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(54) **PSORIASIN EXPRESSION BY BREAST EPITHELIAL CELLS**

PSORIASIN-EXPRESSION DURCH BRUSTEPITHELZELLEN

EXPRESSION DE LA PSORIASINE PAR LES CELLULES EPITHELIALES MAMMAIRES

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Description

STATEMENT REWARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0001] The research described in this application was supported in part by a grant (No. P50 A89393-01) from the U.S. National Cancer Institute of the U.S. National Institutes of Health. Thus the U.S. government has certain rights in the invention.

TECHNICAL FIELD

[0002] This invention relates to cancer diagnosis, and more particularly to breast cancer diagnosis.

BACKGROUND

[0003] Breast carcinoma is the second leading cause of cancer-related death in women of the western world. In the United States alone over 175,000 new cases are diagnosed annually. The natural history of breast cancer involves a sequential progression through defined clinical and pathologic stages starting with initially benign then atypical hyperproliferation, progressing into in situ then invasive carcinomas, and culminating in metastatic disease. Ductal carcinoma in situ (DCIS) is the precursor of invasive ductal carcinoma. Thus, it is important that there be a reliable test for DCIS.

[0004] In Cancer Research, 56, 4606-4609 [1996] Leygue *et al* discuss differential expression of psoriasin mRNA between *in situ* and invasive breast carcinoma. Comedo and non-comedo type DCIS cases are compared. It is concluded that the functional role of the psoriasin protein in breast tumor cells remains to be determined.

[0005] WO 00/26668 is an international patent application that is entitled "S100 proteins and auto-antibodies as serum markers for cancer". The proteins S100-Ag, S100-A7, S100-A8 and S 100-A9 are discussed. DCIS is not mentioned.

SUMMARY

[0006] The invention is based on the observation that human high grade DCIS cells express elevated levels of a protein designated by the inventors HID-5 (high in DCIS-5). This protein is also known as psoriasin. Low and intermediate DCIS express the HID-5 gene at a very low level, if at all. In addition, the inventors discovered that HID-5 is secreted by breast cancer cells. Thus, the invention features methods useful in diagnosing high grade DCIS.

[0007] According to the present invention there is provided a method for assessing whether or not a subject has high grade ductal corcirona in situ (DCIS), said method comprising:

(a) identifying a subject suspected of having or at risk of having high-grade DCIS and
(b) measuring the level of psoriasin or psoriasin gene expression in a sample of a body fluid, a lavage, an aspirate, a cell culture supernatant or a breast tissue sample from the subject;

wherein said method includes determining whether or not said level is above the level of psoriasin or psoriasin gene expression that would be present in such a sample in respect of a subject with intermediate grade DCIS or low grade DCIS.

[0008] The invention also provides a method of discriminating high grade DCIS from intermediate grade DCIS or low grade DCIS, the method comprising measuring the level of psoriasin or psoriasin gene expression in a sample of a body fluid, a lavage, an aspirate,a cell culture supernatant or a breast tissue sample from a subject identified as having DCIS, wherein said method includes determining whether or not said level is above the level that would be present in such a sample in respect of a subject with intermediate grade DCIS or low grade DCIS.

[0009] Various optional features are set out in claims 3 to 10.

[0010] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. In case of conflict, the present document, including definitions, will control. Preferred methods and materials are described below. The materials, methods, and examples disclosed herein are illustrative only and not intended to be limiting.

DESCRIPTION OF DRAWINGS

[0011]

Fig. 1A is a bar graph showing the results of a real-time PCR analysis of HID-5/psoriasin mRNA expression in laser

capture microdissection (LCM) purified primary breast carcinomas and corresponding normal breast epithelium. The data are expressed as the ratio of the HID-5/psoriasin mRNA level in cancerous epithelium to the HID-5/psoriasin mRNA level in the corresponding normal epithelium ("T/N ratio"). Each bar is labeled with the case number and acronym indicating whether the carcinoma was in situ ("is") or invasive ("inv"). Whether the carcinoma was high ("H"), intermediate ("I"), or low ("L") grade and expression ("+") and lack of expression ("-") of estrogen receptor α ("ERα"), progesterone receptor ("PR"), and erbB2 are shown.

Fig. 1B is a series of photomicrographs of histological sections of two high grade comedo DCIS tumors ("DCIS-1" and "DCIS-2") that were stained with hemotoxylin and eosin ("H&E") or subjected *to in situ* hybridization analysis with ^{33}P-labeled antisense or sense HID-5/psoriasin riboprobes. The samples analyzed with the antisense riboprobe were photographed under "Bright-field" and "Dark-field" conditions using objective lens magnifications of 4x and 20x. The samples analyzed with the sense riboprobe were photographed under "Dark-field" conditions only using an objective lens magnification of 20x. Similar *in situ* hybridization analyses of two low and two intermediate grade DCIS tumors failed to detect HID-5/psoriasin mRNA in any of the samples,

Fig. 2A is a pair of photographs of immunoblots showing the specificity of a polyclonal anti-HID-5/psoriasin antibody (left panel) and four individual monoclonal anti-HID-5/psoriasin antibodies (right panel). Lysates of HID-5/psoriasin-expressing ("H") and control HID-5/psoriasin-non-expressing ("C") cells were resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and the resulting gels were blotted onto membranes that were stained with control pre-immune serum ("P.I."), polyclonal anti-HID-5/psoriasin antibody ("αHID5"), or the four monoclonal anti-HID-5/psoriasin antibodies ("Cl 1", "Cl 2", "Cl 3", and "Cl 4"). The lane labeled "M" shows the positions of 17kDa and 7kDa molecular weight markers. The positions of HID-5/psoriasin in both panels are indicated ("HID-5/Psoriasin").

Fig. 2B is a series of three photographs of immunoblots showing the relative levels of HID-5/psoriasin protein expression by MCF10A cells grown in culture medium containing a low ("0.2% serum medium") or high ("5% serum medium") concentration of serum and under confluent ("Confluent cells") or sparse ("Sparse cells") culture conditions. Cells were tested for HID-5/psoriasin protein expression after 0, 2, 4, 8,12, and 16 days in culture. The blots were generated as described for Fig. 2A and stained with a polyclonal antibody specific for HID-5/psoriasin. The positions of HID-5 ("HID-5/Psoriasin") and a control protein ("β-tubulin") on the immunoblots are indicated.

Fig. 2C is a photograph of an immunoblot showing the relative levels of HID-5/psoriasin protein expressed by MCF10A cells grown in suspension culture for 0,1,2, and 3 days. The blots were generated as described for Fig. 2A and stained with a polyclonal antibody specific for HID-5/psoriasin. The positions of HID-5 ("HID-5/Psoriasin") and a control protein ("β-tubulin") on the immunoblot are indicated.

Fig. 2D is a photograph of an autoradiogram from a Northern blot showing the relative levels of HID-5/psoriasin mRNA expressed by MCF10A cells grown in suspension culture ("Suspension") for 1, 2, and 3 days or confluent culture conditions ("Confluency") for 4, 8, and 12 days. RNA was isolated from the cells at the indicated times and the RNA was subjected to Northern blot analysis as previously described [Krop et al. (2001) Proc. Natl. Acad. Sci., U.S.A. 98:9796-9801]. The blots were analyzed sequentially with ^{32}P-labeled HID-5/psoriasin and β-actin cDNA probes and the positions of HID-5 ("HID-5/Psoriasin") and β-actin on the blots are shown.

Fig. 3A is a series of photomicrographs of cultures of MDA-MB-468 breast cancer cells (left panel) and MCF10A normal breast epithelial cells growing exponentially ("Exp. growing") or in the absence of serum ("Serum starved") (right panel). The MDA-MB-468 cells were stained with either an anti-HID-5/psoriasin monoclonal antibody (left panel, bottom photomicrographs) or control normal mouse serum (left panel, top photomicrographs). The MCF10A cells were stained with an anti-HID-5/psoriasin monoclonal antibody. No staining was seen in MCF10A cells stained with control normal mouse serum. The left photomicrographs were taken at an objective lens magnification of 2x and the right photomicrographs at an objective lens magnification of 10x.

Fig. 3B is a photograph of an immunoblot showing intracellular localization and secretion of HID-5/psoriasin by MDA-MB-468 cells in culture. Total MDA-MB-468 cell lysate ("Total") and proteins immunoprecipitated from lysates ("Cells") or culture supernatant ("Medium") of MDA-MB-468 cells by a polyclonal anti-HID-5/psoriasin antibody ("HID-5") or control pre-immune serum ("P.I.") were resolved by SDS-PAGE and subjected to immunoblot analysis. The position of HID-5 ("HID-5/Psoriasin") on the immunoblot is shown.

Fig. 3C is a series of three photomicrographs of sections of a high grade comedo DCIS lesion that were stained with hemotoxylin and eosin ("H&E") (left photograph), anti-HID-5/psoriasin monoclonal antibody ("HID-5") (right photograph), and control normal mouse serum ("Control") (middle photograph).

Fig. 3D is a series of six photomicrographs of samples from a representative breast tumor in a tissue array stained with hemotoxylin and eosin ("H&E"), monoclonal antibodies specific for HID-5/psoriasin, ERα, erbB2, or CD45 , or control normal mouse serum ("Control").

