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(54) **CRAB-PII DIRECTED DIAGNOSTICS FOR NEOPLASTIC DISEASE**

(76) Inventors: **Elias Georges**, Laval (CA); **Anne-Marie Bonneau**, Laval (CA)

Correspondence Address:
WILMERHALE/BOSTON
60 STATE STREET
BOSTON, MA 02109 (US)

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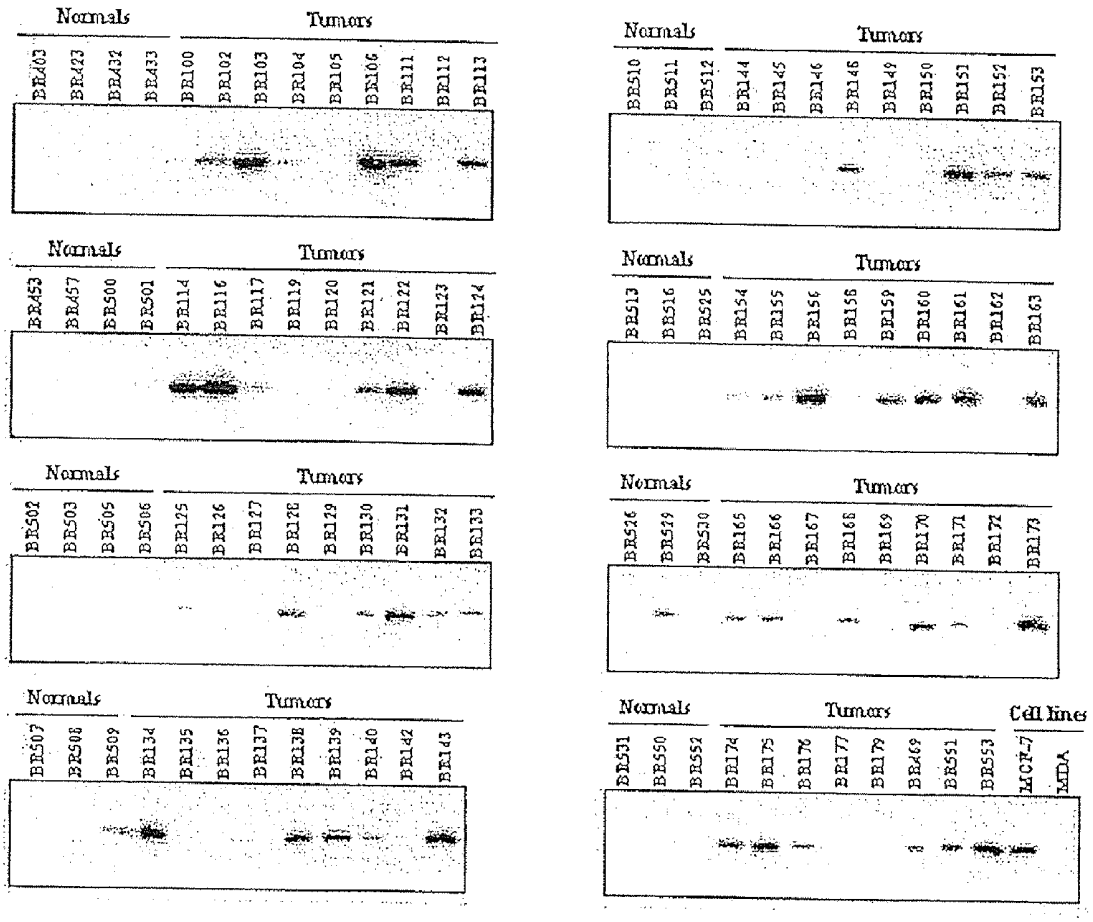
(57) **ABSTRACT**

Disclosed are methods for diagnosing cancer in a test cell sample or fluid sample by detecting an increase in the level of expression of CRAB-PII in the test cell sample or fluid sample as compared to the level of expression of CRAB-PII in a control cell sample or fluid sample isolated from a normal subject.

