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(54) **METHODS AND KITS FOR DIAGNOSING TUMORIGENICITY**

VERFAHREN UND KITS ZUR TUMORIGENIZITÄTSDIAGNOSE

PROCEDES ET TROUSSES POUR DIAGNOSTIQUER UNE CANCEROGENICITE

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Description

BACKGROUND OF THE INVENTION

[0001] This invention relates to cell biology, physiology and medicine, and concerns an 88kDa glycoprotein growth factor ("GP88") PCDGF. More specifically the invention relates to kit products, compositions and methods which are useful for diagnosis of diseases including cancer.

REFERENCES

[0002] Several publications are referenced herein by Arabic numerals within parenthesis. Full citations for these references may be found at the end of the specification immediately preceding the claims.

BACKGROUND OF THE INVENTION

[0003] The proliferation and differentiation of cells in multicellular organisms is subject to a highly regulated process. A distinguishing feature of cancer cells is the absence of control over this process; proliferation and differentiation become deregulated resulting in uncontrolled growth. Significant research efforts have been directed toward better understanding this difference between normal and tumor cells. One area of research focus is growth factors and, more specifically, autocrine growth stimulation.

[0004] Growth factors are polypeptides which carry messages to cells concerning growth, differentiation, migration and gene expression. Typically, growth factors are produced in one cell and act on another cell to stimulate proliferation. However, certain malignant cells, in culture, demonstrate a greater or absolute reliance on an autocrine growth mechanism. Malignant cells which observe this autocrine behavior circumvent the regulation of growth factor production by other cells and are therefore unregulated in their growth.

[0005] Study of autocrine growth control advances understanding of cell growth mechanisms and leads to important advances in the diagnosis and treatment of cancer. Toward this end, a number of growth factors have been studied, including insulin-like growth factors ("IGF1" and "IGF2"), gastrin-releasing peptide ("GRP"), transforming growth factors alpha and beta ("TGF-a" and "TGF-b"), and epidermal growth factor ("EGF").

[0006] The present invention is directed to a recently discovered growth factor. This growth factor was first discovered in the culture medium of highly tumorigenic "PC cells," an insulin-independent variant isolated from the teratoma derived adipogenic cell line 1246. This growth factor is referred to herein as "GP88," as PCDGF. GP88 has been purified and structurally characterized. Amino acid sequencing of GP88 indicates that GP88 has amino acid sequence similarities with the mouse granulin/epithelin precursor.

[0007] Granulins/epithelins ("grn/epi") are 6kDa polypeptides and belong to a novel family of double cysteine rich polypeptides. U.S. Patent No. 5,416,192 (Shoyab et al.) is directed to 6 kDa epithelins, particularly epithelin 1 and epithelin 2. According to Shoyab, both epithelins are encoded by a common 63.5 kDa precursor, which is processed into smaller forms as soon as it is synthesized, so that the only natural products found in biological samples are the 6 kDa forms. Shoyab et al. teaches that the epithelin precursor is biologically inactive.

[0008] Contrary to the teachings of Shoyab et al., the inventor's laboratory has demonstrated that the precursor is not always processed as soon as it is synthesized. Studies, conducted in part by this inventor, have demonstrated that the precursor (*i.e.*, GP88) is in fact secreted as an 88kDa glycoprotein with an N-linked carbohydrate moiety of 20kDa. Analysis of the N-terminal sequence of GP88 indicates that GP88 starts at amino acid 17 of the grn/epi precursor, demonstrating that the first 17 amino acids from the protein sequence deduced from the precursor cDNA correspond to a signal peptide compatible with targeting for membrane localization or for secretion. Also in contrast to the teachings of Shoyab et al., GP88 is biologically active and has growth promoting activity, particularly as an autocrine growth factor for the producer cells.

[0009] Diagnosis of cancer often requires sampling a biopsy of a tissue suspected of being tumorigenic, testing the tissue sample to determine if a tumor marker is present, and determining if the tissue sample is tumorigenic. Biopsy procedures can be risky and painful depending on the location of the tissue and the condition of the patient. In addition, the trauma inflicted by biopsy procedures may increase the risk of malignancy. A study reported in the British Medical Journal identified biopsy as the strongest risk factor for testicular cancer. Swerdlow et al., BMJ 1997;314:1507. Biopsy has also been identified as a risk factor in breast, liver, and other cancers. In addition, a study conducted at the Johns Hopkins University concluded that misdiagnosis following biopsies occurs at a significant rate. Kronz et al., Cancer: Dec. 1, 1999, vol. 86, no. 11 pp 2426-2435. Misdiagnosis may be due, in part, to the small sample sizes obtained from needle biopsies and other procedures that capture only small tissue samples. Small biopsy sample sizes reduce patient risk. Id. However, the risk of misdiagnosis increases when only a small tissue sample is utilized. Id.

US 6,309,826 teaches methods of diagnosing tumorigenicity comprising measuring GP88 nucleic acid.

US 6,309,826 also discloses anti-GP88 antibodies and the fact that GP88 is found in the serum of mice, adipose tissue, brain, testes, ovary, liver and the kidney.

[0010] What is needed are new methods and kits for diagnosis, treatment, and prevention of cancer, and particularly methods and kits that avoid risks associated with biopsy of tissue.

BRIEF SUMMARY OF THE INVENTION

[0011] The inventor has now unexpectedly discovered that a glycoprotein (GP88), which is expressed in a tightly regulated fashion in normal cells, is overexpressed and unregulated in highly tumorigenic cells derived from the normal cells, that GP88 acts as a stringently required growth stimulator for the tumorigenic cells and that inhibition of GP88 expression or action in the tumorigenic cells results in an inhibition of the tumorigenic properties of the overproducing cells.

[0012] The inventor has further discovered methods of detecting GP88 in biological fluids at concentrations as low as about 0.1 nanograms of GP88 per milliliter (ng/ml). The invention provides non-invasive methods and kits for detecting GP88 in a biological fluid (e.g., whole blood, plasma, serum, lymph, saliva, and urine).

In a first aspect, the present invention provides a method of measuring the concentration of GP88/PCDGF in a biological fluid sample comprising contacting said sample with an anti-GP88/PCDGF antibody or antibody-fragment thereof and measuring the concentration of GP88/PCDGF wherein PCDGF is capable of being detected at a concentration as low as 0.1 nanogram of GP88/PCDGF per milliliter, wherein the anti-GP88/PCDGF antibody is produced from a hybridoma cell line selected from the group consisting of ATCC Accession Number PTA-5262 (6B3), ATCC Accession Number PTA-5261 (6B2), ATCC Number PTA-5589 (2A5), ATCC Number PTA-5593 (4D1), ATCC Number PTA-5259 (3F5), ATCC Number PTA-5260 (5B4), and ATCC Number PTA-5591 (3F8). The biological fluid sample may be a serum sample.

The invention further provides a method for diagnosing tumorigenic activity in cells comprising: measuring the level of GP88/PCDGF protein in a first biological fluid sample taken from a patient; measuring the level of GP88/PCDGF protein in a second biological fluid sample taken from a patient; and diagnosing tumorigenic activity in cells by determining whether the measured level of GP88/PCDGF protein in said second sample is higher than the level in said first sample by an amount sufficient to indicate tumorigenic activity in cells, wherein measuring the level of GP88/PCDGF is performed using the method of the first aspect of the invention.

The present invention further provides a method of diagnosing tumorigenic activity in cells comprising measuring the level of GP88/PCDGF in a biological fluid sample using an anti-GP88/PCDGF antibody, and determining whether the level of GP88/PCDGF in said sample is sufficient to indicate tumorigenic activity in cells, wherein measuring the level of GP88/PCDGF is performed using the method of the first aspect of the invention.

The invention yet further provides a method of determining whether a patient is responding or responsive to anti-tumorigenic therapy, comprising measuring the concentration of GP88/PCDGF in a biological fluid sample from a patient and determining whether the concentration of GP88/PCDGF in said sample is sufficient to indicate that said patient is not responding or responsive to anti-tumorigenic therapy, wherein measuring the level of GP88/PCDGF is performed using the method of the first aspect of the invention.

In a second aspect, the present invention provides an anti-GP88/PCDGF antibody produced from a hybridoma cell line selected from the group consisting of ATCC
Accession Number PTA-5262 (6B3), ATCC Accession Number PTA-5261 (682), ATCC Number PTA-5589 (2A5), ATCC Number PTA-5593 (4D1), ATCC Number PTA-5259 (3F5), ATCC Number PTA-5260 (5B4), and ATCC Number PTA-5591 (3F8), wherein said anti-GP88/PCDGF antibody is capable of detecting GP88 at a concentration as low as 0.1 nanogram per millilitre.

The invention further provides a kit for diagnosing tumorigenic activity in cells by measuring PCDGF concentration according to the method of the first aspect of the invention, comprising a container, and an anti-human PCDGF antibody as described in the first aspect of the invention or an antigen-binding fragment thereof.

The invention yet further provides a kit for determining whether a patient is responding or responsive to anti-tumorigenic therapy by measuring PCDGF concentration according to the method of the first aspect of the invention, comprising a container and an anti-human PCDGF antibody as described in the first aspect of the invention or an antigen-binding fragment thereof.

[0013] Use of the term "altered expression" herein means increased expression or overexpression of GP88 by a factor of at least two-fold, and at times by a factor of 10 or more, based on the level of mRNA or protein as compared to corresponding normal cells or surrounding peripheral cells. The term "altered expression" also means expression which became unregulated or constitutive without being necessarily elevated. Use of the terms increased or altered "response" to GP88 means a condition wherein increase in any of the biological functions (e.g., growth, differentiation, viral infectivity) conferred by GP88 results in the same or equivalent condition as altered expression of GP88.

[0014] Use of the term "GP88" as PCDGF herein means epithelin/granulin precursor in cell extracts and extracellular fluids, and is intended to include not only GP88 according to the amino acid sequences included in figures 8 or 9, which are of mouse and human origins, but also GP88 of other species. In addition, the term also includes functional derivatives

thereof having additional components such as a carbohydrate moiety including a glycoprotein or other modified structures.

[0015] Also intended by the term GP88 is any polypeptide fragment having at least 10 amino-acids present in the above mentioned sequences. Sequences of this length are useful as antigens and for making immunogenic conjugates with carriers for the production of antibodies specific for various epitopes of the entire protein. Such polypeptides are useful in screening such antibodies and in the methods directed to detection of GP88 in biological fluids. It is well known in the art that peptides are useful in generation of antibodies to larger proteins (7).

Peptides from 12-19 amino-acids in length have been successfully used to develop antibodies that recognize the full length GP88.

[0016] The polypeptide may exist covalently or non-covalently bound to another molecule. For example, it may be fused to one or more other polypeptides via one or more peptide bonds such as glutathione transferase, poly-histidine, or myc tag.

[0017] The polypeptide is sufficiently large to comprise an antigenetically distinct determinant or epitope which can be used as an immunogen to reproduce or test antibodies against GP88 or a functional derivative thereof.

[0018] A "fragment" of GP88 refers to any subset of the molecule that is a shorter peptide retaining the tumorigenic properties of GP88. This corresponds for example regions such as K19T and S14R for mouse GP88, and E19V and Al4R (equivalent to murine K19T and S14R, respectively) for human GP88.

[0019] A "variant" of GP88 refers to a molecule substantially similar to either the entire peptide or a fragment thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020] FIG. 1A compares the level of expression of GP88 protein in the 1246, 1246-3A and PC cell lines. Cells were cultured in DME-F12 medium supplemented with 2% fetal bovine serum (FBS). GP88 expression levels were measured by immunoprecipitation and Western blot analysis with anti-K19T antibody.

[0021] FIG. 1B compares the level of GP88 mRNA expression in the 1246, 1246-3A and PC cell lines. mRNA for RPL32 is used as an interval control for equal amounts of RNA loading.

[0022] FIG. 1C compares the expression of GP88 mRNA in 1246 cells (left panel) and in PC cells (right panel) in serum-free and serum containing medium. The results show that GP88 expression in 1246 cells is inhibited by the addition of fetal bovine serum whereas such inhibition is not observed in the highly tumorigenic PC cells.

[0023] FIG. 2 illustrates the effect of treatment of the highly tumorigenic PC cells with increasing concentrations of anti-GP88 neutralizing antibody.

[0024] FIG. 3 shows C3H mice injected subcutaneously with 10^6 antisense GP88 transfected PC cells (bottom) and with empty vector transfected control PC cells (top).

[0025] FIG. 4 shows *in vivo* GP88 expression levels in C3H mice tumor tissues and in surrounding normal tissues.

[0026] FIG. 5 shows GP88 mRNA expression levels in estrogen receptor positive and estrogen receptor negative human mammary carcinoma cell lines.

[0027] FIG. 6 shows the effect of increasing concentrations of GP88 on the growth of the mouse mammary epithelial cell line C57.

[0028] FIG. 7 shows the growth properties and tumorigenic ability of PC cells transfected with a cytomegalovirus promoter controlled expression vector containing GP88 in antisense orientation and PC cells transfected with an empty vector.

[0029] FIGS. 8A-8C show the nucleotide and deduced amino-acid sequence of mouse GP88. Peptide regions used as antigens to raise anti-GP88 antibodies K19T and S 14R are underlined. The region cloned in the antisense orientation in the pCMV4 mammalian expression vector is indicated between brackets.

[0030] FIG. 9A shows the nucleotide sequence of human GP88 cDNA. Indicated between brackets is the region cloned in the antisense orientation into the pcDNA3 mammalian expression system; and

[0031] FIG. 9B shows the deduced amino-acid sequence of human GP88. The E19V region used as antigen to develop anti-human GP88 neutralizing antibody is underlined. It also indicates the region A14R equivalent to the mouse S14R region.

[0032] FIG. 10 shows the amino-acid sequence of mouse GP88 arranged to show the 7 and one-half repeats defined as granulins g, f, B, A, C, D and e (right side). This representation shows that the region K19T and S14R used to raise GP88 antibodies for developing anti-GP88 neutralizing antibodies is found between two epithelin/granulin repeats in what is considered a variant region. Indicated on the right hand side is the granulin classification of the repeats according to Bateman et al (6). Granulin B and granulin A are also defined as epithelin 2 and epithelin 1 respectively according to Plowman et al., 1992 (5).