Fig. 3E is a schematic representation summarizing the results of immunochemical analyses of two tissue arrays. Array 1 was composed of five individual samples of normal breast tissue and 30 individual samples of primary invasive breast carcinomas (ten each of low, intermediate, and high grade) and array 2 was composed of six individual

samples of normal breast tissue, three samples of benign hyperproliferative lesions and 49 samples of primary invasive ductal carcinomas. In array 1, three punches (in horizontal rows) of each tumor sample were affixed to the slide and tumors were grouped according to their histologic grade (low, intermediate, and high grade tumors). Array 1 tumor samples were analyzed for expression of HID-5/psoriasin, ER α (ERα), and erbB2 and for the presence of leukocytes using an antibody specific for CD45, a panleukocyte antigen. In array 2, the first vertical row contained the six samples of normal breast tissue, three samples of benign hyperproliferative lesions, and an empty spot (indicated by the hatched square). Array 2 samples were analyzed for expression of ERα, progesterone receptor (PR), and p53; data from the analysis of HID-5/psoriasin and ERα expression are shown. Intensity of staining is indicated by the intensity of shading, with white representing no detectable staining and black being very intense staining. Hatched rectangles represent either empty spots on the arrays or samples lost during the staining procedure.
Fig. 4A is a depiction of the amino acid sequence (SEQ ID NO:1) of HID-5/psoriasin.
Fig. 4B is a depiction of nucleotide sequence (SEQ ID NO:2) of HID-5/psoriasin.

DETAILED DESCRIPTION

[0012] The inventors discovered by Serial Analysis of Gene Expression (SAGE) that HIND-5/psoriasin is differentially and highly expressed in high grade DCIS cells relative to normal breast epithelium and intermediate grade DCIS. Chromosome spread and interphase nuclear FISH analysis indicated that the increased expression of HID-5/psoriasin in the high grade DCIS cells was not due to gene amplification. A real time PCR analysis of a panel of primary breast cancers indicated the presence of higher levels of HID-5/psoriasin in mRNA in high and intermediate grade tumors than in normal mammary epithelium from the same patient. By mRNA *in situ* hybridization, HID-5/psoriasin mRNA was detected in cells of high grade but not low or intermediate grade DCIS cells or normal mammary epithelium.

[0013] *In vitro* experiments with normal mammary epithelial MCF10A cells indicated that:

(a) HID-5/psoriasin protein expression was greatly up-regulated by growing the cells in medium containing a low concentration of serum, under confluent conditions and in suspension; and
(b) HID-5/psoriasin mRNA expression was greatly up-regulated by growing the cells under confluent conditions and in suspension. Since serum deprivation,confluency and lack of cell anchorage also resulted in G1 arrest and apoptosis, cells surviving these conditions are likely to be relatively resistant to apoptosis. Thus HID-5/psoriasin is likely involved in regulation of G1 arrest and relative resistance to apoptosis.

[0014] Nuclear and cytoplasmic staining by HID-5/psoriasin-specific antibody was seen in MDA-MB-468 breast cancer cells and serum deprived MCF10A cells. Moreover testing of cell lysates and culture medium of MDA-MB-468 cells indicated that HID-5/psoriasin is both expressed intracellularly and secreted.

[0015] Immunochemical analyses indicated enhanced expression of HID-5/psoriasin in a significant number of high grade breast cancers versus low and intermediate grade breast cancers and normal mammary epithelium.

[0016] These data provide the bases for the methods of the invention.

[0017] The invention features diagnostic assays. Such assays are based on the findings that:

(1) high grade DCIS cells express high levels of HID-5/psoriasin protein and HID-5/psoriasin mRNA while normal breast cells and low and intermediate grade breast cancer cells express either significantly lower levels or undetectable levels of HID-5/psoriasin protein and HID-5/psoriasin mRNA; and (2) HID-5/psoriasin protein is secreted by breast cancer cells. These findings provide the bases for assays to diagnose high grade DCIS. Such assays can be used on their own or, preferably, in conjunction with other procedures to test for high grade DCIS.

[0018] In the assays of the invention either: (1) the presence of HID-5/psoriasin protein or HID-5/psoriasin mRNA in is tested for or their levels are measured; or (2) the level of HID-5/psoriasin protein is measured in a liquid sample such as a body fluid (e.g., urine, saliva, semen, blood, or serum or plasma derived from blood); a lavage such as a breast duct lavage, lung lavage, a gastric lavage, a rectal or colonic lavage, or a vaginal lavage; an aspirate such as a nipple aspirate; or a fluid such as a supernatant from a cell culture. In order to test for the presence, or measure the level, of HID-5/psoriasin mRNA in cells, the cells can be lysed and total RNA can be purified or semi-purified from lysates by any of a variety of methods known in the art. Methods of detecting or measuring levels of particular mRNA transcripts are also familiar to those in the art. Such assays include, without limitation, hybridization assays using detectably labeled HID-5/psoriasin-specific DNA or RNA probes and quantitative or semi-quantitative RT-PCR methodologies employing appropriate HID-5/psoriasin-specific oligonucleotide primers. Additional methods for quantitating mRNA in cell lysates include RNA protection assays and serial analysis of gene expression (SAGE). Alternatively, qualitative, quantitative, or semi-quantitative *in situ* hybridization assays can be carried out using, for example, tissue sections or unlysed cell suspensions, and detectably (e.g., fluorescently or enzyme) labeled DNA or RNA probes.

[0019] Methods of detecting or measuring the levels of a protein of interest (e.g., HID-5/psoriasin) in cells are known in the art. Many such methods employ antibodies (e.g., polyclonal antibodies or mAbs) that bind specifically to the protein. In such assays, the antibody itself or a secondary antibody that binds to it can be detectably labeled. Alternatively, the antibody can be conjugated with biotin, and detectably labeled avidin (a protein that binds to biotin) can be used to detect the presence of the biotinylated antibody. Combinations of these approaches (including "multi-layer" assays) familiar to those in the art can be used to enhance the sensitivity of assays. Some of these assays (e.g., immunohistological methods or fluorescence flow cytometry) can be applied to histological sections or unlysed cell suspensions. The methods described below for detecting HID-5/psoriasin in a liquid sample can also be used to detect HID-5/psoriasin in cell lysates.

[0020] Methods of detecting HID-5/psoriasin in a liquid sample (see above) basically involve contacting a sample of interest with an antibody that binds to HID-5/psoriasin and testing for binding of the antibody to a component of the sample. In such assays the antibody need not be detectably labeled and can be used without a second antibody that binds to HID-5/psoriasin. For example, by exploiting the phenomenon of surface plasmon resonance, an antibody specific for HID-5/psoriasin bound to an appropriate solid substrate is exposed to the sample. Binding of HID-5/psoriasin to the antibody on the solid substrate results in a change in the intensity of surface plasmon resonance that can be detected qualitatively or quantitatively by an appropriate instrument, e.g., a Biacore apparatus (Biacore International AB, Rapsgatan, Sweden).

[0021] Moreover, assays for detection of HID-5/psoriasin in a liquid sample can involve the use, for example, of: (a) a single HID-5/psoriasin specific antibody that is detectably labeled; (b) an unlabeled HID-5/psoriasin specfic antibody and a detectably labeled secondary antibody; or (c) a biotinylated HID-5/psoriasin 5/psoriasin-specific antibody and detectably labeled avidin. In addition, as described above for detection of proteins in cells, combinations of these approaches (including "multi-layer" assays) familiar to those in the art can be used to enhance the sensitivity of assays. In these assays, the sample or an (aliquot of the sample) suspected of containing HID-5/psoriasin can be immobilized on a solid substrate such as a nylon or nitrocellulose membrane by, for example, "spotting" an aliquot of the liquid sample or by blotting of an electrophoretic gel on which the sample or an aliquot of the sample has been subjected to electrophoretic separation. The presence or amount of HID-5/psoriasin on the solid substrate is then assayed using any of the above-described forms of the HID-5/psoriasin-specific antibody and, where required, appropriate detectably labeled secondary antibodies or avidin.

[0022] The invention also features "sandwich" assays. In these sandwich assays, instead of immobilizing samples on solid substrates by the methods described above, any HID-5/psoriasin that may be present in a sample can be immobilized on the solid substrate by, prior to exposing the solid substrate to the sample, conjugating a second ("capture") HID-5/psoriasin-specific antibody (polyclonal or mAb) to the solid substrate by any of a variety of methods known in the art. In exposing the sample to the solid substrate with the second HID-5/psoriasin-specific antibody bound to it, any HID-5/psoriasin in the sample (or sample aliquot) will bind to the second HID-5/psoriasin-specific antibody on the solid substrate. The presence or amount of HID-5/psoriasin bound to the conjugated second HID-5/psoriasin-specific antibody is then assayed using a "detection" HID-5/psoriasin-specific antibody by methods essentially the same as those described above using a single HID-5/psoriasin-specific antibody. It is understood that in these sandwich assays, the capture antibody should not bind to the same epitope (or range of epitopes in the case of a polyclonal antibody) as the detection antibody. Thus, if a mAb is used as a capture antibody, the detection antibody can be either: (a) another mAb that binds to an epitope that is either completely physically separated from or only partially overlaps with the epitope to which the capture mAb binds; or (b) a polyclonal antibody that binds to epitopes other than or in addition to that to which the capture mAb binds. On the other hand, if a polyclonal antibody is used as a capture antibody, the detection antibody can be either (a) a mAb that binds to an epitope to that is either completely physically separated from or partially overlaps with any of the epitopes to which the capture polyclonal antibody binds; or (b) a polyclonal antibody that binds to epitopes other than or in addition to that to which the capture polyclonal antibody binds. Assays which involve the used of a capture and detection antibody include sandwich ELISA assays, sandwich Western blotting assays, and sandwich immunomagnetic detection assays.

