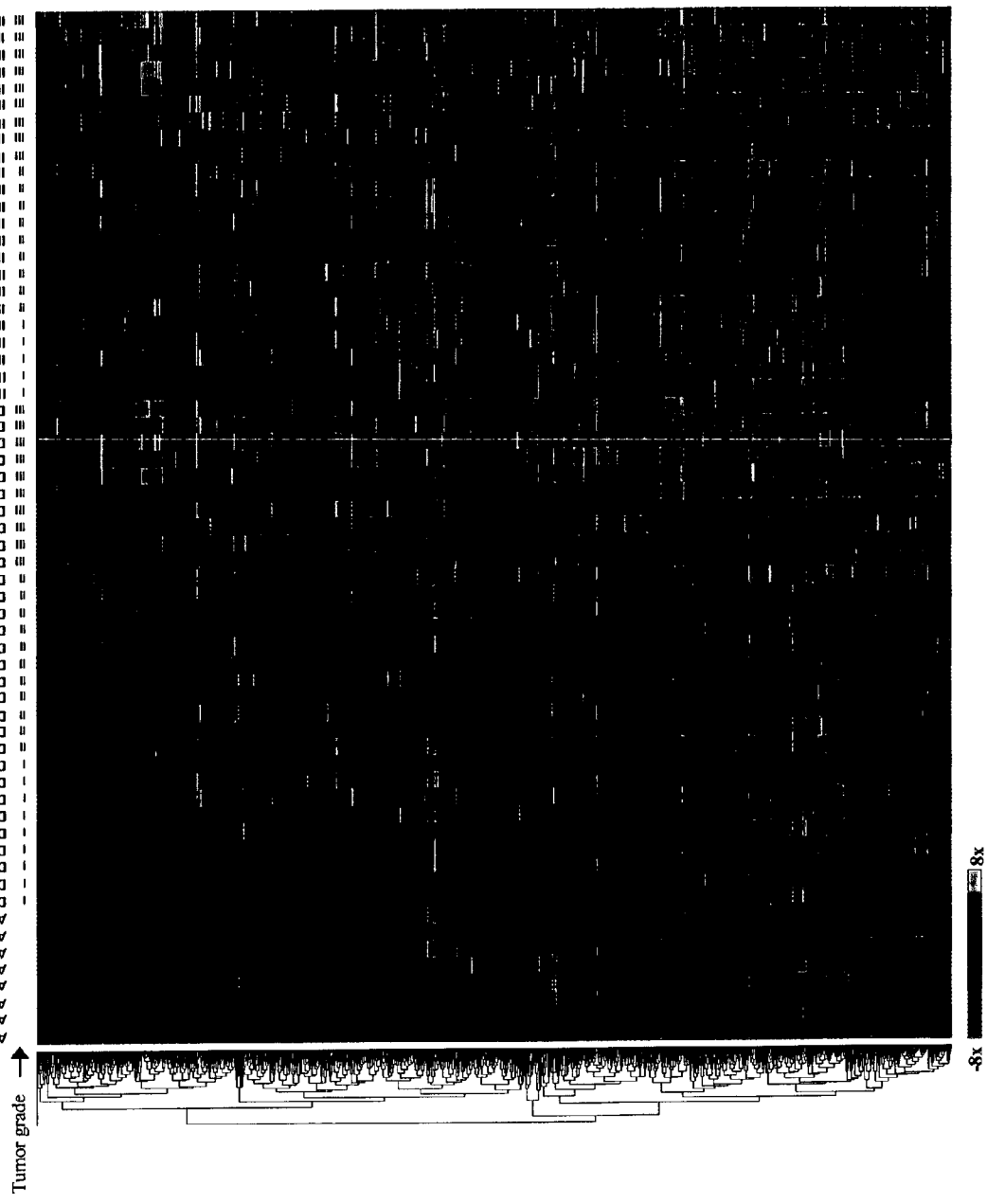
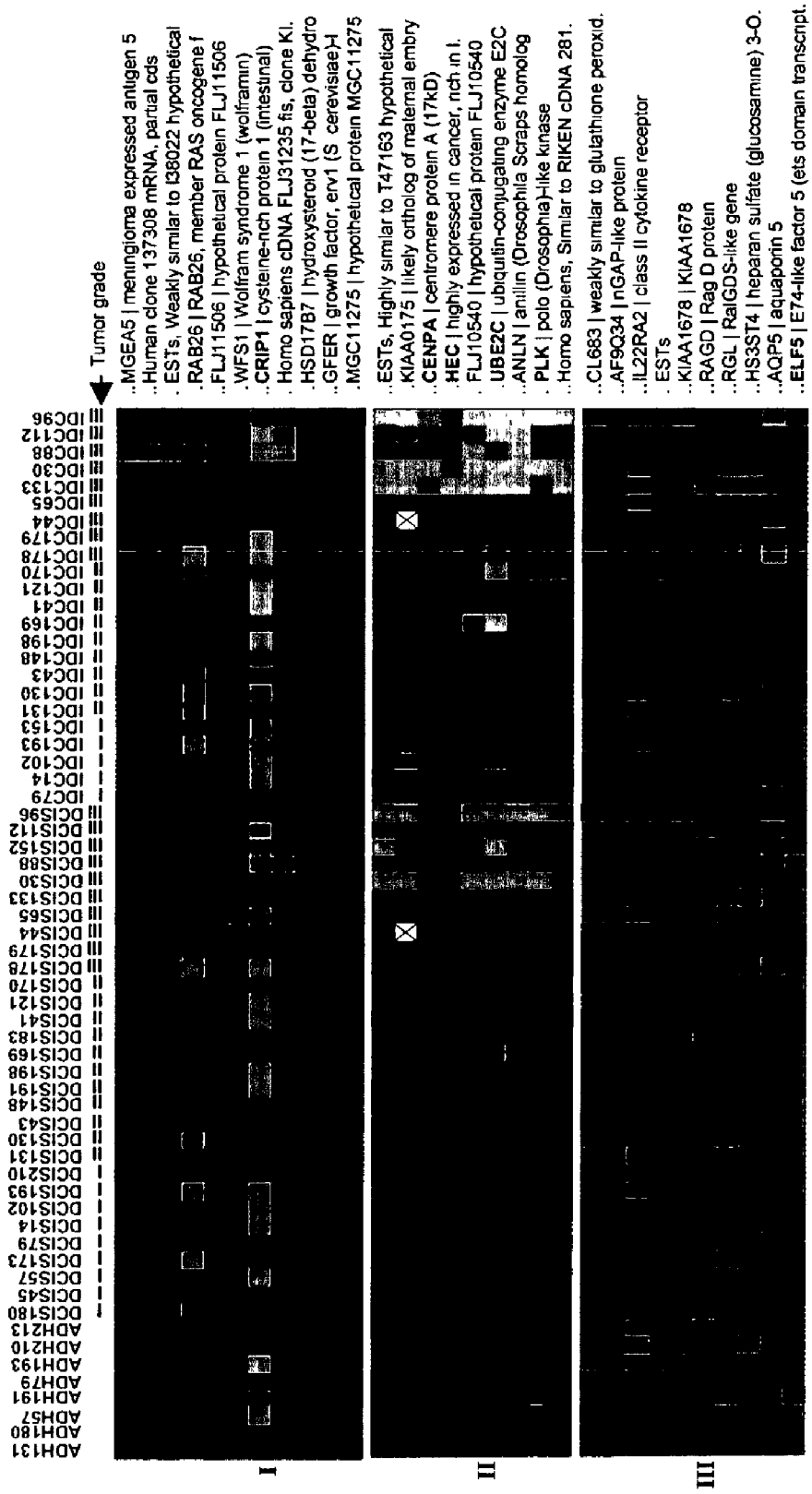

IDC96
IDC112
IDC88
IDC30
IDC133
IDC65
IDC44
IDC179
IDC178
IDC170
IDC121
IDC41
IDC169
IDC198
IDC148
IDC43
IDC130
IDC131
IDC153
IDC193
IDC102
IDC14
IDC79
DCIS96
DCIS112
DCIS152
DCIS88
DCIS30
DCIS133
DCIS65
DCIS44
DCIS179
DCIS178
DCIS170
DCIS121
DCIS41
DCIS183
DCIS169
DCIS198
DCIS191
DCIS148
DCIS43
DCIS130
DCIS131
DCIS210
DCIS193
DCIS102
DCIS14
DCIS79
DCIS173
DCIS57
DCIS45
DCIS180
ADH213
ADH210
ADH193
ADH79
ADH191
ADH57
ADH180
ADH131
Tumor grade
8x
-8x

Fig.2a

Fig.2b


Tumor grade
ADH131
ADH180
ADH57
ADH191
ADH79
ADH193
ADH210
ADH213
DCIS180
DCIS45
DCIS57
DCIS173
DCIS79
DCIS14
DCIS102
DCIS193
DCIS210
DCIS131
DCIS130
DCIS43
DCIS148
DCIS191
DCIS198
DCIS169
DCIS183
DCIS41
DCIS121
DCIS170
DCIS178
DCIS179
DCIS44
DCIS65
DCIS133
DCIS30
DCIS88
DCIS152
DCIS112
DCIS96
DC79
DC14
DC102
DC193
DC153
DC131
DC130
DC43
DC148
DC198
DC169
DC41
DC121
DC170
DC178
DC179
DC44
DC65
DC133
DC30
DC88
DC112
DC96
..MGEA5 | meningioma expressed antigen 5
..Human clone 137308 mRNA, partial cds
. ESTs, Weakly similar to I38022 hypothetical
..RAB26 | RAB26, member RAS oncogene f
..FLJ11506 | hypothetical protein FLJ11506
..WFS1 | Wolfram syndrome 1 (wolframin)
..CRIP1 | cysteine-rich protein 1 (intestinal)
. Homo sapiens cDNA FLJ31235 fis, clone KI.
..HSD17B7 | hydroxysteroid (17-beta) dehydro
..GFER | growth factor, erv1 (S. cerevisiae)-l
..MGC11275 | hypothetical protein MGC11275
..ESTs, Highly similar to T47163 hypothetical
..KIAA0175 | likely ortholog of maternal embry
..CENPA | centromere protein A (17kD)
..HEC | highly expressed in cancer, rich in l.
. FLJ10540 | hypothetical protein FLJ10540
..UBE2C | ubiquitin-conjugating enzyme E2C
..ANLN | anillin (Drosophila Scraps homolog
..PLK | polo (Drosophila)-like kinase
. Homo sapiens, Similar to RIKEN cDNA 281.
..CL683 | weakly similar to glutathione peroxid.
..AF9Q34 | nGAP-like protein
..IL22RA2 | class II cytokine receptor
. ESTs
..KIAA1678 | KIAA1678
..RAGD | Rag D protein
..RGL | RalGDS-like gene
..HS3ST4 | heparan sulfate (glucosamine) 3-O.
..AQP5 | aquaporin 5
..ELF5 | E74-like factor 5 (ets domain transcript.
I
II
III

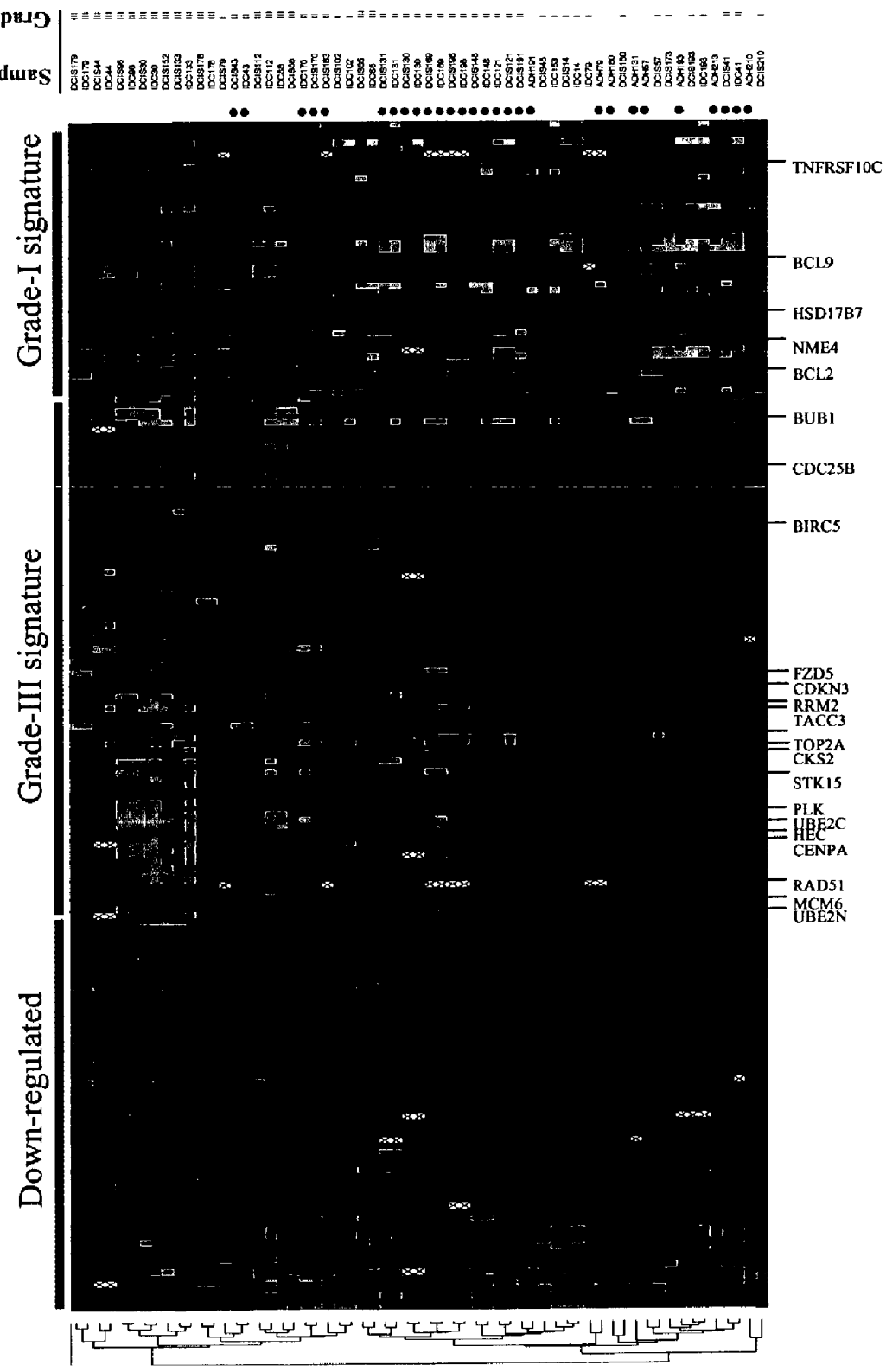

Grade
Sample
Grade-I signature
Grade-III signature
Down-regulated
TNFRSF10C
BCL9
HSD17B7
NME4
BCL2
BUB1
CDC25B
BIRC5
FZD5
CDKN3
RRM2
TACC3
TOP2A
CKS2
STK15
PLK
UBE2C
HEC
CENPA
RAD51
MCM6
UBE2N

Fig. 3

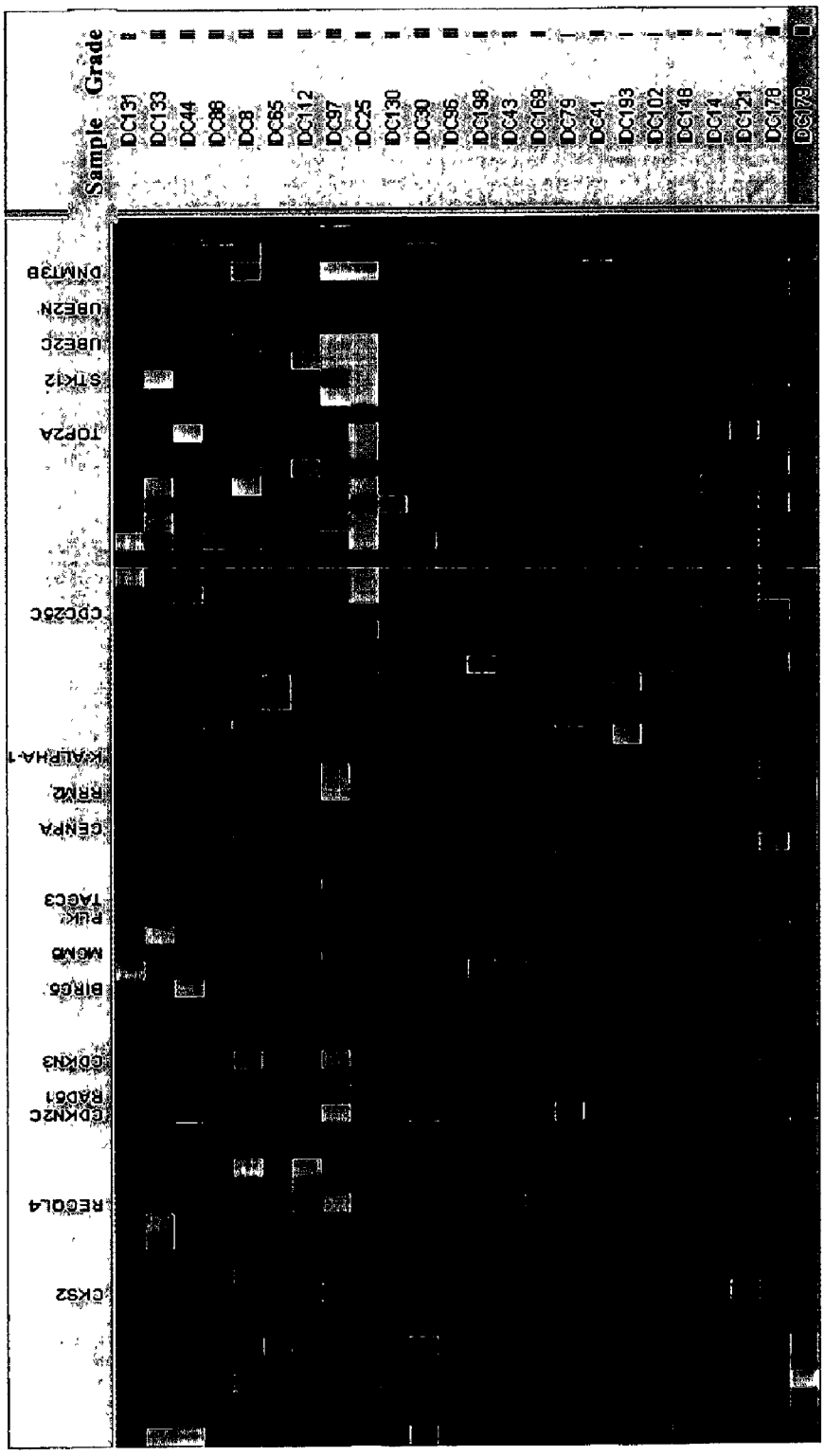
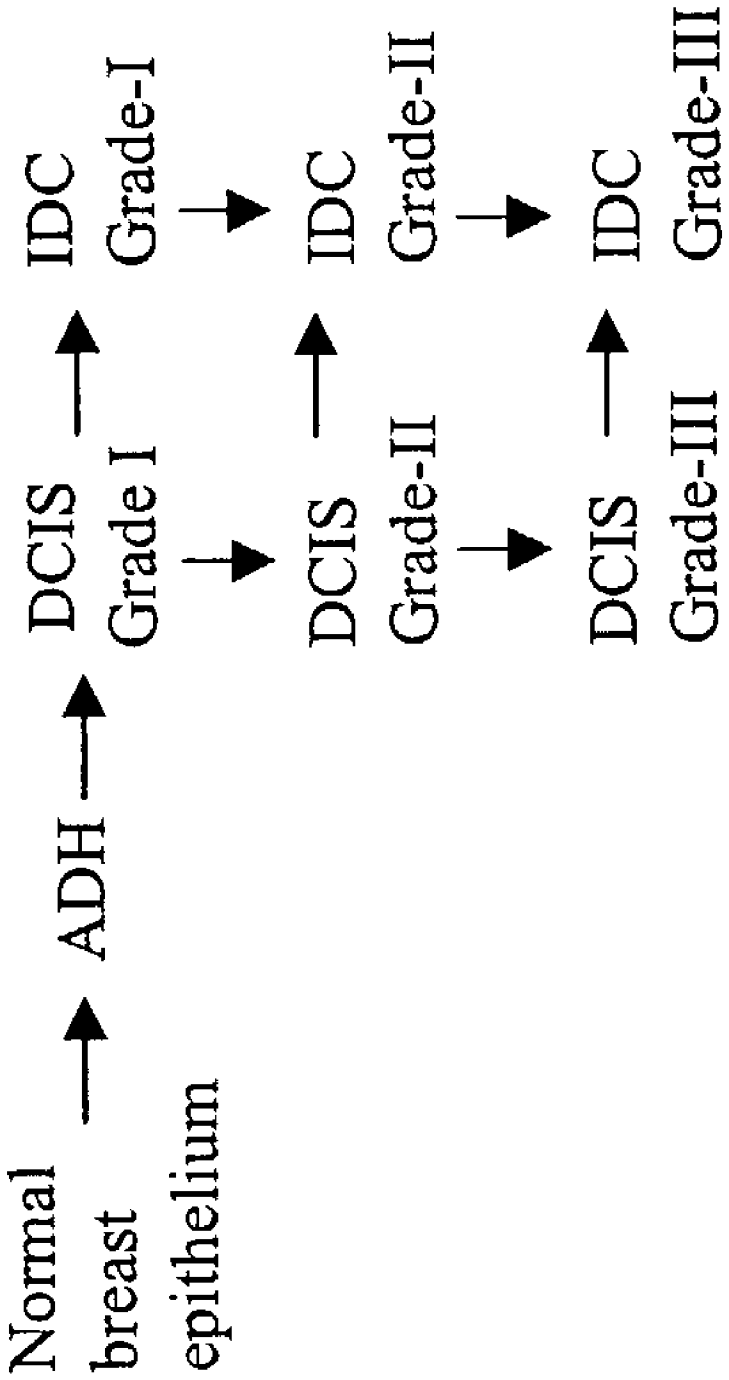

Grade
Sample
DC131
DC133
DC44
DC86
DC8
DC85
DC112
DC97
DC25
DC130
DC30
DC96
DC198
DC43
DC169
DC79
DC41
DC193
DC102
DC148
DC14
DC121
DC178
DC179
DNMT3B
UBE2N
UBE2C
STK12
TOP2A
CDC25C
KIAA1PHA-1
RRM2
CENPA
PLK/TAGC3
MCM6
BIRC6
CDKN3
CDKN2C/RAD51
RECQL4
CKS2
-4 -2 1 2 4

Fig. 4

Fig. 5


Normal breast epithelium
ADH
DCIS Grade I
IDC Grade-I
DCIS Grade-II
IDC Grade-II
DCIS Grade-III
IDC Grade-III

GRADING OF BREAST CANCER

RELATED APPLICATIONS

[0001] This application is a continuation-in-part of U.S. patent application Ser. No. 10/028,018 filed Dec. 21, 2001, which is hereby incorporated in its entirety as if fully set forth.