FIG 1


Normals
Tumors
BR403 BR423 BR432 BR433 BR100 BR102 BR103 BR104 BR105 BR106 BR111 BR112 BR113
Normals
Tumors
BR453 BR457 BR500 BR501 BR114 BR116 BR117 BR119 BR120 BR121 BR122 BR123 BR124
Normals
Tumors
BR502 BR503 BR505 BR506 BR125 BR126 BR127 BR128 BR129 BR130 BR131 BR132 BR133
Normals
Tumors
BR507 BR508 BR509 BR134 BR135 BR136 BR137 BR138 BR139 BR140 BR142 BR143
Normals
Tumors
BR510 BR511 BR512 BR144 BR145 BR146 BR148 BR149 BR150 BR151 BR152 BR153
Normals
Tumors
BR513 BR516 BR525 BR154 BR155 BR156 BR158 BR159 BR160 BR161 BR162 BR163
Normals
Tumors
BR526 BR529 BR530 BR165 BR166 BR167 BR168 BR169 BR170 BR171 BR172 BR173
Normals
Tumors
Cell lines
BR531 BR550 BR552 BR174 BR175 BR176 BR177 BR179 BR469 BR551 BR553 MCF-7 MDA

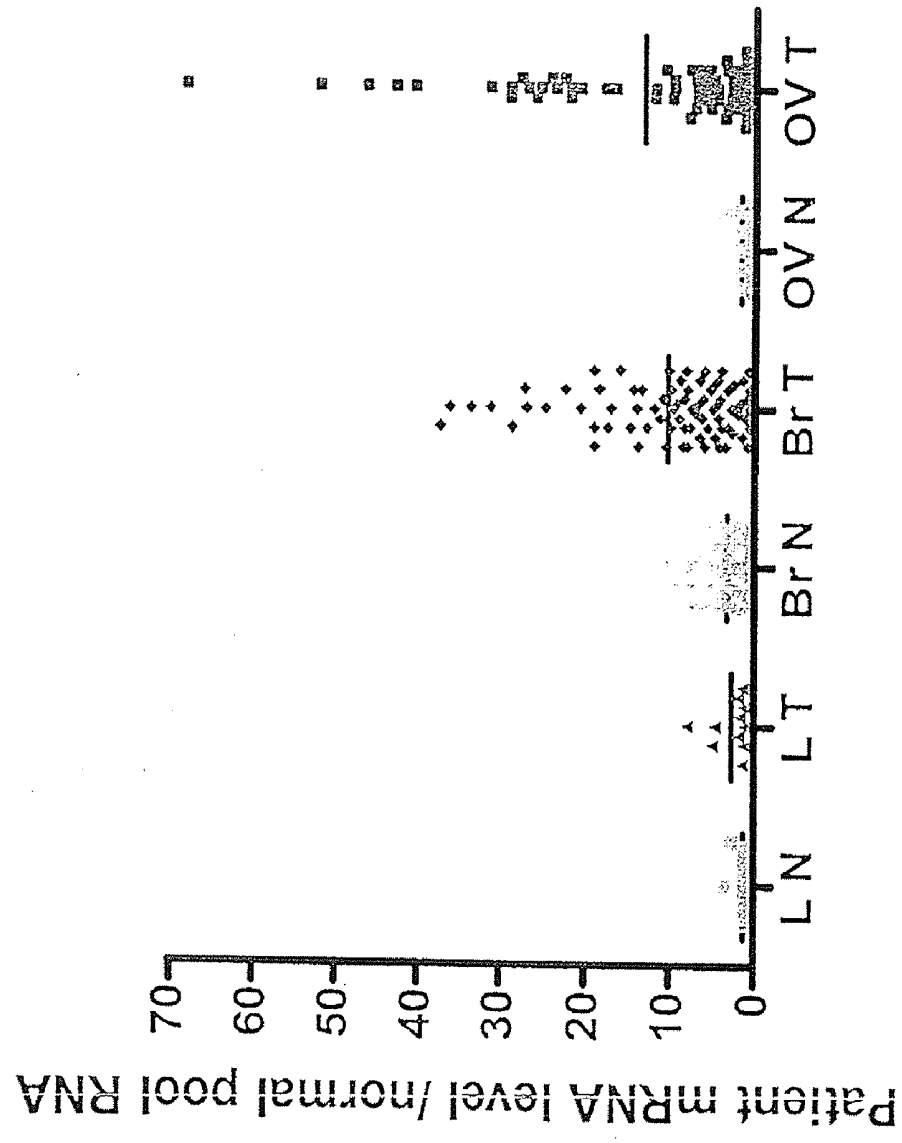

Patient mRNA level /normal pool RNA
70
60
50
40
30
20
10
0
LN
LT
BrN
BrT
OVN
OVT

FIG 2

FIG 3

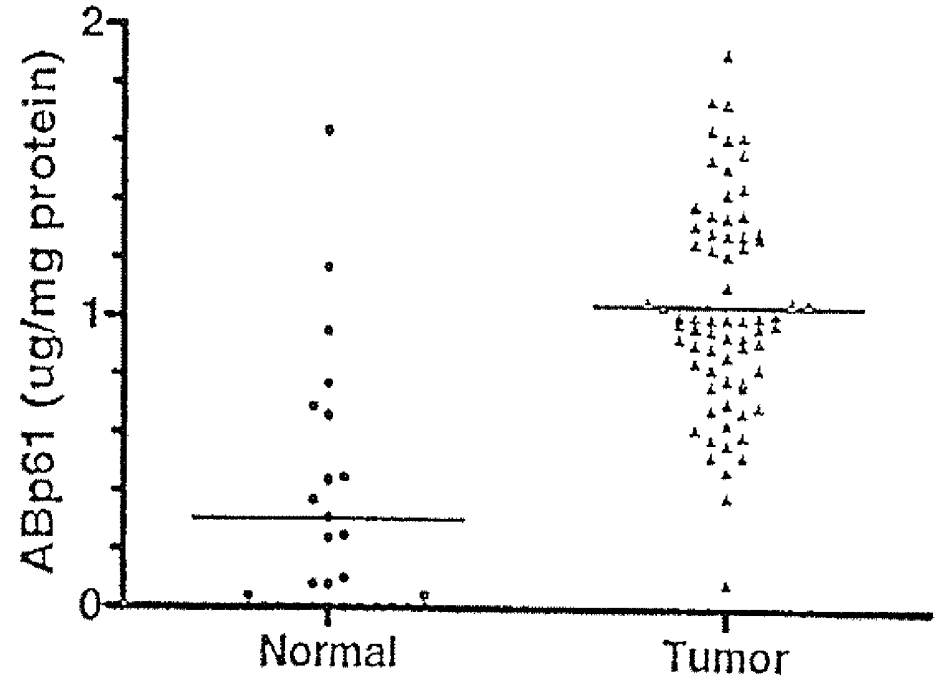
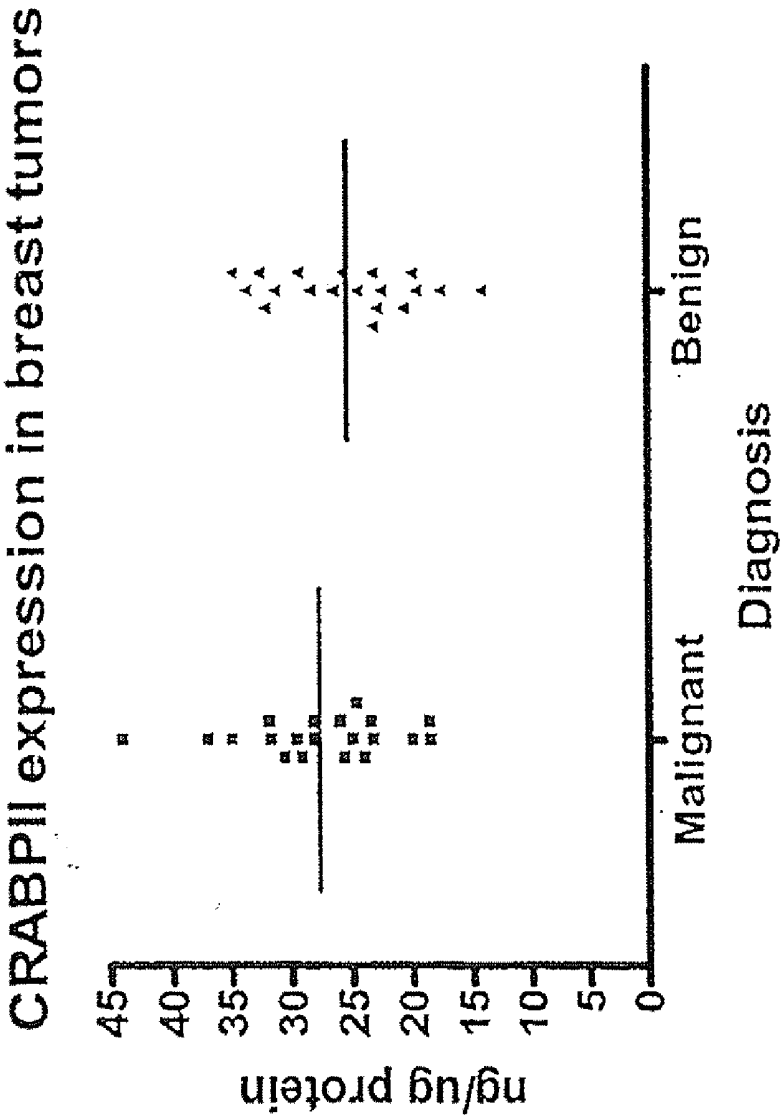