[0023] Suitable solid substrates to which the capture antibody can be bound include, without limitation, the plastic bottoms and sides of wells of microtiter plates, membranes such as nylon or nitrocellulose membranes, polymeric (e.g., without limitation, agarose, cellulose, or polyacrylamide) beads or particles. It is noted that HID-5/psoriasin-specific antibodies bound to such beads or particles can also be used for immunoaffinity purification of HID-5/psoriasin.

[0024] Methods of detecting or for quantifying a detectable label depend on the nature of the label and are known in the art. Appropriate labels include, without limitation, radionuclides (e.g., ^{125}I,^{131}I,^{35}S, ^{3}H, ^{32}P, ^{33}P, or^{14}C), fluorescent moieties (e.g., fluorescein, rhodamine, or phycoerythrin), luminescent moieties (e.g., Qdot™ nanoparticles supplied by the Quantum Dot Corporation, Palo Alto, CA), compounds that absorb light of a defined wavelength, or enzymes (e.g., alkaline phosphatase or horseradish peroxidase). The products of reactions catalyzed by appropriate enzymes can be, without limitation, fluorescent, luminescent, or radioactive or they may absorb visible or ultraviolet light. Examples of detectors include, without limitation, x-ray film, radioactivity counters, scintillation counters, spectrophotometers, colorimeters,

[0025] In assays to diagnose high grade DCIS, the concentration of HID-5/psoriasin in, for example, serum from a patient suspected of having, or at risk of having, high grade DCIS is compared to the mean of the concentrations of HID-5/psoriasin in sera from a control group of subjects, e.g., subjects not having breast cancer, subjects having low grade breast cancer, subjects having intermediate grade breast cancer, or any combination of such subjects. A significantly higher concentration of HID-5/psoriasin in the serum of the patient relative to the mean concentration in sera of the control group would indicate that the patient has high grade DCIS. Alternatively, if a sample of the subject's serum that was obtained at a prior date at which the patient clearly did not have breast cancer is available, the concentration of HID-5/psoriasin in the test serum sample can be compared to the concentration in the prior obtained sample. A higher level in the test serum sample would be an indication that the patient had high grade DCIS.

[0026] It is understood that, while the above descriptions of the diagnostic assays refer to assays on serum, the assays can also be carried out on any of the other fluid samples listed herein. In addition, it is noted that the patients and control subjects referred to above need not be human patients. They can be for example, non-human primates (e.g., monkeys), horses, sheep, cattle, goats, pigs, dogs, guinea pigs, hamsters, rats, rabbits or mice.

[0027] It is understood that, since the SAGE analysis described in Example 2 showed that expression of calgranulin B/S 100A9 and connexin 43 was upregulated in high grade DCIS cells relative to normal breast epithelial cells and intermediate grade DCIS cells, detection and/or measurement of calgranulin B/S 100A9 or connexin 43 expression by test breast cells, by adaption of any of the above-described methods, can be performed to diagnose high grade DCIS.

[0028] The data described below show that the expression of HID-5/psoriasin is upregulated in certain breast cancer patients. Thus, in parents that have the ability to mount an autoimmune response to HID-5/psoriasin, immunization with HID-5/psoriasin or one or more peptide fragments of HID-5/psoriasin could be an effective immunotherapeutic regimen. Without being limited to any particular mechanism of action, therapeutic effect in such a regimen could be due to the action of cytotoxic T lymphocytes (CTL) specific for HID-5/psoriasin peptide fragments or neutralizing antibodies specific for HID-5/psoriasin.

[0029] Antibodies can be polyclonal or monoclonal antibodies; methods for producing both types of antibody are known in the art. The antibodies can be of any class (e.g., IgM, IgG, IgA, IgD, or IgE) and be generated in any of the species recited herein. They are preferably IgG antibodies. Recombinant antibodies specific for HID-5/psoriasin, such as chimeric and humanized monoclonal antibodies comprising both human and non-human portions, can also be used in the methods of the invention. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example, using methods described in Robinson et al., International Patent Publication PCT/US86/02269; Akira et al., European Patent Application 184,187; Taniguchi, European Patent Application 171,496; Morrison et al., European Patent Application 173,494;Neuberger et al., PCT Application WO 86/01533; Cabilly et al., U.S. Patent No. 4,816,567; Cabilly et al., European Patent Application 125,023; Better et al. (1988) Science 240, 1041-43; Liu et al. (1987) J. Immunol. 139, 3521-26; Sun et al. (1987) PNAS 84, 214-18; Nishimura et al. (1987) Canc. Res. 47, 999-1005; Wood et al. (1985) Nature 314, 446-49; Shaw et al. (1988) J. Natl. Cancer Inst. 80, 1553-59; Morrison, (1985) Science 229, 1202-07; Oi et al. (1986) BioTechniques 4, 214; Winter, U.S. Patent No. 5,225,539; Jones et al. (1986) Nature 321,552-25; Veroeyan et al. (1988) Science 239, 1534; and Beidler et al. (1988) J. Immunol. 141, 4053-60.

[0030] Also useful for the invention are antibody fragments and derivatives that contain at least the functional portion of the antigen-binding domain of an antibody that binds to HID-5/psoriasin. Antibody fragments that contain the binding domain of the molecule can be generated by known techniques. Such fragments include, but are not limited to: $F(ab')_2$ fragments that can be produced by pepsin digestion of antibody molecules; Fab fragments that can be generated by reducing the disulfide bridges of $F(ab')_2$ fragments; and Fab fragments that can be generated by treating antibody molecules with papain and a reducing agent. See, e.g., National Institutes of Health, 1 Current Protocols In Immunology, Coligan et al., ed. 2.8, 2.10 (Wiley Interscience, 1991). Antibody fragments also include Fv fragments, i.e., antibody products in which there are few or no constant region amino acid residues. A single chain Fv fragment (scFv) is a single polypeptide chain that includes both the heavy and light chain variable regions of the antibody from which the scFv is derived. Such fragments can be produced, for example, as described in U.S. Patent No. 4,642,334, which is incorporated herein by reference in its entirety. For a human subject, the antibody can be a "humanized" version of a monoclonal antibody originally generated in a different species.

[0031] The invention is illustrated, not limited, by the following examples.

EXAMPLES

Example 1. Materials and Methods

Cell lines and culture conditions

[0032] The MDA-MB468 and MCF10A cell lines were obtained from American Type Culture Collection (ATCC; Manassas, VA) and were maintained in McCoy's medium (Life Technologies, Gaithersburg, MD) containing 10% fetal bovine

serum (FBS) and in DMEM/F12 medium (Life Technologies) containing 5% horse serum and supplemented with 20 ng/ml epidermal growth factor, 100 ng/ml cholera toxin, 0.01 mg/ml insulin, and 500 ng/ml hydrocortisone, respectively. To determine the effect of serum deprivation on HID-5/psoriasin expression in subconfluent or confluent cultures, MCF10A cells were switched to 0.2 % serum containing DMEM/F12 medium and incubated for the indicated time. The effect of confluency was analyzed by maintaining MCF10A cells under confluent conditions for the indicated time with frequent (every other day) medium changes. For suspension cultures, MCF10A cells were trypsinized, resuspended in fresh medium ($1.75x10^5$ cells/ml medium), plated into poly-2-hydroxy-ethylmethacrylate (Aldrich, St. Louis, MO) coated ($1mg/cm^2$ in 100% ethanol) petri dishes, and incubated for the indicated time.

Generation of polyclonal and monoclonal anti-HID-5/psoriasin antibodies

[0033] A rabbit polyclonal anti-HID-5/psoriasin antibody was generated by immunization with a synthetic peptide corresponding to amino acids 83-100 of human HID-5/psoriasin (TDYHKQSHGAAPCSGGSQ) (SEQ ID NO:3). In Fig. 4A is shown the amino acid sequence (SEQ ID NO:1) of full length, human HID-5/psoriasin and in Fig. 4B is shown the nucleotide sequence (SEQ ID NO:2) of cDNA encoding full-length human mature HID-5/psoriasin. For the generation of mouse monoclonal antibodies, a PCR-generated BamHI-HindIII cDNA fragment encoding full-length human HID-5/psoriasin was subcloned into BamHI-HindIII sites of pQE-30 (Qiagen Sciences, Germantown, MD) yielding a construct that encodes HID-5/psoriasin with an N-terminal hexahistidine sequence. The protein was expressed in M15[pREP4] bacteria, purified to homogeneity using denaturing urea buffer and NiNTA beads (Qiagen Sciences). Bound protein was eluted in 50 mM Tris pH 7.5, 500 mM imidazole, 100 mM EDTA, 1 M NaCl, 10% glycerol, 1 mM DTT. In collaboration with Imgenex, San Diego, CA, the protein was used to hyperimmunize BALB/c mice, which provided a source of antibody producing cells for generating HID-5/psoriasin-specific monoclonal antibodies. The resulting anti-HID-5/psoriasin monoclonal antibodies are commercially available from Imgenex.

Western blot analysis, immunohistochemistry, and tissue microarrays

[0034] Western blot analyses of cell lysates and immunohistochemistry were performed using anti-CD45 panleukocyte (Dako, Glostrup, Denmark), anti-estrogen receptor α (ERα), anti-erbB2, and anti-HID-5/psoriasin(clone 1068-1; designated "Cl 1" in the right panel of Fig. 2A) antibodies as previously described [Krop et al. (2001) Proc. Natl. Acad. Sci. U.S.A. 98:9796-9801; Leach et al. (1998) Cancer Res. 56:235-240]. Tissue microarrays were purchased from Imgenex or were generated as previously described [Kononen et al. (1998) Nat. Med. 4:844-847].