FIELD OF THE INVENTION

[0002] The invention relates to the identification and use of gene expression profiles, or patterns, involved in breast cancer progression. In particular, the invention provides the identities of genes that may be used to identify different grades of breast cancer within and between stages thereof. The gene expression profiles, whether embodied in nucleic acid expression, protein expression, or other expression formats, are used in the study and/or diagnosis of cells and tissue during breast cancer progression as well as for the study and/or determination of prognosis of a patient. When used for diagnosis or prognosis, the profiles are used to predict the status and/or phenotype of cells and tissues relative to breast cancer and the treatment thereof.

BACKGROUND OF THE INVENTION

[0003] Breast cancer is by far the most common cancer among women. Each year, more than 180,000 and 1 million women in the U.S. and worldwide, respectively, are diagnosed with breast cancer. Breast cancer is the leading cause of death for women between ages 50-55, and is the most common non-preventable malignancy in women in the Western Hemisphere. An estimated 2,167,000 women in the United States are currently living with the disease (National Cancer Institute, Surveillance Epidemiology and End Results (NCI SEER) program, *Cancer Statistics Review* (CSR), www-seer.ims.nci.nih.gov/Publications/CSR1973 (1998)). Based on cancer rates from 1995 through 1997, a report from the National Cancer Institute (NCI) estimates that about 1 in 8 women in the United States (approximately 12.8 percent) will develop breast cancer during her lifetime (NCI's Surveillance, Epidemiology, and End Results Program (SEER) publication *SEER Cancer Statistics Review* 1973-1997). Breast cancer is the second most common form of cancer, after skin cancer, among women in the United States. An estimated 250,100 new cases of breast cancer are expected to be diagnosed in the United States in 2001. Of these, 192,200 new cases of more advanced (invasive) breast cancer are expected to occur among women (an increase of 5% over last year), 46,400 new cases of early stage (in situ) breast cancer are expected to occur among women (up 9% from last year), and about 1,500 new cases of breast cancer are expected to be diagnosed in men (Cancer Facts & Figures 2001 American Cancer Society). An estimated 40,600 deaths (40,300 women, 400 men) from breast cancer are expected in 2001. Breast cancer ranks second only to lung cancer among causes of cancer deaths in women. Nearly 86% of women who are diagnosed with breast cancer are likely to still be alive five years later, though 24% of them will die of breast cancer after 10 years, and nearly half (47%) will die of breast cancer after 20 years.

[0004] Every woman is at risk for breast cancer. Over 70 percent of breast cancers occur in women who have no identifiable risk factors other than age (U.S. General Accounting Office. Breast Cancer, 1971-1991: Prevention, Treatment and Research. GAO/PEMD-92-12; 1991). Only 5 to 10% of breast cancers are linked to a family history of breast cancer (Henderson I C, Breast Cancer. In: Murphy G P, Lawrence W L, Lenhard R E (eds). *Clinical Oncology.* Atlanta, Ga: American Cancer Society; 1995:198-219).

[0005] Each breast has 15 to 20 sections called lobes. Within each lobe are many smaller lobules. Lobules end in dozens of tiny bulbs that can produce milk. The lobes, lobules, and bulbs are all linked by thin tubes called ducts. These ducts lead to the nipple in the center of a dark area of skin called the areola. Fat surrounds the lobules and ducts. There are no muscles in the breast, but muscles lie under each breast and cover the ribs. Each breast also contains blood vessels and lymph vessels. The lymph vessels carry colorless fluid called lymph, and lead to the lymph nodes. Clusters of lymph nodes are found near the breast in the axilla (under the arm), above the collarbone, and in the chest.

[0006] Breast tumors can be either benign or malignant. Benign tumors are not cancerous, they do not spread to other parts of the body, and are not a threat to life. They can usually be removed, and in most cases, do not come back. Malignant tumors are cancerous, and can invade and damage nearby tissues and organs. Malignant tumor cells may metastasize, entering the bloodstream or lymphatic system. When breast cancer cells metastasize outside the breast, they are often found in the lymph nodes under the arm (axillary lymph nodes). If the cancer has reached these nodes, it means that cancer cells may have spread to other lymph nodes or other organs, such as bones, liver, or lungs.

[0007] Major and intensive research has been focussed on early detection, treatment and prevention. This has included an emphasis on determining the presence of precancerous or cancerous ductal epithelial cells. These cells are analyzed, for example, for cell morphology, for protein markers, for nucleic acid markers, for chromosomal abnormalities, for biochemical markers, and for other characteristic changes that would signal the presence of cancerous or precancerous cells. This has led to various molecular alterations that have been reported in breast cancer, few of which have been well characterized in human clinical breast specimens. Molecular alterations include presence/absence of estrogen and progesterone steroid receptors, HER-2 expression/amplification (Mark H F, et al. HER-2/neu gene amplification in stages I-IV breast cancer detected by fluorescent in situ hybridization. Genet Med; 1(3):98-103 1999), Ki-67 (an antigen that is present in all stages of the cell cycle except G0 and used as a marker for tumor cell proliferation, and prognostic markers (including oncogenes, tumor suppressor genes, and angiogenesis markers) like p53, p27, Cathepsin D, pS2, multi-drug resistance (MDR) gene, and CD31.

[0008] Examination of cells by a trained pathologist has also been used to establish whether ductal epithelial cells are normal (i.e. not precancerous or cancerous or having another noncancerous abnormality), precancerous (i.e. comprising hyperplasia, atypical ductal hyperplasia (ADH)) or cancerous (comprising ductal carcinoma in situ, or DCIS, which includes low grade ductal carcinoma in situ, or LG-DCIS, and high grade ductal carcinoma in situ, or HG-DCIS) or invasive (ductal) carcinoma (IDC). Pathologists may also identify the occurrence of lobular carcinoma in situ (LCIS) or invasive lobular carcinoma (ILC). Breast cancer progression may be viewed as the occurrence of abnormal cells, such as those of ADH, DCIS, IDC, LCIS, and/or ILC, among normal cells.

[0009] It remains unclear whether normal cells become hyperplastic (such as ADH) and then progressing on to

become malignant (DCIS, IDC, LCIS, and/or ILC) or whether normal cells are able to directly become malignant without transitioning through a hyperplastic stage. It has been observed, however, that the presence of ADH indicates a higher likelihood of developing a malignancy. This has resulting in treatment of patients with ADH to begin treatment with an antineoplastic/antitumor agent such as tamoxifen. This is in contrast to the treatment of patients with malignant breast cancer which usually includes surgical removal.

[0010] The rational development of preventive, diagnostic and therapeutic strategies for women at risk for breast cancer would be aided by a molecular map of the tumorigenesis process. Relatively little is known of the molecular events that mediate the transition of normal breast cells to the various stages of breast cancer progression. Similarly, little is known of the molecular events that mediate the transition of cells from one stage of breast cancer to another.

[0011] Molecular means of identifying the differences between normal, non-cancerous cells and cancerous cells (in general) have been the focus of intense study. The use of cDNA libraries to analyze differences in gene expression patterns in normal versus tumorigenic cells has been described (U.S. Pat. No. 4,981,783). DeRisi et al. (1996) describe the analysis of gene expression patterns between two cell lines: UACC-903, which is a tumorigenic human melanoma cell line, and UACC-903(+6), which is a chromosome 6 suppressed non-tumorigenic form of UACC-903. Labeled cDNA probes made from mRNA from these cell lines were applied to DNA microarrays containing 870 different cDNAs and controls. Genes that were preferentially expressed in one of the two cell lines were identified.

[0012] Golub et al. (1999) describe the use of gene expression monitoring as means to cancer class discovery and class prediction between acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL). Their approach to class predictors used a neighborhood analysis followed by cross-validation of the validity of the predictors by withholding one sample and building a predictor based only on the remaining samples. This predictor is then used to predict the class of the withheld sample. They also used cluster analysis to identify new classes (or subtypes) within the AML and ALL.

[0013] Gene expression patterns in human breast cancers have been described by Perou et al. (1999), who studied gene expression between cultured human mammary epithelia cells (HMEC) and breast tissue samples by use of microarrays comprising about 5000 genes. They used a clustering algorithm to identify patterns of expression in HMEC and tissue samples. Perou et al. (2000) describe the use of clustered gene expression profiles to classify subtypes of human breast tumors. Hedenfalk et al. describe gene expression profiles in BRCA1 mutation positive, BRCA2 mutation positive, and sporadic tumors. Using gene expression patterns to distinguish breast tumor subclasses and predict clinical implications is described by Sorlie et al. and West et al.

[0014] All of the above described approaches, however, utilize heterogeneous populations of cells found in culture or in a biopsy to obtain information on gene expression patterns. The use of such populations may result in the inclusion or exclusion of multiple genes from the patterns. For this and the lack of statistical robustness reasons, the gene expression patterns observed by the above described approaches provide little confidence that the differences in gene expression may be meaningfully associated with the stages of breast cancer.

[0015] Citation of documents herein is not intended as an admission that any is pertinent prior art. All statements as to the date or representation as to the contents of documents is based on the information available to the applicant and does not constitute any admission as to the correctness of the dates or contents of the documents.

SUMMARY OF THE INVENTION

[0016] The present invention relates to the identification and use of gene expression patterns (or profiles or "signatures") which are correlated with (and thus able to discriminate between) cells in various stages and/or grades of breast cancer. Broadly defined, these stages are non-malignant versus malignant, but may also be viewed as normal versus atypical (optionally including reactive and pre-neoplastic) versus cancerous. Another definition of the stages is normal versus precancerous (e.g. atypical ductal hyperplasia (ADH) or atypical lobular hyperplasia (ALH)) versus cancerous (e.g. carcinoma in situ such as DCIS and/or LCIS) versus invasive (e.g. carcinomas such as IDC and/or ILC). The invention may also be applied to discriminations between normal and non-normal (including cancerous and other non-normal cells).

[0017] The invention also relates to the identification and use of gene expression patterns (or profiles or "signatures") which are correlated with (and thus able to discriminate between) cells in various grades (within one or more stage) of breast cancer. Grading of breast cancer is normally done for cases of invasive ductal carcinoma (IDC), and may be done for invasive lobular carcinoma (ILC) as well, where cytological criteria such as the Nottingham BSR, nuclear morphology, tissue architecture, proliferation index (such as assays for PCNA or Ki67), and extent of differentiation are used to assign a grade of I, II or III to particular breast cancer samples. Grade I is usually where the cells are still well differentiated and are usually positive for the estrogen receptor (ER). Grade III is usually where the cells are poorly differentiated and usually negative for ER. Grade II is generally where the cells have characteristics intermediate between grades I and III and can make up approximately 60% of all samples assayed. This is rather unfortunate because determination of grade in IDC is used directly for decisions on patient care.

[0018] Grading of cases of ductal carcinoma in situ (DCIS) is also possible, but is not routine in current clinical practice. Grading of lobular carcinoma in situ (LCIS) is also possible. In addition to grades I to III, conventional grading schemes may use the terms "low grade" and/or "high grade".

[0019] The present invention provides a non-subjective means for the identification of grades of various stages of cancer by assaying for the expression patterns associated with particular grades. Thus where subjective interpretation is used in grade assessment by pathologists using cytological criteria, the present invention provides objective gene expression patterns, which may optionally be performed in the absence of grading by histomorphological or cytological criteria, that are correlated with grades I-III (or low to high

grade) to provide a more accurate assessment of breast cancer progression. The expression patterns of the invention thus provide a means to determine breast cancer prognosis. Furthermore, the expression patterns can also be used as a means to assay small, node negative tumors that are not readily graded by conventional means.

[0020] The gene expression patterns comprise one or more than one gene capable of discriminating between various stages and/or grades of breast cancer with significant accuracy. The gene(s) are identified as correlated with various stages and/or grades of breast cancer such that the levels of their expression are relevant to a determination of the stage and/or grade of breast cancer of a cell. Thus in one aspect, the invention provides a method to determine the stage and/or grade of breast cancer of a subject afflicted with, or suspected of having, breast cancer by assaying a cell containing sample from said subject for expression of one or more than one gene disclosed herein as correlated with one or more stages and/or grades of breast cancer.

[0021] Gene expression patterns of the invention are identified by analysis of gene expression in multiple samples of each stage and/or grade to be studied. The overall gene expression profile of a sample is obtained through quantifying the expression levels of mRNA corresponding to approximately 12000 genes. This overall profile is then analyzed to identify genes that are positively, or negatively, correlated, with a stage and/or grade of breast cancer. An expression profile of a subset of human genes may then be identified by the methods of the present invention as correlated with a particular stage and/or grade of breast cancer. The use of multiple samples increases the confidence which a gene may be believed to be correlated with a particular stage and/or grade. Without sufficient confidence, it remains unpredictable whether a particular gene is actually correlated with a stage and/or grade of breast cancer and also unpredictable whether a particular gene may be successfully used to identify the stage and/or grade of an unknown breast cancer cell sample.

[0022] A profile of genes that are highly correlated with one stage and/or grade relative to another may be used to assay an sample from a subject afflicted with, or suspected of having, breast cancer to identify the stage and/or grade of breast cancer to which the sample belongs. Such an assay may be used as part of a method to determine the therapeutic treatment for said subject based upon the stage(s) and/or grade(s) of breast cancer identified. The present invention thus also provides for the advantageous ability to determine grade of a stage of breast cancer in combination with stage information to provide more detailed information in diagnosing and treating breast cancer. This has not always been possible in the diagnosis and treatment of breast cancer using previous protocols, where it was often only possible to determine stage with grade being only occasionally determinable.

[0023] The correlated genes may be used singly with significant accuracy or in combination to increase the ability to accurately discriminate between various stages and/or grades of breast cancer. The present invention thus provides means for correlating a molecular expression phenotype with a physiological (cellular) stage or state. This correlation provides a way to molecularly diagnose and/or monitor a cell's status in comparison to different cancerous versus non-cancerous phenotypes as disclosed herein. Additional uses of the correlated gene(s) are in the classification of cells and tissues; determination of diagnosis and/or prognosis; and determination and/or alteration of therapy.

[0024] The ability to discriminate is conferred by the identification of expression of the individual genes as relevant and not by the form of the assay used to determine the actual level of expression. An assay may utilize any identifying feature of an identified individual gene as disclosed herein as long as the assay reflects, quantitatively or qualitatively, expression of the gene. Identifying features include, but are not limited to, unique nucleic acid sequences used to encode (DNA), or express (RNA), said gene or epitopes specific to, or activities of, a protein encoded by said gene. All that is required is the identity of the gene(s) necessary to discriminate between stages and/or grades of breast cancer and an appropriate cell containing sample for use in an expression assay.

[0025] In one aspect, the invention provides for the identification of the gene expression patterns by analyzing global, or near global, gene expression from single cells or homogenous cell populations which have been dissected away from, or otherwise isolated or purified from, contaminating cells beyond that possible by a simple biopsy. Because the expression of numerous genes fluctuate between cells from different patients as well as between cells from the same patient sample, multiple individual gene expression patterns are used as reference data to generate models which in turn permit the identification of individual gene(s) that are most highly correlated with particular breast cancer stages, and/or grades, and/or have the best the ability to discriminate cells of one stage and/or grade from another.

[0026] Use of the present invention has resulted in the identification of two major changes in gene expression, one of which is associated with the transition of normal breast cells to ADH (and persisting in a majority of DCIS and IDC cells), and the second is associated with tumor grade progression. The invention also provides the identification of a subset of genes that differ quantitatively in expression between DCIS and IDC cells.

[0027] In another aspect, the invention provides physical and methodological means for detecting the expression of gene(s) identified by the models generated by individual expression patterns. These means may be directed to assaying one or more aspect of the DNA template(s) underlying the expression of the gene(s), of the RNA used as an intermediate to express the gene(s), or of the proteinaceous product expressed by the gene(s).

[0028] In a further aspect, the gene(s) identified by a model as capable of discriminating between breast cancer stages and/or grades may be used to identify the cellular state of an unknown sample of cell(s) from the breast. Preferably, the sample is isolated via non-invasive means. The expression of said gene(s) in said unknown sample may be determined and compared to the expression of said gene(s) in reference data of gene expression patterns from the various stages and/or grades of breast cancer. Optionally, the comparison to reference samples may be by comparison to the model(s) constructed based on the reference samples.

[0029] One advantage provided by the present invention is that contaminating, non-breast cells (such as infiltrating

lymphocytes or other immune system cells) are not present to possibly affect the genes identified or the subsequent analysis of gene expression to identify the status of suspected breast cancer cells. Such contamination is present where a biopsy is used to generate gene expression profiles.

[0030] While the present invention has been described mainly in the context of human breast cancer, it may be practiced in the context of breast cancer of any animal known to be potentially afflicted by breast cancer. Preferred animals for the application of the present invention are mammals, particularly those important to agricultural applications (such as, but not limited to, cattle, sheep, horses, and other "farm animals") and for human companionship (such as, but not limited to, dogs and cats).

BRIEF DESCRIPTION OF THE FIGURES

[0031] **FIG. 1**. Laser capture microdissection. Phenotypically normal breast epithelium and phenotypically abnormal epithelium from atypical ductal hyperplasia (ADH), ductal carcinoma in situ (DCIS) and invasive ductal carcinoma (IDC) from a single breast specimen (case 79) were captured from hematoxylin and eosin-stained sections (8 m in thickness). Panels A, B and C show the images of pre-capture, post-capture, and the captured epithelial compartments, respectively.

[0032] **FIGS. 2***a* and **2***b*. Expression profiles of breast cancer progression. **2***a*. Data matrix of 1940 genes by breast cancer samples of different pathological stages. Columns represent samples of tissues identified as ADH; grades I, II, or III of DCIS; and grades I, II, or III of IDC. Rows represent genes. Color scale shown at left bottom. Genes are ordered by hierarchical clustering, and samples are ordered by pathological stage and tumor grade. **2***b*. Examples of interesting clusters I, II and III.

[0033] **FIG. 3**. Two-dimensional clustering of 62 samples and 200 genes correlated with tumor grade. Genes (columns) and samples (rows) were clustered independently using a hierarchical clustering algorithm. Red dots indicate ADH samples and green dots indicate grade II samples (DCIS or IDC). Three main clusters (down regulated, Grade III signature, and Grade I signature) are highlighted by color bars. See **FIG. 2A** for color scale.

[0034] **FIG. 4**. Genes with increased expression in IDC relative to DCIS. Two dimensional clustering was applied to 1688 genes and 24 IDC samples and a portion of the data matrix is shown to highlight a cluster of genes with higher expression in IDC than its corresponding DCIS from the same patient. Expression values are expressed as log-ratios of expression in IDC to that in DCIS. Color scheme shown at left bottom.

[0035] **FIG. 5**. Breast cancer progression model Breast cancer initiates within normal epithelium evolving into ADH, which progresses into grade I DCIS. A simultaneous 2-dimensional process drives tumor grade progression from I to II to III and stage progression from DCIS to IDC.

DETAILED DESCRIPTION OF THE SPECIFIC EMBODIMENTS

[0036] Definitions of Terms as Used Herein:

[0037] A gene expression "pattern" or "profile" or "signature" refers to the relative expression of a gene between two or more stages of breast cancer which is correlated with being able to distinguish between said stages.

[0038] A "gene" is a polynucleotide that encodes a discrete product, whether RNA or proteinaceous in nature. It is appreciated that more than one polynucleotide may be capable of encoding a discrete product. The term includes alleles and polymorphisms of a gene that encodes the same product, or a functionally associated (including gain, loss, or modulation of function) analog thereof, based upon chromosomal location and ability to recombine during normal mitosis.

[0039] A "stage" or "stages" (or equivalents thereof) of breast cancer refer to a physiologic state of a breast cell as defined by known cytological or histological (including immunohistology, histochemistry, and immunohistochemistry) procedures and are readily known to skilled in the art. Non-limiting examples include normal versus abnormal, non-cancerous versus cancerous, the different stages described herein (e.g. hyperplastic, carcinoma, and invasive), and grades within different stages (e.g. grades I, II, or III or the equivalents thereof within cancerous stages).

[0040] The terms "correlate" or "correlation" or equivalents thereof refer to an association between expression of one or more genes and a physiologic state of a breast cell to the exclusion of one or more other stages and/or identified by use of the methods as described herein. A gene may be expressed at higher or lower levels and still be correlated with one or more breast cancer stages.