[0033] FIG. 11 shows a schematic representation of pCMV4 and a GP88 cDNA clone indicating the restriction sites used to clone GP88 antisense cDNA into the expression vector.

[0034] FIG. 12 shows the cross-linking of ^{125}I-rGP88 to GP88 cell surface receptors on CCL-64 cells. The cross-linking reaction was carried out with disuccinimidyl suberate (DSS). Reaction products were analyzed by SDS-PAGE on a 7%

polyacrylamide gel.

[0035] FIG. 13 shows the cross-linking of ^{125}I-rGP88 to GP88 cell surface receptors on 3T3 fibroblasts, PC cells and C57MG mammary epithelial cells. The results show that these various cell lines display GP88 cell surface receptors of similar molecular weight as the ones on CCL64 cells (FIG. 12).

[0036] FIG. 14 shows GP88 expression levels in non-tumorigenic MCF 10A and in malignant (MCF 7, MDA-468) human mammary epithelial cells.

[0037] FIG. 15 shows that GP88 expression is inhibited by antisense GP88 cDNA transfection in human breast carcinoma MDA-468 cells.

[0038] FIG. 16 is a graph showing the optical density (y-axis) of serum samples containing known quantities of GP88 (x-axis). The graph can be used as a reference to determine the concentration of GP88 in a biological fluid sample such as blood serum.

DETAINED DESCRIPTION OF THE INVENTION

Biological Activity of GP88

[0039] The invention relates to GP88 and methods and kits useful for aiding in diagnosing diseases linked to altered (increased) expression of GP88. Using a murine model system consisting of three cell lines, the inventor has shown that cells which overexpress GP88 form tumors. The parent cell line, 1246, is a C3H mouse adipogenic cell line which proliferates and differentiates into adipocytes in a defined medium under stringent regulation by insulin. The 1246 cells cannot form tumors in a syngeneic animal (C3H mouse) even when injected at a high cell density. An insulin independent cell line, 1246-3A, was isolated from 1246 cells maintained in insulin-free medium. The 1246-3A cells lost the ability to differentiate and form tumors when 10^6 are injected subcutaneously in syngeneic mice. A highly tumorigenic cell line, PC, was developed from 1246-3A cells by an *in vitro-in vivo* shuttle technique. The PC cells formed tumors when 10^4 cells were injected into syngeneic mice.

[0040] GP88 is overexpressed in the insulin-independent tumorigenic cell lines relative to the parent non-tumorigenic insulin-dependent cell line. Moreover, the degree of overexpression of GP88 positively correlates with the degree of tumorigenicity of these cells, demonstrating for the first time that GP88 is important in tumorigenesis (FIG. 1). With reference to FIG. 1, since GP88 is synthesized by cells but also secreted in culture medium, the level of GP88 was determined in cell lysates and in culture medium (CM). All cells were cultivated in DME/F12 nutrient medium supplemented with 2% fetal bovine serum. When cells reached confluency, culture medium (CM) was collected and cell lysates were prepared by incubation in buffer containing detergent followed by a 10,000 x g centrifugation. Cell lysate and conditioned medium were normalized by cell number. Samples from cell lysate and conditioned medium were analyzed by Western blot analysis using an anti-GP88 antibody, as explained below.

[0041] The development of a neutralizing antibody confirmed GP88's key role in tumorigenesis. When an anti-GP88 antibody directed to the K19T region of mouse GP88 was added to the culture medium, the growth of highly tumorigenic PC cells was inhibited in a dose dependent fashion (FIG. 2). With reference to FIG. 2, PC cells were cultivated in 96 well plates at a density 2 x 10^4 cells/well in DME/F12 medium supplemented with human fibronectin (2 μg/ml) and human transferrin (10 μg/ml). Increasing concentrations of anti-GP88 IgG fraction were added to the wells after the cells were attached. Control cells were treated with equivalent concentrations of non-immune IgG. Two days later, 0.25 mCi of ^{3}H-thymidine was added per well for 6 hrs. Cells were then harvested to count ^{3}H-thymidine incorporated into DNA as a measure for cell proliferation.

[0042] Moreover, when the expression of GP88 was specifically inhibited by antisense GP88 cDNA in PC cells, the production of GP88 was reduced and these PC cells could no longer form tumors in syngeneic C3H mouse. In addition, these PC cells regained responsiveness to insulin. With reference to FIG. 3 and Tables 1 and 2, C3H female mice were injected subcutaneously with 10^6 antisense GP88 transfected PC cells (as explained below) or 10^6 empty vector transfected PC cells. Mice were monitored daily for tumor appearance. Photographs were taken 45 days after injection of the cells. The results show that mice injected with antisense GP88 PC cells do not develop tumors, in contrast to the mice injected with empty vector transfected PC cells used as control.

Table 1. COMPARISON OF TUMORIGENIC PROPERTIES OF GP88 ANTISENSE TRANSFECTED CELLS, CONTROL TRANSFECTED CELLS AND PC CELLS

CELLS INJECTED	AVERAGE DAY OF TUMOR DETECTION	NUMBER OF MICE WITH TUMORS	AVERAGE TUMOR WEIGHT (g)
PC	15±3.0	5/5	9.0±3.2
P14	15±3.7	5/5	7.8±2.7
ASGP88	---	0/5	---

PC: Control non-transfected cells
P-14: Empty vector control transfected PC cells
ASGP88: PC cells transfected with expression vector containing GP88 antisense cDNA

Tumors were excised and weighed at 45 days. -- indicates no tumor formation.

Table 2. COMPARISON OF PROPERTIES OF 1246, PC CELLS AND GP88 ANTISENSE CELLS

1246 cells	insulin independence PC cells	GP88 antisense transfection Antisense GP 88 cells
insulin responsive for growth and differentiation	insulin-independent for growth differentiation deficient autocrine production of insulin-related factor	recovery of insulin responsiveness for growth (differentiation?)
cell surface insulin receptor expression high	cell surface insulin receptor expression very low	cell surface insulin receptor expression elevated
GP88 expression low	GP88 expression constitutively high	GP88 expression inhibited by antisense
GP88 expression inhibited by serum	No inhibition by serum	
GP88 expression regulated by insulin	GP88 expression constitutive	recovery of insulin regulation for endogenous GP88 expression
non-tumorigenic	highly tumorigenic	non-tumorigenic

[0043] Comparison of the expression of GP88 indicates that *in vivo* GP88 levels in tumors is dramatically higher than in normal tissues (FIG. 4). C3H mice were injected with 10^6 PC cells. Tumor bearing mice were euthanized. Tumors, fat pads and connective tissue were collected. Cell lysates were prepared by incubation in buffer containing detergent as described above for FIG. 1. Protein concentration of tissue extracts was determined, and equivalent amounts of proteins for each sample were analyzed by SDS-PAGE followed by Western blot analysis using anti-GP88 antibody to measure the content of GP88 in tissue extracts. The results showed that the level of GP88 in tumor extracts is at least 10-fold higher than in surrounding connective and fat tissues.

[0044] In normal cells (1246 cells, fibroblasts), the expression of GP88 is regulated, in particular by insulin, and inhibited by fetal bovine serum. In tumorigenic cells, a loss of regulation of normal growth leads to the increased expression of GP88 and the acquisition of GP88 dependence for growth. Therefore, inhibition of GP88 expression and/or action is an effective approach to suppression of tumorigenesis. Detection of an elevated GP88 expression in biopsies provides diagnostic analysis of tumors that are responsive to GP88 inhibition therapy.

[0045] GP88 is also a tumor-inducing factor in human cancers. As seen in the 1246-3A cell line, a loss of responsiveness to insulin (or to IGF-I) and a concurrent increase in malignancy has been well documented in several human cancers including but not limited to breast cancers. Specifically, breast carcinoma is accompanied by the acquisition of an

insulin/IGF-I autocrine loop, which is also the starting point of the development of tumorigenic properties in the mouse model system discussed above. Furthermore, GP88 expression is elevated in human breast carcinomas. More specifically, with reference to FIG. 5, human GP88 was highly expressed in estrogen receptor positive and also in estrogen receptor negative insulin/IGF-I independent highly malignant cells. Also, GP88 is a potent growth factor for mammary epithelial cells (FIG. 6). The data in FIG. 5 was obtained by cultivating MCF7, MDA-MB-453 and MDA-MB-468 cells in DME/F12 medium supplemented with 10% fetal bovine serum (FBS). RNA was extracted from each cell line by the RNAzol method and poly-A^+ RNA prepared. GP88 mRNA expression was examined by Northern blot analysis with 3 μg of poly-A^+ RNA for each cell line using a ^{32}P-labeled GP88 cDNA probe.

[0046] For Northern blot analysis of GP88 mRNA expression in rodent cells or tissues (mouse and rats), we used a mouse GP88 cDNA probe 311 bp in length starting at nucleotide 551 to 862 (corresponding to amino-acid sequence 160 to 270). RNA can be extracted by a variety of methods (Sambrook, Molecular Biology manual: 35) well known to people of ordinary skill in the art. The method of choice was to extract RNA using RNAzol (Cinnabiotech) or Trizol (Gibco-BRL) solutions which consists of a single step extraction by guanidinium isothiocyanate and phenol-chloroform.

[0047] For Northern blot analysis of GP88 mRNA expression in human cell lines, a 672 bp human GP88 cDNA probe was developed corresponding to nucleotide 1002 to 1674 (corresponding to amino-acid sequence 334-558) of human GP88.

[0048] With respect to FIG. 6, C57MG cells were cultivated in the presence of increasing concentrations of GP88 purified from PC cells conditioned medium (top panel), and recombinant GP88 expressed in insect cells (bottom panel), to demonstrate the growth stimulating effect of increasing concentrations of GP88 on the growth of the mouse mammary epithelial cell line C57MG.

[0049] A correlation between IGF-1 autocrine production and increased malignancy has also been well established for glioblastomas, teratocarcinomas and breast carcinomas. In these cancers, GP88 expression is also elevated in human tumors when compared to non-tumorigenic human fibroblasts and other human cell lines. GP88 promotes the growth of mammary carcinoma cells.

Anti-GP88 Antibodies

[0050] The invention provides methods for measuring GP88 in biological fluids which can be used for diagnosis of diseases linked to increased responsiveness to GP88.

[0051] We provide an antibody specific for an epitope of GP88. Such antibody can be used to detect the presence or measure the quantity or concentration of GP88 molecule, a functional derivative thereof or a homologue from different animal species in a cell, a cell or tissue extract, culture medium or biological fluid (e.g., whole blood, serum, plasma, lymph, and urine).

[0052] For use as antigen for development of antibodies, the GP88 protein naturally produced or expressed in recombinant form or functional derivative thereof, preferably having at least 9 amino-acids, is obtained and used to immunize an animal for production of polyclonal or monoclonal antibody. An antibody is said to be capable of binding a molecule if it is capable of reacting with the molecule to thereby bind the molecule to the antibody. The specific reaction is meant to indicate that the antigen will react in a highly selective manner with its corresponding antibody and not with the multitude of other antibodies which may be evoked by other antigens.

[0053] The term antibody herein includes human and non-human polyclonal antibodies, human and non-human monoclonal antibodies (mAbs), chimeric antibodies, anti-idiotypic antibodies (anti-IdAb) and humanized antibodies. Polyclonal antibodies are heterogeneous populations of antibody molecules derived either from sera of animals immunized with an antigen or from chicken eggs. Monoclonal antibodies ("mAbs") are substantially homogeneous populations of antibodies to specific antigens. mAbs may be obtained by methods know to those skilled in the art (U.S. Patent No. 4,376,110). Such antibodies may be of any immunological class including IgG, IgM, IgE, IgA, IgD and any subclass thereof. The hybridoma producing human and non-human antibodies to GP88 may be cultivated *in vitro* or *in vivo*. For production of a large amount of mAbs, *in vivo* is the presently preferred method of production. Briefly, cells from the individual hybridomas are injected intraperitoneally into pristane primed Balb/c mice or Nude mice to produce ascites fluid containing high concentrations of the desired mAbs. mAbs may be purified from such ascites fluids or from culture supernatants using standard chromatography methods well known to those of skill in the art.

[0054] Human monoclonal Ab to human GP88 can be prepared by immunizing transgenic mice expressing human immunoglobulin genes. Hybridoma produced by using lymphocytes from these transgenic animals will produce human immunoglobulin instead of mouse immunoglobulin.

[0055] Since most monoclonal antibodies are derived from murine source and other non-human sources, their clinical efficiency may be limited due to the immunogenicity of rodent mAbs administered to humans, weak recruitment of effector function and rapid clearance from serum. To circumvent these problems, the antigen-binding properties of murine antibodies can be conferred to human antibodies through a process called humanization. A humanized antibody contains the amino-acid sequences for the 6 complementarity-determining regions (CDRs) of the parent murine mAb which are

grafted onto a human antibody framework. The low content of non-human sequences in humanized antibodies (around 5%) has proven effective in both reducing the immunogenicity and prolonging the serum half life in humans. Methods such as the ones using monovalent phage display and combinatorial library strategy for humanization of monoclonal antibodies are now widely applied to the humanization of a variety of antibodies and are known to people skilled in the art. These humanized antibodies and human antibodies developed with transgenic animals as described above are of great therapeutic use for several diseases including cancer.

[0056] Hybridoma supernatants and sera are screened for the presence of antibody specific for GP88 by any number of immunoassays including dot blots and standard immunoassays (EIA or ELISA) which are well known in the art. Once a supernatant has been identified as having an antibody of interest, it may be further screened by Western blotting to identify the size of the antigen to which the antibody binds. One of ordinary skill in the art will know how to prepare and screen such hybridomas without undue experimentation in order to obtain a desired polyclonal or mAb.

[0057] Chimeric antibodies have different portions derived from different animal species. For example, a chimeric antibody might have a variable region from a murine mAb and a human immunoglobulin constant region. Chimeric antibodies and methods for their production are also known to those skilled in the art.

[0058] Accordingly, mAbs generated against GP88 may be used to induce human and non-human anti-IdAbs in suitable animals. Spleen cells from such immunized mice are used to produce hybridomas secreting human or non-human anti-Id mAbs. Further, the anti-Id mAbs can be coupled to a carrier such as Keyhole Limpet Hemocyanin (KLH) or bovine serum albumin (BSA) and used to immunize additional mice. Sera from these mice will contain human or non-human anti-anti-IdAb that have the binding properties of the original mAb specific for a GP88 polypeptide epitope. The anti-Id mAbs thus have their own idiotypic epitopes or idiotypes structurally similar to the epitope being evaluated.