Fluorescence in situ hybridization (FISH), real-time PCR, northern blots and mRNA in situ hybridization

[0035] FISH analysis of metaphase chromosome preparations from peripheral blood lymphocytes obtained from normal human males was performed according to a previously described method [Ney et al. (1993) Mol. Cell. Biol. 13: 5604-5612]. Interphase nuclei from disaggregated formalin fixed, paraffin embedded tumor tissue were prepared and FISH was performed according to previously described methods [Kuchinka et al. (19950 Mod. Pathol. 8:183-186]. Metaphase chromosomes and interphase nuclei were counterstained with 4,6-diamidino-2-phenylindole-dihydrochloride (DAPI). Laser capture microdissection, real-time PCR analysis, RNA isolation, and Northern blot analysis were performed as previously described [Krop et al. (2001) Proc. Natl. Acad. Sci. U.S.A. 98:9796-9801]. mRNA *in situ* hybridizations using ^{33}P-labeled sense (control) or anti-sense HID-5 ribo-probes were performed as previously described [Rosen et al. (1999) Mol. Cell. 4:611-617].

Example 2. Genes Aberrantly Expressed in DCIS and Psoriatic Lesions

[0036] The generation of SAGE libraries has been previously described [e.g., Porter et al. (2001) Cancer Res. 61: 5697-5702]. Comparison of SAGE libraries generated from two normal breast epithelial samples ("Normal 1" and "Normal 2"), estrogen receptor (ER)-expressing, intermediate grade DCIS cells ("IM DCIS (ER+)") and ER-non-expressing, high grade DCIS ("HG DCIS (ER)") revealed that HID-5/psoriasin is among the most highly differentially expressed transcripts and is one of the most abundant mRNAs in high grade DCIS (Table 1). In addition to mRNA transcribed from the gene encoding psoriasin mRNA, S100A9, another S100 protein, was also highly expressed in high grade DCIS (Table 1). Both genes are located on the long (q) arm of chromosome 1 and the expression of both is up-regulated in psoriatic keratinocytes.

TABLE 1. Genes aberrantly expressed in psoriatic keratinocytes and high-grade DCIS
Number of SAGE tags detected

SAGE tag	SEQ ID NO:	Gene	Unigene ID	Normal 1	Normal 2	HG DCIS (ER-)	IM DCIS (ER+)
GAGCAGCGCC	4	Psoriasin/S100A7	11.2408	10	0	568	1
GTGGCCACGG	5	Calgranulin B/S100A9	112405	16	16	111	0
TGTTCTGGAG	6	Connexin 43	74471	1	2	28	1
TGGAAGCACT	7	Interleukin 8	624	205	196	4	21
CGAATGTCCT	8	Keratin 6	91539	29	27	0	0
CTATAGCATA	9	Amphiregulin	1257	16	13	0	1
GGCACCTCAG	10	Interleukin 6	93913	17	2	0	0
GTGGCCCACG	11	Interleukin 6 receptor	193400	5	3	0	0
CCTGTAATCC	12	SCCA1	227948	0	0	3	0

SCCA1-Squamous Cell Carcinoma Antigen-1 1

[0037] The chromosomal localization of other highly differentially expressed genes and the expression level of genes implicated in psoriatic lesions was examined (Table 1). Surprisingly, a significant fraction (13 out of 46 reliably mapped genes) of genes specifically overexpressed in high grade DCIS is located on the long arm of chromosome 1 (Table 1). Structural abnormalities of chromosome 1 are among the most frequent cytogenetic abnormalities in breast carcinomas and several genes involved in epidermal differentiation map to chromosome 1q [Volz et al. (1993) Genomics 18:92-99; Tirkkonen et al. (1998) Genes Chrom. Cancer 21:177-184].

[0038] To determine if the overexpression of these 13 genes in high grade DCIS is due to aneuploidy/aneusomy of chromosome 1q, FISH analysis was carried out using as probes two non-overlapping BACs (bacterial artificial chromosomes) containing the psoriasin and the ephrin A4 genes, respectively. The analysis was performed on metaphase spreads from a normal individual and interphase nuclei from the DCIS used for SAGE (data not shown). Following the confirmation of the chromosomal assignment of the HID-5/psoriasin gene to the long arm of chromosome 1 in band q21 by metaphase spread analysis, interphase nuclei from the DCIS tumor tissue were hybridized with the BAC containing the gene (data not shown). Two hybridization signals were noted in 31/33 (94%) nuclei examined, consistent with a normal number of copies for the genomic region tested. This result indicated that the aberrant expression of psoriasin/HID-5 in high grade DCIS lesion is not caused by amplification of the psoriasin/HID-5 locus.

[0039] In addition to psoriasin, several other genes known to be up-regulated in psoriatic keratinocytes were aberrantly expressed in high grade DCIS (Table 1) [Celis et al. (1990) Electrophoresis 11:242-254; Labarthe et al. (1998) J. Invest. Dermatol. 111:72-76; Rivas et al. (1997) J. Invest. Dermatol. 108:188-194). These genes included those encoding S100A9, connexin 43, interleukins 6 and 8, interleukin 6 receptor, amphiregulin, and keratin 6. SCCA1 (squamous cell carcinoma antigen 1) mRNA expression was also slightly up-regulated in high grade DCIS although, due to the low abundance of this mRNA, the detected difference did not reach statistical significance. The aberrant expression of these genes in high grade DCIS and psoriatic keratinocytes could be due to hyperproliferation, abnormal differentiation, or lymphocytic infiltration characteristic of both types of lesions [Bos et al. (1999) Immunol. Today 20:40-46; Page et al. (2000) Curr. Opin. Oncol. 12:526-531].

Example 3. HID-5/Psoriasin Expression in Mammary Epithelial Cells *in vivo* and *in vitro*

[0040] To evaluate the expression of HID-5/psoriasin in primary breast carcinomas, real-time PCR analysis of 11 LCM (Laser Capture Microdissection) purified primary tumors and corresponding normal mammary epithelium samples (Fig. 1A). Thus each tumor sample from a patient was compared to normal mammary epithelium from the same patient. In all cases, except for an in situ, ER-expressing, progesterone receptor-expressing low grade lesion (sample 57) and an invasive, ER-expressing, progesterone receptor expressing, intermediate grade lesion (sample 65), HID-5/psoriasin mRNA levels were significantly (≥10 fold) increased relative to corresponding normal mammary epithelium (Fig. 1A).

[0041] To confirm HID-5/psoriasin expression in high grade DCIS epithelial cells at the cellular level, a mRNA *in situ* hybridization analysis of two low, two intermediate, and two high grade DCIS tumors and corresponding normal epithelium was performed (Fig. 1B and data not shown). HID-5/psoriasin is highly and specifically expressed by the tumor cells of the two high grade comedo DCIS (Fig. 1B). In contrast, no hybridization signal was detected in low and intermediate grade DCIS and normal mammary epithelial cells (Fig.1B and data not shown).

[0042] To analyze the expression of HID-5/psoriasin protein, polyclonal and monoclonal antibodies specific for human HID-5/psoriasin were generated and characterized. Both the polyclonal antibody and the monoclonal antibodies bound to recombinant and the endogenous HID-5/psoriasin proteins migrating as a~11 kDa single band in sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Fig. 2A). A series of experiments were performed to test whether HID-5/psoriasin expression can be detected under various growth conditions in MCF10A cells. MCF10A cells are normal immortalized human mammary epithelial cells that demonstrate no (or a very low level of) HID-5/psoriasin expression in sparse, exponentially growing cultures. In order to mimic the conditions likely to occur *in vivo* in high grade DCIS and psoriatic skin lesions, the MCF10A cells were cultured in medium serum containing a low concentration of serum (0.2% versus high (5%)) and under confluent (versus sparse) conditions. Culture of the cells in a low concentration of serum and under confluent conditions (regardless of the serum concentration) led to dramatic up-regulation of HID-5/psoriasin protein levels (Fig. 2B). The highest HID-5/psoriasin protein levels were observed in confluent, serum deprived cells (Fig. 2B).

[0043] The effect of cell detachment from extracellular matrix was tested by culturing MCF10A cells in suspension for several days. Lack of cell anchorage also dramatically increased HID-5/psoriasin protein levels (Fig. 2C). Northern blot analysis indicated that the up-regulation of HID-5/psoriasin expression by cell suspension and confluency occurred at the mRNA level (Fig. 2D). Cell cycle analysis of MCF10A cells revealed that serum deprivation, confluency and lack of cell anchorage ultimately results in G1 arrest followed by apoptosis (data not shown). The dramatic up-regulation of HID-5/psoriasin expression by these extracellular signals indicates that HID-5/psoriasin may play a role in the regulation of these cellular processes. Interestingly, keratinocytes derived from psoriatic lesions have been shown to be resistant to apoptosis compared to those derived from normal skin [Wrone-Smith et al. (1997) Am J. Pathol. 151:1321-1329]. High grade DCIS tumors demonstrate high apoptotic rates [Page et al. (2000) Curr. Opin. Oncol. 12:526-531] and surviving tumor cells are likely to be relatively resistant to apoptosis.