[0041] A "polynucleotide" is a polymeric form of nucleotides of any length, either ribonucleotides or deoxyribonucleotides. This term refers only to the primary structure of the molecule. Thus, this term includes double- and single-stranded DNA and RNA. It also includes known types of modifications including labels known in the art, methylation, "caps", substitution of one or more of the naturally occurring nucleotides with an analog, and internucleotide modifications such as uncharged linkages (e.g., phosphorothioates, phosphorodithioates, etc.), as well as unmodified forms of the polynucleotide.

[0042] The term "amplify" is used in the broad sense to mean creating an amplification product can be made enzymatically with DNA or RNA polymerases. "Amplification," as used herein, generally refers to the process of producing multiple copies of a desired sequence, particularly those of a sample. "Multiple copies" mean at least 2 copies. A "copy" does not necessarily mean perfect sequence complementarity or identity to the template sequence.

[0043] By corresponding is meant that a nucleic acid molecule shares a substantial amount of sequence identity with another nucleic acid molecule. Substantial amount means at least 95%, usually at least 98% and more usually at least 99%, and sequence identity is determined using the BLAST algorithm, as described in Altschul et al. (1990), J. Mol. Biol. 215:403-410 (using the published default setting, i.e. parameters w=4, t=17). Methods for amplifying mRNA are generally known in the art, and include reverse transcription PCR (RT-PCR) and those described in U.S. Patent Application (number to be assigned) entitled "Nucleic Acid Amplification" filed on Oct. 25, 2001 as attorney docket number 485772002900 as well as U.S. Provisional Patent Applications 60/298,847 (filed Jun. 15, 2001) and 60/257,

801 (filed Dec. 22, 2000), all of which are hereby incorporated by reference in their entireties as if fully set forth. Another method which may be used is quantitative PCR (or Q-PCR). Alternatively, RNA may be directly labeled as the corresponding cDNA by methods known in the art.

[0044] A "microarray" is a linear or two-dimensional array of preferably discrete regions, each having a defined area, formed on the surface of a solid support such as, but not limited to, glass, plastic, or synthetic membrane. The density of the discrete regions on a microarray is determined by the total numbers of immobilized polynucleotides to be detected on the surface of a single solid phase support, preferably at least about 50/cm^2, more preferably at least about 100/cm^2, even more preferably at least about 500/cm^2, but preferably below about 1,000/cm^2. Preferably, the arrays contain less than about 500, about 1000, about 1500, about 2000, about 2500, or about 3000 immobilized polynucleotides in total. As used herein, a DNA microarray is an array of oligonucleotides or polynucleotides placed on a chip or other surfaces used to hybridize to amplified or cloned polynucleotides from a sample. Since the position of each particular group of primers in the array is known, the identities of a sample polynucleotides can be determined based on their binding to a particular position in the microarray.

[0045] Because the invention relies upon the identification of genes that are over- or under-expressed, one embodiment of the invention involves determining expression by hybridization of mRNA, or an amplified or cloned version thereof, of a sample cell to a polynucleotide that is unique to a particular gene sequence. Preferred polynucleotides of this type contain at least about 20, at least about 22, at least about 24, at least about 26, at least about 28, at least about 30, or at least about 32 consecutive basepairs of a gene sequence that is not found in other gene sequences. The term "about" as used in the previous sentence refers to an increase or decrease of 1 from the stated numerical value. Even more preferred are polynucleotides of at least or about 50, at least or about 100, at least about or 150, at least or about 200, at least or about 250, at least or about 300, at least or about 350, or at least or about 400 basepairs of a gene sequence that is not found in other gene sequences. The term "about" as used in the preceding sentence refers to an increase or decrease of 10% from the stated numerical value. Such polynucleotides may also be referred to as polynucleotide probes that are capable of hybridizing to sequences of the genes, or unique portions thereof, described herein. Preferably, the sequences are those of mRNA encoded by the genes, the corresponding cDNA to such mRNAs, and/or amplified versions of such sequences. In preferred embodiments of the invention, the polynucleotide probes are immobilized on an array, other devices, or in individual spots that localize the probes.

[0046] Alternatively, and in another embodiment of the invention, gene expression may be determined by analysis of expressed protein in a cell sample of interest by use of one or more antibodies specific for one or more epitopes of individual gene products (proteins) in said cell sample. Such antibodies are preferably labeled to permit their easy detection after binding to the gene product.

[0047] The term "label" refers to a composition capable of producing a detectable signal indicative of the presence of the labeled molecule. Suitable labels include radioisotopes, nucleotide chromophores, enzymes, substrates, fluorescent molecules, chemiluminescent moieties, magnetic particles, bioluminescent moieties, and the like. As such, a label is any composition detectable by spectroscopic, photochemical, biochemical, immunochemical, electrical, optical or chemical means.

[0048] The term "support" refers to conventional supports such as beads, particles, dipsticks, fibers, filters, membranes and silane or silicate supports such as glass slides.

[0049] As used herein, a "breast tissue sample" or "breast cell sample" refers to a sample of breast tissue or fluid isolated from an individual suspected of being afflicted with, or at risk of developing, breast cancer. Such samples are primary isolates (in contrast to cultured cells) and may be collected by any non-invasive means, including, but not limited to, ductal lavage, fine needle aspiration, needle biopsy, the devices and methods described in U.S. Pat. No. 6,328,709, or any other suitable means recognized in the art. Alternatively, the "sample" may be collected by an invasive method, including, but not limited to, surgical biopsy.

[0050] "Expression" and "gene expression" include transcription and/or translation of nucleic acid material.

[0051] As used herein, the term "comprising" and its cognates are used in their inclusive sense; that is, equivalent to the term "including" and its corresponding cognates.

[0052] Conditions that "allow" an event to occur or conditions that are "suitable" for an event to occur, such as hybridization, strand extension, and the like, or "suitable" conditions are conditions that do not prevent such events from occurring. Thus, these conditions permit, enhance, facilitate, and/or are conducive to the event. Such conditions, known in the art and described herein, depend upon, for example, the nature of the nucleotide sequence, temperature, and buffer conditions. These conditions also depend on what event is desired, such as hybridization, cleavage, strand extension or transcription.

[0053] Sequence "mutation," as used herein, refers to any sequence alteration in the sequence of a gene disclosed herein interest in comparison to a reference sequence. A sequence mutation includes single nucleotide changes, or alterations of more than one nucleotide in a sequence, due to mechanisms such as substitution, deletion or insertion. Single nucleotide polymorphism (SNP) is also a sequence mutation as used herein. Because the present invention is based on the relative level of gene expression, mutations in non-coding regions of genes as disclosed herein may also be assayed in the practice of the invention.

[0054] "Detection" includes any means of detecting, including direct and indirect detection of gene expression and changes therein. For example, "detectably less" products may be observed directly or indirectly, and the term indicates any reduction (including the absence of detectable signal). Similarly, "detectably more" product means any increase, whether observed directly or indirectly.

[0055] Unless defined otherwise all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this invention belongs.

[0056] Specific Embodiments

[0057] The present invention relates to the identification and use of gene expression patterns (or profiles or "signa-

tures") which discriminate between (or are correlated with) cells in various stages and/or grades of breast cancer. Such patterns may be determined by the methods of the invention by use of a number of reference cell or tissue samples, such as those reviewed by a pathologist of ordinary skill in the pathology of breast cancer, which reflect various stages and/or grades of breast cancer. Because the overall gene expression profile differs from person to person, cancer to cancer, and cancer cell to cancer cell, correlations between certain cell states and genes expressed or underexpressed may be made as disclosed herein to identify genes that are capable of discriminating between different breast cancer states.

[0058] The present invention may be practiced with any number of genes believed, or likely to be, differentially expressed in breast cancer cells. Approximately 12,000 genes were used to identify hundreds of genes capable of discriminating between various stages and/or grades of breast cancer as shown in the following Examples. The identification may be made by using expression profiles of various homogenous normal and breast cancer cell populations, which were isolated by microdissection, such as, but not limited to, laser capture microdissection (LCM) of 100-1000 cells. Each gene of the expression profile may be assigned weights based on its ability to discriminate between two or more stages and/or grades of breast cancer. The magnitude of each assigned weight indicates the extent of difference in expression between the two groups and is an approximation of the ability of expression of the gene to discriminate between the two groups (and thus stages and/or grades). The magnitude of each assigned weight also approximates the extent of correlation between expression of individual gene(s) and particular breast cancer stages and/or grades.

[0059] It should be noted that merely high levels of expression in cells from a particular stage or grade does not necessarily mean that a gene will be identified as having a high absolute weight value.

[0060] Genes with top ranking weights (in absolute terms) may be used to generate models of gene expressions that would maximally discriminate between the two groups. Alternatively, genes with top ranking weights (in absolute terms) may be used in combination with genes with lower weights without significant loss of ability to discriminate between groups. Such models may be generated by any appropriate means recognized in the art, including, but not limited to, cluster analysis, supported vector machines, neural networks or other algorithm known in the art. The models are capable of predicting the classification of a unknown sample based upon the expression of the genes used for discrimination in the models. "Leave one out" cross-validation may be used to test the performance of various models and to help identify weights (genes) that are uninformative or detrimental to the predictive ability of the models. Cross-validation may also be used to identify genes that enhance the predictive ability of the models.

[0061] The gene(s) identified as correlated with particular breast cancer stages and/or grades by the above models provide the ability to focus gene expression analysis to only those genes that contribute to the ability to identify a cell as being in a particular stage and/or grade of breast cancer relative to another stage or grade. The expression of other genes in a breast cancer cell would be relatively unable to provide information concerning, and thus assist in the discrimination of, different stages of breast cancer. For example, the cysteine-rich protein 1 (intestinal), identified by I.M.A.G.E. Consortium CloneID 1323448 ("The I.M.A.G.E. Consortium: An Integrated Molecular Analysis of Genomes and their Expression," Lennon et al., 1996, Genomics 33:151-152; see also image.llnl.gov) has been found to be useful in discriminations between normal and ADH cells (with persistence through DCIS and IDC). Thus expression of this gene would be utilized in models to discriminate between the above listed stages but not for discerning between other stages. This type of analysis is readily incorporated into algorithms used to generate models with reference gene expression data.

[0062] As will be appreciated by those skilled in the art, the models are highly useful with even a small set of reference gene expression data and can become increasingly accurate with the inclusion of more reference data although the incremental increase in accuracy will likely diminish with each additional datum. The preparation of additional reference gene expression data using genes identified and disclosed herein for discriminating between different stages and/or grades of breast cancer is routine and may be readily performed by the skilled artisan to permit the generation of models as described above to predict the status of an unknown sample based upon the expression levels of those genes.

[0063] To determine the (increased or decreased) expression levels of genes in the practice of the present invention, any method known in the art may be utilized. In one preferred embodiment of the invention, expression based on detection of RNA which hybridizes to the genes identified and disclosed herein is used. This is readily performed by any RNA detection or amplification+detection method known or recognized as equivalent in the art such as, but not limited to, reverse transcription-PCR, the methods disclosed in U.S. Patent Application (number to be assigned) entitled "Nucleic Acid Amplification" filed on Oct. 25, 2001 as attorney docket number 485772002900 as well as U.S. Provisional Patent Applications 60/298,847 (filed Jun. 15, 2001) and 60/257,801 (filed Dec. 22, 2000), and methods to detect the presence, or absence, of RNA stabilizing or destabilizing sequences.

[0064] Alternatively, expression based on detection of DNA status may be used. Detection of the DNA of an identified gene as methylated or deleted may be used for genes that have decreased expression in correlation with a particular breast cancer stage and/or grade. This may be readily performed by PCR based methods known in the art, including, but not limited to, Q-PCR. Conversely, detection of the DNA of an identified gene as amplified may be used for genes that have increased expression in correlation with a particular breast cancer stage and/or grade. This may be readily performed by PCR based, fluorescent in situ hybridization (FISH) and chromosome in situ hybridization (CISH) methods known in the art.

[0065] Expression based on detection of a presence, increase, or decrease in protein levels or activity may also be used. Detection may be performed by any immunohistochemistry (IHC) based, blood based (especially for secreted proteins), antibody (including autoantibodies

against the protein) based, ex foliate cell (from the cancer) based, mass spectroscopy based, and image (including used of labeled ligand) based method known in the art and recognized as appropriate for the detection of the protein. Antibody and image based methods are additionally useful for the localization of tumors after determination of cancer by use of cells obtained by a non-invasive procedure (such as ductal lavage or fine needle aspiration), where the source of the cancerous cells is not known. A labeled antibody or ligand may be used to localize the carcinoma(s) within a patient.

[0066] A preferred embodiment using a nucleic acid based assay to determine expression is by immobilization of one or more of the genes identified herein on a solid support, including, but not limited to, a solid substrate as an array or to beads or bead based technology as known in the art. Alternatively, solution based expression assays known in the art may also be used. The immobilized gene(s) may be in the form of polynucleotides that are unique or otherwise specific to the gene(s) such that the polynucleotide would be capable of hybridizing to a DNA or RNA corresponding to the gene(s). These polynucleotides may be the full length of the gene(s) or be short sequences of the genes (up to one nucleotide shorter than the full length sequence known in the art by deletion from the 5' or 3' end of the sequence) that are optionally minimally interrupted (such as by mismatches or inserted non-complementary basepairs) such that hybridization with a DNA or RNA corresponding to the gene(s) is not affected.

[0067] The immobilized gene(s) may be used to determine the state of nucleic acid samples prepared from sample breast cell(s) for which the pre-cancer or cancer status is not known or for confirmation of a status that is already assigned to the sample breast cell(s). Without limiting the invention, such a cell may be from a patient suspected of being afflicted with, or at risk of developing, breast cancer. The immobilized polynucleotide(s) need only be sufficient to specifically hybridize to the corresponding nucleic acid molecules derived from the sample. While even a single correlated gene sequence may to able to provide adequate accuracy in discriminating between two breast cancer cell stages and/or grades, two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, ten or more, or eleven or more of the genes identified herein may be used as a subset capable of discriminating may be used in combination to increase the accuracy of the method. The invention specifically contemplates the selection of more than one, two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, ten or more, or eleven or more of the genes disclosed in the tables and figures herein for use as a subset in the identification of whether an unknown or suspicious breast cancer sample is normal or is in one or more stages and/or grades of breast cancer. Optionally, the genes used will not include CloneID 809507, which is also known as GenBank accession number AA454563, described as an EST with high similarity to CD63 but of unknown function.

[0068] In embodiments where only one or a few genes are to be analyzed, the nucleic acid derived from the sample breast cancer cell(s) may be preferentially amplified by use of appropriate primers such that only the genes to be analyzed are amplified to reduce contaminating background signals from other genes expressed in the breast cell. Alternatively, and where multiple genes are to be analyzed or where very few cells (or one cell) is used, the nucleic acid from the sample may be globally amplified before hybridization to the immobilized polynucleotides. Of course RNA, or the cDNA counterpart thereof may be directly labeled and used, without amplification, by methods known in the art.

[0069] The above assay embodiments may be used in a number of different ways to identify or detect the breast cancer stage and/or grade, if any, of a breast cancer cell sample from a patient. In many cases, this would reflect a secondary screen for the patient, who may have already undergone mammography or physical exam as a primary screen. If positive, the subsequent needle biopsy, ductal lavage, fine needle aspiration, or other analogous methods may provide the sample for use in the above assay embodiments. The present invention is particularly useful in combination with non-invasive protocols, such as ductal lavage or fine needle aspiration, to prepare a breast cell sample. The current analysis of ductal lavage samples is by cytological examination by a trained pathologist who classifies the samples in terms that are at least partly subjective: unsatisfactory (too few cells), benign (including fibrocystic change), atypical (or mild atypia), suspicious (or marked atypia), or malignant.

[0070] The present invention provides a more objective set of criteria, in the form of gene expression profiles of a discrete set of genes, to discriminate (or delineate) between meaningful stages and/or grades (or classes) of breast cancer cells. In particularly preferred embodiments of the invention, the assays are used to discriminate between the three grades (I, II, III) of carcinomas in situ as well as invasive carcinomas. With the use of alternative algorithms, such as neural networks, comparisons that discriminate between multiple (more than pairwise) classes may also be performed.

[0071] In one embodiment of the invention, the isolation and analysis of a breast cancer cell sample may be performed as follows:

[0072] (1) Ductal lavage or other non-invasive procedure is performed on a patient to obtain a sample.

[0073] (2) Sample is prepared and coated onto a microscope slide. Note that ductal lavage results in clusters of cells that are cytologically examined as stated above.

[0074] (3) Pathologist or image analysis software scans the sample for the presence of non-normal and/or atypical cells.

[0075] (4) If non-normal and/or atypical cells are observed, those cells are harvested (e.g. by microdissection such as LCM).

[0076] (5) RNA is extracted from the harvested cells.

[0077] (6) RNA is purified, amplified, and labeled.

[0078] (7) Labeled nucleic acid is contacted with a microarray containing polynucleotides of the genes identified herein as correlated to discriminations between two or more stages of breast cancer under hybridization conditions, then processed and scanned to obtain a pattern of intensities of each spot

(relative to a control for general gene expression in cells) which determine the level of expression of the gene(s) in the cells.

[0079] (8) The pattern of intensities is analyzed by comparison to the expression patterns of the genes in known samples of normal and breast cancer cells (relative to the same control).

[0080] A specific example of the above method would be performing ductal lavage following a primary screen, observing and collecting non-normal and/or atypical cells for analysis. The comparison to known expression patterns, such as that made possible by a model generated by an algorithm (such as, but not limited to nearest neighbor type analysis, SVM, or neural networks) with reference gene expression data for the different breast cancer stages and/or grades, identifies the cells as being most likely grade III IDC.

[0081] Alternatively, the sample may permit the collection of both normal as well as non-normal and/or atypical cells for analysis. The gene expression patterns for each of these two samples will be compared to each other as well as the model and the normal versus individual abnormal comparisons therein based upon the reference data set. This approach can be significantly more powerful that the non-normal and/or atypical cells only approach because it utilizes significantly more information from the normal cells and the differences between normal and non-normal/atypical cells (in both the sample and reference data sets) to determine the status of the non-normal and/or atypical cells from the sample.

[0082] By appropriate selection of the genes used in the analysis, identification of the relative amounts of non-normal and/or atypical cells may also be possible, although in most clinical settings, the identification of the highest grade of breast cancer with confidence makes identification of lower grades less important. Stated differently, the identification of invasive cancer determines the clinical situation regardless of the presence of carcinoma in situ or hyperplastic cells, or the identification of carcinoma in situ makes determines the clinical situation regardless of the presence of hyperplastic cells. Similarly, the identification of a higher grade of cancer cells determines the clinical situation regardless of the presence of lower grades of cancer cells.

[0083] With use of the present invention, skilled physicians may prescribe treatments based on non-invasive samples that they would have prescribed for a patient which had previously received a diagnosis via a solid tissue biopsy.

[0084] The above discussion is also applicable where a palpable lesion is detected followed by fine needle aspiration or needle biopsy of cells from the breast. The cells are plated and reviewed by a pathologist or automated imaging system which selects cells for analysis as described above. This again provides a means of linking visual to molecular cytology and provides a less subjective means of identifying the physiological state of breast cancer cells without the need for invasive solid tissue biopsies.

[0085] The present invention may also be used, however, with solid tissue biopsies. For example, a solid biopsy may be collected and prepared for visualization followed by determination of expression of one or more genes identified herein to determine the stage of breast cancer, if any. One preferred means is by use of in situ hybridization with polynucleotide or protein identifying probe(s) for assaying expression of said gene(s).

[0086] In an alternative method, the solid tissue biopsy may be used to extract molecules followed by analysis for expression of one or more gene(s). This provides the possibility of leaving out the need for visualization and collection of only those cells suspected of being non-normal and/or atypical. This method may of course be modified such that only cells suspected of being non-normal and/or atypical are collected and used to extract molecules for analysis. This would require visualization and selection as an prerequisite to gene expression analysis.

[0087] In a further modification of the above, both normal cells and cells suspected of being non-normal and/or atypical are collected and used to extract molecules for analysis of gene expression. The approach, benefits and results are as described above using non-invasive sampling.

[0088] In a further alternative to all of the above, the gene(s) identified herein may be used as part of a simple PCR or array based assay simply to determine the presence of non-normal and/or atypical cells in a sample from a non-invasive sampling procedure. This is simple to perform and utilizes genes identified to be the best discriminators of normal versus abnormal cells without the need for any cytological examination. If no non-normal and/or atypical cells are identified, no cytological examination is necessary. If non-normal and/or atypical cells are identified, cytological examination follows, and a more comprehensive analysis, as described above, may follow.

[0089] The genes identified herein may be used to generate a model capable of predicting the breast cancer stage and/or grade (if any) of an unknown breast cell sample based on the expression of the identified genes in the sample. Such a model may be generated by any of the algorithms described herein or otherwise known in the art as well as those recognized as equivalent in the art using gene(s) (and subsets thereof) disclosed herein for the identification of whether an unknown or suspicious breast cancer sample is normal or is in one or more stages and/or grades of breast cancer. The model provides a means for comparing expression profiles of gene(s) of the subset from the sample against the profiles of reference data used to build the model. The model can compare the sample profile against each of the reference profiles or against model defining delineations made based upon the reference profiles. Additionally, relative values from the sample profile may be used in comparison with the model or reference profiles.

[0090] In a preferred embodiment of the invention, breast cell samples identified as normal and non-normal and/or atypical from the same subject may be analyzed for their expression profiles of the genes used to generate the model. This provides an advantageous means of identifying the stage of the abnormal sample based on relative differences from the expression profile of the normal sample. These differences can then be used in comparison to differences between normal and individual abnormal reference data which was also used to generate the model.

[0091] The detection of gene expression from the samples may be by use of a single microarray able to assay gene expression from all pairwise comparisons disclosed herein for convenience and accuracy.