[0059] The term antibody is also meant to include both intact molecules as well as fragments thereof such as, for example, Fab and F(ab')2, which are capable of binding to the antigen. Fab and F(ab')2 fragments lack the Fc fragment of intact antibody, clear more rapidly from the circulation and may have less non-specific tissue binding than an intact antibody. Such fragments are typically produced by proteolytic cleavage, using enzymes such as papain (to generate Fab fragments) and pepsin (to generate F(ab')2 fragments). It will be appreciated that Fab and F(ab')2 and other fragments of the antibodies useful in the present invention may be used for the detection or quantitation of GP88.

[0060] The antibodies or fragments of antibodies useful in the present invention may also be used to quantitatively or qualitatively detect the presence of cells which express the GP88 protein. This can be accomplished by immunofluorescence techniques employing a fluorescently labeled antibody (see below) with fluorescent microscopic, flow cytometric, or fluorometric detection. The reaction of antibodies and polypeptides of the present invention may be detected by immunoassay methods well known in the art.

[0061] The antibodies of the present invention may be employed histologically as in light microscopy, immunofluorescence or immunoelectron microscopy, for *in situ* detection of the GP88 protein in tissues samples, biopsies, and biological fluids. *In situ* detection may be accomplished by removing a histological specimen from a patient and applying the appropriately labeled antibody of the present invention. The antibody (or fragment) is preferably provided by applying or overlaying the labeled antibody (or fragment) to the biological sample. Through the use of such a procedure, it is possible to determine not only the presence of the GP88 protein but also its distribution in the examined tissue or concentration in a biological fluid. Using the present invention, those of ordinary skill in the art will readily perceive that any wide variety of histological methods (such as staining procedures) can be modified in order to achieve such *in situ* detection.

[0062] Assays for GP88 typically comprise incubating a biological sample such as a biological fluid, a tissue extract, freshly harvested or cultured cells or their culture medium in the presence of a detectably labeled antibody capable of identifying the GP88 protein and detecting the antibody by any of a number of techniques well known in the art.

[0063] The biological sample may be treated with a solid phase support or carrier such as nitrocellulose or other solid support capable of immobilizing cells or cell particles or soluble proteins. The support may then be washed followed by treatment with the detectably labeled anti-GP88 antibody. This is followed by wash of the support to remove unbound antibody. The amount of bound label on said support may then be detected by conventional means. By solid phase support is intended any support capable of binding antigen or antibodies such as but not limited to glass, polystyrene polypropylene, nylon, modified cellulose, or polyacrylamide.

[0064] The binding activity of a given lot of antibody to the GP88 protein may be determined according to well known methods. Those skilled in the art will be able to determine operative and optimal assay conditions for each determination by employing routine experimentation.

[0065] Detection of the GP88 protein or functional derivative thereof and of a specific antibody for the protein may be accomplished by a variety of immunoassays well known in the art such as enzyme linked immunoassays (EIA) or radioimmunoassays (RIA). Such assays are well known in the art and one of skill will readily know how to carry out such assays using the anti-GP88 antibodies and GP88 protein of the present invention.

[0066] Such immunoassays are useful to detect and quantitate GP88 protein in serum or other biological fluid as well as in tissues, cells, cell extracts, or biopsies. In a preferred embodiment, the concentration of GP88 is measured in a

tissue specimen as a means for diagnosing cancer or other disease associated with increased expression of GP88. In another preferred embodiment, the concentration of GP88 in a biological fluid sample is used to determine if a patient is likely to be responsive, or is responding to, anti-tumorigenic therapy.

[0067] The presence of certain types of cancers (e.g., breast cancer) and the degree of malignancy are said to be "proportional" to an increase in the level of the GP88 protein. The term "proportional" as used herein is not intended to be limited to a linear or constant relationship between the level of protein and the malignant properties of the cancer. The term "proportional" as used herein, is intended to indicate that an increased level of GP88 protein is related to appearance, recurrence or display of malignant properties of a cancer or other disease associated with increased expression of GP88 at ranges of concentration of the protein that can be readily determined by one skilled in the art.

[0068] Another embodiment of the invention relates to evaluating the efficacy of anti-cancer or anti-viral drug or agent by measuring the ability of the drug or agent to inhibit the expression or production of GP88. The antibodies of the present invention are useful in a method for evaluating anti-cancer or anti-viral drugs in that they can be employed to determine the amount of the GP88 protein in one of the above-mentioned immunoassays. Alternatively, the amount of the GP88 protein produced is measured by bioassay (cell proliferation assay) as described herein. The bioassay and immunoassay can be used in combination for a more precise assessment.

Detection of GP88 in Biological Fluids

[0069] Preferred embodiments of the invention are directed to methods and kits for detecting GP88 in biological fluids. As described above, cancer cells express elevated levels of GP88. The present invention demonstrates that GP88 can be detected in biological fluids at a concentration as low as about 0.1 ng/ml. As described above, GP88 is overexpressed in cancer cells and elevated levels of GP88 are indicative of tumorigenicity. Typically, a tissue sample or biopsy is required to detect the presence of a tumor marker. For example, breast cancer patients are often subjected to needle biopsy procedures in order to remove samples of breast tissue for examination to determine whether a particular tumor marker is present. Biopsy procedures, like any surgical procedure, are associated with increased risk to the patient. Biopsy procedures, in particular, have been associated with increased risk of tumor formation.

[0070] Unlike tissue biopsy procedures, blood sampling is a routine and safe procedure that can be carried out by a patient if necessary. For example, a small sterile lance can be used to prick a patient's fingertip and obtain a small sample of blood. The blood sample can be processed by any suitable procedure to isolate the serum or plasma fractions. Alternatively, a whole blood sample can be used. An assay, for example an enzyme-linked immunoabsorption assay (ELISA), utilizing anti-GP88 antibodies can be used to detect the presence and quantitate the amount of GP88 in the serum sample. Blood sampling avoids the risks associated with tissue biopsies. In addition, obtaining blood samples from the same patient on a regular basis (e.g., weekly examinations) permits monitoring of the patient to determine the level of GP88 in the serum over time. If the level of GP88 increases, the physician can treat the patient accordingly (e.g., administering GP88 antagonists) before significant tumor growth can occur.

[0071] In carrying out a method of measuring the concentration of GP88 in a biological fluid, a biological fluid (e.g., whole blood, plasma, serum, lymph, saliva, and urine) is contacted with an anti-GP88 antibody and the concentration of GP88 is measured. GP88 can be detected, for example, at a concentration as low as about 0.1 ng/ml. As described above, anti-GP88 antibodies can bind to GP88 and be used to determine the amount of GP88 in a sample. Anti-GP88 antibodies that can be used to measure the concentration of GP88 in a biological fluid sample include antibodies produced from the following hybridoma cell lines ATCC Accession Nos. 6B3 PTA-5262 and 6B2 PTA-5261 (10801 University Blvd., Manassas, VA 20110).

[0072] ELISAs are rapid, sensitive, and reproducible assays for quantifying the amount of an antigen in a sample. A "sandwich" ELISA utilizes a primary antibody to bind to or "capture" its antigen (e.g., a protein) and a labeled secondary antibody to also bind to the antigen. The addition of a substrate for the detection moiety results in a signal (e.g., color change, or radioactivity) that is proportional to the amount of antigen present in the sample. In an exemplary sandwich ELISA, the primary antibody is adsorbed to a support such as a well of a microtiter plate. A sample containing the antigen is incubated with the attached primary antibody and the antigen is permitted to bind to the antibody. Next, a secondary antibody labeled with a detection molecule (e.g., enzyme, radionuclide) is also permitted to bind to the antigen. Examples of labels include alkaline phosphatase and horseradish peroxidase. Alternatively, the secondary antibody is unlabeled and a third antibody (e.g., a labeled anti-IgG antibody) is also used in the assay. The labeled third antibody binds to the constant region of immunoglobulin G (IgG). The addition of a substrate for the enzyme (e.g., 2,2-azo-bis(3-ethylbenz-thiazoline-6-sulfonic acid) (ABTS), o-phenylenediamine (OPD), and 3,3,-5,5-tetramethylbenzidine base (TMB)) results in a color change in the sample solution that is proportional to the amount of antigen present in the sample. The color change can be detected using a spectrophotometer at a wavelength suitable for detecting the color change induced by the enzyme label (e.g., 624 nm using TMB as a substrate).

[0073] Alternatively, the secondary antibody or third antibody can be labeled with a radioactive moiety (e.g., ^{125}I, ^{35}S, ^{32}P, ^{12}C). A radioactivity detector (e.g., gamma counter) can be used to measure the radioactivity emitted

by the secondary antibody after binding to the antigen. The level of radioactivity is proportional to the amount of antigen present in the sample. In yet another alternative, the level of GP88 in a biological fluid sample can be determined using a Western blot procedure. In an exemplary Western blot procedure, the biological fluid sample is loaded on to an SDS Polyacrylamide Gel (SDS-PAGE) which is subjected to an electric current (e.g., 20 mA) to separate the proteins in the biological fluid sample by molecular weight. The proteins are transferred to a nitrocellulose membrane and incubated with a labeled anti-GP88 antibody. If the anti-GP88 antibody is labeled with an enzyme, the nitrocellulose membrane is exposed to the substrate for the enzyme which induces a color change in the GP88 protein band located on the nitrocellulose membrane. The amount of GP88 in the sample is proportional to the degree of color change.

[0074] In one embodiment of the invention, GP88 monoclonal antibody 6B3, produced by hybridoma cell line (ATCC Number PTA-5262) is used as the primary antibody in a sandwich ELISA. Various antibodies raised against GP88 can be used in an ELISA to detect GP88 (e.g., GP88 monoclonal antibodies 6B3, 6B2, 2A5, 4D1, 3F5, 5B4, 3F8 produced from following hybridoma cell lines respectively ATCC Number PTA-5262, ATCC Number PTA-5261, ATCC Number PTA-5589, ATCC Number PTA-5593, ATCC Number PTA-5259, ATCC Number PTA-5260, ATCC Number PTA-5591). GP88 antibodies labeled with horseradish peroxidase can be used as the secondary antibody, and TMB can be used as the substrate. Alternatively, a labeled antibody capable of binding the constant region of an immunoglobulin can be used as the secondary antibody in an ELISA. (e.g., anti-IgG antibody). Samples containing known quantities of GP88 protein (0.1, 2, 5, 10, 20 nanograms) in a carrier (e.g., buffer solution) can be used to generate a standard curve based on the results of an ELISA. The optical density of the sample can be plotted against the known amount of GP88 in the sample to generate a standard curve (e.g., FIG. 16). The concentration of GP88 in an unknown sample can be determined by measuring the optical density of an unknown sample and using the standard curve to calculate the GP88 concentration.

[0075] An exemplary protocol for a sandwich ELISA includes the following steps:

[0076] (1) Add a solution containing the primary antibody to the bottom of the well of a microtiter plate (e.g., 50 microliters of an antibody solution containing 20 mg/ml of antibody).

[0077] (2) incubate the microtiter plate overnight at 4° C to allow for complete binding of the antibody to the well.

[0078] (3) wash the wells with a buffer solution (e.g., phosobuffered saline (PBS))

[0079] (4) block non-specific protein binding by saturating the wells with a blocking buffer (e.g., Bovine Serum Albumin (BSA) in PBS)

[0080] (5) wash the wells with a buffer solution

[0081] (6) add a solution containing the antigen and incubate the microtiter plate at room temperature for at least 2 hours.

[0082] (7) wash the wells with a buffer solution

[0083] (8) add the labeled secondary antibody and incubate the microtiter plate for at least two hours at room temperature

[0084] (9) wash the wells with a buffer solution

[0085] (10) add the desired substrate diluted in a buffer solution, allow sufficient time for a reaction to occur

[0086] (11) read the optical density of resulting substrate solution in an ELISA reader at the appropriate wavelength for the substrate.

[0087] FIG. 16 shows an exemplary curve that can be used for determining the concentration of GP88 in a blood serum biological fluid. Samples containing known quantities of recombinant GP88 protein were prepared and measured using an ELISA assay. The optical densities of the samples were plotted against known quantities of GP88 protein. FIG. 16 shows a linear relationship between the OD (optical density) of a biological fluid sample (y-axis) and the concentration of GP88 (x-axis). The concentration of GP88 in a biological fluid sample can be determined by using anti-GP88 antibodies in a suitable detection technique (e.g., ELISA, RIA, Western blot) as described above. In one embodiment, the optical density of the biological fluid sample is measured and the concentration of GP88 in the biological fluid sample is determined by comparing the measured optical density to a standard curve (e.g., FIG. 16). For example, using the curve of FIG. 16, if the optical density of a biological fluid sample contacted with anti-GP88 antibody and subjected to an immunoassay is 0.6, the concentration of GP88 in the biological fluid sample would be 15 ng/ml.

[0088] Serum concentrations of GP88 in healthy humans vary between about 23 ng/ml and 44 ng/ml in healthy humans. Measurement of the level of GP88 in plasma from healthy human volunteers gave similar results. Human breast cancer patients showed elevated levels of GP88 in serum. Three out of twenty breast cancer patients showed elevated levels of GP88 (49, 51, and 56 ng/ml). However, patients with progressive disease (e.g., metastatic) who showed no response to therapy had dramatically increased serum levels of GP88 over time (from 27 to 233 ng/ml in 6 months). Another patient with advanced disease who was non-responsive to anti-tumorigenic therapy had a GP88 serum concentration of 158 ng/ml on March 21, 2002 and 148 ng/ml on May 23, 2002. Patients initially diagnosed with non-metastatic breast cancer and patients in remission had GP88 serum concentrations within the normal range. Thus, elevated serum concentrations of GP88 (e.g., about 40 to 50 ng/ml) are indicative of tumorigenicity. Highly elevated levels of serum GP88 (e.g., about 100 to 300 ng/ml) are indicative of progressive disease and resistance to anti-tumorigenic therapy.