Example 4. HID-5/Psoriasin is a PartiallySecreted Cytoplasmic Protein

[0044] To determine the subcellular localization of the HID-5/psoriasin protein, immunohistochemistry on MDA-MB468 breast cancer cells and exponentially growing and serum starved MCF10A cells was performed using the monoclonal anti-HID-5/psoriasin antibody designated "Cl 1" in Fig. 2A (Fig. 3A). Both nuclear and cytoplasmic staining were detected in MDA-MB468 cells and in serum-starved MCF10A cells, whereas no staining was seen using a negative control antiserum or in exponentially growing MCF10A cells (Fig. 3A). Previous results demonstrated that psoriasin can be detected in the urine of bladder cancer patients and is partially secreted by psoriatic keratinocytes even though it contains no signal peptide [Madsen et al. (1991) J. Invest. Dermatol. 97:701-712; Ostergaard et al. (1999) Electrophoresis 20: 349-354]. To determine if HID-5/psoriasin is also secreted by breast cancer cells, immunoprecipitations were performed on cell lysate and culture medium of MDA-MB468 cells using an anti-HID-5/psoriasin polyclonal antibody. Immunoprecipitates were resolved by SDS-PAGE and analyzed by western blotting with a polyclonal anti-HID-5 antibody. HID-5/psoriasin protein was precipitated from both cell lysate and the culture medium with the anti-HID-5/psoriasin antibody ("HID-5"), whereas no protein was precipitated by control pre-immune serum ("P.I.") (Fig. 3B). Thus, HID-5/psoriasin protein is partially secreted or released by breast cancer cells. It is thus likely that it is detectable in the body fluids (e.g., blood and urine) of breast cancer patients. Detection of HID-5/psoriasin in such body fluids can therefore be a test for high grade breast cancer, e.g., high grade DCIS.

Example 5. Immunohistohemical Analysis of HID-5/Psoriasin

Protein Levels in Primary Breast Carcinomas

[0045] To analyze the *in* vivo expression of the HID-5/psoriasin protein, immunohistochemical analysis of formalin-fixed, paraffin-embedded breast carcinomas was performed using monoclonal anti-HID-5/psoriasin antibodies. To assess the reliability of the staining, a high grade comedo DCIS tumor previously shown by mRNA *in situ* hybridization to express HID-5/psoriasin was analyzed. Intense immunohistochemical staining was detected in the tumor cells using anti-HID-5/psoriasin antibody ("HID-5"), while no staining was seen using isotype control serum ("Control") (Fig. 3C).

[0046] Two tissue microarrays were examined. Array 1 was composed of five individual samples of normal breast tissue and 30 individual samples of primary invasive breast carcinomas (ten each of low, intermediate, and high grade) and array 2 was composed of six individual samples of normal breast tissue, three samples of benign hyperproliferative lesions and 49 samples of primary invasive ductal carcinomas. Diagrammatic representations of the two arrays are shown in Fig. 3E. In array 1, three punches (in horizontal rows) of each tumor sample were affixed to the slide and tumors were grouped according to their histologic grade (low, intermediate, and high grade tumors). Array 1 tumor samples were also analyzed for expression of ER α (ERα) and erbB2 and for the presence of leukocytes using an antibody specific for CD45, a panleukocyte antigen. In array 2, the first vertical row contained the six samples of normal

breast tissue, the three samples of benign hyperproliferative lesions, and an empty spot (indicated by the hatched square). Array 2 samples were analyzed for expression of ERα, progesterone receptor (PR), and p53. Staining from a representative tumor is shown in Fig. 3D and the results are summarized in Fig. 3E.

[0047] As expected, low grade tumors were mostly ERα positive, erbB2 negative and CD45 low, while high grade ones were mostly ERα negative, erbB2 positive, and CD45 high. No significant HID-5/psoriasin expression was detected in any of the normal breast tissue samples nor in the benign hyperproliferative lesions (Fig. 3E). HID-5/psoriasin positive invasive tumors were mostly ERα negative. Among the 78 tumors examined, 38 were HID-5/psoriasin positive (15 ERα+ and 23 ERα-) and 40 were HID-5/psoriasin negative (26 ERα+ and 14 ERα-). Based on these results HID-5/psoriasin positive tumors are more likely to be ERα negative (P=0.04, Fisher exact test). In one of three punches from a low grade tumor, a high level of HID-5/psoriasin was observed (Fig. 3E, Array 1); however, this tumor was later found to be a high grade DCIS lesion.

[0048] HID-5/psoriasin is a putative chemoattractant for lymphocytes, and both psoriatic skin and high grade DCIS lesions are frequently unfiltrated by lymphocytes [Bos et al. (1999) Immunol. Today 20:40-46; Page et al. (2000) Curr. Opin. Oncol. 12:526-531]. Although lymphocytic infiltration, as indicated by CD45 staining, was frequent in high-grade tumors, no clear association was seen between CD45 and HID-5/psoriasin positivity (Fig. 3E). This could be due to the relatively small sample size or to the fact that the carcinoma were invasive and not *in situ.*

[0049] To determine if the expression of HID-5/psoriasin correlates with histopathologic or clinical characteristics of breast tumors, a separate immunohistochemical analysis of 722 breast tumors was performed. Overall, approximately 30 % of the tumors were HID-5/psoriasin positive.. Statistical analysis of the immunohistochemistry data showed that expression of HID-5/psoriasin was statistically significantly different in *in situ* and primary invasive tumors, and distant metastasis. Specifically, *in situ* and primary invasive tumors were more likely to be HID5/psoriasin positive than distant metastases (p=0.008). Logistic regression model analysis of HID5/psoriasin expression in primary invasive breast tumors showed a statistically significant positive correlation between HID5/psoriasin positivity and lack of estrogen receptor (Odds ratio [OR]=6.25 and likelihood ratio [LR] p=0.001), high histologic grade (OR=20.85 LR p=0.0007), and ≥4 positive lymph nodes (OR=10.025 LR p=0.01). In other words, HID5/psoriasin positive primary invasive breast tumors are more likely to be estrogen receptor (ER) negative and high histologic grade with ≥4 positive lymph nodes. In a subset of tumor samples (156 Korean patients) HID5/psoriasin expression correlated positively with erbB2 expression (OR=5.29 LR p<0.0001), but this was not true in the combined data set possibly indicating ethnicity related differences. This study, using Fisher's exact test, also showed that, in breast cancer cells, the expression of S100A7 was associated with a higher likelihood of expression of FASN (fatty acid synthase) ($p= 9.95 \times 10^{-6}$) and trefoil factor 3 (TFF3) (p=0.002), and a lower likelihood of expression of connective tissue growth factor (CTGF) (p=0.005). In addition, the expression in breast cancer cells of FASN was associated with that of TFF3 ($p=3.Sx10^{-6}$) and SPARC ($p=4x10^{-5}$).

[0050] Since ER negative, high-grade tumors, with multiple positive lymph nodes in general tend to have worse clinical outcome, the expression of HID5/psoriasin in relation to overall and distant metastasis free survival was analyzed. Clinical follow up data was available only for a subset of patients (156 Korean patients) and this was for only up to 7 years. Based on this analysis, patients with HID5/psoriasin positive tumors had somewhat decreased >5 year overall survival; however this decrease was not statistically significant.

[0051] In summary, SAGE analysis of gene expression profiles of normal mammary epithelial cells and DCIS tumors revealed that several genes implicated in psoriasis are aberrantly expressed in high grade DCIS, with HID-5/psoriasin being one of the most abundant transcripts in these tumors. Dramatic up-regulation of HID-5/psoriasin in mammary epithelial cells *in vitro* is induced by growth factor deprivation, cell confluency, and lack of attachment to extracellular matrix. Because all these conditions are likely to occur in psoriatic skin lesions and high grade DCIS characterized by high proliferation rates, the high expression of HID-5/psoriasin in these cells could be due to the same signals and HID-5/psoriasin may play a role in the acquisition of apoptosis resistance of these cells.

Claims

1. A method for assessing whether or not a subject has high grade ductal carcinona in situ (DCIS), said method comprising:

   (a) identifying a subject suspected of having or at risk of having high-grade DCIS; and
   (b) measuring the level of psoriasin or psoriasin gene expression in a sample of a body fluid, a lavage, an aspirate, a cell culture supernatant, or a breast tissue sample from the subject;

   wherein said method includes determining whether or not said level is above the level of psoriasin or psoriasin gene expression that would be present in such a sample in respect of a subject with intermediate grade DCIS or low grade DCIS.

2. A method of discriminating high grade DCIS from intermediate grade DCIS or low grade DCIS, the method comprising measuring the level of psoriasin or psoriasin gene expression in a sample of a body fluid, a lavage, an aspirate, a cell culture supernatant, or a breast tissue sample from a subject identified as having DCIS, wherein said method includes determining whether or not said level is above the level that would be present in such a sample in respect of a subject with intermediate grade DCIS or low grade DCIS.

3. The method of claim 1 or claim 2, wherein the body fluid is blood or urine, the lavage is a breast duct lavage, or the aspirate is a nipple aspirate.