FIG 4


CRABPII expression in breast tumors
ng/ug protein
45
40
35
30
25
20
15
10
5
0
Malignant
Benign
Diagnosis

CRAB-PII DIRECTED DIAGNOSTICS FOR NEOPLASTIC DISEASE

[0001] This Application claims the benefit of priority to U.S. Provisional Application No. 60/993,582, filed Sep. 12, 2007, and to U.S. Provisional Application No. 60/993,576, filed Sep. 13, 2007, the specifications of which are incorporated by reference in their entirety.

FIELD OF THE INVENTION

[0002] The present invention relates generally to the field of medicine. More specifically, the invention pertains to methods and devices for detecting the development of cancer in a subject.

BACKGROUND OF THE INVENTION

[0003] Cancer is one of the deadliest illnesses in the United States. It accounts for nearly 600,000 deaths annually in the United States, and costs billions of dollars for those who suffer from the disease. This disease is in fact a diverse group of disorders, which can originate in almost any tissue of the body. In addition, cancers may be generated by multiple mechanisms including pathogenic infections, mutations, and environmental insults (see, e.g., Pratt et al. (2005) *Hum Pathol.* 36(8): 861-70). The variety of cancer types and mechanisms of tumorigenesis add to the difficulty associated with treating a tumor, increasing the risk posed by the cancer to the patient's life and wellbeing.

[0004] Cancers manifest abnormal growth and the ability to move from an original site of growth to other tissues in the body (hereinafter termed "metastasis"), unlike most non-cancerous cells. These clinical manifestations are therefore used to diagnose cancer because they are applicable to all cancers. Additionally, a cancer diagnosis is made based on identifying cancer cells by their gross pathology through histological and microscopic inspection of the cells. Although the gross pathology of the cells can provide accurate diagnoses of the cells, the techniques used for such analysis are hampered by the time necessary to process the tissues and the skill of the technician analyzing the samples. These methodologies can lead to unnecessary delay in treating a growing tumor, thereby increasing the likelihood that a benign tumor will acquire metastatic characteristics. It is thus necessary to accurately diagnose potentially cancerous growths as quickly as possible to avoid the development of a potentially life threatening illness.