[0089] In another embodiment of the invention, a method of diagnosing tumorigenicity is provided comprising measuring the level of GP88 protein in a first biological fluid sample, measuring the level of GP88 protein in a second biological

fluid sample, and diagnosing tumorigenicity by determining whether the measured level of GP88 protein in the second biological fluid sample is higher than the level of GP88 protein in the initial biological fluid sample by an amount sufficient to indicate tumorigenicity. An initial biological fluid sample can be taken from a patient suspected of having cancer or cell growth-related disease. The level of GP88 in the initial biological fluid sample can be measured and compared to the level of GP88 in a second biological fluid sample taken at a different time. Biological fluid samples can be taken at regular intervals and the measured concentration of GP88 in subsequent samples can be compared to the GP88 level in the initial sample. If the results indicate an increase in the level of GP88 in the biological fluid over time, the physician can initiate or modify the patient's treatment in order to reduce or eliminate tumor growth.

[0090] The invention also provides methods of determining whether a patient is responsive or responding to anti-tumorigenic therapy comprising measuring the concentration of GP88 in a biological fluid sample from a patient receiving anti-tumorigenic therapy, wherein a concentration of GP88 of at least about 100 ng/ml indicates that the patient is not responding to anti-tumorigenic therapy. The term "anti-tumorigenic therapy" refers to any medicament, drug, therapy, or method of administering a medicament, drug, or therapy for the purpose of treating cancer or a growth-related disease. Examples of anti-tumorigenic therapy include antiestrogen therapy, the use of anti-tumor antibodies (e.g., anti-GP88 antibodies), antisense therapy (e.g., anti-GP88 nucleic acids), chemotherapy, radiation treatment, and gene therapy. The GP88 serum concentration of a patient undergoing anti-tumorigenic therapy can be monitored by analyzing serum samples at regular intervals (e.g., daily, weekly, monthly). A GP88 serum level of at least about 100 ng/ml can indicate that the patient will not be responsive to or is not responding to, anti-tumorigenic therapy.

[0091] Certain anti-tumorigenic therapies pose undesirable side effects or additional risk to a patient. For example, treatment or prevention of breast cancer with antiestrogens (e.g., tamoxifen, raloxifene) is associated with increased risk of ovarian cancer. Elevated levels of GP88 indicate that a patient will not be responsive to treatment with antiestrogens. If a patient has elevated levels of GP88 (e.g., greater than about 100 ng/ml), the patient would likely not be responsive to antiestrogen therapy and the additional risk posed by antiestrogen therapy may outweigh any benefit. Chemotherapy also is associated with many undesirable side effects including, nausea, weakness, hair loss, appetite loss etc. If a patient is not likely to respond to a particular type of chemotherapy, an anti-tumorigenic therapy with fewer side effects may be more effective and not subject the patient to additional trauma. The present invention is useful for determining if a patient will be responsive or is responding to antitumorigenic therapy.

[0092] The invention also provide kits for diagnosing tumorigenicity and determining whether a patient is responsive or responding to anti-tumorigenic therapy. Such kits preferably comprise a container and a compound or compounds for detecting GP88 (e.g., anti-GP88 antibodies or antibody fragments). The anti-GP88 antibody or antibody fragment can be labeled (e.g., enzymatic, radioisotopic, fluorescent, and chemical labels) for use in a suitable detection method (e.g., ELISA, radioimmunoassay). In one embodiment, the kits contain at least one primary antibody (e.g., anti-GP88 monoclonal antibody 6B3), at least one labeled secondary antibody (e.g., anti-human GP88 polyclonal antibody labeled with a detection enzyme such as HRP), and at least one substrate (e.g., TMB). Alternatively, the kits can contain radiolabeled secondary antibody in place of the secondary antibody labeled with an enzyme. The kits may also contain disposable supplies for carrying out detection assays (e.g., microtiter plates, pipettes).

EXAMPLE 1

DETERMINING THE CONCENTRATION OF GP88 IN A BIOLOGICAL FLUID SAMPLE

[0093] Serum samples were obtained from 17 normal, healthy, human volunteers. GP88 concentrations in human serum samples were measured in triplicate by enzyme-linked immunoabsorbance assay (ELISA). Standard GP88 samples were prepared from recombinant GP88 diluted in a solution of 30% glycerol and 1% milk-PBS at concentrations of 0, 0.1, 0.25, 0.5, 1, 3, 10, and 20 ng/ml. 100 microliter wells on a microtiter plate were coated with 10 microgram per milliliter of anti-human GP88 monoclonal antibody 6B3 (0.78 mg/ml of 6B3 antibody in phospho buffered saline (PBS)) and incubated overnight at 4°C. The wells were washed with PBS followed by the addition of anti-human PCDGF polyclonal (IgG fraction) to each well at a concentration of 3 micrograms/ml at 37°C for 1. 5 hours. The wells were washed in PBS before the addition of detection antibody (horseradish peroxidase (HRP)-goat-rabbit-IgG) to each well. TMB (substrate) was added and allowed to incubate with the samples for 1 hour. The optical density of the samples was determined using an ELISA spectrometer reader set at a wavelength of 620 nanometer. Plotting the optical density of the standard GP88 samples (y-axis) against the amount of GP88 in each sample (x-axis) generated a standard curve (FIG. 16). The GP88 concentration of the unknown samples was determined by measuring the optical density and using the standard curve (FIG. 16) to determine the GP88 concentration. Table 1 provides the GP88 serum sample concentration for each of the seventeen healthy human volunteers:

Table 1

Patient #	GP88 Conc.(ng/ml)
1	36.415
2	31.534
3	42.342
4	27.109
5	37.85
6	23.793
7	32.837
8	42.208
9	32.089
10	42.792
11	36.213
12	31.902
13	26.383
14	32.823
15	28.028
16	34.1

[0094] Table 2 shows the GP88 concentration of serum samples taken from a patient with advanced, progressive breast cancer who did not respond to anti-tumorigenic therapy. The GP88 serum concentration increased from 27 to 233 ng/ml in a six-month period.

Table 2

Date	GP88 Conc.(ng/ml)
8/1/2001	27
10/10/2001	128.26
3/21/2002	233

Claims

1. A method of measuring the concentration of GP88/PCDGF in a biological fluid sample comprising contacting said sample with an anti-GP88/PCDGF antibody or antibody-fragment thereof and measuring the concentration of GP88/PCDGF wherein PCDGF is capable of being detected at a concentration as low as 0.1 nanogram of GP88/PCDGF per milliliter, wherein the anti-GP88/PCDGF antibody is produced from a hybridoma cell line selected from the group consisting of ATCC Accession Number PTA-5262 (6B3), ATCC Accession Number PTA-5261 (6B2), ATCC Number PTA-5589 (2A5), ATCC Number PTA-5593 (4D1), ATCC Number PTA-5259 (3F5), ATCC Number PTA-5260 (5B4), and ATCC Number PTA-5591 (3F8).

2. A method for diagnosing tumorigenic activity in cells comprising: measuring the level of GP88/PCDGF protein in a first biological fluid sample taken from a patient; measuring the level of GP88/PCDGF protein in a second biological fluid sample taken from a patient; and diagnosing tumorigenic activity in cells by determining whether the measured level of GP88/PCDGF protein in said second sample is higher than the level in said first sample by an amount sufficient to indicate tumorigenic activity in cells, wherein measuring the level of GP88/PCDGF is performed using the method of claim 1.

3. A method of diagnosing tumorigenic activity in cells comprising measuring the level of GP88/PCDGF in a biological fluid sample using an anti-GP88/PCDGF antibody, and determining whether the level of GP88/PCDGF in said sample is sufficient to indicate tumorigenic activity in cells, wherein measuring the level of GP88/PCDGF is performed using the method of claim 1.

4. A method of determining whether a patient is responding or responsive to anti-tumorigenic therapy, comprising measuring the concentration of GP88/PCDGF in a biological fluid sample from a patient and determining whether the concentration of GP88/PCDGF in said sample is sufficient to indicate that said patient is not responding or responsive to anti-tumorigenic therapy, wherein measuring the level of GP88/PCDGF is performed using the method of claim 1.

5. An anti-GP88/PCDGF antibody produced from a hybridoma cell line selected from the group consisting of ATCC Accession Number PTA-5262 (6B3), ATCC Accession Number PTA-5261 (6B2), ATCC Number PTA-5589 (2A5), ATCC Number PTA-5593 (4D1), ATCC Number PTA-5259 (3F5), ATCC Number PTA-5260 (5B4), and ATCC Number PTA-5591 (3F8), wherein said anti-GP88/PCDGF antibody is capable of detecting GP88 at a concentration as low as 0.1 nanogram per millilitre.

6. A method according to claim 1, wherein said sample is a serum sample.

7. A kit for diagnosing tumorigenic activity in cells by measuring PCDGF concentration according to the method of claim 1, comprising a container, and an anti-human PCDGF antibody as described in claim 5 or an antigen-binding fragment thereof.

8. A kit for determining whether a patient is responding or responsive to anti-tumorigenic therapy by measuring PCDGF concentration according to the method of claim 1, comprising a container and an anti-human PCDGF antibody as described in claim 5 or an antigen-binding fragment thereof.

Patentansprüche

1. Verfahren zum Messen der Konzentration von GP88/PCDGF in einer Probe biologischer Flüssigkeit, welches umfasst das Kontaktieren genannter Probe mit einem Anti-GP88/PCDGF Antikörper oder einem Antikörperfragment davon und das Messen der Konzentration von GP88/PCDGF, wobei PCDGF fähig ist, mit einer Konzentration bis hinunter zu 0,1 Nanogramm von GP88/PCDGF pro Milliliter nachgewiesen zu werden, wobei der Anti-GP88/PCDGF Antikörper hergestellt ist aus einer Hybridomazelllinie ausgewählt aus der Gruppe bestehend aus ATCC Aufnahmenummer PTA-5262 (6B3), ATCC Aufnahmenummer PTA-5261 (6B2), ATCC Nummer PTA-5589 (2A5), ATCC Nummer PTA-5593 (4D1), ATCC Nummer PTA-5259 (3F5), ATCC Nummer PTA-5260 (5B4), und ATCC Nummer PTA-5591 (3F8).

2. Verfahren zur Diagnose tumorigener Aktivitäten in Zellen, welches umfasst: Messen Gehalts von GP88/PCDGF Protein in einer ersten Probe biologischer Flüssigkeit, die von einem Patienten genommen ist; Messen des Gehalts von GP88/PCDGF Protein in einer zweiten Probe biologischer Flüssigkeit, die von einem Patienten genommen ist; und Diagnose tumorigener Aktivität in Zellen durch Bestimmen, ob der gemessene Gehalt von GP88/PCDGF Protein in genannter zweiter Probe höher ist als der Gehalt in genannter erster Probe um einen Betrag, der ausreicht, die tumorigene Aktivität in Zellen anzuzeigen, wobei das Messen des Gehalts von GP88/PCDGF unter Anwendung des Verfahrens nach Anspruch 1 durchgeführt wird.

3. Verfahren zur Diagnose tumorigener Aktivität in Zellen, welches umfasst das Messen des Gehalts von GP88/PCDGF in einer Probe biologischer Flüssigkeit unter Verwendung eines Anti-GP88/PCDGF Antikörpers, und Bestimmen, ob der Gehalt von GP88/PCDGF in genannter Probe ausreichend ist, um tumorigene Aktivitäten anzuzeigen, wobei das Messen des Gehalts von GP88/PCDGF unter Verwendung des Verfahrens nach Anspruch 1 durchgeführt wird.

4. Verfahren zur Bestimmung, ob ein Patient auf eine anti-tumorigene Therapie anspricht oder empfänglich ist, welches umfasst das Messen der Konzentration von GP88/PCDGF in einer Probe biologischer Flüssigkeit von einem Patienten und Bestimmen, ob die Konzentration von GP88/PCDGF in genannter Probe ausreichend ist, um anzuzeigen, dass genannter Patient nicht auf eine Anti-tumorigene Therapie anspricht oder empfänglich ist, wobei das Messen des Gehalts von GP88/PCDGF durchgeführt wird unter Anwendung des Verfahrens nach Anspruch 1.

5. Anti-GP88/PCDGF Antikörper, hergestellt aus einer Hybridomazelllinie, die aus einer Gruppe ausgewählt ist, die aus ATCC Aufnahmenummer PTA-5262 (6B3), ATCC Aufnahmenummer PTA-5261 (6B2), ATCC Nummer PTA-5589 (2A5), ATCC Nummer PTA-5593 (4D1), ATCC Nummer PTA-5259 (3F5), ATCC Nummer PTA-5260 (5B4), und ATCC Nummer PTA-5591 (3F8) ausgewählt ist, wobei genannter Anti-GP88/PCDGF Antikörper fähig ist GP88/PCDGF mit einer Konzentration bis hinunter zu 0,1 Nanogramm pro Milliliter zu nachzuweisen.

6. Verfahren nach Anspruch 1, bei dem genannte Probe eine Serumprobe ist.

7. Kit zur Diagnose tumorigener Aktivität in Zellen durch Messen der PCDGF Konzentration nach dem Verfahren gemäß Anspruch 1, umfassend einen Behälter, und einen Antikörper gegen menschliches PCDGF wie im Anspruch 5 beschrieben oder ein Antigen-bindendes Fragment davon.

8. Kit zur Bestimmung, ob ein Patient auf eine Anti-tumorigene Therapie anspricht oder empfänglich ist, durch Messen der PCDGF Konzentration gemäß dem Verfahren nach Anspruch 1, umfassend einen Behälter und einen Antikörper gegen menschliches PCDGF wie in Anspruch 5 beschrieben oder ein Antigen-bindendes Fragment davon.

Revendications

1. Procédé de mesure de la concentration en GP88/PCDGF dans un échantillon de fluide biologique comprenant la mise en contact dudit échantillon avec un anticorps anti-GP88/PCDGF ou un fragment d'anticorps de celui-ci et la mesure de la concentration en GP88/PCDGF, dans lequel le PCDGF est capable d'être détecté à une concentration aussi faible que 0,1 nanogramme de GP88/PCDGF par millilitre, dans lequel l'anticorps anti-GP88/PCDGF est produit à partir d'une lignée cellulaire d'hybridome choisie dans le groupe constitué par le numéro d'accès ATCC PTA-5262 (6B3), le numéro d'accès ATCC PTA-5261 (6B2), le numéro d'accès ATCC PTA-5589 (2A5), le numéro d'accès ATCC PTA-5593 (4D1), le numéro d'accès ATCC PTA-5259 (3F5), le numéro d'accès ATCC PTA-5260 (5B4) et le numéro d'accès ATCC PTA-5591 (3F8).