4. A method according to claim 1 or claim 2, wherein said sample is a sample of breast tissue from the subject.

5. The method of any preceding claim, wherein psoriasin protein is measured.

6. The method of claim 5, wherein the psoriasin protein is measured using an antibody that binds to psoriasin.

7. The method of any of claims 1 to 4, wherein psoriasin mRNA is measured.

8. The method of claim 7, wherein psoriasin mRNA is measured using RT-PCR.

9. The method according to any preceding claim wherein the subject is a mammal.

10. The method according to any preceding claim wherein the subject is a human.

Patentansprüche

1. Verfahren zur Beurteilung davon, ob ein Individuum an hochgradigem duktalem Karzinom in situ (DCIS) leidet oder nicht, wobei man in dem Verfahren

   (a) ein Individuum identifiziert, bei dem hochgradiges DCIS oder ein Risiko dafür vermutet wird, und
   (b) das Niveau von Psoriasin oder der Psoriasin-Genexpression in einer Probe einer Körperflüssigkeit, einer Lavage, eines Aspirats, eines Zellkulturüberstands oder einer Brustgewebsprobe aus dem Individuum misst,

   wobei das Verfahren die Bestimmung davon beinhaltet, ob das Niveau oberhalb des Niveaus von Psoriasin oder der Psoriasin-Genexpression, das in einer solchen Probe bezüglich eines Individuums mit mittelgradigem DCIS oder niedriggradigem DCIS vorläge, liegt oder nicht.

2. Verfahren zur Unterscheidung von hochgradigem DCIS von mittelgradigem DCIS oder niedriggradigem DCIS, wobei man in dem Verfahren das Niveau von Psoriasin oder der Psoriasin-Genexpression in einer Probe einer Körperflüssigkeit, einer Lavage, eines Aspirats, eines Zellkulturüberstands oder einer Brustgewebsprobe aus einem Individuum, bei dem DCIS gefunden wurde, misst, wobei das Verfahren die Bestimmung davon beinhaltet, ob das Niveau oberhalb des Niveaus, das in einer solchen Probe bezüglich eines Individuums mit mittelgradigem DCIS oder niedriggradigem DCIS vorläge, liegt oder nicht.

3. Verfahren nach Anspruch 1 oder Anspruch 2, wobei es sich bei der Körperflüssigkeit um Blut oder Urin, der Lavage um eine Milchgangspülung bzw. dem Aspirat um ein Brustwarzenaspirat handelt.

4. Verfahren gemäß Anspruch 1 oder Anspruch 2, wobei es sich bei der Probe um eine Probe von Brustgewebe aus dem Individuum handelt.

5. Verfahren nach einem der vorhergehenden Ansprüche,
   wobei Psoriasin-Protein gemessen wird.

6. Verfahren nach Anspruch 5, wobei das Psoriasin-Protein mittels eines an Psoriasin bindenden Antikörpers gemessen wird.

7. Verfahren nach einem der Ansprüche 1 bis 4, wobei Psoriasin-mRNA gemessen wird.

8. Verfahren nach Anspruch 7, wobei Psoriasin-mRNA mittels RT-PCR gemessen wird.

9. Verfahren gemäß einem der vorhergehenden Ansprüche, wobei es sich bei dem Individuum um einen Säuger handelt.

10. Verfahren gemäß einem der vorhergehenden Ansprüche, wobei es sich bei dem Individuum um einen Menschen handelt.

Revendications

1. Méthode pour déterminer si un sujet a ou non un carcinome intracanalaire in situ (DCIS) de haut grade, ladite méthode comprenant :

   (a) l'identification d'un sujet suspecté d'avoir ou d'être à risque d'avoir le DCIS de haut grade ; et
   (b) la mesure du taux de psoriasine ou d'expression du gène de psoriasine dans un échantillon d'un liquide corporel, un liquide de lavage, un échantillon aspiré, un surnageant de culture cellulaire ou un échantillon de tissu mammaire du sujet ;
   ladite méthode comprenant le fait de déterminer si ledit taux est supérieur ou non au taux de psoriasine ou d'expression du gène de psoriasine qui serait présent dans un tel échantillon par rapport à un sujet ayant un DCIS de grade intermédiaire ou un DCIS de bas grade.

2. Méthode de distinction entre le DCIS de haut grade et le DCIS de grade intermédiaire ou le DCIS de bas grade, la méthode comprenant la mesure du taux de psoriasine ou d'expression du gène de psoriasine dans un échantillon d'un liquide corporel, un liquide de lavage, un échantillon aspiré, un surnageant de culture de cellulaire ou un échantillon de tissu mammaire d'un sujet identifié comme ayant le DCIS, ladite méthode comprenant le fait de déterminer si ledit taux est supérieur ou non au taux qui serait présent dans un tel échantillon par rapport à un sujet ayant le DCIS de grade intermédiaire ou le DCIS de bas grade.

3. Méthode selon la revendication 1 ou la revendication 2, **caractérisée en ce que** le liquide corporel est le sang ou l'urine, le liquide de lavage est un liquide de lavage du canal mammaire, ou l'échantillon aspiré est un échantillon aspiré du mamelon.

4. Méthode selon la revendication 1 ou la revendication 2, **caractérisée en ce que** ledit échantillon est un échantillon de tissu mammaire du sujet.

5. Méthode selon l'une quelconque des revendications précédentes, **caractérisée en ce que** la protéine psoriasine est mesurée.

6. Méthode selon la revendication 5, **caractérisée en ce que** la protéine psoriasine est mesurée en utilisant un anticorps qui se lie à la psoriasine.

7. Méthode selon l'une quelconque des revendications 1 à 4, **caractérisée en ce que** l'ARNm de la psoriasine est mesuré.

8. Méthode selon la revendication 7, **caractérisée en ce que** l'ARNm est mesuré par RT-PCR.

9. Méthode selon l'une quelconque des revendications précédentes, **caractérisée en ce que** le sujet est un mammifère.

10. Méthode selon l'une quelconque des revendications précédentes, **caractérisée en ce que** le sujet est un être humain.

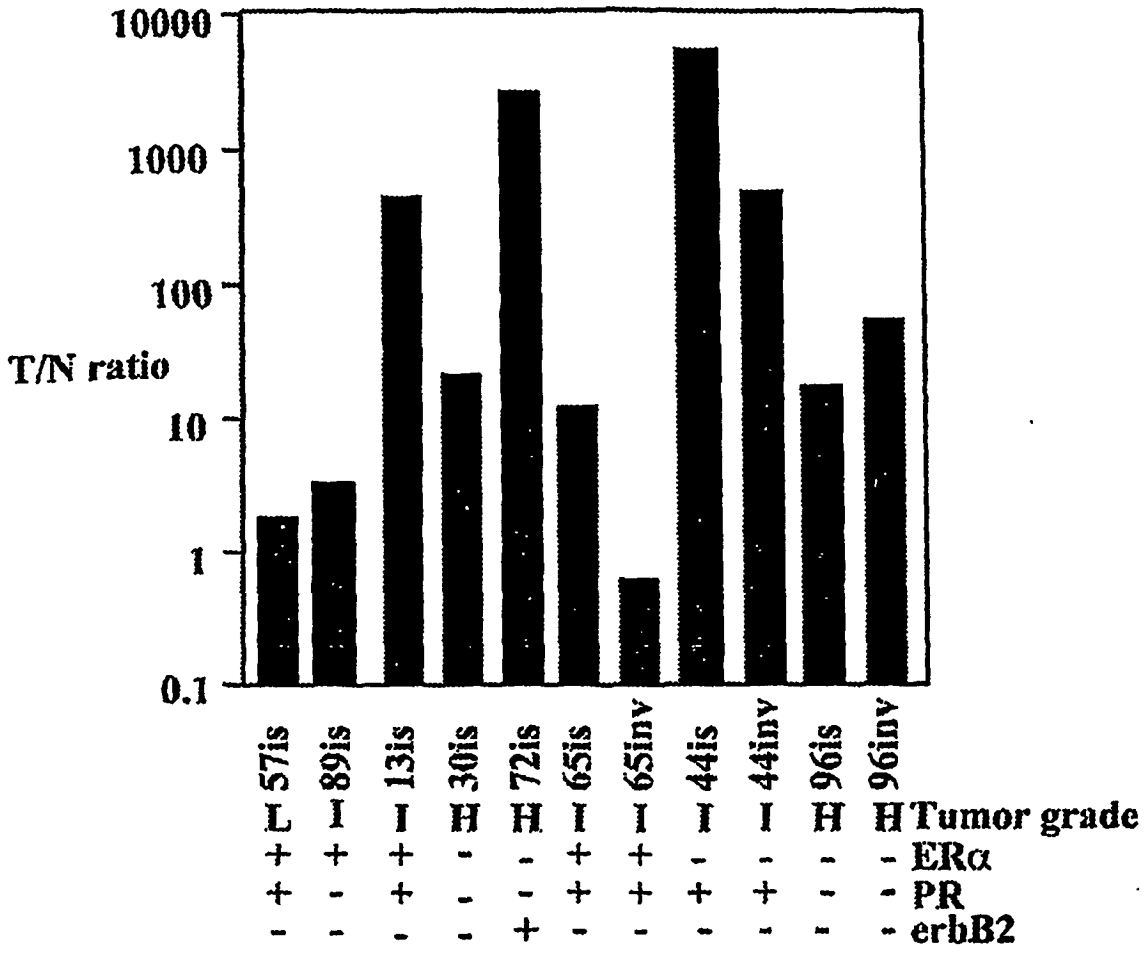

10000
1000
100
T/N ratio
10
1
0.1
57is
89is
13is
30is
72is
65is
65inv
44is
44inv
96is
96inv
L I I H H I I I I H H Tumor grade
+ + + - - + + + + - - ERα
+ - + - - + - + - - - PR
- - - - + - - - - - - erbB2