[0092] Other uses of the present invention include providing the ability to identify breast cancer cell samples as being those of a particular stage and/or grade of cancer for further research or study. This provides a particular advantage in many contexts requiring the identification of breast cancer stage and/or grade based on objective genetic or molecular criteria rather than cytological observation. It is of particular utility to distinguish different grades of a particular breast cancer stage for further study, research or characterization because no objective criteria for such delineation was previously available.

[0093] The materials for use in the methods of the present invention are ideally suited for preparation of kits produced in accordance with well known procedures. The invention thus provides kits comprising agents for the detection of expression of the disclosed genes for identifying breast cancer stage. Such kits optionally comprising the agent with an identifying description or label or instructions relating to their use in the methods of the present invention, is provided. Such a kit may comprise containers, each with one or more of the various reagents (typically in concentrated form) utilized in the methods, including, for example, pre-fabricated microarrays, buffers, the appropriate nucleotide triphosphates (e.g., dATP, dCTP, dGTP and dTTP; or rATP, rCTP, rGTP and UTP), reverse transcriptase, DNA polymerase, RNA polymerase, and one or more primer complexes of the present invention (e.g., appropriate length poly(T) or random primers linked to a promoter reactive with the RNA polymerase). A set of instructions will also typically be included.

[0094] The methods provided by the present invention may also be automated in whole or in part. All aspects of the present invention may also be practiced such that they consist essentially of a subset of the disclosed genes to the exclusion of material irrelevant to the identification of breast cancer stages in a cell containing sample.

[0095] Gene Expression Profiles of Pathological Stage and Histological Grade Progression of Human Breast Cancer

[0096] To identify gene expression changes that occur during breast cancer progression, isolation via LCM phenotypically of abnormal epithelium from ADH, DCIS and IDC and phenotypically normal epithelium (henceforth referred to as normal) from 36 breast cancer patients and 3 healthy mammoplasty reduction patients (**FIG. 1A** and Table 1) was performed. The resulting 300 independently microdissected samples were used to interrogate a microarray containing approximately 12,000 human genes. Genes showing significant differences in the pair-wise comparisons of normal vs. ADH, normal vs. DCIS and normal vs. IDC were selected by linear discriminant analysis, resulting in a total of 1940 unique genes for further exploration.

TABLE 1

Patient and tumor characteristics of clinical samples in this study

Case ID	Stages Microdissected	Age	ER	PR	HER2	Node[a]
8	DCIS (III), IDC (III)	48	Pos	Pos	Pos	Pos
14	N, DCIS (I), IDC (I)	44	Pos	Pos	ND	Pos
22	ADH, DCIS (I)	44	ND	ND	ND	Pos
25	DCIS (I), IDC (II)	81	Pos	Neg	ND	ND

TABLE 1-continued

Patient and tumor characteristics of clinical samples in this study

Case ID	Stages Microdissected	Age	ER	PR	HER2	Node[a]
30	N, DCIS (III), IDC (III)	47	Neg	Neg	Neg	Pos
41	N, DCIS (II), IDC (II)	55	Pos	Pos	ND	Neg
43	N, DCIS (II), IDC (II)	53	Pos	Neg	Neg	Pos
44	N, DCIS (III), IDC (III)	28	Pos	Pos	Neg	Neg
45	N, DCIS (I)	36	Pos	Neg	Neg	Neg
[b]57	N, ADH, DCIS (I)	34	ND	ND	ND	Neg
65	N, DCIS (III), IDC (III)	39	Pos	Pos	Neg	Neg
78	MPR	46				
79	N, ADH, DCIS (I), IDC (I)	54	Pos	Pos	Neg	Pos
88	N, DCIS (III), IDC (III)	35	Pos	Pos	ND	Pos
95	MPR	16				
96	N, DCIS (III), IDC (III)	31	Neg	Neg	Neg	Pos
97	DCIS (III), IDC (III)	79	Neg	Neg	Pos	Pos
102	N, DCIS (I), IDC (I)	55	Pos	Neg	Neg	Pos
112	N, DCIS (III), IDC (III)	31	Neg	Pos	Neg	Pos
121	N, DCIS (II), IDC (II)	45	Pos	Pos	Pos	Pos
130	N, DCIS (II), IDC (II)	54	Pos	Pos	Neg	Pos
131	N, ADH, DCIS (II), IDC (II)	37	Pos	Pos	Pos	Pos
133	N, DCIS (III), IDC (III)	44	Neg	Neg	Pos	Pos
148	N, DCIS (II), IDC (II)	42	Pos	Pos	Neg	Pos
[b]152	N, DCIS (III)	55	ND	ND	ND	Neg
153	N, IDC (I)	46	Pos	Pos	Pos	Pos
169	N, DCIS (II), IDC (II)	34	Pos	Pos	Neg	Pos
170	N, DCIS (II), IDC (II)	44	Pos	Pos	Pos-FISH	Pos
173	N, DCIS (I), IDC (I)	52	Pos	Pos	Neg	Neg
178	N, DCIS (III), IDC (III)	43	Pos	Pos	Pos	Pos
179	N, DCIS (III), IDC (III)	37	Neg	Neg	Pos-FISH	Pos
180	N, ADH, DCIS (I), IDC (I)	46	Pos	Pos	Neg	Pos
183	N, DCIS (II)	46	ND	ND	ND	Pos
191	N, ADH, DCIS (II)	43	ND	ND	ND	
193	N, ADH, DCIS (I), IDC (I)	45	Pos	Pos	Neg	Pos
198	N, DCIS (II), IDC (II)	30	Pos	Pos	Neg	Neg
[b]210	N, ADH, DCIS (I)	62	ND	ND	ND	Neg
[b]213	N, ADH	45	ND	ND	ND	Neg
215	MPR	30				

[a]Nodal status. Tumor grades indicated by roman numerals in parenthesis after the pathological stage of the specimen. Abbreviations used for pathological stages: N, normal; ADH, atypical ductal hyperplasia; DCIS, ductal carcinoma in situ; IDC, invasive ductal carcinoma; MPR, mammoplasty reduction. Abbreviations used for tumor marker status: ND, not determined; Pos, positive; Neg, negative; Pos-Fish, HER2-positivity by fluorescent in situ hybridization (FISH).
[b]Individuals with pre-invasive breast cancer only.

[0097] One important advantage of LCM is the ability to procure both normal and diseased cell populations from the same biopsy. Therefore, the expression level of each gene in a disease state (ADH or DCIS or IDC) is represented as the ratio to the patient-matched normal, which highlights differences due to disease state as opposed to the genetic background of a particular patient. Unsupervised hierarchical clustering of the 1940 genes based on the resulting data across all samples reveals two main clusters (See **FIG. 2*a***). One cluster demonstrates increased expression in a majority of the diseased samples, and another cluster shows a relatively uniform decrease in expression across all samples. Importantly, most of these alterations (both increases and decreases) occur in the earliest stage of progression (ADH) and persist throughout later stages of DCIS and IDC. In addition, closer examination of this global view suggests

that some of these genes increase their expression in DCIS/IDC of higher tumor grade. See Example II below.

[0098] Three example clusters of genes, further illustrate these points (**FIG. 2*b***). Cluster I consists of genes whose expression levels increase in ADH and persist in a majority of DCIS and IDC samples. The gene CRIP1 is especially prominent and thus may be a potential biomarker for the detection of breast cancer including the pre-malignant stage of ADH. The genes of Cluster I along with their I.M.A.G.E. Consortium CloneID number and descriptive identifiers are listed in Table 2.

TABLE 2

IMAGE CloneID	Description
729975	MGEA5 | meningioma expressed antigen 5 (hyaluronidase)
241043	Human clone 137308 mRNA, partial cds
1556859	ESTs, Weakly similar to I38022 hypothetical protein [*H. sapiens*]
1911343	RAB26 | RAB26, member RAS oncogene family
589232	FLJ11506 | hypothetical protein FLJ11506
138189	WFS1 | Wolfram syndrome 1 (wolframin)
1323448	CRIP1 | cysteine-rich protein 1 (intestinal)
488202	*Homo sapiens* cDNA FLJ31235 fis, clone KIDNE2004681, moderately similar to *Mus musculus* peroxisomal long chain acyl-CoA thioesterase Ib (Pte1b) gene
256619	HSD17B7 | hydroxysteroid (17-beta) dehydrogenase 7
810063	GFER | growth factor, erv1 (*S. cerevisiae*)-like (augmenter of liver regeneration)
824879	MGC11275 | hypothetical protein MGC11275

[0099] Genes in cluster II display an expression pattern that correlate with tumor grade with the highest expression in grade III DCIS/IDC. Cluster II includes several genes important in the cell cycle (CENPA, HEC, UBE2C and PLK), and their elevated expression in grade III DCIS/IDC may reflect the higher proliferative index of high-grade tumors. The genes of Cluster II along with their I.M.A.G.E. Consortium CloneID number and descriptive identifiers are listed in Table 3.

TABLE 3

IMAGE CloneID	Description
66406	ESTs, Highly similar to T47163 hypothetical protein DKFZp762E1312.1 [*H. sapiens*]
1517595	KIAA0175 | likely ortholog of maternal embryonic leucine zipper kinase
2017415	CENPA | centromere protein A (17 kD)
345787	HEC | highly expressed in cancer, rich in leucine heptad repeats
504308	FLJ10540 | hypothetical protein FLJ10540
769921	UBE2C | ubiquitin-conjugating enzyme E2C
128711	ANLN | anillin (Drosophila Scraps homolog), actin binding protein
744047	PLK | polo (Drosophia)-like kinase
128695	*Homo sapiens*, Similar to RIKEN cDNA 1810054O13 gene, clone IMAGE: 3845933, mRNA, partial cds

[0100] Genes in cluster III demonstrate decreased expression in all three pathological stages. The epithelium-specific transcription factor ELF5 is noteworthy since it maps to chromosome 11 p13-15, a region subject to frequent loss of heterozygosity and rearrangement in multiple carcinoma including breast cancer (Zhou, J. et al. (1998a)). Therefore, loss of expression of ELF5 in ADH may be an important first step in the initiation of breast malignancy. Taken together, these results demonstrate that the normal to ADH transition is associated with extensive gene expression alterations and support the notion that ADH is a direct precursor to DCIS and IDC. The genes of Cluster III along with their I.M.A.G.E. Consortium CloneID number and descriptive identifiers are listed in Table 4.

TABLE 4

IMAGE CloneID	Description
768007	CL683 | weakly similar to glutathione peroxidase 2
877621	| nGAP-like protein
1570670	IL22RA2 | class II cytokine receptor

TABLE 4-continued

IMAGE CloneID	Description
1881774	KIAA1678 | KIAA1678
1686766	| Rag D protein
505864	RGL | RalGDS-like gene
1569187	HS3ST4 | heparan sulfate (glucosamine) 3-O-

TABLE 4-continued

IMAGE CloneID	Description
	sulfotransferase 4
755881	AQP5 | aquaporin 5
1864302	ELF5 | E74-like factor 5 (ets domain transcription factor)

[0101] To gain further insight into the observation that different histological grades may be associated with distinct gene expression signatures (**FIG. 2***b*, cluster II), two sets of genes were identified. Each comprised 100 genes correlating with grade I and grade III samples respectively using discriminant analysis. Again, to cancel out potential differences in the absolute levels of expression among individuals, gene expression values were expressed as ratios of ADH, DCIS or IDC to the corresponding normal. Unsupervised two-dimensional clustering revealed three major gene clusters (**FIG. 3**). One cluster of genes demonstrated decreased expression in all samples with subtle quantitative differences between grade I and grade III (green bar). A second cluster of genes (denoted as the grade III signature) shows markedly increased expression in grade III samples (red bar), whereas a third cluster (grade I signature) demonstrates increased expression primarily in grade I samples (blue bar). The genes of "green bar" genes along with their I.M.A.G.E. Consortium CloneID number, chromosomal location and descriptive identifiers (if known) are listed in Table 5.

TABLE 5

Clone ID	location	Description
471196	2q37	ITM3 | integral membrane protein 3
796904	6q24–q25	PLAGL1 | pleiomorphic adenoma gene-like 1
32493	2q31.1	ITGA6 | "integrin, alpha 6"
1534700	11q21	KIAA0830 | KIAA0830 protein
712139	2q37.2	ARL7 | ADP-ribosylation factor-like 7
291478	1p36	RUNX3 | runt-related transcription factor 3
150897	19p13.1	B3GNT3 | "UDP-GlcNAc: betaGal beta-1,3-N-acetylglucosaminyltransferase 3"
1653105	3p14–p12	TSP50 | testes-specific protease 50
665384	16	KIAA1609 | KIAA1609 protein
842818	16q23–q24	KARS | lysyl-tRNA synthetase
37671	18q11.2	FLJ21610 | hypothetical protein FLJ21610
773301	16q22.1	CDH3 | "cadherin 3, type 1, P-cadherin (placental)"
503671	6	*Homo sapiens* cDNA FLJ14368 fis, clone HEMBA1001122
3172883	11	ESTs, Weakly similar to S24195 dopamine receptor D4 [*H. sapiens*]
684890	16p12.1	FLJ20274 | hypothetical protein FLJ20274
593840	17q11.2	DKFZP564K1964 | DKFZP564K1964 protein
121454	17p13.1	ALOX12 | arachidonate 12-lipoxygenase
197913	1p34.2	SFPQ | splicing factor proline/glutamine rich (polypyrimidine tract-binding protein-associated)
43090	20q13.12	H-L(3)MBT | lethal (3) malignant brain tumor l(3)mbt protein (Drosophila) homolog
814826	2	ESTs
1635062	12q13.13	DKFZP586A011 | DKFZP586A011 protein
814815		
1601845	7q22–q31.1	CAPRI | Ca2+-promoted Ras inactivator
190059	19p13.3	GNG7 | "guanine nucleotide binding protein (G protein), gamma 7"
277044	19q13.32	KIAA1183 | KIAA1183 protein
1592530	3p21.31	IP6K2 | mammalian inositol hexakisphosphate kinase 2
431231	11q13	EFEMP2 | EGF-containing fibulin-like extracellular matrix protein 2
267254	17	ESTs, Highly similar to LOX2_HUMAN ARACHIDONATE 12-LIPOXYGENASE [*H. sapiens*]
43679	10	ESTs
295572	12q24.21	KIAA0682 | KIAA0682 gene product
46129	12q13.1	HDAC7A | histone deacetylase 7A
1569077	6	EST
138242	1	ESTs, Moderately similar to MAS2_human mannan-binding lectin serine protease 2 precursor [*H. sapiens*]
417637	4p16	KIAA1276 | KIAA1276 protein
248631	3p21.2–p21.1	AMT | aminomethyltransferase (glycine cleavage system protein T)
1553530	2	KIAA0788 | KIAA0788 protein
307029		
1883169	5p15.32	FLJ20303 | hypothetical protein FLJ20303
345764	3p23	SATB1 | special AT-rich sequence binding protein 1 (binds to nuclear matrix/scaffold-associating DNA's)
703964	11q23	INPPL1 | inositol polyphosphate phosphatase-like 1
70349	Xq13.1	MLLT7 | "myeloid/lymphoid or mixed-lineage leukemia (trithorax (Drosophila) homolog); translocated to, 7"
1868349	15q11.2–q21.3	PLA2G4B | "phospholipase A2, group IVB (cytosolic)"
126466	1p34.1	KIAA0467 | KIAA0467 protein
1631682	1p32	PPIE | peptidylprolyl isomerase E (cyclophilin E)
172783	19	ZNF358 | zinc finger protein 358
1566877	11q13	C11orf2 | chromosome 11 open reading frame2
1630990	3p21.3–p21.2	RPL29 | ribosomal protein L29
283124	19	*Homo sapiens*, clone IMAGE: 3917549, mRNA, partial cds
126415	10	*Homo sapiens* mRNA; cDNA DKFZp566H0124 (from clone DKFZp566H0124)
344168	10q23	POLL | "polymerase (DNA directed), lambda"
823634	10	ESTs
325583		EST
810741	17p13.2	GABARAP | GABA(A) receptor-associated protein
511831	3	MGC12936 | hypothetical protein MGC12936

TABLE 5-continued

Clone ID	location	Description
180561	1p13.3	GSTM1 | glutathione S-transferase M1
206217	11p11.2	NR1H3 | "nuclear receptor subfamily 1, group H, member 3"
108667	22q12.2	SF3A1 | "splicing factor 3a, subunit 1, 120 kD"
839796	12p13.31	LOC51147 | candidate tumor suppressor p33 ING1 homolog
502518	3p21	LAMB2 | "laminin, beta 2 (laminin S)"
810981	22q13	FLJ20699 | hypothetical protein FLJ20699
1635059	9	*Homo sapiens*, clone MGC: 16638 IMAGE: 4121964, mRNA, complete cds
767176	17p13.1	TNFSF13 | "tumor necrosis factor (ligand) superfamily, member 13"
810358	17p13-p11	ACADVL | "acyl-Coenzyme A dehydrogenase, very long chain"
2757710	10p11.2	ZNF37A | zinc finger protein 37a (KOX 21)
1652259	7q31.3	LKR/SDH | lysine-ketoglutarate reductase/saccharopine dehydrogenase

[**0102**] The genes of "red bar" genes along with their I.M.A.G.E. Consortium CloneID number, chromosomal location and descriptive identifiers (if known) are listed in Table 6.

TABLE 6

IMAGE Clone ID	Chromosomal location	Description
293727	22q13.2	MGC861 | hypothetical protein MGC861
843121	6p22.1–p21.2	CLIC1 | chloride intracellular channel 1
839682	12q22	UBE2N | ubiquitin-conjugating enzyme E2N (homologous to yeast UBC13)
815501	19p13.3	MGC2721 | hypothetical protein MGC2721
1587847	2q21	MCM6 | "minichromosome maintenance deficient (mis5, *S. pombe*) 6"
1416055	8	KIAA0165 | "extra spindle poles, *S. cerevisiae*, homolog of"
2018131	12p13.2–p13.1	RACGAP1 | Rac GTPase activating protein 1
1476053	15q15.1	RAD51 | RAD51 (*S. cerevisiae*) homolog (*E coli* RecA homolog)
869375	15q26.1	IDH2 | "isocitrate dehydrogenase 2 (NADP+), mitochondrial"
951241	15q13.3	ANKT | nucleolar protein ANKT
743810	12p13	MGC2577 | hypothetical protein MGC2577
292936	1p34.3	FLJ10468 | hypothetical protein FLJ10468
66406	2	ESTs, Highly similar to T47163 hypothetical protein DKFZp762E1312.1 [*H. sapiens*]
1517595	9p11.2	KIAA0175 | likely ortholog of maternal embryonic leucine zipper kinase
2017415	2p24–p21	CENPA | centromere protein A (17 kD)
345787	18p11.31	HEC | "highly expressed in cancer, rich in leucine heptad repeats"
504308	10cen-q26.11	FLJ10540 | hypothetical protein FLJ10540
769921	20q13.12	UBE2C | ubiquitin-conjugating enzyme E2C
128711	7p15–p14	ANLN | "anillin (Drosophila Scraps homolog), actin binding protein"
744047	16p12.3	PLK | polo (Drosophia)-like kinase
564981	18	*Homo sapiens*, Similar to RIKEN cDNA 2810433K01 gene, clone MGC: 10200 IMAGE: 3909951, mRNA, complete cds
259950	8q23	CML66 | chronic myelogenous leukemia tumor antigen 66
825606	10q24.1	KNSL1 | kinesin-like 1
814270	4q27	PMSCL1 | polymyositis/scleroderma autoantigen 1 (75 kD)
785368	8p21–p12	TOPK | PDZ-binding kinase; T-cell originated protein kinase
209066	20q13.2–q13.3	STK15 | serine/threonine kinase 15
739450	1q21.2	LASS2 | "longevity assurance(LAG1, *S. cerevisiae*) homolog 2"
1702742	16q24.3	SLC7A5 | "solute carrier family 7 (cationic amino acid transporter, y+ system), member 5"
1631634	9q34.11	MGC3038 | "hypothetical protein similar to actin related protein 2/3 complex, subunit 5"
725454	9q22	CKS2 | CDC28 protein kinase 2
825470	17q21–q22	TOP2A | topoisomerase (DNA) II alpha (170 kD)
796469	1q32.1	HSPC150 | HSPC150 protein similar to ubiquitin-conjugating enzyme
705064	4p16.3	TACC3 | "transforming, acidic coiled-coil containing protein 3"
471568	17q25	HN1 | hematological and neurological expressed 1
742707	7	ESTs, Weakly similar to MUC2_HUMAN MUCIN 2 PRECURSOR [*H. sapiens*]
624667	9q34.13	LOC51117 | CGI-92 protein
1422338	2p25–p24	RRM2 | ribonucleotide reductase M2 polypeptide
700792	14q22	CDKN3 | cyclin-dependent kinase inhibitor 3 (CDK2-associated dual specificity phosphatase)
280375	8p22	PRO2000 | PRO2000 protein
122241	1p34.2	PSMB2 | "proteasome (prosome, macropain) subunit, beta type, 2"
2309073	2q33–q34	FZD5 | frizzled (Drosophila) homolog 5
2322367	2p14–p13	RTN4 | reticulon 4
796694	17q25	BIRC5 | baculoviral IAP repeat-containing 5 (survivin)
74677		*Homo sapiens*, Similar to RIKEN cDNA A430107J06 gene, clone MGC: 21416 IMAGE: 4452699, mRNA, complete cds
824524	17q21.32	UGTREL1 | UDP-galactose transporter related
825282		DKFZP586L0724 | DKFZP586L0724 protein
824962	17q23.1–q23.3	KPNA2 | "karyopherin alpha 2 (RAG cohort 1, importin alpha 1)"
42831	11q11–q12	NTKL | N-terminal kinase-like