2. Procédé de diagnostic d'une activité tumorigène dans des cellules comprenant : la mesure du taux de protéine GP88/PCDGF dans un premier échantillon de fluide biologique prélevé chez un patient ; la mesure du taux de protéine GP88/PCDGF dans un second échantillon de fluide biologique prélevé chez un patient ; et le diagnostic d'une activité tumorigène dans les cellules en déterminant si le taux mesuré de protéine GP88/PCDGF dans ledit second échantillon est plus élevé que le taux dans ledit premier échantillon d'une quantité suffisante pour indiquer une activité tumorigène dans les cellules, dans lequel la mesure du taux de GP88/PCDGF est effectuée au moyen du procédé de la revendication 1.

3. Procédé de diagnostic d'une activité tumorigène dans des cellules comprenant la mesure du taux de GP88/PCDGF dans un échantillon de fluide biologique à l'aide d'un anticorps anti-GP88/PCDGF, et la détermination consistant à établir si le taux de GP88/PCDGF dans ledit échantillon est suffisant pour indiquer une activité tumorigène dans les cellules, dans lequel la mesure du taux de GP88/PCDGF est effectuée au moyen du procédé de la revendication 1.

4. Procédé de détermination consistant à savoir si un patient répond ou est réactif à une thérapie anti-tumorigène, comprenant la mesure de la concentration de GP88/PCDGF dans un échantillon de fluide biologique provenant d'un patient et la détermination consistant à établir si la concentration de GP88/PCDGF dans ledit échantillon est suffisante pour indiquer que ledit patient ne répond pas ou n'est pas réactif à une thérapie anti-tumorigène, où la mesure du taux de GP88/PCDGF est effectuée au moyen du procédé de la revendication 1.

5. Anticorps anti-GP88/PCDGF produit à partir d'une lignée cellulaire d'hybridome choisie dans le groupe constitué par le numéro d'accès ATCC PTA-5262 (6B3), le numéro d'accès ATCC PTA-5261 (6B2), le numéro d'accès ATCC PTA-5589 (2A5), le numéro d'accès ATCC PTA-5593 (4D1), le numéro d'accès ATCC PTA-5259 (3F5), le numéro d'accès ATCC PTA-5260 (5B4) et le numéro d'accès ATCC PTA-5591 (3F8), où ledit anticorps anti-GP88/PCDGF est capable de détecter du GP88 à une concentration aussi faible que 0,1 nanogramme par millilitre.

6. Procédé selon la revendication 1, dans lequel ledit échantillon est un échantillon de sérum.

7. Kit pour diagnostiquer une activité tumorigène dans des cellules par mesure de la concentration en PCDGF selon le procédé de la revendication 1, comprenant un récipient, et un anticorps anti-PCDGF humain tel que décrit dans la revendication 5 ou un fragment de celui-ci se liant à l'antigène.

8. Kit pour déterminer si un patient répond ou est réactif à une thérapie anti-tumorigène par mesure de la concentration en PCDGF selon le procédé de la revendication 1, comprenant un récipient, et un anticorps anti-PCDGF humain tel que décrit dans la revendication 5 ou un fragment de celui-ci se liant à l'antigène.

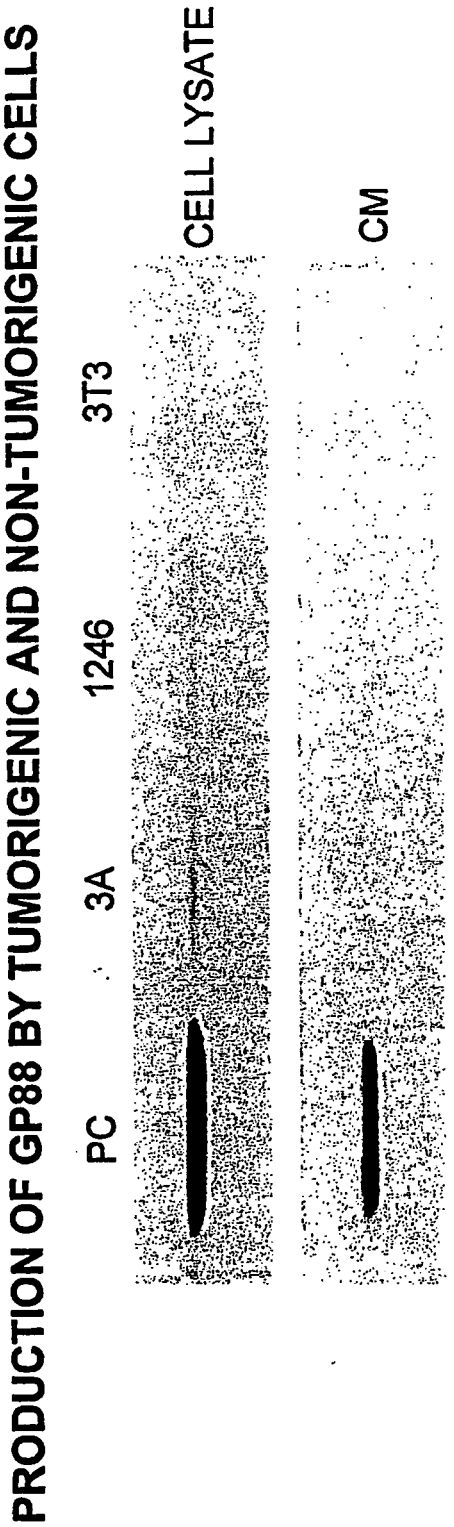

PRODUCTION OF GP88 BY TUMORIGENIC AND NON-TUMORIGENIC CELLS
PC
3A
1246
3T3
CELL LYSATE
CM

FIG. 1A

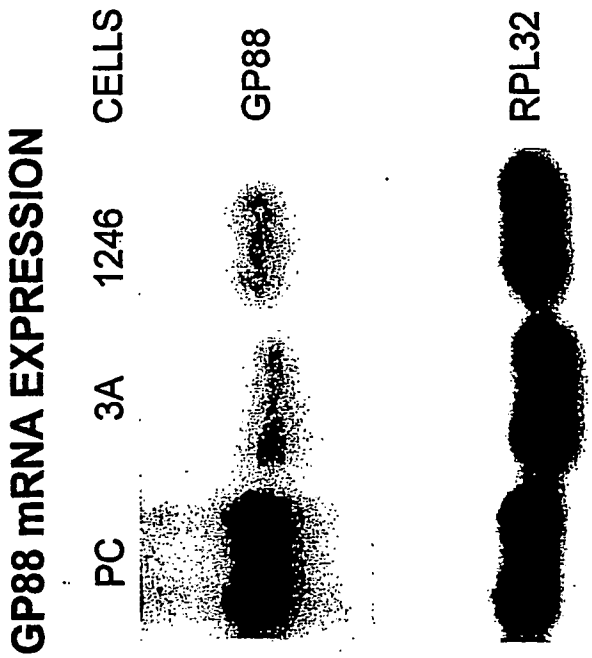


FIG.1B

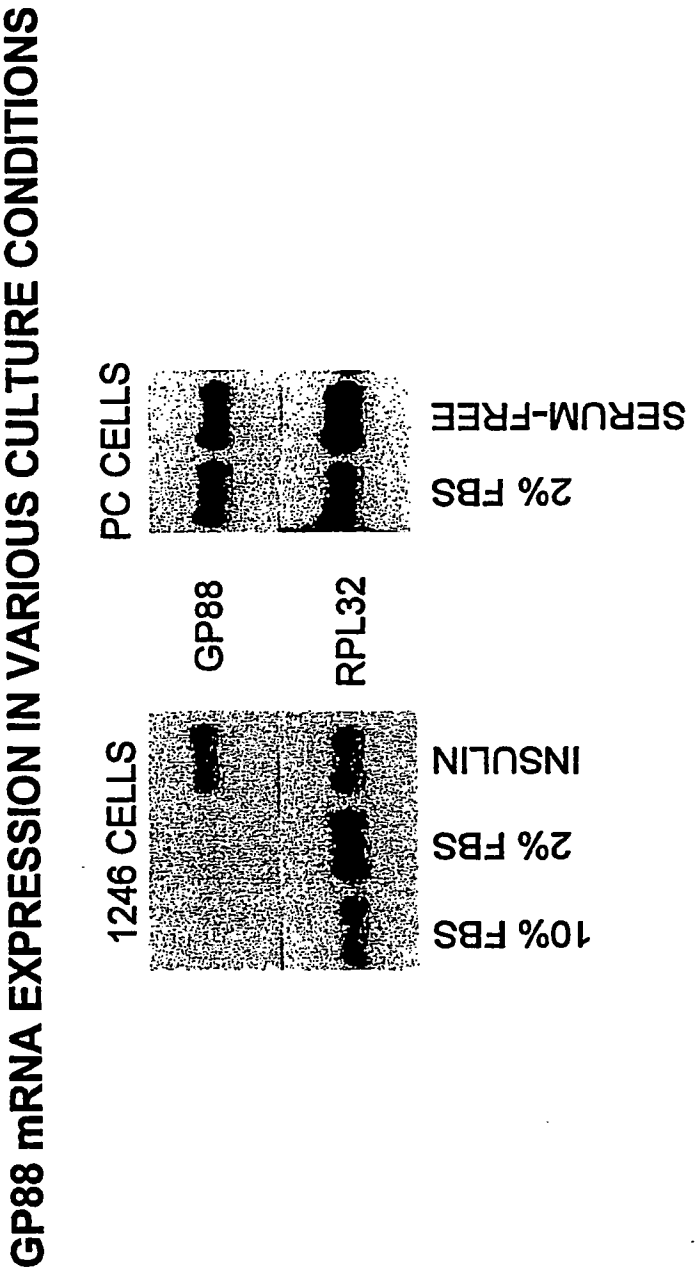

GP88 mRNA EXPRESSION IN VARIOUS CULTURE CONDITIONS
1246 CELLS
PC CELLS
GP88
RPL32
10% FBS
2% FBS
INSULIN
2% FBS
SERUM-FREE

FIG.1C

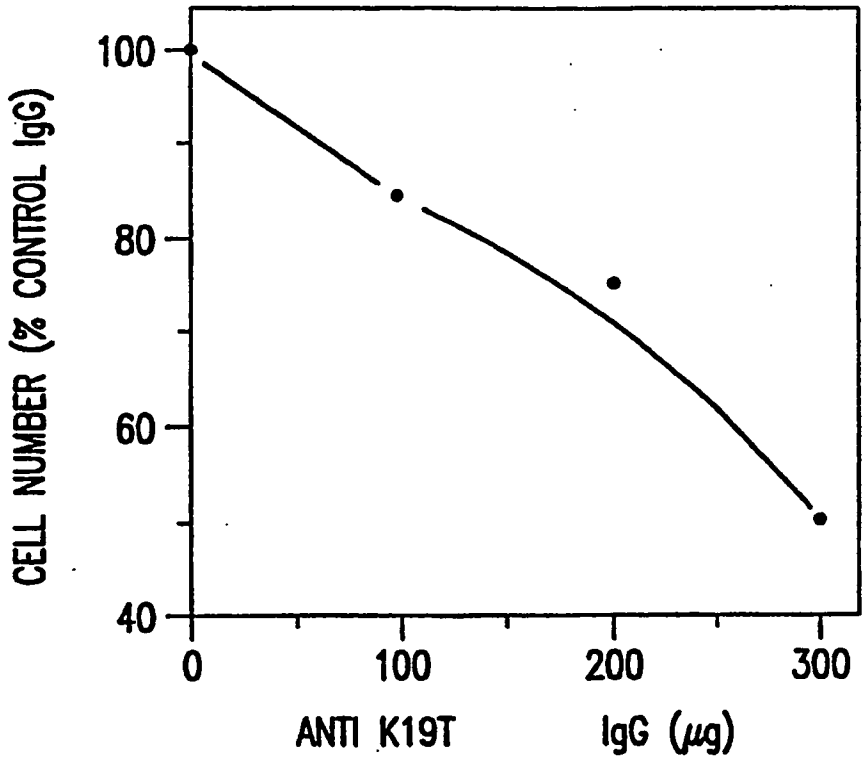
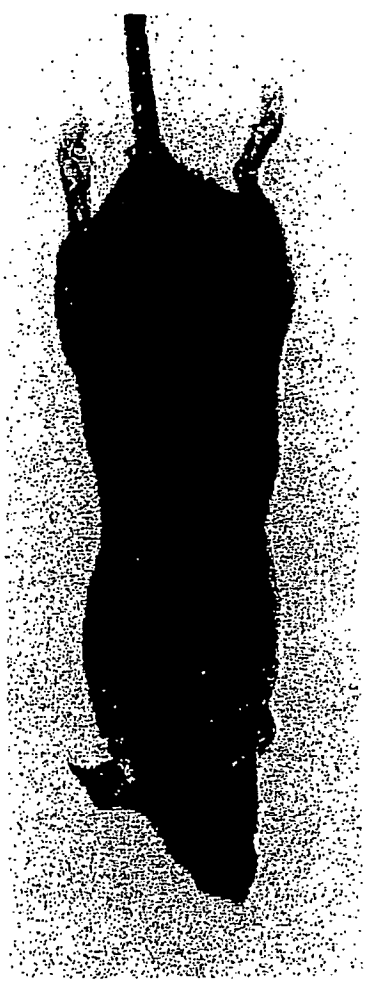

CELL NUMBER (% CONTROL IgG)
100
80
60
40
0
100
200
300
ANTI K19T IgG (μg)