FIG. 1A

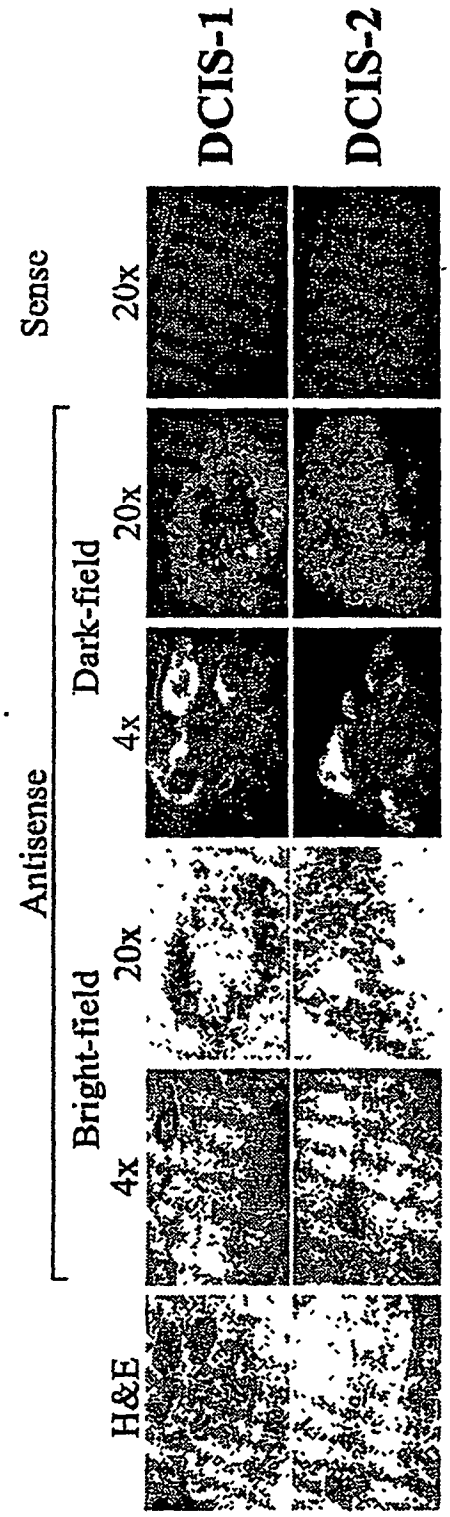

DCIS-1
DCIS-2
Sense
Antisense
Bright-field
Dark-field
H&E
4x
20x
4x
20x
20x

FIG. 1B

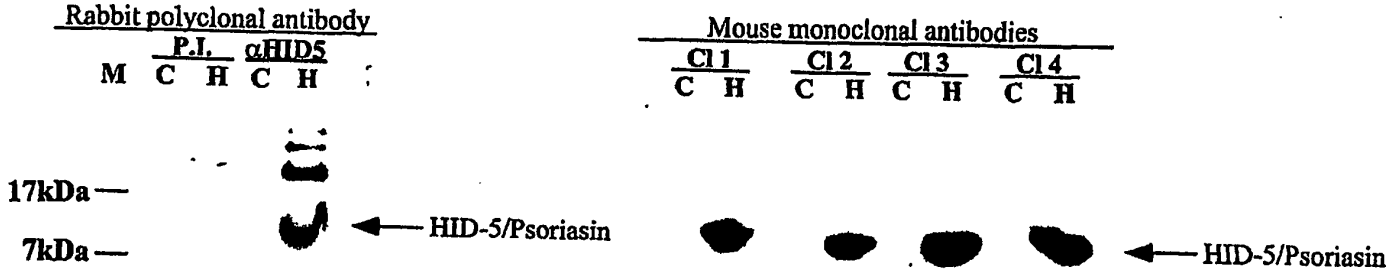


FIG. 2A

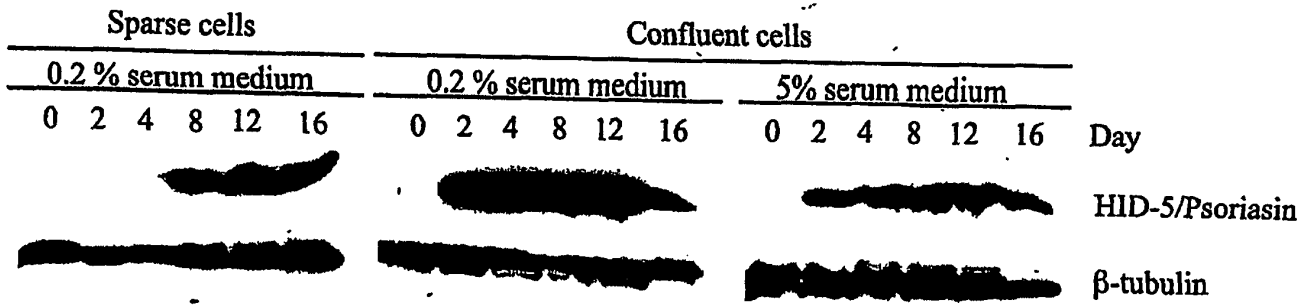


FIG. 2B

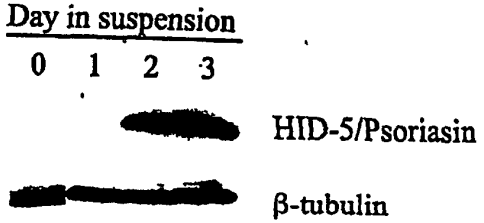


FIG. 2C

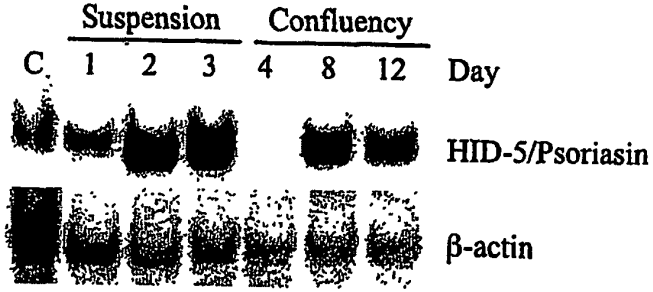


FIG. 2D

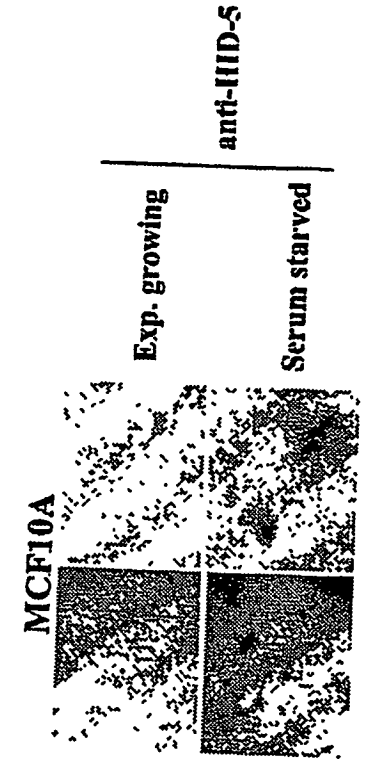
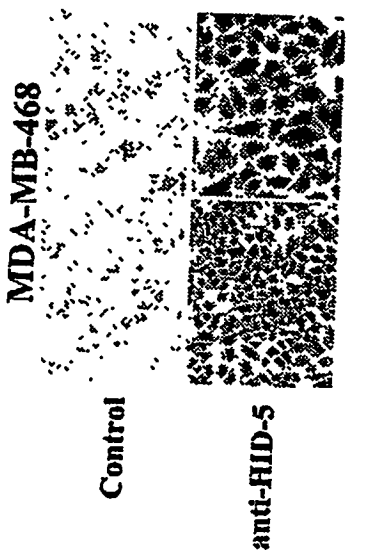