TABLE 6-continued

IMAGE Clone ID	Chromosomal location	Description
814054	1q24–25	KIAA0040 | KIAA0040 gene product
2054635	20q13.33	PSMA7 | "proteasome (prosome, macropain) subunit, alpha type, 7"
210862	17q24–17q25	ACOX1 | "acyl-Coenzyme A oxidase 1, palmitoyl"
897997	Xp11.22–p11.21	SMC1L1 | "SMC1 (structural maintenance of chromosomes 1, yeast)-like 1"
769890	14q13.1	NP | nucleoside phosphorylase
756595	1q21	S100A10 | "S100 calcium-binding protein A10 (annexin II ligand, calpactin I, light polypeptide (p11))"
951233	2q35	PSMB3 | "proteasome (prosome, macropain) subunit, beta type, 3"
529827	Xp22.31	SYAP1 | reserved
1660666	Xp21.1	CA5B | "carbonic anhydrase VB, mitochondrial"
1696757	13q22.2	KIAA1165 | hypothetical protein KIAA1165
361922	1p34	ZMPSTE24 | "zinc metalloproteinase, STE24 (yeast, homolog)"
823598		PSMD12 | "proteasome (prosome, macropain) 26S subunit, non-ATPase, 12"
772220	3q21.2	PDIR | for protein disulfide isomerase-related
703707	8q12.1	ASPH | aspartate beta-hydroxylase
78869	20q13.33	GP110 | "cell membrane glycoprotein, 110000M(r) (surface antigen)"
1474424	17	*Homo sapiens* cDNA FLJ31911 fis, clone NT2RP7004751
1947647	17q23.3	LOC51651 | CGI-147 protein
897609	12q23.2	FLJ10074 | hypothetical protein FLJ10074
753378	4q34.1	FLJ22649 | hypothetical protein FLJ22649 similar to signal peptidase SPC22/23
124331	16	CPSF5 | "cleavage and polyadenylation specific factor 5, 25 kD subunit"
327506	15	*Homo sapiens* mRNA full length insert cDNA clone EUROIMAGE 327506
345538	9q21–q22	CTSL | cathepsin L
753320	8q13.3	FLJ20533 | hypothetical protein FLJ20533
823907	8q12.2	FLJ10511 | hypothetical protein FLJ10511
149355	8q13.1	TRAM | translocating chain-associating membrane protein
347373	8q13.3	TCEB1 | "transcription elongation factor B (SIII), polypeptide 1 (15 kD, elongin C)"
2028949	17q21.31	PRO1855 | hypothetical protein PRO1855
624627	2p25–p24	RRM2 | ribonucleotide reductase M2 polypeptide
731023	9q34	WDR5 | WD repeat domain 5
786067	20p13	CDC25B | cell division cycle 25B
878330	3	*Homo sapiens* cDNA: FLJ22044 fis, clone HEP09141
1631132	11q12.1	PHT2 | peptide transporter 3
756442	7q11.2	POR | P450 (cytochrome) oxidoreductase
823930	7q22.1	ARPC1A | "actin related protein 2/3 complex, subunit 1A (41 kD)"
268946	2	*Homo sapiens* cDNA FLJ31861 fis, clone NT2RP7001319
1914863	2p13.3–p13.1	DYSF | "dysferlin, limb girdle muscular dystrophy 2B (autosomal recessive)"
789012	3p25-p24	FBLN2 | fibulin 2
781047	2q14	BUB1 | budding uninhibited by benzimidazoles 1 (yeast homolog)
753428	8	*Homo sapiens*, Similar to RIKEN cDNA 1110014B07 gene, clone MGC: 20766 IMAGE: 4586039, mRNA, complete cds

[**0103**] The genes of "blue bar" genes along with their I.M.A.G.E. Consortium CloneID number, chromosomal location and descriptive identifiers (if known) are listed in Table 7.

TABLE 7

IMAGE Clone ID	Chromosomal location	Description
286378	19q13.4	ZNF135 | zinc finger protein 135 (clone pHZ-17)
854763	2q31.1	MGC20702 | hypothetical protein MGC20702
344959	4p16.2	HSA250839 | gene for serine/threonine protein kinase
278222	18	*Homo sapiens*, clone MGC: 10083 IMAGE: 3897118, mRNA, complete cds
1679977	18	*Homo sapiens*, clone MGC: 10083 IMAGE: 3897118, mRNA, complete cds
504959	11	*Homo sapiens* mRNA; cDNA DKFZp586G0321 (from clone DKFZp586G0321)
342181	18q21.3	BCL2 | B-cell CLL/lymphoma 2
502988	19p13.3–p13.2	ZNF20 | zinc finger protein 20 (KOX 13)
590310	2	*Homo sapiens*, clone MGC: 17393 IMAGE: 3914851, mRNA, complete cds
186301	11	*Homo sapiens* cDNA FLJ12924 fis, clone NT2RP2004709
357120	16	*Homo sapiens*, clone IMAGE: 3538007, mRNA, partial cds
203003	16p13.3	NME4 | "non-metastatic cells 4, protein expressed in"
725649	14q11.2	NFATC4 | "nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 4"
2014373	2q11.2	HNK-1ST | HNK-1 sulfotransferase
183440	22q13.33	ARSA | arylsulfatase A
2014856	1q25.3	HLALS | "major histocompatibility complex, class I-like sequence"
256619	10p11.2	HSD17B7 | hydroxysteroid (17-beta) dehydrogenase 7
768570	1q21.2	FLJ11280 | hypothetical protein FLJ11280
2975668	11p13	RAG2 | recombination activating gene 2

TABLE 7-continued

IMAGE Clone ID	Chromosomal location	Description
278430	2q23.3	KIF5C | kinesin family member 5C
1558233	3	ESTs
627248	5q23.2	SBBI31 | SBBI31 protein
1517171	10p15–p14	IL2RA | "interleukin 2 receptor, alpha"
1492468	1p32.3	KIAA0452 | DEME-6 protein
292770	1	*Homo sapiens*, clone IMAGE: 3627860, mRNA, partial cds
1456701	1q21	BCL9 | B-cell CLL/lymphoma 9
743146	18p11.21	FLJ23403 | hypothetical protein FLJ23403
1557637	5	ESTs
1583198	5	ESTs, Weakly similar to S65824 reverse transcriptase homolog [*H. sapiens*]
741891	6p21.3	RAB2L | "RAB2, member RAS oncogene family-like"
179572	1	*Homo sapiens* cDNA FLJ14227 fis, clone NT2RP3004095
1569902	16p11.2	KIAA0556 | KIAA0556 protein
127646	18	ESTs, Weakly similar to T00365 hypothetical protein KIAA0670 [*H. sapiens*]
782688	1p35.1	P28 | "dynein, axonemal, light intermediate polypeptide"
1883630	15	KIAA1547 | KIAA1547 protein
725340	4p16.3	TETRAN | tetracycline transporter-like protein
726890	10q24.2	MGC4643 | hypothetical protein MGC4643
82322	2p23.3	RBSK | ribokinase
839382	9	*Homo sapiens*, Similar to RIKEN cDNA 1700017I11 gene, clone MGC: 26847 IMAGE: 4821517, mRNA, complete cds
49630	3p14.3	CACNA1D | "calcium channel, voltage-dependent, L type, alpha 1D subunit"
32050	2	*Homo sapiens* mRNA; cDNA DKFZp586P1124 (from clone DKFZp586P1124)
110226		TNFRSF10C | "tumor necrosis factor receptor superfamily, member 10c, decoy without an intracellular domain"
1932725	1q32.1	ZNF281 | zinc finger protein 281
279720	11	*Homo sapiens*, Similar to RIKEN cDNA 1700008D07 gene, clone MGC: 9830 IMAGE: 3863323, mRNA, complete cds
1733262	3p21.3	BLu | BLu protein
197903	1	ESTs, Moderately similar to unnamed protein product [*H. sapiens*]
1556859	17	ESTs, Weakly similar to I38022 hypothetical protein [*H. sapiens*]
726699	16	*Homo sapiens*, clone MGC: 9889 IMAGE: 3868330, mRNA, complete cds

[**0104**] Most striking is the existence of reciprocal gradients in the intensities of these two signatures from grade I to grade III with most grade II lesions exhibiting both signatures to varying degrees (e.g., cases 130, 169, 198). Interestingly, some grade II lesions show an expression pattern that is most similar to either grade I or grade III lesions (case 41 and 43, respectively), and some grade III samples also express the grade I signature (e.g., cases 65, 88 and 112). Histological grade is an important characteristic of breast cancer with proven utility in patient prognostication and treatment (Fitzgibbons, P. L. et al.). For example, tumors of grade III are more likely to recur and are more likely to respond to chemotherapy than those of grade I (Page, D. L. et al. (2001)). However, the current tumor grading system relies mainly on histomorphological criteria, which, although highly successful in differentiating grade I from grade III tumors, are inadequate to score grade II tumors consistently (Dalton, L. W. et al.). This difficulty may be explained by the existence of a transcriptional continuum from grade I to grade III as we observed here. Therefore, a gene expression-based molecular grading system may allow greater precision in classifying breast cancer and provide greater insight into the state of progression of a particular tumor.

[**0105**] An expanded set of 250 genes that display increased expression in Grade I samples in comparison to Grade III samples are identified in Table 8 by use of their I.M.A.G.E. Consortium CloneID numbers along with their chromosomal location and descriptive identifiers (if known) and relative weights.

TABLE 8

IMAGE Clone ID	Weight	Chromosome Location	Description
344959	1.451333	4p16.2	HSA250839 | gene for serine/threonine protein kinase
504959	1.28687	11	*Homo sapiens* mRNA; cDNA DKFZp586G0321 (from clone DKFZp586G0321)
814815	1.2414		
743146	1.221818	18p11.21	FLJ23403 | hypothetical protein FLJ23403
417637	1.208243	4p16	KIAA1276 | KIAA1276 protein
502988	1.133964	19p13.3–p13.2	ZNF20 | zinc finger protein 20 (KOX 13)
1679977	1.131337	18	*Homo sapiens*, clone MGC: 10083 IMAGE: 3897118, mRNA, complete cds
342181	1.12098	18q21.3	BCL2 | B-cell CLL/lymphoma 2
1932725	1.11409	1q32.1	ZNF281 | zinc finger protein 281
70349	1.110469	Xq13.1	MLLT7 | myeloid/lymphoid or mixed-lineage leukemia (trithorax (Drosophila) homolog); translocated to, 7

TABLE 8-continued

IMAGE Clone ID	Weight	Chromosome Location	Description
180561	1.077508	1p13.3	GSTM1 | glutathione S-transferase M1
186301	1.068369	11	*Homo sapiens* cDNA FLJ12924 fis, clone NT2RP2004709
278222	1.065646	18	*Homo sapiens*, clone MGC: 10083 IMAGE: 3897118, mRNA, complete cds
357120	1.062902	16	*Homo sapiens*, clone IMAGE: 3538007, mRNA, partial cds
248631	1.04971	3p21.2–p21.1	AMT | aminomethyltransferase (glycine cleavage system protein T)
43090	1.021857	20q13.12	H-L(3)MBT | lethal (3) malignant brain tumor I(3)mbt protein (Drosophila) homolog
1631682	1.021091	1p32	PPIE | peptidylprolyl isomerase E (cyclophilin E)
767176	1.003495	17p13.1	TNFSF13 | tumor necrosis factor (ligand) superfamily, member 13
325583	1.00279		EST
1883630	0.979795	15	KIAA1547 | KIAA1547 protein
32050	0.979642	2	*Homo sapiens* mRNA; cDNA DKFZp586P1124 (from clone DKFZp586P1124)
502518	0.962484	3p21	LAMB2 | laminin, beta 2 (laminin S)
126415	0.957069	10	*Homo sapiens* mRNA; cDNA DKFZp566H0124 (from clone DKFZp566H0124)
82322	0.946458	2p23.3	RBSK | ribokinase
2975668	0.936737	11p13	RAG2 | recombination activating gene 2
1558233	0.931636	3	ESTs
256619	0.928002	10p11.2	HSD17B7 | hydroxysteroid (17-beta) dehydrogenase 7
206217	0.92794	11p11.2	NR1H3 | nuclear receptor subfamily 1, group H, member 3
726890	0.926526	10q24.2	MGC4643 | hypothetical protein MGC4643
2014373	0.906969	2q11.2	HNK-1ST | HNK-1 sulfotransferase
283124	0.89695	19	*Homo sapiens*, clone IMAGE: 3917549, mRNA, partial cds
741891	0.887613	6p21.3	RAB2L | RAB2, member RAS oncogene family-like
49630	0.885374	3p14.3	CACNA1D | calcium channel, voltage-dependent, L type, alpha 1D subunit
1592530	0.871817	3p21.31	IP6K2 | mammalian inositol hexakisphosphate kinase 2
277044	0.868338	19q13.32	KIAA1183 | KIAA1183 protein
1566877	0.867336	11q13	C11orf2 | chromosome 11 open reading frame2
839796	0.867221	12p13.31	LOC51147 | candidate tumor suppressor p33 ING1 homolog
279720	0.864865	11	*Homo sapiens*, Similar to RIKEN cDNA 1700008D07 gene, clone MGC:9830 IMAGE: 3863323, mRNA, complete cds
511831	0.854961	3	MGC12936 | hypothetical protein MGC12936
2014856	0.849103	1q25.3	HLALS | major histocompatibility complex, class I-like sequence
1652259	0.845966	7q31.3	LKR/SDH | lysine-ketoglutarate reductase/saccharopine dehydrogenase
172783	0.844046	19	ZNF358 | zinc finger protein 358
267254	0.838823	17	ESTs, Highly similar to LOX2_HUMAN ARACHIDONATE 12-LIPOXYGENASE [*H. sapiens*]
725340	0.826253	4p16.3	TETRAN | tetracycline transporter-like protein
593840	0.82327	17q11.2	DKFZP564K1964 | DKFZP564K1964 protein
179572	0.819502	1	*Homo sapiens* cDNA FLJ14227 fis, clone NT2RP3004095
854763	0.818371	2q31.1	MGC20702 | hypothetical protein MGC20702
286378	0.818288	19q13.4	ZNF135 | zinc finger protein 135 (clone pHZ-17)
1733262	0.815457	3p21.3	BLu | BLu protein
1517171	0.812481	10p15–p14	IL2RA | interleukin 2 receptor, alpha
814826	0.807648	2	ESTs
126466	0.797965	1p34.1	KIAA0467 | KIAA0467 protein
110226	0.796159		TNFRSF10C | tumor necrosis factor receptor superfamily, member 10c, decoy without an intracellular domain
344168	0.795755	10q23	POLL | polymerase (DNA directed), lambda
108667	0.79402	22q12.2	SF3A1 | splicing factor 3a, subunit 1, 120kD
295572	0.792031	12q24.21	KIAA0682 | KIAA0682 gene product
823634	0.789164	10	ESTs
138242	0.787686	1	ESTs, Moderately similar to MAS2_HUMAN MANNAN-BINDING LECTIN SERINE PROTEASE 2 PRECURSOR [*H. sapiens*]
197903	0.785879	1	ESTs, Moderately similar to unnamed protein product [*H. sapiens*]
292770	0.784314	1	*Homo sapiens*, clone IMAGE: 3627860, mRNA, partial cds
810981	0.784118	22q13	FLJ20699 | hypothetical protein FLJ20699
197913	0.777546	1p34.2	SFPQ | splicing factor proline/glutamine rich (polypyrimidine tract-binding protein-associated)
190059	0.77474	19p13.3	GNG7 | guanine nucleotide binding protein (G protein), gamma 7
782688	0.77051	1p35.1	P28 | dynein, axonemal, light intermediate polypeptide
121454	0.76967	17p13.1	ALOX12 | arachidonate 12-lipoxygenase
1569902	0.764217	16p11.2	KIAA0556 | KIAA0556 protein
726699	0.760736	16	*Homo sapiens*, clone MGC: 9889 IMAGE: 3868330, mRNA, complete cds
1601845	0.759847	7q22–q31.1	CAPRI | Ca2+-promoted Ras inactivator
703964	0.759625	11q23	INPPL1 | inositol polyphosphate phosphatase-like 1
183440	0.757148	22q13.33	ARSA | arylsulfatase A
431231	0.756281	11q13	EFEMP2 | EGF-containing fibulin-like extracellular matrix protein 2
810358	0.750312	17p13–p11 ACADVL	| acyl-Coenzyme A dehydrogenase, very long chain
1583198	0.749857	5	ESTs, Weakly similar to S65824 reverse transcriptase homolog [*H. sapiens*]
1630990	0.748442	3p21.3–p21.2	RPL29 | ribosomal protein L29
1868349	0.746257	15q11.2–q21.3	PLA2G4B | phospholipase A2, group IVB (cytosolic)
627248	0.744679	5q23.2	SBBI31 | SBBI31 protein
127646	0.743672	18	ESTs, Weakly similar to T00365 hypothetical protein KIAA0670 [*H. sapiens*]
1635059	0.739062	9	*Homo sapiens*, clone MGC: 16638 IMAGE: 4121964, mRNA, complete cds

TABLE 8-continued

IMAGE Clone ID	Weight	Chromosome Location	Description
1456701	0.732349	1q21	BCL9 | B-cell CLL/lymphoma 9
345764	0.72889	3p23	SATB1 | special AT-rich sequence binding protein 1 (binds to nuclear matrix/scaffold-associating DNA's)
278430	0.728595	2q23.3	KIF5C | kinesin family member 5C
1492468	0.72665	1p32.3	KIAA0452 | DEME-6 protein
590310	0.725531	2	*Homo sapiens*, clone MGC: 17393 IMAGE: 3914851, mRNA, complete cds
768570	0.720983	1q21.2	FLJ11280 | hypothetical protein FLJ11280
1883169	0.716948	5p15.32	FLJ20303 | hypothetical protein FLJ20303
1635062	0.716142	12q13.13	DKFZP586A011 | DKFZP586A011 protein
2757710	0.715294	10p11.2	ZNF37A | zinc finger protein 37a (KOX 21)
810741	0.709032	17p13.2	GABARAP | GABA(A) receptor-associated protein
1569077	0.708429	6	EST
1653105	0.708359	3p14–p12	TSP50 | testes-specific protease 50
1553530	0.707954	2	KIAA0788 | KIAA0788 protein
43679	0.707235	10	ESTs
725649	0.706826	14q11.2	NFATC4 | nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 4
684890	0.705934	16p12.1	FLJ20274 | hypothetical protein FLJ20274
1556859	0.702746	17	ESTs, Weakly similar to I38022 hypothetical protein [*H. sapiens*]
1557637	0.698307	5	ESTs
203003	0.697573	16p13.3	NME4 | non-metastatic cells 4, protein expressed in
46129	0.694321	12q13.1	HDAC7A | histone deacetylase 7A
839382	0.693177	9	*Homo sapiens*, Similar to RIKEN cDNA 1700017I11 gene, clone MGC:26847 IMAGE: 4821517, mRNA, complete cds
307029	0.690207		
184022	0.689767	11p15	APBB1 | amyloid beta (A4) precursor protein-binding, family B, member 1 (Fe65)
745077	0.681153	19	*Homo sapiens* mRNA; cDNA DKFZp566J2324 (from clone DKFZp566J2324); partial cds
769600	0.68017	5p15.2–p13.1	UNG2 | uracil-DNA glycosylase 2
280776	0.677821	15	MGC5139 | hypothetical protein MGC5139
810947	0.674861	16p13.11	NUDE1 | LIS1-interacting protein NUDE1, rat homolog
824879	0.674702	16p13.3	MGC11275 | hypothetical protein MGC11275
454503	0.669502	12	*Homo sapiens*, clone IMAGE: 3346451, mRNA, partial cds
811920	0.658971	9p13	IL11RA | interleukin 11 receptor, alpha
1636360	0.658963	15q21.1–q21.2	FLJ14957 | hypothetical protein FLJ14957
2502722	0.658146	11q23	LOH11CR2A | loss of heterozygosity, 11, chromosomal region 2, gene A
1609372	0.657294	14q11.2	RIPK3 | receptor-interacting serine-threonine kinase 3
346977	0.655725	3p24.3	KIAA0210 | KIAA0210 gene product
293569	0.653314	1q25	C1orf21 | chromosome 1 open reading frame 21
1635307	0.651746	12	*Homo sapiens*, clone IMAGE: 3833472, mRNA
240505	0.65172	14q11.2	KIAA0323 | KIAA0323 protein
52724	0.648958		FLJ20241 | hypothetical protein FLJ20241
120138	0.648579	10q21.1	JDP1 | domain containing protein 1
74070	0.648244	1q21.2	ENSA | endosulfine alpha
186626	0.644915	6	ESTs, Weakly similar to CYP4_HUMAN 40 KDA PEPTIDYL-PROLYL CIS-TRANS ISOMERASE [*H. sapiens*]
296679	0.644155	5	*Homo sapiens* clone TCCCTA00151 mRNA sequence
2119838	0.64368	11q25	ADAMTS8 | a disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif, 8
813488	0.643211	1q32.1	LOC51235 | hypothetical protein
742094	0.639857	14q32.12	FLJ20950 | hypothetical protein FLJ20950
705274	0.638802	2q37.2	DGKD | diacylglycerol kinase, delta (130 kD)
826285	0.633833		*Homo sapiens* cDNA FLJ32001 fis, clone NT2RP7009373
358217	0.631361	Xq26.1	GPC4 | glypican 4
796723	0.629143		*Homo sapiens* clone CDABP0014 mRNA sequence
529843	0.628435	19	ESTs
262251	0.627736	16p13	CLCN7 | chloride channel 7
490449	0.623346	5q31	RAD50 | RAD50 (*S. cerevisiae*) homolog
788334	0.622909	11p15.5–p15.4	MRPL23 | mitochondrial ribosomal protein L23
1909935	0.62043	8	ESTs
250883	0.61921	3p21	UBE1L | ubiquitin-activating enzyme E1-like
1707667	0.618023	17	*Homo sapiens* cDNA FLJ31065 fis, clone HSYRA2001142
68103	0.617869	12	MLC1SA | myosin light chain 1 slow a
773381	0.617263	19q13.33	NAPA | N-ethylmaleimide-sensitive factor attachment protein, alpha
1559596	0.616776	11	ESTs, Highly similar to AF175283 1 zinc metalloendopeptidase [*H. sapiens*]
825296	0.616769	1q42.11–q42.3	LDLC | low density lipoprotein receptor defect C complementing
866866	0.616211	3p21.3	RASSF1 | Ras association (RalGDS/AF-6) domain family 1
490668	0.613699	3	*Homo sapiens*, clone IMAGE: 4182947, mRNA
824052	0.613652	6p21.3	C6orf1 | chromosome 6 open reading frame 1
505243	0.612671	12p11	ITPR2 | inositol 1,4,5-triphosphate receptor, type 2
1911343	0.612387	16p13.3	RAB26 | RAB26, member RAS oncogene family
1637296	0.60612	10q22–q23	RPS24 | ribosomal protein S24
753252	0.604292	17q21.31	MGC4251 | hypothetical protein MGC4251
1518890	0.602612	11q13.2–q13.3	MTL5 | metallothionein-like 5, testis-specific (tesmin)