FIG.2

ABSENCE OF TUMOR FORMATION IN C3H MICE BY INHIBITION OF GP88 EXPRESSION



GP88 ANTISENSE TRANSFECTED PC CELLS

CONTROL TRANSFECTED PC CELLS

FIG.3

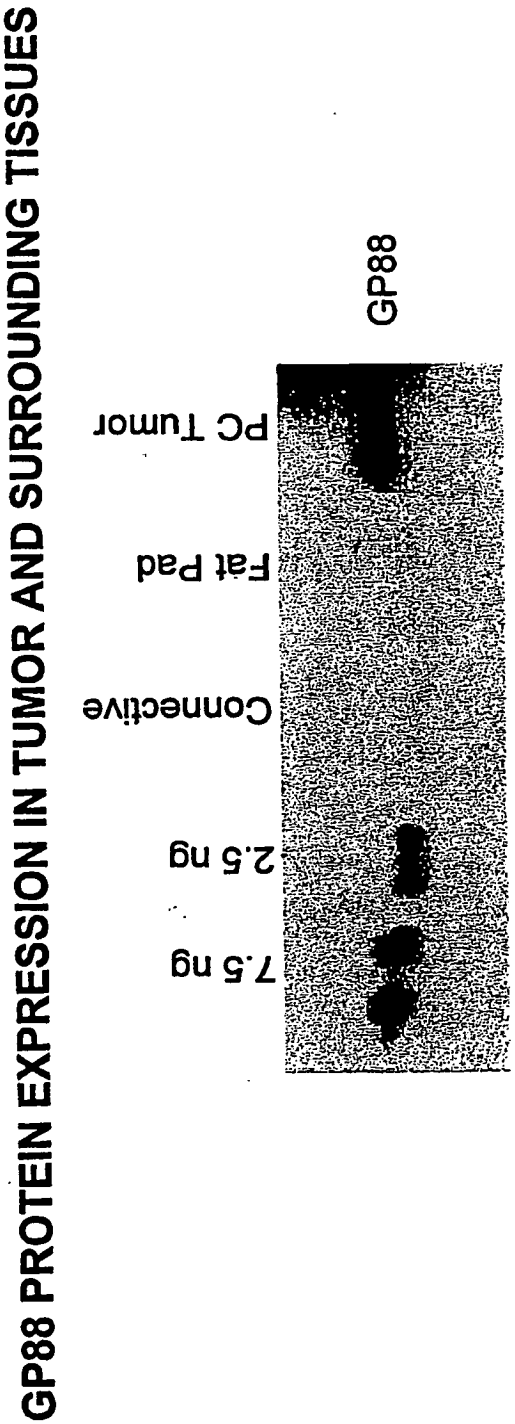

GP88 PROTEIN EXPRESSION IN TUMOR AND SURROUNDING TISSUES
GP88
PC Tumor
Fat Pad
Connective
2.5 ng
7.5 ng

FIG.4

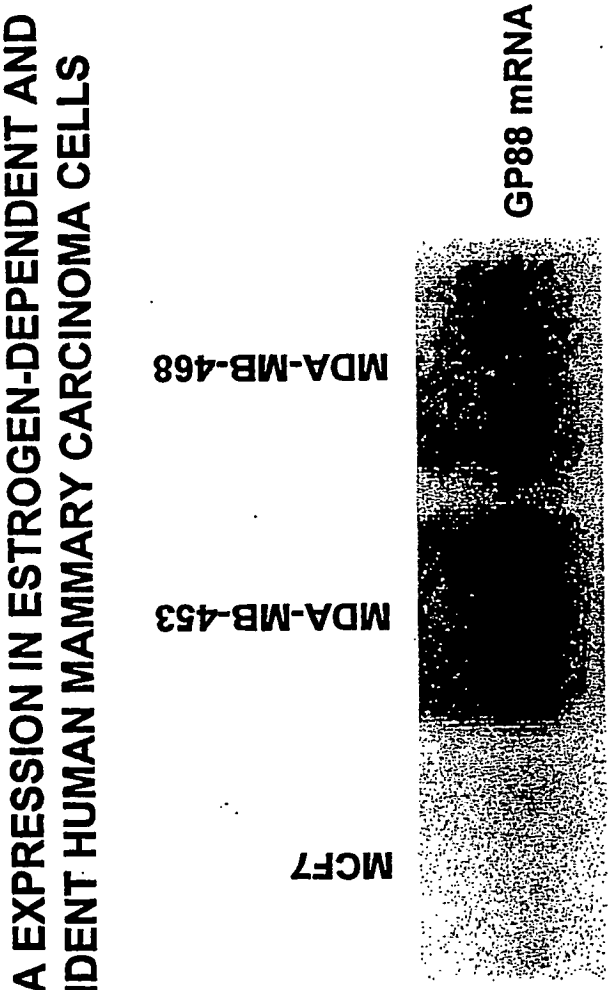

GP88 mRNA EXPRESSION IN ESTROGEN-DEPENDENT AND INDEPENDENT HUMAN MAMMARY CARCINOMA CELLS
GP88 mRNA
MDA-MB-468
MDA-MB-453
MCF7

FIG.5

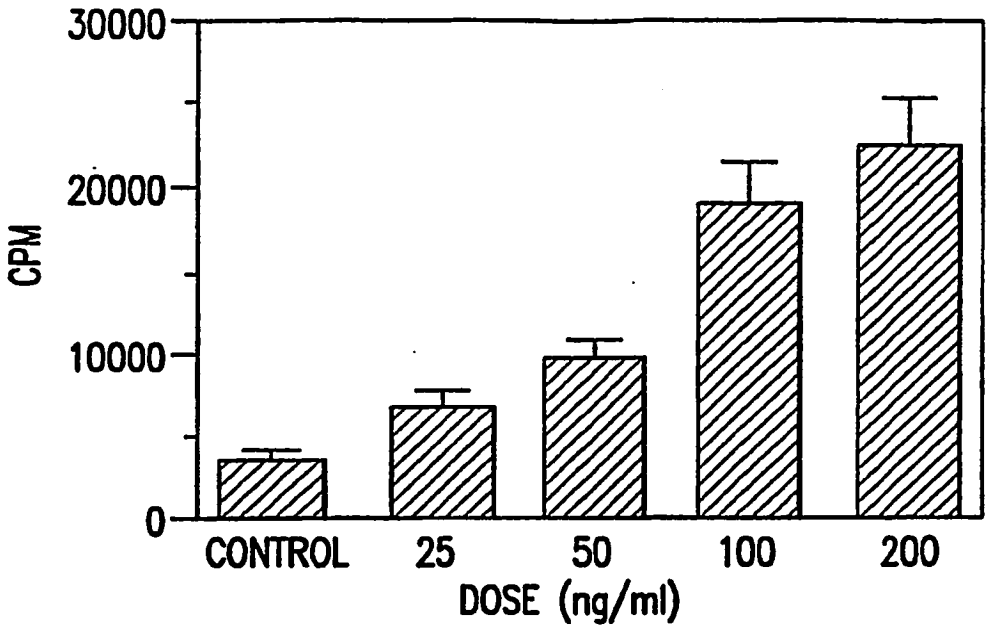

30000
20000
10000
0
CPM
CONTROL
25
50
100
200
DOSE (ng/ml)

FIG.6A

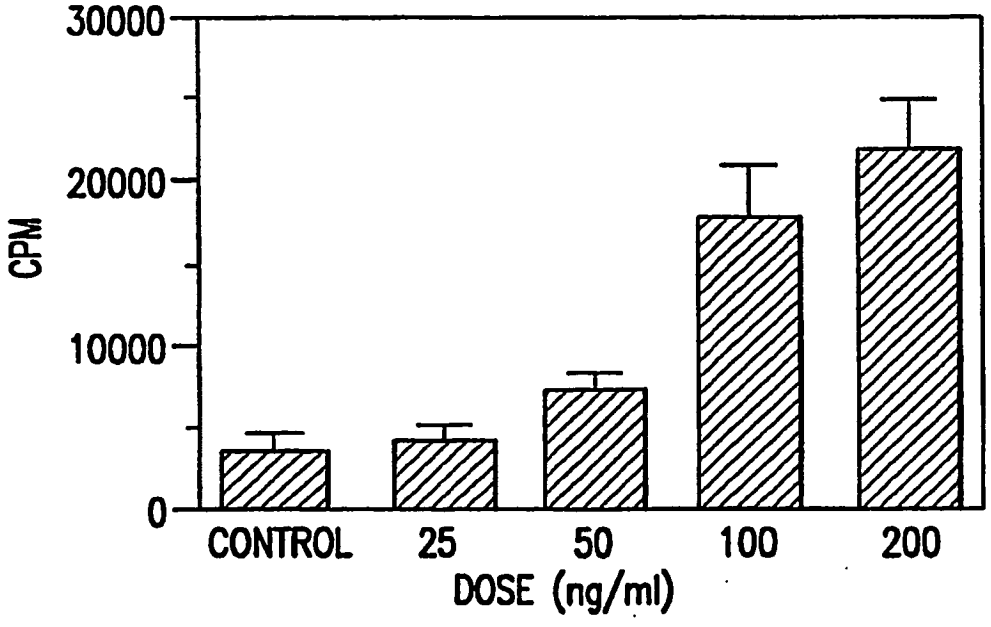

30000
20000
10000
0
CPM
CONTROL
25
50
100
200
DOSE (ng/ml)

FIG.6B

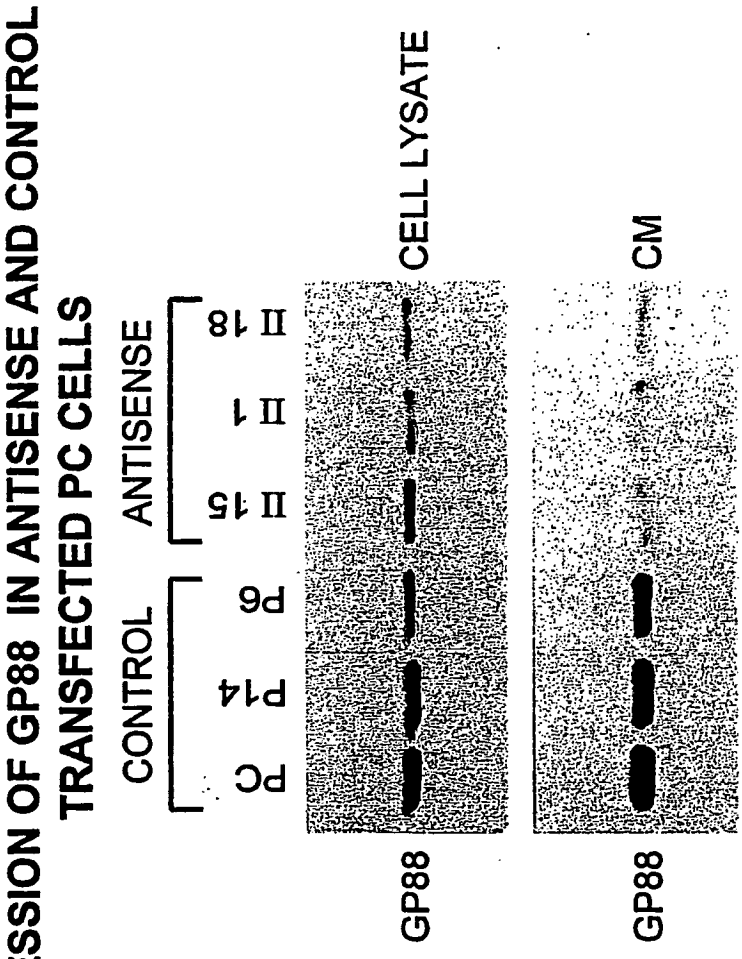

EXPRESSION OF GP88 IN ANTISENSE AND CONTROL TRANSFECTED PC CELLS
CONTROL
ANTISENSE
PC
P14
P6
II 15
II 1
II 18
CELL LYSATE
CM
GP88
GP88

FIG.7

Mouse GP88 cDNA

```
[C] GGA CCC CGA CGC AGA CAG ACC ATG TGG GTC CTG ATG AGC TGG CTG      46
                            M   W   V   L   M   S   W   L        8

GCC TTC GCG GCA GGG CTG GTA GCC GGA ACA CAG TGT CCA GAT GGG CAG      94
A   F   A   A   G   L   V   A   G   T   Q   C   P   D   G   Q      24

TTC TGC CCT GTT GCC TGC TGC CTT GAC CAG GGA GGA GCC AAC TA[C] AGC    142
F   C   P   V   A   C   C   L   D   Q   G   G   A   N   Y   S      40

TGC TGT AAC CCT CTT CTG GAC ACA TGG CCT AGA ATA ACG AGC CAT CAT     190
C   C   N   P   L   L   D   T   W   P   R   I   T   S   H   H      56

CTA GAT GGC TCC TGC CAG ACC CAT GGC CAC TGT CCT GCT GGC TAT TCT     238
L   D   G   S   C   Q   T   H   G   H   C   P   A   G   Y   S      72

TGT CTT CTC ACT GTG TCT GGG ACT TCC AGC TGC TGC CCG TTC TCT AAG     286
C   L   L   T   V   S   G   T   S   S   C   C   P   F   S   K      88

GGT GTG TCT TGT GGT GAT GGC TAC CAC TGC TGC CCC CAG GGC TTC CAC     334
G   V   S   C   G   D   G   Y   H   C   C   P   Q   G   F   H     104

TGT AGT GCA GAT GGG AAA TCC TGC TTC CAG ATG TCA GAT AAC CCC TTG     382
C   S   A   D   G   K   S   C   F   Q   M   S   D   N   P   L     120

GGT GCT GTC CAG TGT CCT GGG AGC CAG TTT GAA TGT CCT GAC TCT GCC     430
G   A   V   Q   C   P   G   S   Q   F   E   C   P   D   S   A     136

ACC TGC TGC ATT ATG GTT GAT GGT TCG TGG GGA TGT TGT CCC ATG CCC     478
T   C   C   I   M   V   D   G   S   W   G   C   C   P   M   P     152

CAG GCC TCT TGC TGT GAA GAC AGA GTG CAT TGC TGT CCC CAT GGG GCC     526
Q   A   S   C   C   E   D   R   V   H   C   C   P   H   G   A     168

TCC TGT GAC CTG GTT CAC ACA CGA TGC GTT TCA CCC ACG GGC ACC CAC     574
S   C   D   L   V   H   T   R   C   V   S   P   T   G   T   H     184

ACC CTA CTA AAG AAG TTC CCT GCA CAA AAG ACC AAC AGG GCA GTG TCT     622
T   L   L   K   K   F   P   A   Q   K   T   N   R   A   V   S     200

TTG CCT TTT TCT GTC GTG TGC CCT GAT GCT AAG ACC CAG TGT CCC GAT     670
L   P   F   S   V   V   C   P   D   A   K   T   Q   C   P   D     216
```