anti-HID-5
Exp. growing
Serum starved
MCF10A

FIG. 3A


Control
anti-HID-5
MDA-MB-468

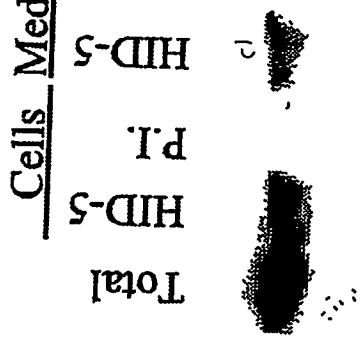

Cells Medium
Total
HID-5
P.I.
HID-5
P.I.
HID-5/Psoriasin

FIG. 3B

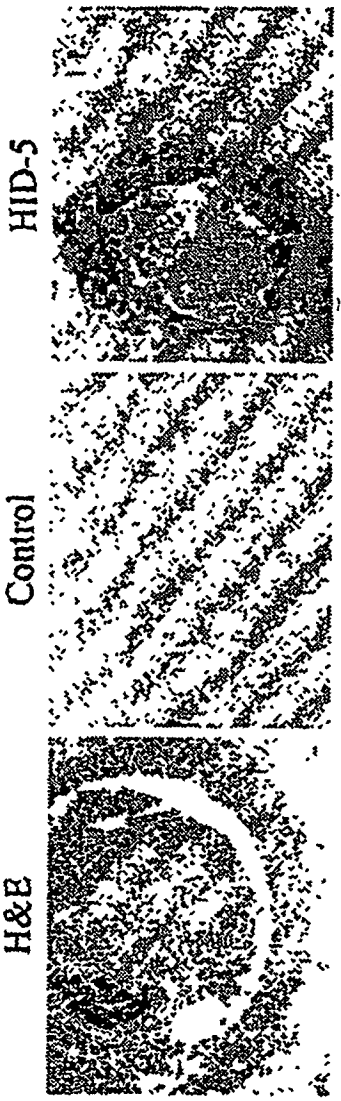


FIG. 3C

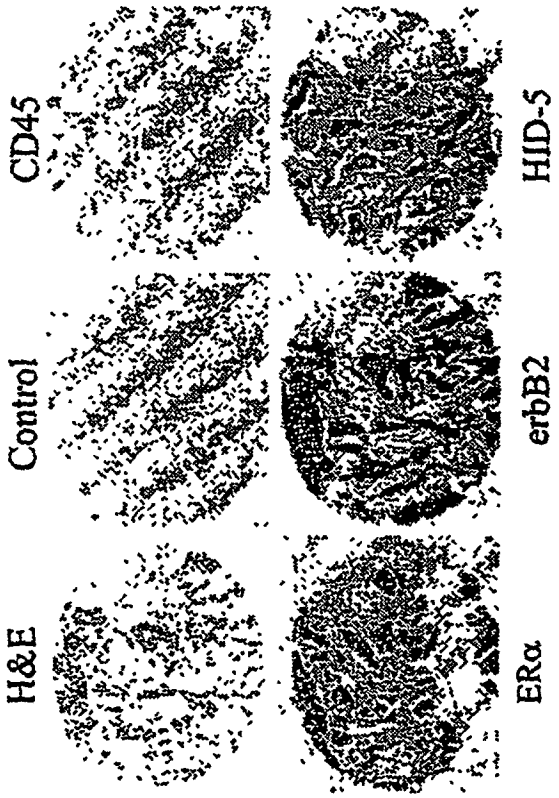
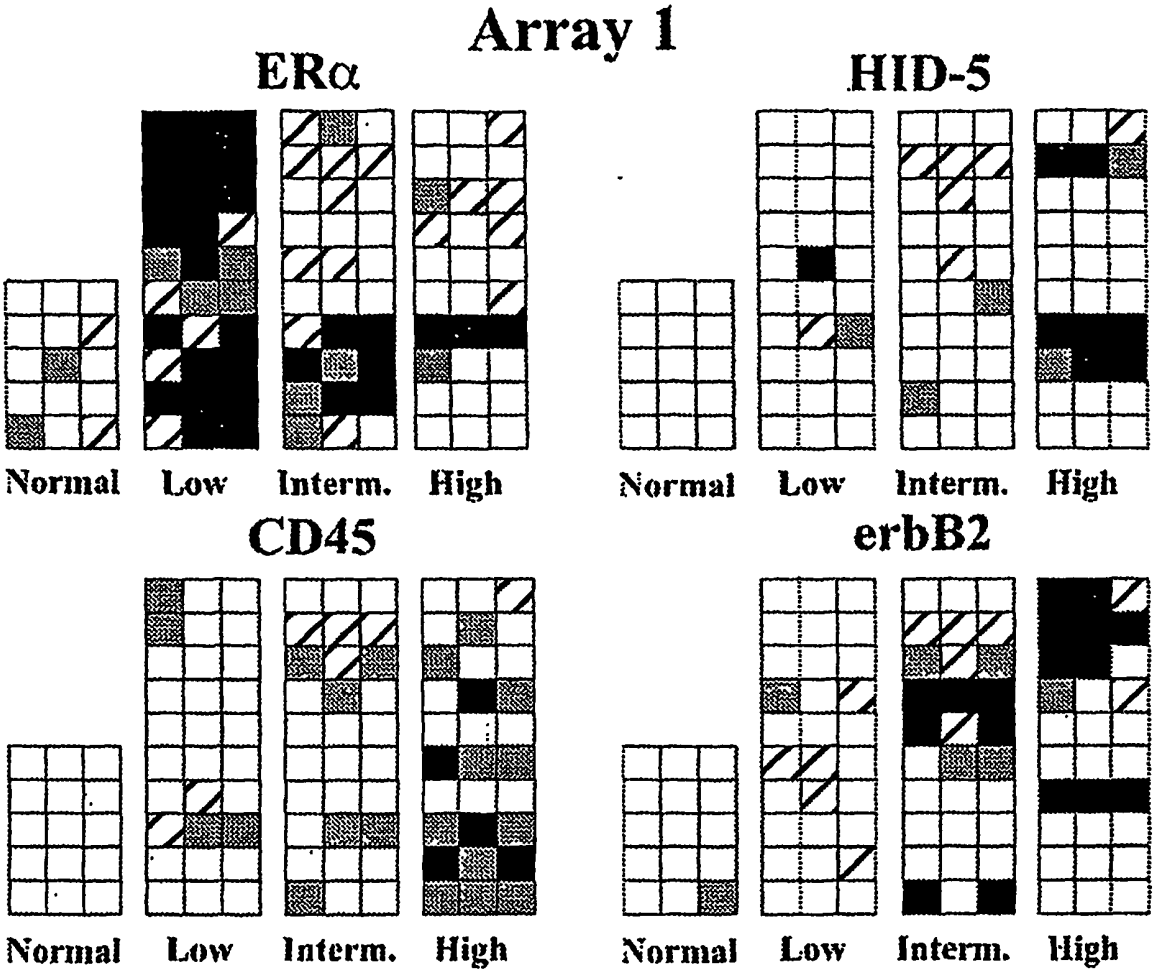


FIG. 3D


Array 1
ERα
HID-5
Normal
Low
Interm.
High
Normal
Low
Interm.
High
CD45
erbB2
Normal
Low
Interm.
High
Normal
Low
Interm.
High

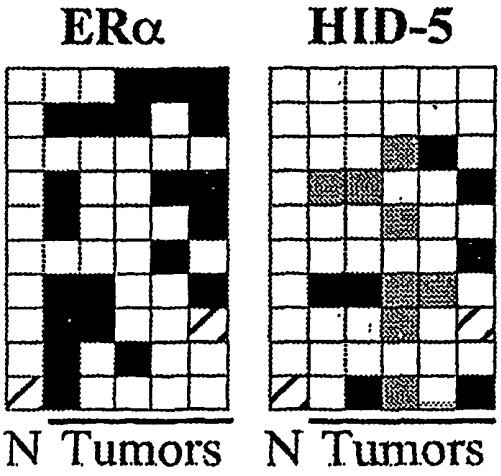

Array 2
ERα
HID-5
N Tumors
N Tumors

FIG 3F

MSNTQAERSIIGMIDMFHKYTRRDDKIDKPSLLTMMKENFPNFLSACDKKGTNYLADVFEKKDKNEDKKID
FSEFLSLLGDIATDYHKQSHGAAPCSGGSQ

FIG. 4A

atgagcaacactcaagctgagaggtccataataggcatgatcgacatgtttcacaaatacaccagacgtga
tgacaagattgacaagccaagcctgctgacgatgatgaaggagaacttccccaacttccttagtgcctgtg
acaaaaagggcacaaattacctcgccgacgtctttgagaaaaaggacaagaatgaggataagaagattgat
ttttctgagtttctgtccttgctgggagacatagccacagactaccacaagcagagccatggagcagcgcc
ctgttccgggggcagccag

FIG. 4B

REFERENCES CITED IN THE DESCRIPTION

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专利名称(译)	牛皮癣表达由乳腺上皮细胞表达		
公开(公告)号	EP1470239B1	公开(公告)日	2009-12-30
申请号	EP2002806283	申请日	2002-12-30
[标]申请(专利权)人(译)	达那-法伯癌症研究所		
申请(专利权)人(译)	Dana-Farber癌症研究所INC.		
当前申请(专利权)人(译)	Dana-Farber癌症研究所INC.		
[标]发明人	POLYAK KORNELIA ENERBACK CHARLOTTA		
发明人	POLYAK, KORNELIA ENERBÄCK, CHARLOTTA		
IPC分类号	C12Q1/00 C12Q1/68 G01N33/53 A61K38/00 A61K48/00 A61K39/395 G01N33/68 A61K31/7088 A61K45/00 A61P15/00 A61P35/00 A61P37/02 C12N5/06 C12N15/09 C12Q1/02 G01N33/15 G01N33/50 G01N33/574		
CPC分类号	A61P15/00 A61P35/00 A61P37/02 G01N33/57415 G01N33/57488		
优先权	60/345740 2001-12-31 US		
其他公开文献	EP1470239A4 EP1470239A2		
外部链接	Espacenet		

摘要(译)

本发明的特征在于诊断高级别导管原位癌（DCIS）的方法。这些方法涉及测量：（1）被怀疑患有或处于危险中的受试者的体液（例如血液或尿液）中HID-5的水平。 具有高级DCIS的；（2）怀疑患有高等级DCIS或有患高等级DCIS的受试者的乳房组织中HID-5基因表达的水平。 本发明还体现了抑制DCIS细胞中HID-5蛋白表达的方法和治疗怀疑患有高等级DCIS或有患高等级DCIS风险的受试者的方法。

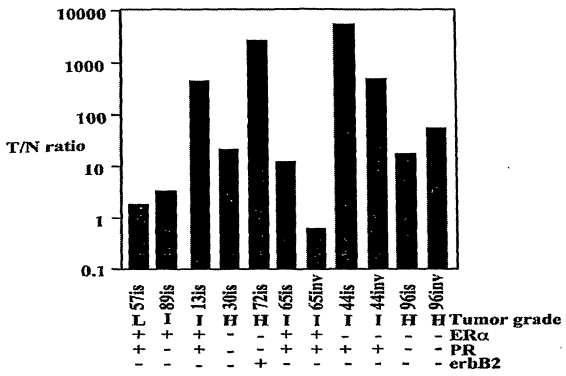

FIG. 1A