TABLE 8-continued

IMAGE Clone ID	Weight	Chromosome Location	Description
234522	0.601183	1q21.3	KIAA1535 | KIAA1535 protein
52419	0.598962	9q13–q21	X123 | Friedreich ataxia region gene X123
278483	0.598475	18p11.32	TYMS | thymidylate synthetase
877664	0.598243	20	FLJ14987 | hypothetical protein FLJ14987
826622	0.594938	16p13.12	KIAA0430 | KIAA0430 gene product
701112	0.591773	3p25	XPC | xeroderma pigmentosum, complementation group C
1859625	0.591377	8q24	BAI1 | brain-specific angiogenesis inhibitor 1
812975	0.586956	9p23	KIAA0172 | KIAA0172 protein
214068	0.585918	10p15	GATA3 | GATA-binding protein 3
1587863	0.581689	3p23–p22	ACAA1 | acetyl-Coenzyme A acyltransferase 1 (peroxisomal 3-oxoacyl-Coenzyme A thiolase)
1518402	0.576275	17q11.1	KIAA1361 | KIAA1361 protein
796996	0.57565	Xq13.1–q13.3	IGBP1 | immunoglobulin (CD79A) binding protein 1
1323448	0.575218	7q11.23	CRIP1 | cysteine-rich protein 1 (intestinal)
2388571	0.574109	19p13.1–q12	AKAP8 | A kinase (PRKA) anchor protein 8
75078	0.573276	12	ESTs
1604642	0.572299	6	*Homo sapiens* cDNA FLJ32724 fis, clone TESTI2000951
66532	0.572179	20q13.2–q13.3	EDN3 | endothelin 3
2273445	0.571917	20q11.2	GHRH | growth hormone releasing hormone
346643	0.567626	10	ESTs
595297	0.563887	1q21.3	SNAPAP | SNARE associated protein snapin
971399	0.561448	12cen–q21	SYT1 | synaptotagmin I
897550	0.561065	17q21.2	MGC2744 | hypothetical protein MGC2744
215000	0.560663	3p22	VIPR1 | vasoactive intestinal peptide receptor 1
155896	0.560564	11cen–q12.1	LOC51035 | ORF
1700429	0.56053	10q26	GFRA1 | GDNF family receptor alpha 1
277463	0.560068	18p11.2	C18orf1 | chromosome 18 open reading frame 1
1587710	0.556854	17p13.1–17p12	PER1 | period (Drosophila) homolog 1
565849	0.55621	1q32.1	C3IP1 | kelch-like protein C3IP1
126851	0.555748	10q22.1	FLJ11160 | hypothetical protein FLJ11160
2413337	0.554359	11q23.2–q24.2	SORL1 | sortilin-related receptor, L(DLR class) A repeats-containing
824753	0.554027	13	FLJ22624 | hypothetical protein FLJ22624
50471	0.553058	11	*Homo sapiens* cDNA FLJ14242 fis, clone OVARC1000678
33500	0.552872		*Homo sapiens* clone 23556 mRNA sequence
752547	0.551916	15	*Homo sapiens* mRNA; cDNA DKFZp586G1520 (from clone DKFZp586G1520)
83358	0.550166		ESTs
2096306	0.55013	8q24.3	ARC | activity-regulated cytoskeleton-associated protein
196189	0.548574	18q23	CYB5 | cytochrome b-5
2018808	0.546276	11q14	PRCP | prolylcarboxypeptidase (angiotensinase C)
1500542	0.544517	16p13.3	RGS11 | regulator of G-protein signalling 11
470061	0.544311	3q25	SIAH2 | seven in absentia (Drosophila) homolog 2
1762111	0.543871	5p14–p13	NPR3 | natriuretic peptide receptor C/guanylate cyclase C (atrionatriuretic peptide receptor C)
2116188	0.543472	17q21	HDAC5 | histone deacetylase 5
826668	0.542351	6q21	KIAA0274 | KIAA0274 gene product
26736	0.540638	20	*Homo sapiens* cDNA FLJ30872 fis, clone FEBRA2004293
669379	0.540325	7	*Homo sapiens*, clone IMAGE: 3463399, mRNA, partial cds
221776	0.536594	14	ESTs, Weakly similar to T20410 hypothetical protein E02A10.2 —Caenorhabditis elegans [C.elegans]
264632	0.535737	19	ESTs
741790	0.53497	2p13.3	FLJ20080 | hypothetical protein FLJ20080
1626087	0.53252	3p21.31	DKFZP434A236 | DKFZP434A236 protein
812033	0.532407	2q35–q37	GPC1 | glypican 1
950574	0.531092	17q25	H3F3B | H3 histone, family 3B (H3.3B)
284022	0.531011	8p23	ARHGEF10 | Rho guanine nucleotide exchange factor (GEF) 10
35828	0.528716	5q23	DTR | diphtheria toxin receptor (heparin-binding epidermal growth factor-like growth factor)
2284619	0.528522	19q13.4	ZNF132 | zinc finger protein 132 (clone pHZ-12)
681992	0.528384	7	*Homo sapiens* cDNA FLJ13384 fis, clone PLACE1001062, highly similar to *Homo sapiens* mRNA for lysine-ketoglutarate reductase/saccharopine dehydrogenase
43933	0.52806	Xp11.4–p11.3	MAOA | monoamine oxidase A
785538	0.527955		*Homo sapiens* cDNA FLJ32293 fis, clone PROST2001739
343760	0.526569	6q13–15	SH3BGRL2 | SH3 domain binding glutamic acid-rich protein like 2
785571	0.525679	10	DNAJL1 | hypothetical protein similar to mouse Dnajl1
809507	0.525406	16p13.3	FLJ20568 | hypothetical protein FLJ20568
1895664	0.524227	15q26.1	PRO2198 | hypothetical protein PRO2198
823661	0.521218	14	*Homo sapiens* cDNA FLJ31768 fis, clone NT2RI2007891, moderately similar to DMR-N9 PROTEIN
842980	0.519909	22q12.2	DRG1 | developmentally regulated GTP-binding protein 1
126419	0.517789	1q21–q22	NIT1 | nitrilase 1
1926023	0.516851	7	ESTs, Weakly similar to T42727 proliferation potential-related protein-mouse [*M. musculus*]
132857	0.516382	17	*Homo sapiens* mRNA; cDNA DKFZp586N1323 (from clone DKFZp586N1323)

TABLE 8-continued

IMAGE Clone ID	Weight	Chromosome Location	Description
855586	0.515352	5q31	NR3C1 | nuclear receptor subfamily 3, group C, member 1
810331	0.515056	1q24	QSCN6 | quiescin Q6
265103	0.512718	1p36	MMEL2 | membrane metallo-endopeptidase-like 2
1521361	0.511233	8p21.2	KIAA0717 | KIAA0717 protein
432072	0.508774	18q23	NFATC1 | nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1
2069602	0.506115	16q24.3	MC1R | melanocortin 1 receptor (alpha melanocyte stimulating hormone receptor)
283173	0.505655	4	*Homo sapiens* PAC clone RP1-130H16 from 22q12.1-qter
1404841	0.501049	19q13.4	ZNF175 | zinc finger protein 175
1871116	0.500004	2	*Homo sapiens* mRNA; cDNA DKFZp434C1714 (from clone DKFZp434C1714); partial cds
758365	0.4988	12q13–q15	OS4 | conserved gene amplified in osteosarcoma
1641894	0.498542	10	ESTs
1492147	0.498131	Xq13.1	RPS4X | ribosomal protein S4, X-linked
1558642	0.497736	2q37.3	MLPH | melanophilin
1641245	0.497723	18q21.1	LOC51320 | hypothetical protein
1635649	0.497647	20p13	CDS2 | CDP-diacylglycerol synthase (phosphatidate cytidylyltransferase) 2
414999	0.496855	17q21	ETV4 | ets variant gene 4 (E1A enhancer-binding protein, E1AF)
1535957	0.496325	5p15.3	SEC6 | similar to *S. cerevisiae* Sec6p and R. norvegicus rsec6
774082	0.495883	12q22–q23	ASCL1 | achaete-scute complex (Drosophila) homolog-like 1
811013	0.494705	1p13.3	AMPD2 | adenosine monophosphate deaminase 2 (isoform L)
809998	0.493372	1p21	AMY2A | amylase, alpha 2A; pancreatic
2018084	0.48899	2q24.3	SPAK | Ste-20 related kinase
161373	0.485425	7q11–q22	PMS2L4 | postmeiotic segregation increased 2-like 4
178137	0.485162	4q25	RPL34 | ribosomal protein L34
75886	0.484926	4	ESTs, Weakly similar to E54024 protein kinase [*H. sapiens*]
429387	0.484053	7p15.3	CHN2 | chimerin (chimaerin) 2
742977	0.481369	7p13	DKFZP761I2123 | KIAA1886 protein
240637	0.480946	1p33–p32.1	MGC8974 | hypothetical protein MGC8974
838366	0.480888	1p36.1–p35	HMGCL | 3-hydroxymethyl-3-methylglutaryl-Coenzyme A lyase (hydroxymethylglutaricaciduria)
796181	0.480102	13q34	GAS6 | growth arrest-specific 6
23776	0.479727	4p15.31	QDPR | quinoid dihydropteridine reductase
1909433	0.478064	17	*Homo sapiens* cDNA FLJ30754 fis, clone FEBRA2000438
2160920	0.477446	1p13	PHTF1 | putative homeodomain transcription factor 1
1500536	0.475933	12pter-p13.31	MDS028 | uncharacterized hematopoietic stem/progenitor cells protein MDS028
294537	0.474189	2q37.3	RAB17 | RAB17, member RAS oncogene family
784085	0.469813	6q25–q26	TUSP | tubby super-family protein
239877	0.469171	5q31	HDAC3 | histone deacetylase 3
626861	0.468844	11p15	EIF4G2 | eukaryotic translation initiation factor 4 gamma, 2
741977	0.466816	6p21.3	BF | B-factor, properdin

[0106] Some of the genes within the tumor grade I/III signatures have been previously reported to be associated with breast cancer. Within the grade I signature, two genes, BCL2 and TNFRSF10C, are inhibitors of apoptosis. Various reports in the literature link BCL2 expression to ER-positive, low-grade tumors (van Slooten, H. J. et al.). TNFRSF10C is a decoy receptor (DcR1) for TRAIL, an apoptosis-inducing cytokine of the tumor necrosis factor (TNF) family (Sheridan, J. P. et al.). Without being bound by theory, presence of DcR1 on the surface of breast cancer cells would be expected to block signaling through the cell death receptors activated by TRAIL, thus inhibiting apoptosis.

[0107] Similarly, an expanded set of 250 genes that display increased expression in Grade III samples in comparison to Grade I samples are identified in Table 9 by use of their I.M.A.G.E. Consortium CloneID numbers along with their chromosomal location and descriptive identifiers (if known) and relative weights (which are expressed with a negative sign solely due to the relative comparison).

TABLE 9

IMAGE Clone ID	Weight	Chromosome Location	Description
769921	−1.53568	20q13.12	UBE2C | ubiquitin-conjugating enzyme E2C
951241	−1.33815	15q13.3	ANKT | nucleolar protein ANKT
1517595	−1.3332	9p11.2	KIAA0175 | likely ortholog of maternal embryonic leucine zipper kinase
1474424	−1.32072	17	*Homo sapiens* cDNA FLJ31911 fis, clone NT2RP7004751
2309073	−1.29533	2q33–q34	FZD5 | frizzled (Drosophila) homolog 5
796469	−1.27516	1q32.1	HSPC150 | HSPC150 protein similar to ubiquitin-conjugating enzyme

TABLE 9-continued

IMAGE Clone ID	Weight	Chromosome Location	Description
823598	−1.26568		PSMD12 | proteasome (prosome, macropain) 26S subunit, non-ATPase, 12
700792	−1.25232	14q22	CDKN3 | cyclin-dependent kinase inhibitor 3 (CDK2-associated dual specificity phosphatase)
2018131	−1.23217	12p13.2–p13.1	RACGAP1 | Rac GTPase activating protein 1
292936	−1.20973	1p34.3	FLJ10468 | hypothetical protein FLJ10468
1422338	−1.20922	2p25–p24	RRM2 | ribonucleotide reductase M2 polypeptide
504308	−1.18743	10cen-q26.11	FLJ10540 | hypothetical protein FLJ10540
796694	−1.16444	17q25	BIRC5 | baculoviral IAP repeat-containing 5 (survivin)
869375	−1.15363	15q26.1	IDH2 | isocitrate dehydrogenase 2 (NADP+), mitochondrial
814270	−1.14538	4q27	PMSCL1 | polymyositis/scleroderma autoantigen 1 (75 kD)
42831	−1.12878	11q11–q12	NTKL | N-terminal kinase-like
1476053	−1.10462	15q15.1	RAD51 | RAD51 (*S. cerevisiae*) homolog (*E coli* RecA homolog)
32493	−1.10275	2q31.1	ITGA6 | integrin, alpha 6
149355	−1.10225	8q13.1	TRAM | translocating chain-associating membrane protein
824962	−1.09918	17q23.1–q23.3	KPNA2 | karyopherin alpha 2 (RAG cohort 1, importin alpha 1)
1702742	−1.09644	16q24.3	SLC7A5 | solute carrier family 7 (cationic amino acid transporter, y^+ system), member 5
824524	−1.07854	17q21.32	UGTREL1 | UDP-galactose transporter related
128711	−1.07401	7p15–p14	ANLN | anillin (Drosophila Scraps homolog), actin binding protein
843121	−1.06508	6p22.1–p21.2	CLIC1 | chloride intracellular channel 1
2017415	−1.06388	2p24–p21	CENPA | centromere protein A (17 kD)
753378	−1.0364	4q34.1	FLJ22649 | hypothetical protein FLJ22649 similar to signal peptidase SPC22/23
825470	−1.03507	17q21–q22	TOP2A | topoisomerase (DNA) II alpha (170 kD)
705064	−1.02376	4p16.3	TACC3 | transforming, acidic coiled-coil containing protein 3
2054635	−1.02042	20q13.33	PSMA7 | proteasome (prosome, macropain) subunit, alpha type, 7
781047	−1.0153	2q14	BUB1 | budding uninhibited by benzimidazoles 1 (yeast homolog)
1534700	−1.01343	11q21	KIAA0830 | KIAA0830 protein
1587847	−1.01171	2q21	MCM6 | minichromosome maintenance deficient (mis5, *S. pombe*) 6
743810	−1.0099	12p13	MGC2577 | hypothetical protein MGC2577
897609	−0.99379	12q23.2	FLJ10074 | hypothetical protein FLJ10074
66406	−0.98421	2	ESTs, Highly similar to T47163 hypothetical protein DKFZp762E1312.1 [*H. sapiens*]
1631634	−0.98233	9q34.11	MGC3038 | hypothetical protein similar to actin related protein 2/3 complex, subunit 5
624627	−0.96436	2p25–p24	RRM2 | ribonucleotide reductase M2 polypeptide
814054	−0.95575	1q24–25	KIAA0040 | KIAA0040 gene product
773301	−0.91294	16q22.1	CDH3 | cadherin 3, type 1, P-cadherin (placental)
1416055	−0.91005	8	KIAA0165 | extra spindle poles, S. cerevisiae, homolog of
345787	−0.89554	18p11.31	HEC | highly expressed in cancer, rich in leucine heptad repeats
624667	−0.88376	9q34.13	LOC51117 | CGI-92 protein
786067	−0.87714	20p13	CDC25B | cell division cycle 25B
785368	−0.87699	8p21–p12	TOPK | PDZ-binding kinase; T-cell originated protein kinase
564981	−0.85513	18	*Homo sapiens*, Similar to RIKEN cDNA 2810433K01 gene, clone MGC: 10200 IMAGE: 3909951, mRNA, complete cds
753320	−0.85505	8q13.3	FLJ20533 | hypothetical protein FLJ20533
529827	−0.85016	Xp22.31	SYAP1 | reserved
122241	−0.84842	1p34.2	PSMB2 | proteasome (prosome, macropain) subunit, beta type, 2
712139	−0.84823	2q37.2	ARL7 | ADP-ribosylation factor-like 7
259950	−0.83947	8q23	CML66 | chronic myelogenous leukemia tumor antigen 66
772220	−0.83895	3q21.2	PDIR | for protein disulfide isomerase-related
124331	−0.83664	16	CPSF5 | cleavage and polyadenylation specific factor 5, 25 kD subunit
842818	−0.83338	16q23–q24	KARS | lysyl-tRNA synthetase
150897	−0.82922	19p13.1	B3GNT3 | UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 3
823930	−0.82876	7q22.1	ARPC1A | actin related protein 2/3 complex, subunit 1A (41 kD)
210862	−0.82312	17q24–17q25	ACOX1 | acyl-Coenzyme A oxidase 1, palmitoyl
731023	−0.82276	9q34	WDR5 | WD repeat domain 5
665384	−0.82232	16	KIAA1609 | KIAA1609 protein
815501	−0.82108	19p13.3	MGC2721 | hypothetical protein MGC2721
769890	−0.81864	14q13.1	NP | nucleoside phosphorylase
209066	−0.81121	20q13.2–q13.3	STK15 | serine/threonine kinase 15
471568	−0.81026	17q25	HN1 | hematological and neurological expressed 1
725454	−0.80701	9q22	CKS2 | CDC28 protein kinase 2
951233	−0.80178	2q35	PSMB3 | proteasome (prosome, macropain) subunit, beta type, 3
268946	−0.79976	2	*Homo sapiens* cDNA FLJ31861 fis, clone NT2RP7001319
2028949	−0.78651	17q21.31	PRO1855 | hypothetical protein PRO1855
1914863	−0.78621	2p13.3–p13.1	DYSF | dysferlin, limb girdle muscular dystrophy 2B (autosomal recessive)
744047	−0.77737	16p12.3	PLK | polo (Drosophia)-like kinase
703707	−0.77579	8q12.1	ASPH | aspartate beta-hydroxylase
78869	−0.76948	20q13.33	GP110 | cell membrane glycoprotein, 110000M(r) (surface antigen)
742707	−0.7686	7	ESTs, Weakly similar to MUC2_HUMAN MUCIN 2 PRECURSOR [*H. sapiens*]
825606	−0.75817	10q24.1	KNSL1 | kinesin-like 1
361922	−0.7559	1p34	ZMPSTE24 | zinc metalloproteinase, STE24 (yeast, homolog)