FIG.8A

Mouse GP88 cDNA (continued)

```
GAT TCT ACC TGC TGT GAG CTA CCC ACT GGG AAG TAT GGC TGC TGT CCA   718
D   S   T   C   C   E   L   P   T   G   K   Y   G   C   C   P   232

ATG CCC AAT GCC ATC TGC TGT TCC GAC CAC CTG CAC TGC TGC CCC CAG   766
M   P   N   A   I   C   C   S   D   H   L   H   C   C   P   Q   248

GAC ACT GTA TGT GAC CTG ATC CAG AGT AAG TGC CTA TCC AAG AAC TAC   814
D   T   V   C   D   L   I   Q   S   K   C   L   S   K   N   Y   264

ACC ACG GAT CTC CTG ACC AAG CTG CCT GGA TAC CCA GTG AAG GAG GTG   862
T   T   D   L   L   T   K   L   P   G   Y   P   V   K   E   V   280

AAG TGC GAC ATG GAG GTG AGC TGC CCT GAA GGA TAT ACC TGC TGC CGC   910
K   C   D   M   E   V   S   C   P   E   G   Y   T   C   C   R   296

CTC AAC ACT GGG GCC TGG GGC TGC TGT CCA TTT GCC AAG GCC GTG TGT   958
L   N   T   G   A   W   G   C   C   P   F   A   K   A   V   C   312

TGT GAG GAT CAC ATT CAT TGC TGC CCG GCA GGG TTT CAG TGT CAC ACA  1006
C   E   D   H   I   H   C   C   P   A   G   F   Q   C   H   T   328

GAG AAA GGA ACC TGC GAA ATG GGT ATC CTC CAA GTA CCC TGG ATG AAG  1054
E   K   G   T   C   E   X   G   I   L   Q   V   P   W   M   K   344

AAG GTC ATA GCC CCC CTC CGC CTG CCA GAC CCA CAG ATC TTG AAG AGT  1102
K   V   I   A   P   L   R   L   P   D   P   Q   I   L   K   S   360

GAT ACA CCT TGT GAT GAC TTC ACT AGG TGT CCT ACA AAC AAT ACC TGC  1150
D   T   P   C   D   D   F   T   R   C   P   T   N   N   T   C   376

TGC AAA CTC AAT TCT GGG GAC TGG GGC TGC TGT CCC ATC CCA GAG GCT  1198
C   K   L   N   S   G   D   W   G   C   C   P   I   P   E   A   392

GTC TGC TGC TCA GAC AAC CAG CAT TGC TGC CCT CAG GGC TTC ACA TGT  1246
V   C   C   S   D   N   Q   H   C   C   P   Q   G   F   T   C   408

CTG GCT CAG GGG TAC TGT CAG AAG GGA GAC ACA ATG GTG GCT GGC CTG  1294
L   A   Q   G   Y   C   Q   K   G   D   T   M   V   A   G   L   424

GAG AAG ATA CCT GCC CGC CAG ACA ACC CCG CTC CAA ATT GGA GAT ATC  1342
E   K   I   P   A   R   Q   T   T   P   L   Q   I   G   D   I   440
```

FIG.8B

Mouse GP88 cDNA (continued)

```
GGT TGT GAC CAG CAT ACC AGC TGC CCA GTA GGG CAA ACC TGC TGC CCA  1390
G   C   D   Q   H   T   S   C   P   V   G   Q   T   C   C   P    456

AGC CTC AAG GGA AGT TGG GCC TGC TGC CAG CTG CCC CAT GCT GTG TGC  1438
S   L   K   G   S   W   A   C   C   Q   L   P   H   A   V   C    472

TGT GAG GAC CGG CAG CAC TGT TGC CCG GCC GGG TAC ACC TGC AAC GTG  1486
C   E   D   R   Q   H   C   C   P   A   G   Y   T   C   N   V    488

AAG GCG AGG ACC TGT GAG AAG GAT GTC GAT TTT ATC CAG CCT CCC GTG  1534
K   A   R   T   C   E   K   D   V   D   F   I   Q   P   P   V    504

CTC CTG ACC CTC GGC CCT AAG GTT GGG AAT GTG GAG TGT GGA GAA GGG  1582
L   L   T   L   G   P   K   V   G   N   V   E   C   G   E   G    520

CAT TTC TGC CAT GAT AAC CAG ACC TGT TGT AAA GAC AGT GCA GGA GTC  1630
H   F   C   H   D   N   Q   T   C   C   K   D   S   A   G   V    536

TGG GCC TGC TGT CCC TAC CTA AAG GGT GTC TGC TGT AGA GAT GGA CGT  1678
W   A   C   C   P   Y   L   K   G   V   C   C   R   D   G   R    552

CAC TGT TGC CCC GGT GGC TTC CAC TGT TCA GCC AGG GGA ACC AAG TGT  1726
H   C   C   P   G   G   F   H   C   S   A   R   G   T   K   C    568

TTG CGA AAG AAG ATT CCT CGC TGG GAC ATG TTT TTG AGG GAT CCG GTC  1774
L   R   K   K   I   P   R   W   D   M   F   L   R   D   P   V    584

CCA ACA CCG CTA CTG TAA GGA AGG GCT ACA GAC TTA AGG AAC TCC ACA  1822
P   R   P   L   L   *                                            589

GTC CTG GGA ACC CTG TTC CGA GGG TAC CCA CTA CTC AGG CCT CCC TAG  1870
CGC CTC CTC CCC TAA CGT CTC CCC GGC CTA CTC ATC CTG AGT CAC CCT  1918
ATC ACC ATG GGA GGT GGA GCC TCA AAC TAA AAC CTT CTT TTA TGG AAA  1966
GAA GGC TGT GGC CAA AAG CCC CGT ATC AAA CTG CCA TTT CTT CCG GTT  2014
TCT GTG GAC CTT GTG GCC AGG TGC TCT TCC CGA GCC ACA GGT GTT CTG  2062
TGA GCT TGC TTG TGT GTG TGT GCG CGT GTG CGT GTG TTG CTC CAA TAA  2110
AGT TTG TAC GCT TTC TGA AAA AAA AAA                            2137
```

FIG.8C

Nucleotide sequence of human granulin/epithelin precursor (human GP88). Human Granulin Genbank M75161$

cgcaggcaga ccatgtggac cttggtgagc tgggtggcct taacagcagg gctggtggct
ggaacgcggt gcccagatgg tcagttctgc cctgtggcct gctgcctgga ccccggagga
gccagctaca gctgctgccg tccccttctg gacaaatggc ccacaacact gagcaggcat
ctgggtggcc cctgccaggt tgatgcccac tgctctgccg gccactcctg catctttacc
gtctcaggga cttccagttg ctgccccttc ccagaggccg tggcatgcgg ggatggccat
cactgctgcc cacggggctt ccactgcagt gcagacggga gatcctgctt ccaaagatca
ggtaacaact ccgtgggtgc catccagtgc cctgatagtc agttcgaatg cccggacttc
tccacgtgct gtgttatggt cgatggctcc tgggggtgct gccccatgcc ccaggcttcc
tgctgtgaag acagggtgca ctgctgtccg cacggtgcct tctgcgacct ggttcacacc
cgctgcatca cacccacggg cacccacccc ctggcaaaga agctccctgc ccagaggact
aacagggcag tggccttgtc cagctcggtc atgtgtccgg acgcacggtc ccggtgccct
gatggttcta cctgctgtga gctgcccagt gggaagtatg gctgctgccc aatgcccaac
gccacctgct gctccgatca cctgcactgc tgcccccaag acactgtgtg tgacctgatc
cagagtaagt gcctctccaa ggagaacgct accacggacc tcctcactaa gctgcctgcg
cacacagtgg gcgatgtgaa atgtgacatg gaggtgagct gcccagatgg ctatacctgc
tgccgtctac agtcgggggc ctggggctgc tgccctttta cccaggctgt gtgctgtgag
gaccacatac actgctgtcc cgcggggttt acgtgtgaca cgcagaaggg tacctgtgaa
caggggcccc accaggtgcc ctggatggag aaggccccag ctcacctcag cctgccagac
ccacaagcct tgaagagaga tgtcccctgt gataatgtca gcagctgtcc ctcctccgat
acctgctgcc aactcacgtc tggggagtgg ggctgctgtc caatcccaga ggctgtctgc
tgctcggacc accagcactg ctgcccccag cgatacacgt gtgtagctga ggggcagtgt
cagcgaggaa gcgagatcgt ggctggactg gagaagatgc ctgcccgccg cggttcctta
tcccacccca gagacatcgg ctgtgaccag cacaccagct gcccggtggg cggaacctgc
tgcccgagcc agggtgggag ctgggcctgc tgccagttgc cccatgctgt gtgctgcgag
gatcgccagc actgctgccc ggctggctac acctgcaacg tgaaggctcg atcctgcgag
aaggaagtgg tctctgccca gcctgccacc ttcctggccc gtagccctca cgtgggtgtg
aaggacgtgg agtgtgggga aggacacttc tgccatgata accagacctg ctgccgagac
aaccgacagg gctgggcctg ctgtccctac gcccagggcg tctgttgtgc tgatcggcgc
cactgctgtc ctgctggctt ccgctgcgca cgcaggggta ccaagtgttt gcgcagggag
gccccgcgct gggacgcccc tttgagggac ccagccttga gacagctgct gtgagggaca
gtactgaaga ctctgcagcc ctcgggaccc cactcggagg gtgccctctg ctcaggcctc
gtactgaaga ctctgcagcc ctcgggaccc cactcggagg gtgccctctg ctcaggcctc
cctagcacct ccccctaacc aaattctccc tggaccccat tctgagctcc ccatcaccat
gggaggtggg gcctcaatct aaggcccttc cctgtcagaa gggggttgag gcaaaagccc
attacaagct gccatcccct ccccgtttca gtggaccctg tggccaggtg cttttcccta
tccacagggg tgtttgtgtg ttgggtgtgc tttcaataaa gtttgtcact ttctt*

FIG.9A

Amino-acid sequence of human granulin/epithelin precursor (human GP88).

MWTLVSWVALTAGLVAGTRCPDGQFCPVACCLDPGGASYSCCRP
LLDKWPTTLSRHLGGPCQVDAHCSAGHSCIFTVSGTSSCCPFPEAVACGDGHHCCPRG
FHCSADGRSCFQRSGNNSVGAIQCPDSQFECPDFSTCCVMVDGSWGCCPMPQASCCED
RVHCCPHGAFCDLVHTRCITPTGTHPLAKKLPAQRTNRAVALSSSVMCPDARSRCPDG
STCCELPSGKYGCCPMPNATCCSDHLHCCPQDTVCDLIQSKCLSKENATTDLLTYLPA
HTVGDVKCDMEVSCPDGYTCCRLQSGAWGCCPFTQAVCCEDHIHCCPAGFTCDTQKGT
CEQGPHQVPWMEKAPAHLSLPDPQALKRDVPCDNVSSCPSSDTCCQLTSGEWGCCPIP
EAVCCSDHQHCCPQRYTCVAEGQCQRGSEIVAGLEKMPARRGSLSHPRDIGCDQHTSC
PVGGTCCPSQGGSWACCQLPHAVCCEDRQHCCPAGYTCNVKARSCEKEVVSAQPATFL
ARSPHVGVKDVECGEGHFCHDNQTCCRDNRQGWACCPYAQGVCCADRRHCCPAGFRCA
RRGTKCLRREAPRWDAPLRDPALRQLL*

FIG.9B

Mouse GP88 protein sequence

```
MWVLMSWLAFAAGLVAG   17

  TQCPDGQF-CPVA--CCLDQG-GANYSCCNPLLDTWPRITSHHL   57
    :      ::     ::    :   :::
  DGSC-QTHGHCPAGY-SCLLTVSGTS-SCCPFSKGVSCGDGYHCCPQGFHCSADGKSCFQMSDNPL   120                    g
    :      ::     :     : :  :::      : :  :::: :  :      :
 GAVQCPGSQFECPDSATCCIMVD-G-SWGCCPMPQASCCEDRVHCCPHGASCDLVHTRCVSPTGTHTLLKKFPAQKTNAAVSLPFS   204      f
  : ::     :::  :::     :   :::::: : :: :  ::::    :::    : :   :  :: : :
  VVCPDAKTQCPDDSTCCELP-TGK-YGCCPMPNAICCSDHLHCCPQDTVCDLIQSKCLSKNYTTDLLTKLPGYPVK   278          2,B
  : : :    ::   ::: :  ::   ::::   : :: :: ::::    :      :  •      :
  EVKC-DMEVSCPEGYTCCALN-TGA-WGCCPFAKAVCCEDHIHCCPAGFQCHTEKGTCEMGILQVPWMKKVIAPRRLPDPQILKS   360     1,A
    : :    ::   ::: ::  :  :::::   :::: :  :::: :: .      :  :   :    :  :    :
  DTPCDDFTR-CPTNNTCCKLN-SGD-WGCCPIPEAVCCSDNQHCCPQGFTCLAQGY-CQKGDTMVAGLEKIPARQTTPLQIG   438       C
  :  ::  :  ::   :::     :  : ::  : :::: : ::::: : ::      : :
  DIGCDuHT-SCPVGOTCCPSLK-G-SWACCQLPHAVCCEDRQHCCPAGYTCNVKARTCEKDVDFIQPPVLLTLGPKVG   513          D
    :      :  ::::.    :  ::::     ::: :  :::: :  :      :          :    :
  NVECGEGHF-CHDNQTCCKDSA-GV-WACCPYLKGVCCRDGRHCCPGGFHCSARGTKCLAKKIPRWDMFLADPVPRPLL   589          e
             •
```

consensus sequence:

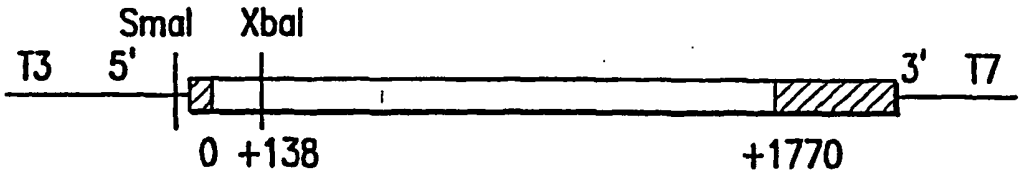
```
   C......C.....CC.....G....CC......CC.D..HCCP....C......C
```

1,2:mouse epithelin 1,2.
A,B,C,D,e,f,g: granulin A,B,C,D,E,F,G;N-terminus of granulin A,B,C,D have been sequenced.
Mouse epithelin precursor sequence is from Plowman et al.(1992).

FIG.10

EP 1 563 308 B1

GP88 cDNA CLONE in SK



STRUCTURE OF pCMV4 EXPRESSION VECTOR

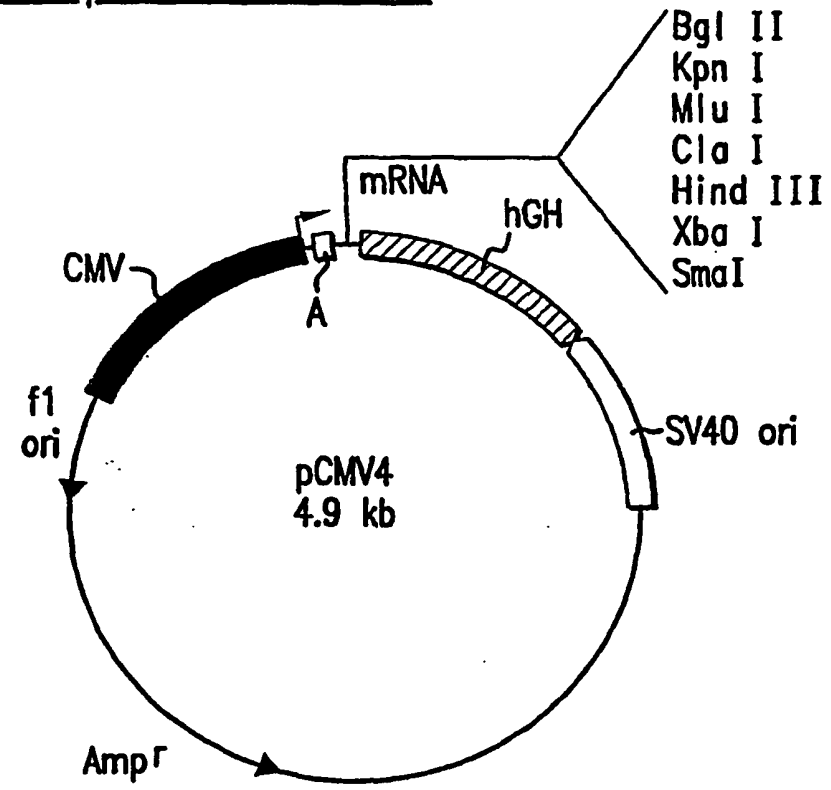


FIG.11

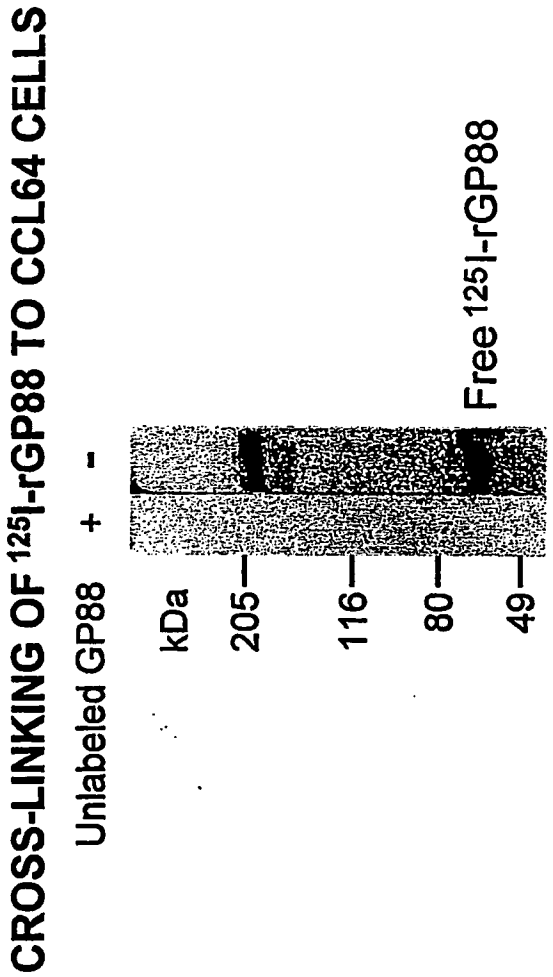

CROSS-LINKING OF 125I-rGP88 TO CCL64 CELLS
Unlabeled GP88
+
-
kDa
205
116
80
49
Free 125I-rGP88

FIG.12

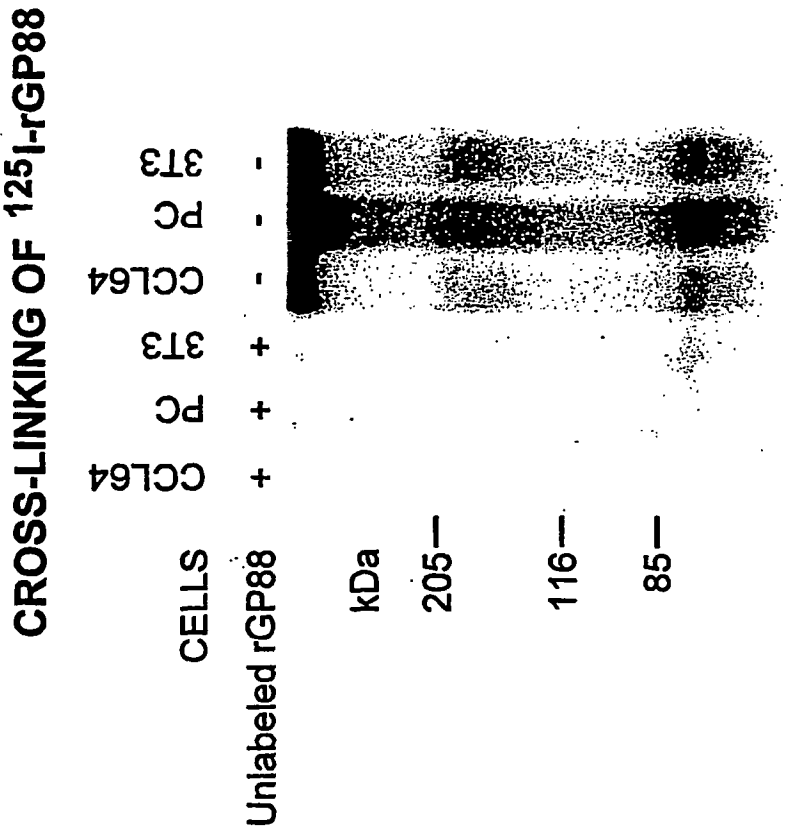

CROSS-LINKING OF 125I-rGP88
CELLS
Unlabeled rGP88
CCL64
PC
3T3
CCL64
PC
3T3
kDa
205
116
85

FIG.13

GP88 EXPRESSION IN NON TUMORIGENIC (MCF 10A) AND MALIGNANT (MCF 7, MDA-468) HUMAN MAMMARY EPITHELIAL CELLS

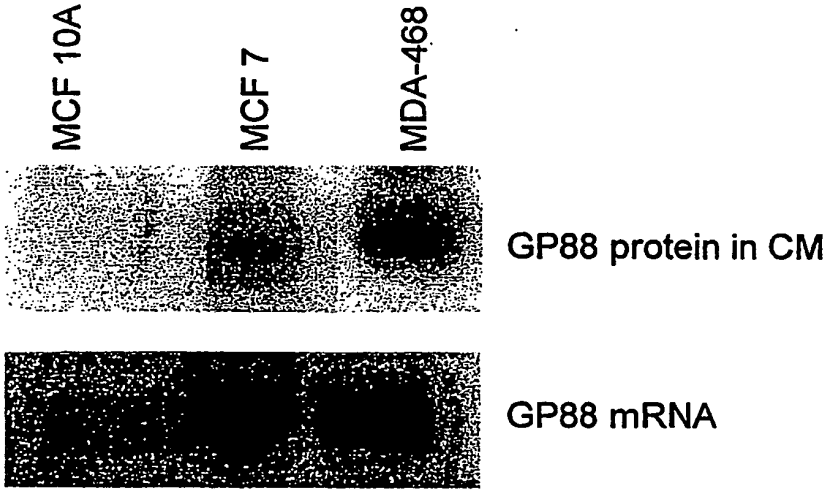


FIG.14

GP88 EXPRESSION IS INHIBITED BY ANTISENSE GP88 cDNA TRANSFECTION IN HUMAN BREAST CARCINOMA MDA-468

FIG.15

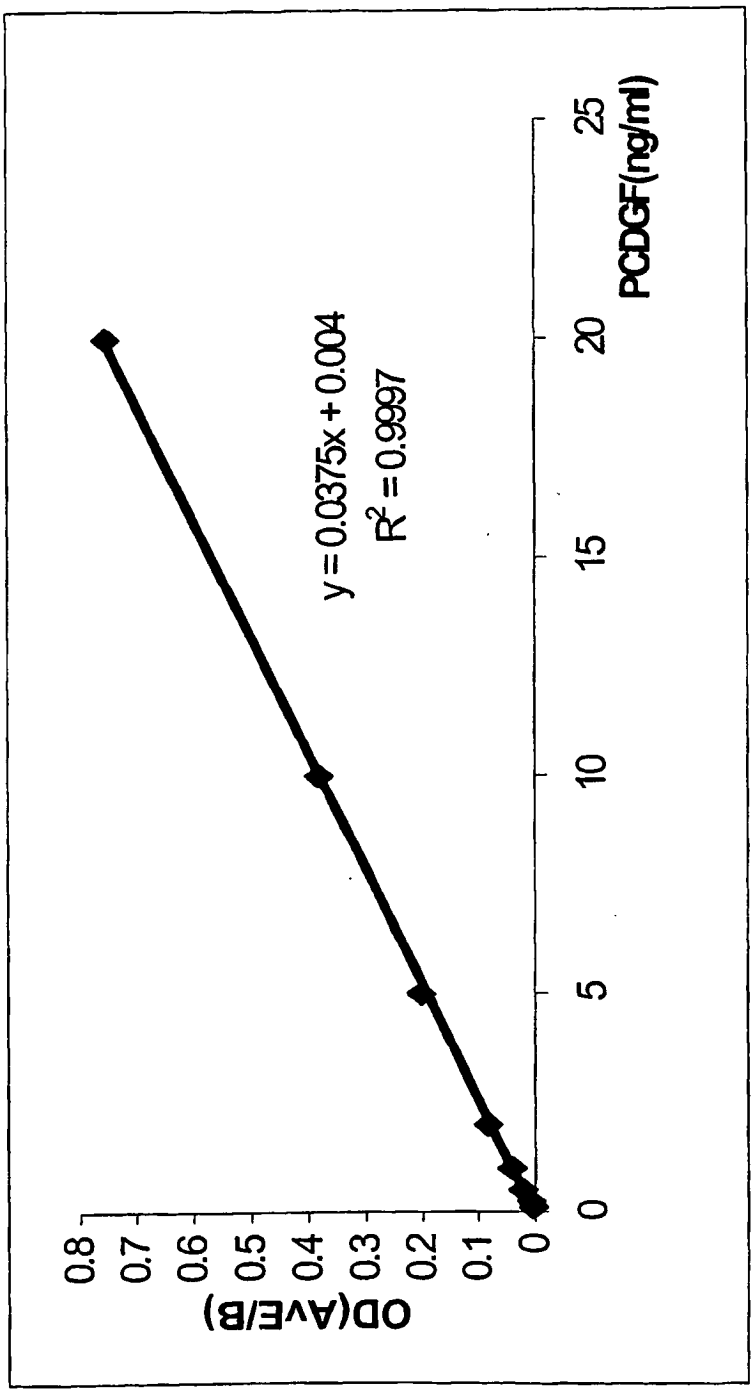

OD(A^E/B)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
5
10
15
20
25
PCDGF(ng/ml)
y = 0.0375x + 0.004
R2 = 0.9997

Figure 16

REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

- US 5416192 A, Shoyab **[0007]**
- US 6309826 B **[0009]**
- US 4376110 A **[0053]**

Non-patent literature cited in the description

- **Swerdlow et al.** British Medical Journal identified biopsy as the strongest risk factor for testicular cancer. *BMJ,* 1997, vol. 314, 1507 **[0009]**
- **Kronz et al.** *Cancer,* 01 December 1999, vol. 86 (11), 2426-2435 **[0009]**

专利名称(译)	用于诊断致瘤性的方法和试剂盒		
公开(公告)号	EP1563308B1	公开(公告)日	2011-10-19
申请号	EP2003776580	申请日	2003-10-27
[标]申请(专利权)人(译)	A&G药品公司		
申请(专利权)人(译)	A＆G制药公司.		
当前申请(专利权)人(译)	A＆G制药，INC.		
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外部链接	Espacenet		

摘要(译)

通过测量血液，血浆，血清，唾液，尿液和其他生物体液中GP88浓度来诊断致瘤性的方法和试剂盒。该方法和试剂盒检测生物体液中的GP88，浓度低至约0.1至10毫微克/毫升，可用于确定患者是否具有致瘤性病症，患者是否可能对抗肿瘤发生疗法有反应，以及通过测量患者血清或其他生物液体中GP88的浓度，治疗患者是否对抗肿瘤发生疗法有反应。

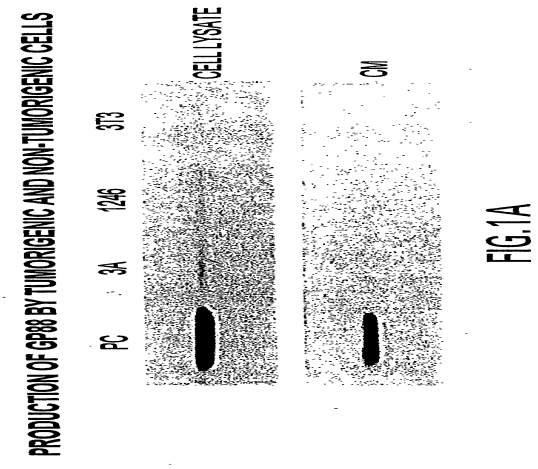

FIG.1A