TABLE 9-continued

IMAGE Clone ID	Weight	Chromosome Location	Description
756595	−0.75094	1q21	S100A10 | S100 calcium-binding protein A10 (annexin II ligand, calpactin I, light polypeptide (p11))
756442	−0.7508	7q11.2	POR | P450 (cytochrome) oxidoreductase
823907	−0.74968	8q12.2	FLJ10511 | hypothetical protein FLJ10511
471196	−0.74806	2q37	ITM3 | integral membrane protein 3
753428	−0.74668	8	*Homo sapiens*, Similar to RIKEN cDNA 1110014B07 gene, clone MGC: 20766 IMAGE: 4586039, mRNA, complete cds
739450	−0.74247	1q21.2	LASS2 | longevity assurance (LAG1, S. cerevisiae) homolog 2
1696757	−0.73849	13q22.2	KIAA1165 | hypothetical protein KIAA1165
293727	−0.73213	22q13.2	MGC861 | hypothetical protein MGC861
839682	−0.731	12q22	UBE2N | ubiquitin-conjugating enzyme E2N (homologous to yeast UBC13)
1631132	−0.73053	11q12.1	PHT2 | peptide transporter3
327506	−0.72966	15	*Homo sapiens* mRNA full length insert cDNA clone EUROIMAGE 327506
1660666	−0.72774	Xp21.1	CA5B | carbonic anhydrase VB, mitochondrial
280375	−0.72588	8p22	PRO2000 | PRO2000 protein
796904	−0.71939	6q24–q25	PLAGL1 | pleiomorphic adenoma gene-like 1
503671	−0.71201	6	*Homo sapiens* cDNA FLJ14368 fis, clone HEMBA1001122
74677	−0.71194		*Homo sapiens*, Similar to RIKEN cDNA A430107 J06 gene, clone MGC: 21416 IMAGE: 4452699, mRNA, complete cds
291478	−0.71127	1p36	RUNX3 | runt-related transcription factor 3
825282	−0.7096		DKFZP586L0724 | DKFZP586L0724 protein
878330	−0.70859	3	*Homo sapiens* cDNA: FLJ22044 fis, clone HEP09141
37671	−0.70374	18q11.2	FLJ21610 | hypothetical protein FLJ21610
789012	−0.7019	3p25–p24	FBLN2 | fibulin 2
347373	−0.70161	8q13.3	TCEB1 | transcription elongation factor B (SIII), polypeptide 1 (15 kD, elongin C)
2322367	−0.69997	2p14–p13	RTN4 | reticulon 4
897997	−0.69961	Xp11.22–p11.21	SMC1L1 | SMC1 (structural maintenance of chromosomes 1, yeast)-like 1
345538	−0.69527	9q21–q22	CTSL | cathepsin L
1947647	−0.69371	17q23.3	LOC51651 | CGI-147 protein
3172883	−0.69164	11	ESTs, Weakly similar to S24195 dopamine receptor D4 [*H. sapiens*]
1035796	−0.68832	1	ESTs, Weakly similar to T33068 hypothetical protein C35E7.9 - Caenorhabditis elegans [*C.elegans*]
746163	−0.67918	8	ESTs, Weakly similar to ALU1¯HUMAN ALU SUBFAMILY J SEQUENCE CONTAMINATION WARNING ENTRY [*H. sapiens*]
810711	−0.67743	10q23–q24	SCD | stearoyl-CoA desaturase (delta-9-desaturase)
462926	−0.67573	1q32.2–q41	NEK2 | NIMA (never in mitosis gene a)-related kinase 2
1614140	−0.67368	15q11.2–q22.33	LOC51285 | Ris
124781	−0.66984	8q24.1	SQLE | squalene epoxidase
1642496	−0.66639	2p24.1	MGC11266 | hypothetical protein MGC11266
113300	−0.66053	9q22.32	TRIM14 | tripartite motif-containing 14
2014034	−0.65845	2p12	MTHFD2 | methylene tetrahydrofolate dehydrogenase (NAD+ dependent), methenyltetrahydrofolate cyclohydrolase
1946448	−0.65464	7q31.1	CAV2 | caveolin 2
1635352	−0.65164	4q12	TPARL | TPA regulated locus
753400	−0.64918	3q27.1	BAF53A | BAF53
1605426	−0.64391	4q12	FLJ13352 | hypothetical protein FLJ13352
565319	−0.64374	8	MAL2 | mal, T-cell differentiation protein 2
489755	−0.64095	10q26.3	ADAM12 | a disintegrin and metalloproteinase domain 12 (meltrin alpha)
1916461	−0.63993	22	*Homo sapiens*, clone IMAGE: 3605655, mRNA
359887	−0.63379	1q32.1	TIM17 | translocase of inner mitochondrial membrane 17 homolog A (yeast)
629944	−0.63018	18q12	MYO5B | myosin VB
150314	−0.62891	6q13	LYPLA1 | lysophospholipase I
770355	−0.62829	21q22.3	LSS | lanosterol synthase (2,3-oxidosqualene-lanosterol cyclase)
489594	−0.6245	Xq22.2	FLJ11565 | hypothetical protein FLJ11565
212640	−0.6219	Xq28	ARHGAP4 | Rho GTPase activating protein 4
30170	−0.62007	4q34	CASP3 | caspase 3, apoptosis-related cysteine protease
51773	−0.61957	7p15–p14	MGC3077 | hypothetical protein MGC3077
490777	−0.61906		
1858892	−0.61695	Xp22.13	MGC4825 | hypothetical protein MGC4825
358456	−0.61552	7p11.2	SEC61G | Sec61 gamma
840894	−0.61414	12q24.2	COX6A1 | cytochrome c oxidase subunit VIa polypeptide 1
241348	−0.61157		PCL1 | prenylcysteine lyase
1505038	−0.61123	8q22.2	FLJ20171 | hypothetical protein FLJ20171
144880	−0.60976	19p13.3	LOC56932 | hypothetical protein from EUROIMAGE 1759349
454896	−0.60575	16q11.1–q11.2	DNAJA2 | DnaJ (Hsp40) homolog, subfamily A, member 2
753236	−0.60461		6 ESTs, Weakly similar to S71512 hypothetical protein T2 - mouse [*M. musculus*]
266218	−0.60106		
418159	−0.60025	22q13.1	SYNGR1 | synaptogyrin 1
208718	−0.59862	9q12–q21.2	ANXA1 | annexin A1
781097	−0.59718	11q13	RTN3 | reticulon 3
469383	−0.59434	8q21	C8orf1 | chromosome 8 open reading frame 1
725152	−0.59375	11q11	DKFZp762A227 | hypothetical protein DKFZp762A227

TABLE 9-continued

IMAGE Clone ID	Weight	Chromosome Location	Description
845363	−0.59313	17q21.3	NME1 | non-metastatic cells 1, protein (NM23A) expressed in
1460110	−0.59206	14q11.2	PSMB5 | proteasome (prosome, macropain) subunit, beta type, 5
769959	−0.5913	13q34	COL4A2 | collagen, type IV, alpha 2
796527	−0.59108	7q34	DKFZp761N0624 | hypothetical protein DKFZp761N0624
108425	−0.59009	1	ESTs, Weakly similar to JC5314 CDC28/cdc2-like kinase associating arginine-serine cyclophilin [*H. sapiens*]
32231	−0.58516		FLJ12442 | hypothetical protein FLJ12442
502690	−0.58445	3q21.3–q25.2	RPN1 | ribophorin I
135221	−0.58203	4p16	S100P | S100 calcium-binding protein P
897813	−0.58167	17p11.1	PAIP1 | polyadenylate binding protein-interacting protein 1
824352	−0.58119	9q31.2	RAD23B | RAD23 (*S. cerevisiae*) homolog B
897751	−0.58057	17q23	TLK2 | tousled-like kinase 2
343607	−0.57727	15q14–q24.3	LOC55829 | AD-015 protein
51899	−0.5772	16q24.1	KIAA0513 | KIAA0513 gene product
726645	−0.57716	16q23	CLECSF1 | C-type (calcium dependent, carbohydrate-recognition domain) lectin, superfamily member 1 (cartilage-derived)
1591264	−0.57558	11p15.5–p15.4	TALDO1 | transaldolase 1
290841	−0.57171	6p21.3	H2BFA | H2B histone family, member A
486626	−0.57063	8	*Homo sapiens*, clone IMAGE: 4332938, mRNA
221846	−0.56769	14q24.3–q31	CHES1 | checkpoint suppressor 1
772913	−0.56751	5	*Homo sapiens* cDNA FLJ31951 fis, clone NT2RP7007177, weakly similar to *Homo sapiens* multiple membrane spanning receptor TRC8 mRNA
1686766	−0.56178	6q15–q16	RAGD | Rag D protein
37708	−0.56053	16q24.3	MGC3101 | hypothetical protein MGC3101
825740	−0.56021	2q32.1	DKFZP434J1813 | DKFZp434J1813 protein
741139	−0.56009	20q13.1	EYA2 | eyes absent (Drosophila) homolog 2
754293	−0.55369	2p12	C2orf6 | chromosome 2 open reading frame 6
83363	−0.55322	6q24–q25	PCMT1 | protein-L-isoaspartate (D-aspartate) O-methyltransferase
686552	−0.55207	1q42.13	GOLPH1 | golgi phosphoprotein 1
950429	−0.54962	12q	KIAA1708 | KIAA1708 protein
813419	−0.54843	Xp11.2	HADH2 | hydroxyacyl-Coenzyme A dehydrogenase, type II
2043167	−0.54694	10q25.2–q26.2	BAG3 | BCL2-associated athanogene 3
701115	−0.54546	6	PRO2013 | hypothetical protein PRO2013
795498	−0.54391	15q26.1	HS1-2 | putative transmembrane protein
965223	−0.54333	17q23.2–q25.3	TK1 | thymidine kinase 1, soluble
377191	−0.53874	8p22–q22.1	LOC51123 | HSPC038 protein
233679	−0.53609	2p23.3	FLJ22362 | hypothetical protein FLJ22362
590759	−0.53571	4q32–q34	SC4MOL | sterol-C4-methyl oxidase-like
358083	−0.53534	3q29	KIAA0226 | KIAA0226 gene product
810612	−0.53335	1q21	S100A11 | S100 calcium-binding protein A11 (calgizzarin)
220395	−0.52987	2p22.3	FLJ23293 | likely ortholog of mouse ADP-ribosylation-like factor 6 interacting protein 2
280699	−0.52812	7	UCC1 | upregulated in colorectal cancer gene 1
2016775	−0.52687	16p12	GPRC5B | G protein-coupled receptor, family C, group 5, member B
470124	−0.52331	5p13.2	RAD1 | RAD1 (*S. pombe*) homolog
154707	−0.51926	2p23–p21	MPV17 | MpV17 transgene, murine homolog, glomerulosclerosis
785933	−0.51884	Xp21.1	SRPX | sushi-repeat-containing protein, X chromosome
2062825	−0.51819	20q11.23	KIAA0964 | KIAA0964 protein
2009491	−0.51791	4q22.1–q23	LOC51191 | cyclin-E binding protein 1
1534493	−0.51765	8	ESTs
150003	−0.5167	8q22.2	FLJ13187 | phafin 2
950600	−0.51409	1	*Homo sapiens* mRNA; cDNA DKFZp586C1019 (from clone DKFZp586C1019)
1455394	−0.51333	7p15.2	HCS | cytochrome c
811918	−0.51318	20p12.1	KIAA0952 | KIAA0952 protein
415191	−0.51	2p25.3	KIAA0161 | KIAA0161 gene product
32927	−0.50974	8q24.3	FBXL6 | f-box and leucine-rich repeat protein 6
1845744	−0.50818		
325160	−0.50752	3q13.13	NP25 | neuronal protein
812048	−0.50542	20pter-p12	PRNP | prion protein (p27–30) (Creutzfeld-Jakob disease, Gerstmann-Strausler-Scheinker syndrome, fatal familial insomnia)
84161	−0.50451		DKFZP434F195 | DKFZP434F195 protein
897806	−0.50236	14q21–q24	HIF1A | hypoxia-inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor)
814378	−0.50026	19q13.1	SPINT2 | serine protease inhibitor, Kunitz type, 2
188335	−0.49789		EMR2 | egf-like module containing, mucin-like, hormone receptor-like sequence 2
1585492	−0.49501	9	ESTs
133213	−0.49332	11q21	FUT4 | fucosyltransferase 4 (alpha (1,3) fucosyltransferase, myeloid-specific)
73009	−0.49156	5	*Homo sapiens*, clone MGC: 9628 IMAGE: 3913311, mRNA, complete cds
785707	−0.49031	15q26.1	PRC1 | protein regulator of cytokinesis 1
84464	−0.49025	1q42.12	FLJ12806 | hypothetical protein FLJ12806
770066	−0.48965	11q25	KIAA0056 | KIAA0056 protein
41208	−0.48944	8p21	BMP1 | bone morphogenetic protein 1

TABLE 9-continued

IMAGE Clone ID	Weight	Chromosome Location	Description
1698036	−0.48904	20q13.2	UBE2V1 | ubiquitin-conjugating enzyme E2 variant 1
1435862	−0.4889	Xp22.32	MIC2 | antigen identified by monoclonal antibodies 12E7, F21 and O13
768452	−0.48449	21	*Homo sapiens* EST from clone 491476, full insert
824426	−0.48229	7q22.1	PDAP1 | PDGFA associated protein 1
768561	−0.48129	17q11.2–q21.1	SCYA2 | small inducible cytokine A2 (monocyte chemotactic protein 1, homologous to mouse Sig-je)
377275	−0.48092	11q22–q23	TRIM29 | tripartite motif-containing 29
470128	−0.48	15q21–q22	MYO1E | myosin IE
809901	−0.47984	9q21–q22	COL15A1 | collagen, type XV, alpha 1
50772	−0.47983	7p14–p13	MGC3251 | hypothetical protein MGC3251
1843843	−0.47902	12q14.1	SRGAP1 | KIAA1304 protein
823940	−0.47897	17q21	TOB1 = transducer of ERBB2, 1
564492	−0.47749	11p11.12	MTCH2 | mitochondrial carrier homolog 2
290101	−0.47734	X	ESTs
263894	−0.47704	16p12.1	QPRT | quinolinate phosphoribosyltransferase (nicotinate-nucleotide pyrophosphorylase (carboxylating))
202901	−0.47699	9q34.1	VAV2 | vav 2 oncogene
1607229	−0.47609	6q22–q23	TPD52L1 | tumor protein D52-like 1
812050	−0.47584	8q24	TRC8 | patched related protein translocated in renal cancer
1637756	−0.47545	1p36.3–p36.2	ENO1 | enolase 1, (alpha)
813410	−0.47231	8q22.3	POLR2K | polymerase (RNA) II (DNA directed) polypeptide K (7.0 kD)
358162	−0.47197	11q13.1	HSU79266 | protein predicted by clone 23627
2062238	−0.47184	2q37.1	PSMD1 | proteasome (prosome, macropain) 26S subunit, non-ATPase, 1
753215	−0.47119	7q21	GNAI1 | guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 1
739126	−0.46952	8q24.3	TSTA3 | tissue specific transplantation antigen P35B
1917941	−0.46918	7p13	H2AV | histone H2A.F/Z variant
111362	−0.46893	20q11.2	OSBPL2 | oxysterol-binding protein-like 2
1456348	−0.46742	9p24.1–p23	SAS | N-acetylneuraminic acid phosphate synthase; sialic acid synthase
263716	−0.46636	21q22.3	COL6A1 | collagen, type VI, alpha 1
810156	−0.46594	2	DTYMK | deoxythymidylate kinase (thymidylate kinase)
115443	−0.46519		HSPC216 | hypothetical protein
32299	−0.46427	18p11.2	IMPA2 | inositol(myo)-1(or 4)-monophosphatase 2
1434897	−0.46024	2q14–q32	COL5A2 | collagen, type V, alpha 2
2028916	−0.45905	10	*Homo sapiens* mRNA for Hmob33 protein, 3'untranslated region
2020898	−0.45878	7q22	PLOD3 | procollagen-lysine, 2-oxoglutarate 5-dioxygenase 3
487797	−0.45837	1p22.1	DR1 | down-regulator of transcription 1, TBP-binding (negative cofactor 2)
284734	−0.45795	6q21–q22	WASF1 | WAS protein family, member 1
79520	−0.45632	8q12.1	RAB2 | RAB2, member RAS oncogene family
812977	−0.45368	12	*Homo sapiens* mesenchymal stem cell protein DSC96 mRNA, partial cds
810899	−0.45368	ESTs	
428163	−0.45286	3	ESTs, Weakly similar to NAH6_HUMAN SODIUM/HYDROGEN EXCHANGER 6 [*H. sapiens*]
613056	−0.45261	11p13	RCN1 | reticulocalbin 1, EF-hand calcium binding domain
741474	−0.45249	19q13.1	GPI | glucose phosphate isomerase
768989	−0.45147	14	*Homo sapiens* cDNA FLJ12874 fis, clone NT2RP2003769
754702	−0.45087	2p25.1–p24.1	KIAA0846 | KIAA0846 protein
246800	−0.45055	7p13	FLJ10803 | hypothetical protein FLJ10803
246304	−0.4503	21q21.1	BTG3 | BTG family, member 3

[**0108**] The grade III signature contains genes known to be involved in cell cycle control (CKS2, CDC25B, MCM6), chromosomal segregation (STK15, CENPA and TACC3), and DNA recombination and repair (RAD51, UBE2N, TOP2A, RRM2). In particular, CDC25B, a potential oncogene, transforms murine diploid fibroblasts into high-grade tumors (Galaktionov, K. et al.). STK15, a centrosomal protein kinase, is frequently amplified in breast cancer, and its quantitative expression levels positively correlate with tumor grade (Zhou, H. et al. (1998b)). RAD51 has recently been shown to interact with the tumor suppressor BRCA1 (Chen, J. J., et al. (1999)), and its expression also positively correlates with tumor grade in breast cancer (Maacke, H. et al.). It has not been previously known or suspected, however, whether the expression of these genes would be capable of differentiating grade III breast cancer cells from grade I breast cancer cells. Without being bound by theory, abnormal expression of the genes associated with DNA recombination and repair and those associated with centrosomal function may result in greater genome instability, thus driving the evolution of aggressively growing and high-grade cancer cells. The data thus verified the association of several known genes with breast tumorigenesis and uncovered additional novel associations, which together may underlie the molecular basis of current tumor grading systems in breast cancer.

[**0109**] The question of whether unique gene expression changes are associated with stage progression, specifically, the transition from noninvasive (DCIS) to invasive (IDC) growth, is also addressed by the present invention. The inventors have noticed that these two pathological stages are highly similar to each other with no striking differences at the level of gene expression (FIGS. **2-3**). To increase our sensitivity in detecting differential gene expression between

DCIS and IDC, each IDC sample was compared directly to its corresponding patient-matched DCIS sample where available. 1,688 genes showing at least a 2-fold difference between IDC and DCIS in at least 3 different sample pairs were selected and subjected to unsupervised two-dimensional hierarchical clustering. One prominent cluster of genes demonstrated elevated expression in IDC as compared with DCIS, predominately amongst the grade III IDC samples (**FIG. 4**). These genes, along with their I.M.A.G.E. Consortium CloneID number, along with their chromosomal location and descriptive identifiers (if known) are listed in Table 10.

TABLE 10

IMAGE Clone ID	Chromosome Location Description
795498	15q26.1 HS1-2 | putative transmembrane protein
431505	15q26.1 HS1-2 | putative transmembrane protein
741139	20q13.1 EYA2 | eyes absent (Drosophila) homolog 2
1534592	2p12 C2orf6 | chromosome 2 open reading frame 6
290422	9q13–q21 ZNF216 | zinc finger protein 216
1609836	1q31 GLUL | glutamate-ammonia ligase (glutamine synthase)
505575	2q35 FLJ10116 | hypothetical protein FLJ10116
141852	11q13.5–q14.1 P2RY2 | purinergic receptor P2Y, G-protein coupled, 2
121251	12q13.1 MGC5576 | hypothetical protein MGC5576
610326-10	12q12–12q14.3 K-ALPHA-1 | tubulin, alpha, ubiquitous
725454	9q22 CKS2 | CDC28 protein kinase 2
756502	7p22 NUDT1 | nudix (nucleoside diphosphate linked moiety X)-type motif 1
504308	10cen-q26.11 FLJ1054O | hypothetical protein FLJ10540
2062329	6q13–q21 TTK | TTK protein kinase
564981	18 *Homo sapiens*, Similar to RIKEN cDNA 2810433K01 gene, clone MGC:10200 IMAGE:3909951, mRNA, complete cds
951080	8q24.3 RECQL4 | RecQ protein-like 4
280375	8p22 PRO2000 | PRO2000 protein
530219	8 *Homo sapiens cDNA FLJ32554 fis, clone SPLEN1000106*
594438	1q12–1q21.2 DJ328E19.C1.1 | hypothetical protein
470232	7 ESTs, Weakly similar to |37356 epithelial microtubule-associated protein, 115K [*H.sapiens*]
291057	1p32 CDKN2C | cyclin-dependent kinase inhibitor 2C (p18, inhibits CDK4)
1476053	15q15.1 RAD51 | RAD51 (*S. cerevisiae*) homolog (*E coli* RecA homolog)
121436	2q11.2 MGC4677 | hypothetical protein MGC4677
700792	14q22 CDKN3 | cyclin-dependent kinase inhibitor 3 (CDK2-associated dual specificity phosphatase)
308633	10q23–q24 HELLS | helicase, lymphoid-specific
809588	8q12.1 GGH | gamma-glutamyl hydrolase (conjugase, folylpolygammaglutamyl hydrolase)
1455394	7p15.2 HCS | cytochrome c
796694	17q25 BIRC5 | baculoviral IAP repeat-containing 5 (survivin)
2018131	12p13.2–p13.1 RACGAP1 | Rac GTPase activating protein 1
1587847	2q21 MCM6 | minichromosome maintenance deficient (mis5, *S. pombe*) 6
743810	12p13 MGC2577 | hypothetical protein MGC2577
744047	16p12.3 PLK | polo (Drosophia)-like kinase
705064	4p16.3 TACC3 | transforming, acidic coiled-coil containing protein 3
1518591	
810899	ESTs
2018976	5q35.1 PTTG1 | pituitary tumor-transforming 1
2017415	2p24–p21 CENPA | centromere protein A (17kD)
815501	19p13.3 MGC2721 | hypothetical protein MGC2721
624627	2p25–p24 RRM2 | ribonucleotide reductase M2 polypeptide
1422338	2p25–p24 RRM2 | ribonucleotide reductase M2 polypeptide
610326-8	12q12–12q14.3 K-ALPHA-1 | tubulin, alpha, ubiquitous
79761	12q22 TMPO | thymopoietin
610326-2	12q12–12q14.3 K-ALPHA-1 | tubulin, alpha, ubiquitous
610326-4	12q12–12q14.3 K-ALPHA-1 | tubulin, alpha, ubiquitous
610326-3	12q12–12q14.3 K-ALPHA-1 | tubulin, alpha, ubiquitous
1476065	1p36.1–p35 STMN1 | stathmin 1/oncoprotein 18
293785	11 ESTs, Weakly similar to A46010 X-linked retinopathy protein [*H.sapiens*]
47781	17 TEM7 | tumor endothelial marker 7 precursor
415102	5q31 CDC25C | cell division cycle 25C
869375	15q26.1 IDH2 | isocitrate dehydrogenase 2 (NADP+), mitochondrial
951241	15q13.3 ANKT | nucleolar protein ANKT
814270	4q27 PMSCL1 | polymyositis/scleroderma autoantigen 1 (75kD)
785368	8p21–p12 TOPK | PDZ-binding kinase; T-cell originated protein kinase
66406	2 ESTs, Highly similar to T47163 hypothetical protein DKFZp762E1312.1 [*H.sapiens*]
292936	1p34.3 FLJ10468 | hypothetical protein FLJ10468
1517595	9p11.2 KIAA0175 | likely ortholog of maternal embryonic leucine zipper kinase
128711	7p15–p14 ANLN | anillin (Drosophila Scraps homolog), actin binding protein
200402	20q11.22–q12 DJ616B8.3 | hypothetical protein dJ616B8.3
825470	17q21–q22 TOP2A | topoisomerase (DNA) II alpha (170kD)
769890	14q13.1 NP | nucleoside phosphorylase
796469	1q32.1 HSPC15O | HSPC150 protein similar to ubiquitin-conjugating enzyme
531319	17p13.1 STK12 | serine/threonine kinase 12
1416055	8 KIAA0165 | extra spindle poles, *S. cerevisiae*, homolog of

TABLE 10-continued

IMAGE Clone ID	Chromosome Location Description
769921	20q13.12 UBE2C | ubiquitin-conjugating enzyme E2C
770992	
839682	12q22 UBE2N | ubiquitin-conjugating enzyme E2N (homologous to yeast UBC13)
840364	20cen-q13.1 AHCY | S-adenosylhomocysteine hydrolase
276915	20q11.2 DNMT3B | DNA (cytosine-5-)-methyltransferase 3 beta

[0110] Interestingly, many of the genes in this cluster have been identified already within the grade III signature cluster (**FIG. 3**). These include genes involved in the cell cycle (e.g., MCM6, TOP2A, CKS2, CDC25C), centrosomal function (TACC3, CENPA), and DNA repair (RAD51, RRM2). Thus, a subset of genes that are expressed at high levels in grade III DCIS are further elevated in IDC, suggesting an intriguing link between the two lines of cancer progression, i.e., tumor grade and invasion. Indeed, and without being bound by theory, RRM2, the M2 subunit of ribonucleotide reductase (RR), which catalyzes a rate-limiting step in DNA synthesis and repair, may play a dual role in both proliferative growth and invasion; overexpression of RRM2 in human cancer cells enhances their invasive potential (Zhou, B. S. et al. (1998c)), whereas its decreased expression inhibits cancer cell proliferation (Chen, S. et al. (2000)). In addition, centrosome amplification (e.g., induced by overexpression of STK15, Zhou et al. 1998b) may result in both high tumor grade and increased invasion potential due to altered cytoskeletal architecture (Lingle, W. L. et al.). However, these genes are not associated with the transition of grade I DCIS to grade I IDC, suggesting that the latter may employ a different mechanism(s) to gain invasion potential.

[0111] Without being bound by theory, and offered for the purposes of improving the understanding of the present invention and its possible applications, the above LCM-derived gene expression profiles of the various phenotypic stages of breast cancer are consistent with a modified model of breast cancer progression (**FIG. 5**). In this model, breast cancer develops along two dimensions, one of which consists of stage transitions from normal to ADH to DCIS to IDC and another consists of tumor grade progression from grade I to II to III. This model is supported by existing histopathological and clinical data (see Dupont, W. D. et al.; Marshall, L. M. et al.; Betsill, W. L. et al.; and Page, D. L. et al. (1982)) and the following lines of evidence presented above. First, extensive changes in gene expression occur in ADH and persist in DCIS/IDC, suggesting a molecular linkage between ADH and DCIS/IDC. Second, the identified 200 genes whose expression levels quantitatively correlate with tumor grade progression in both DCIS and IDC indicate a transcriptional continuum from low to high-grade tumors. Finally, grade III DCIS and IDC differ quantitatively in the expression of the same genes associated with tumor grade progression. It is thus proposed that the various subtypes (e.g., ER+ and ER− subtypes) of breast cancer represent snapshots of this two-dimensional progression scheme; for example (and without limiting the invention), during the progression from grade I through grade III, ER-positive lesions evolve into ER-negative ones. The present invention thus provides the identity, and thus sequences, of various genes associated with the initiation and progression of breast cancer, and so provides for novel diagnostic, preventative and therapeutic strategies for women with breast cancer. This includes the ability to utilize the grade of DCIS/IDC breast cancer, irrespective of which stage of breast cancer is actually present, as a criterion for decisions concerning breast cancer diagnosis and treatment.

[0112] The following Table 11 summarizes the contents of Tables 2-10

TABLE 11

Table	Description
2	Genes with elevated expression in ADH and persisting through DCIS and IDC cells compared to normal cells
3	Genes with highest expression in grade III DCIS or IDC cells
4	Genes with decreased expression in ADH, DCIS and IDC cells compared to normal cells
5	Genes correlated with grade I and III samples and decreased expression in all samples
6	Genes with increased expression in grade III (DCIS and/or IDC) samples
7	Genes with increased expression in grade I (DCIS and/or IDC) samples
8	250 genes with increased expression in grade I (DCIS and/or IDC) samples
9	250 genes with increased expression in grade III (DCIS and/or IDC) samples
10	Genes with quantitative differences in expression between DCIS and IDC samples

[0113] Having now generally described the invention, the same will be more readily understood through reference to the following examples which are provided by way of illustration, and are not intended to be limiting of the present invention, unless specified.

EXAMPLES

Example I

[0114] Materials and Methods

[0115] Clinical specimen collection and clinicopathological parameters. All breast specimens were obtained from the Massachusetts General Hospital between 1998 and 2001. Thirty-six breast cancer patients were selected, 31 of which were diagnosed with two or more pathological stages of breast cancer progression, and 5 of which were diagnosed with pre-invasive disease only. Three healthy women who underwent elective mammoplasty reduction were selected as disease-free normal controls. Tissue specimens that demonstrated one or more pathological lesions (ADH, DCIS and IDC) were selected for the study. Cases of ADH were selected as proliferative epithelial lesions that possessed some, but not all, of the features of carcinoma in situ (Page,

D. L. et al. (1992)) and most closely resemble those lesions described as CAPSS (Oyama, T. et al. and Fraser, J. L. et al.). DCIS and IDC were classified (histological grade) according to the European classification (Holland, R. et al.) and by the Nottingham combined histological grade (Elston, C. W. et al.), respectively. ER and PR expression were determined by immunohistochemical staining (negative when none of the tumor cell nuclei showed staining), and Her-2 expression determined by immunohistochemistry or FISH. This study was approved the Massachusetts General Hospital human research committee in accordance with NIH human research study guidelines.

[0116] LCM and RNA isolation and amplification. Each component (Normal, ADH, DCIS or IDC) was laser capture microdissected in triplicate (from consecutive tissue sections) as described (Sgroi et al.) using a PixCell II LCM system (Arcturus Engineering Inc., Mountain View, Calif.). Total RNA was extracted from the captured cells using the Picopure™ RNA Isolation Kit (Arcturus). T7-based RNA amplification was carried out using the RiboAmp™ kit (Arcturus). Briefly, the RNA from each sample was primed with an oligo-dT primer containing a T7 promoter sequence, reverse transcribed and then converted to double stranded cDNA. The cDNA templates were then used in an in vitro transcription reaction using T7 RNA polymerase to generate amplified RNA (aRNA). To obtain enough aRNA for a microarray experiment, a second round of RNA amplification was performed on all samples. To serve as reference in microarray hybridizations, a human universal reference RNA from Stratagene (La Jolla, Calif.) was amplified identically.

[0117] Fabrication of microarrays. Sequence-verified human cDNA clones were obtained from Research Genetics (Huntsville, Ala.). cDNA clones (from the I.M.A.G.E. Consortium via Research Genetics) inserts were amplified by PCR, gel-purified, and spotted onto a 1×3-inch SuperAmine™ (TeleChem International, Sunnyvale, Calif.) glass microscope slide using an OmniGrid™ robotic arrayer (GeneMachines, San Carlos, Calif.). As used herein, the I.M.A.G.E. Consortium CloneID, or the IMAGE CloneID, lists the identifiers of the cDNA clones on the microarrays according to the I.M.A.G.E. Consortium and Research Genetics (www.resgen.com/). This provides a unique single identifier for each clone. Descriptive names of clones (or genes) use the UniGene symbols and titles (www.ncbi.nlm-.nih.gov/UniGene/).

[0118] Probe labeling and hybridization. cDNA was transcribed from aRNA in the presence of 5-(3-aminoallyl)-2'-deoxyuridine 5'-triphosphate (aminoallyl dUTP) using Stratagene's FairPlay kit™ (La Jolla, Calif.). Cy3 or Cy5 monoreactive dye (Amersham, Piscataway, N.J.) was conjugated onto purified cDNA and the residual dye was removed using QiaQuick PCR Purification columns (Qiagen, Valencia, Calif.). Each Cy5-labeled cDNA was hybridized together with the Cy3-labeled reference probe to a microarray in 40 μL hybridizationr solution (5×SSC, 0.1 μg/pL COT I, 0.2% SDS, 50% formamide) at a concentration of 25 ng/μL per channel for 17 hrs at 42° C. in >60% relative humidity.

[0119] Washing, scanning and image analysis. After hybridization, slides were washed as follows: 1×SSC, 0.2% SDS at 42° for 5 min (two times), 1×SSC, 0.2% SDS at 55° C. for 5 min, 0.1×SSC, 0.2% SDS at 55° C. for 5 min and 0.1×SSC at RT for 2 min. Washed slides were scanned using ScanArray 5000 (PerkinElmer, Billerica, Mass.), and Cy5/Cy3-signals were quantitated using ImaGene 4.2 (BioDiscovery, Los Angeles, Calif.).

[0120] Data processing. Fluorescent intensities of Cy5 and Cy3 channels on each slide were subjected to spot filtering and normalization. Spots flagged by ImaGene were excluded from further analysis. Normalization was performed using a robust nonlinear local regression method (Yang, Y. H. et al.). The normalized ratios of Cy5/Cy3 were used to represent the relative gene expression levels in the experimental samples. Measurements from replicate samples were averaged after normalization.

[0121] Cluster and discriminant analysis. Hierarchical cluster analysis was performed in GeneMaths (v1.5, Applied-Maths, Austin, Tex.) using the cosine correlation coefficient as a measure of similarity between two genes or samples and complete linkage. Linear discriminant analysis with variance was performed within GeneMaths.

Example II

[0122] Genes Showing Significant Differences in the Pair-Wise Comparisons of Normal vs. ADH, Normal vs. DCIS and Normal vs. IDC by Linear Discriminant Analysis

[0123] 2-3 independent LCM captures were made from the same breast biopsy for each disease state (normal, ADH, DCIS or IDC), and RNA from each capture was amplified, labeled, and hybridized to 2 identical 12,000-element microarrays, resulting in from 4 to 6 data points per gene per disease state. The replicate data points were averaged to represent the expression level of each gene at each cellular state, which was further transformed as data points which are the log2 value of the ratio of data from patient matched disease/normal samples or the log2 value of the ratio of data from patient matched IDC/DCIS samples.

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[0163] All references cited herein, including patents, patent applications, and publications, are hereby incorporated by reference in their entireties, whether previously specifically incorporated or not.

[0164] Having now fully described this invention, it will be appreciated by those skilled in the art that the same can be performed within a wide range of equivalent parameters, concentrations, and conditions without departing from the spirit and scope of the invention and without undue experimentation.

[0165] While this invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications. This application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth.

We claim:

1. An array comprising polynucleotide probes, capable of hybridizing to nucleic acid molecules of more than one of the genes listed in Table 2, hybridized to nucleic acids derived from a cell suspected of being non-normal.

2. The array of claim 1 comprising 3-11 of the genes.

3. The array of claim 2 comprising 5-11 of the genes.

4. The array of claim 3 comprising all 11 genes.

5. The array of claim 1 wherein said cell is from a subject afflicted with, or suspected of having, breast cancer.

6. The array of claim 1 wherein said cell is a human cell.

7. The array of claim 1 wherein said nucleic acids derived from a cell are prepared by quantitative PCR.

8. The array of claim 1 wherein at least one polynucleotide probe hybridizes to CRIP1 nucleic acid molecules.

9. The array of claim 1 wherein said non-normal cells are selected from ADH, DCIS, and IDC.

10. An array comprising polynucleotide probes, capable of hybridizing to nucleic acid molecules of more than one of the genes in Table 3, hybridized to nucleic acids derived from a cell suspected of being DCIS or IDC.

11. The array of claim 10 comprising 3-9 of the genes.

12. The array of claim 11 comprising 5-9 of the genes.

13. The array of claim 12 comprising all 9 genes.

14. The array of claim 10 wherein said cell is from a subject afflicted with, or suspected of having, breast cancer.

15. The array of claim 10 wherein said cell is a human cell.

16. The array of claim 10 wherein said nucleic acids derived from a cell are prepared by quantitative PCR.

17. An array comprising polynucleotide probes, capable of hybridizing to nucleic acid molecules of more than one of the genes in Table 4, hybridized to nucleic acids derived from a cell suspected of being non-normal.

18. The array of claim 17 comprising 3-9 of the genes.

19. The array of claim 18 comprising 5-9 of the genes.

20. The array of claim 19 comprising all 9 genes.

21. The array of claim 17 wherein said cell is from a subject afflicted with, or suspected of having, breast cancer.

22. The array of claim 17 wherein said cell is a human cell.

23. The array of claim 17 wherein said nucleic acids derived from a cell are prepared by quantitative PCR.

24. The array of claim 17 wherein at least one polynucleotide probe hybridizes to ELF5 nucleic acid molecules.

25. The array of claim 17 wherein said non-normal cells are selected from ADH, DCIS, and IDC

26. A method to determine the presence, grade or stage of breast cancer cells in a sample from a subject comprising assaying said sample for expression of one or more genes in Table 2 and/or one or more genes in Table 4.

27. The method of claim 26 wherein said assaying comprises preparing RNA from said sample.

28. The method of claim 27 wherein said RNA is used for quantitative PCR.

29. The method of claim 28 wherein said assaying comprises using an array.

30. The method of claim 26 wherein said sample is a ductal lavage or fine needle aspiration sample.

31. The method of claim 30 wherein said sample is microdissected to isolate one or more cells suspected of being breast cancer cells.

32. The method of claim 26 wherein said non-normal cells are selected from ADH, DCIS, and IDC.

33. A method to determine the presence, grade or stage of breast cancer cells in a sample from a subject comprising

assaying said sample for expression of one or more genes selected from Table 2 and/or Table 4 and

identifying said sample as containing non-normal breast cancer cells, optionally without grading by histomorphological criteria.

34. The method of claim 33 wherein said assaying comprises preparing RNA from said sample.

35. The method of claim 34 wherein said RNA is used for quantitative PCR.

36. The method of claim 33 wherein said assaying comprises using an array.

37. The method of claim 33 wherein said sample is a ductal lavage or fine needle aspiration sample.

38. The method of claim 37 wherein said sample is microdissected to isolate one or more cells suspected of being breast cancer cells.

39. The method of claim 33 wherein said non-normal cells are selected from ADH, DCIS, and IDC.

40. A method to determine the presence of DCIS and/or IDC breast cancer cells of grade III in a sample from a subject comprising

assaying said sample for expression of one or more genes selected from Table 3, Table 6, and/or Table 9 and

identifying said sample as containing breast cancer cells of grade III, optionally without grading by histomorphological criteria.

41. The method of claim 40 wherein said assaying comprises preparing RNA from said sample.

42. The method of claim 41 wherein said RNA is used for quantitative PCR.

43. The method of claim 40 wherein said assaying comprises using an array.

44. The method of claim 40 wherein said sample is a ductal lavage or fine needle aspiration sample.

45. The method of claim 44 wherein said sample is microdissected to isolate one or more cells suspected of being breast cancer cells.

46. A method to determine the grade of either DCIS or IDC cancer progression in a sample from a subject comprising assaying said sample for expression of one or more genes listed in Table 10.

47. The method of claim 46 wherein said assaying comprises preparing RNA from said sample.

48. The method of claim 47 wherein said RNA is used for quantitative PCR.

49. The method of claim 46 wherein said assaying comprises using an array.

50. The method of claim 49 wherein said sample is a ductal lavage or fine needle aspiration sample.

51. The method of claim 50 wherein said sample is microdissected to isolate one or more cells suspected of being breast cancer cells.

52. A method to determine the presence of DCIS and/or IDC breast cancer cells of grade I in a sample from a subject comprising

assaying said sample for expression of one or more genes selected from Table 7 and/or Table 8 and

identifying said sample as containing breast cancer cells of grade I, optionally without grading by histomorphological criteria.

53. The method of claim 52 wherein said assaying comprises preparing RNA from said sample.

54. The method of claim 53 wherein said RNA is used for quantitative PCR.

55. The method of claim 52 wherein said assaying comprises using an array.

56. The method of claim 55 wherein said sample is a ductal lavage or fine needle aspiration sample.

57. The method of claim 56 wherein said sample is microdissected to isolate one or more cells suspected of being breast cancer cells.

58. A method to determine the presence of DCIS and/or IDC breast cancer cells of grade I and/or grade III in a sample from a subject comprising assaying said sample for decreased expression of one or more genes selected from Table 5.

59. The method of claim 58 wherein said assaying comprises preparing RNA from said sample.

60. The method of claim 59 wherein said RNA is used for quantitative PCR.

61. The method of claim 58 wherein said assaying comprises using an array.

62. The method of claim 58 wherein said sample is a ductal lavage or fine needle aspiration sample.

63. The method of claim 62 wherein said sample is microdissected to isolate one or more cells suspected of being breast cancer cells.

64. An array comprising polynucleotide probes, capable of hybridizing to nucleic acid molecules of more than one of the genes listed in Table 5, hybridized to nucleic acids derived from a cell suspected of being DCIS or IDC.

65. The array of claim 41 comprising 3-11 of the genes.

66. The array of claim 64 wherein said cell is from a subject afflicted with, or suspected of having, breast cancer.

67. The array of claim 64 wherein said cell is a human cell.

68. The array of claim 64 wherein said nucleic acids derived from a cell are prepared by quantitative PCR.

69. An array comprising polynucleotide probes, capable of hybridizing to nucleic acid molecules of more than one of the genes listed in Table 6 and/or Table 9, hybridized to nucleic acids derived from a cell suspected of being grade III DCIS and/or IDC.

70. The array of claim 69 comprising 3-11 of the genes.

71. The array of claim 69 wherein said cell is from a subject afflicted with, or suspected of having, breast cancer.

72. The array of claim 69 wherein said cell is a human cell.

73. The array of claim 69 wherein said nucleic acids derived from a cell are prepared by quantitative PCR.

74. An array comprising polynucleotide probes, capable of hybridizing to nucleic acid molecules of more than one of the genes listed in Table 7 and/or Table 8, hybridized to nucleic acids derived from a cell suspected of being grade I DCIS and/or IDC.

75. The array of claim 74 comprising 3-11 of the genes.

76. The array of claim 74 wherein said cell is from a subject afflicted with, or suspected of having, breast cancer.

77. The array of claim 74 wherein said cell is a human cell.

78. The array of claim 74 wherein said nucleic acids derived from a cell are prepared by quantitative PCR.

79. An array comprising polynucleotide probes, capable of hybridizing to nucleic acid molecules of more than one of the genes listed in Table 10, hybridized to nucleic acids derived from a cell suspected of being DCIS or IDC.

80. The array of claim 79 comprising 3-11 of the genes.

81. The array of claim 79 wherein said cell is from a subject afflicted with, or suspected of having, breast cancer.

82. The array of claim 79 wherein said cell is a human cell.

83. The array of claim 79 wherein said nucleic acids derived from a cell are prepared by quantitative PCR.

84. A method to determine therapeutic treatment for a patient having cells suspected of being non-normal in a sample therefrom comprising

identifying said patient as having grade III DCIS and/or IDC after assaying said cells for expression of more than one gene listed in Table 3 and

selecting the appropriate treatment for a patient having cells of such a grade.

85. The method of claim 84 wherein said assaying comprises preparing RNA from said cells.

86. The method of claim 85 wherein said RNA is used for quantitative PCR.

87. The method of claim 84 wherein said assaying comprises using an array.

88. The method of claim 87 wherein said sample is a ductal lavage or fine needle aspiration sample.

89. The method of claim 88 wherein said sample is microdissected to isolate one or more cells suspected of being breast cancer cells.

90. The method of claim 84 wherein said non-normal cells are selected from ADH, DCIS, and IDC.

91. A method to determine therapeutic treatment for a patient having non-normal cells in a sample therefrom comprising

identifying said patient as having grade III DCIS and/or IDC after assaying said cells for expression of more than one gene listed in Table 6 and/or Table 9, and

selecting the appropriate treatment for a patient having cells of such a grade.

92. The method of claim 91 wherein said assaying comprises preparing RNA from said cells.

93. The method of claim 92 wherein said RNA is used for quantitative PCR.

94. The method of claim 91 wherein said assaying comprises using an array.

95. The method of claim 91 wherein said sample is a ductal lavage or fine needle aspiration sample.

96. The method of claim 95 wherein said sample is microdissected to isolate one or more cells suspected of being breast cancer cells.

97. The method of claim 91 wherein said non-normal cells are selected from ADH, DCIS, and IDC.

98. A method to determine therapeutic treatment for a patient having non-normal cells in a sample therefrom comprising

identifying said patient as having grade I DCIS and/or IDC after assaying said cells for expression of more than one gene listed in Table 7 and/or Table 8, and

selecting the appropriate treatment for a patient having cells of such a grade.

99. The method of claim 98 wherein said assaying comprises preparing RNA from said cells.

100. The method of claim 99 wherein said RNA is used for quantitative PCR.

101. The method of claim 98 wherein said assaying comprises using an array.

102. The method of claim 98 wherein said sample is a ductal lavage or fine needle aspiration sample.

103. The method of claim 102 wherein said sample is microdissected to isolate one or more cells suspected of being breast cancer cells.

104. The method of claim 98 wherein said non-normal cells are selected from ADH, DCIS, and IDC.

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