[0005] One potential method of increasing the speed and accuracy of cancer diagnoses is the examination of genes as markers for neoplastic potential. Recent advances in molecular biology have identified genes involved in cell cycle control, apoptosis, and metabolic regulation (see, e.g., Isoldi et al. (2005)*Mini Rev. Med. Chem.* 5(7): 685-95). Mutations in many of these genes have also been shown to increase the likelihood that a normal cell will progress to a malignant state (see, e.g., Soejima et al. (2005) *Biochem. Cell Biol.* 83(4): 429-37). For example, mutations in p53, which is a well-known tumor suppressor gene, have been associated with aberrant cell growth leading to neoplastic potential (see Li et al. (2005) *World J. Gastroenterol.* 11(19): 2998-3001). Many mutations can affect the levels of expression of certain genes in the neoplastic cells as compared to normal cells.

[0006] There remains a need to identify an accurate and rapid means for diagnosing cancer in patients. Treatment efficacy would be improved by more efficient diagnoses of tissue samples. Furthermore, rapid diagnoses of cancerous tissues would allow clinicians to treat potential tumors prior to the metastasis of the cancer to other tissues of the body. Finally, a test that did not rely upon a particular technician's skill at identifying abnormal histological characteristics would improve the reliability of cancer diagnoses. There is, therefore, a need for new methods of diagnoses for cancer that are accurate, fast, and relatively easy to interpret.

SUMMARY OF THE INVENTION

[0007] The subject matter disclosed herein is based, in part, upon the discovery that differential expression of Cellular Retinoic Acid Binding Protein II ("CRAB-PII") at the protein and RNA levels occurs when a cell progresses to a neoplastic state. These expression patterns are therefore diagnostic for the presence of cancer in a cell sample. This discovery has been exploited to provide methods and compositions that uses such patterns of expression to diagnose the presence of neoplastic cells in the cell sample.

[0008] In one aspect, a method of detecting a neoplasm is provided. The method comprises obtaining a test cell sample and a non-neoplastic control cell sample. The method includes detecting a level of expression of CRAB-PII in the test cell sample, and detecting a level of expression of CRAB-PII in the control cell sample. The method further includes comparing the level of expression of CRAB-PII in the test cell sample to the level of expression of CRAB-PII in the control cell sample. The test cell sample is neoplastic if the level of expression of CRAB-PII in the test cell sample is greater than the level of expression of CRAB-PII in the control cell sample.

[0009] In some embodiments, the method includes isolating cellular cytoplasmic fractions from the test cell sample and the control cell sample, and then separately detecting the levels of expression of CRAB-PII in the cytoplasmic fractions. In other embodiments, the method includes the level of expression of CRAB-PII protein is detected by contacting the test cell sample and the control cell sample with a protein binding agent selected from the group consisting of antibody and retinoic acid. In other embodiments, the method comprises detecting the level of protein binding agents bound to CRAB-PII protein by detecting a detectable label such as immunofluorescent label, radiolabel, and chemiluminescent label.

[0010] In some embodiments, the protein-binding agent is immobilized on a solid support. In other embodiments, the detecting step comprises detecting the level of expression of CRAB-PII RNA is detected by contacting the test fluid and the non-neoplastic ovarian control fluid with a nucleic acid binding agent such as RNA, cDNA, cRNA, or RNA-DNA hybrids. In certain embodiments, the level of nucleic acid binding agent hybridized to CRAB-PII RNA is detected by a detectable label such as an immunofluorescent label, a radiolabel, or a chemiluminescent label. In still other embodiments, the nucleic acid binding agent is immobilized on a solid support.

[0011] In some embodiments, the level of expression of anti-CRAB-PII antibody is detected in a test fluid sample and a control fluid sample. In certain embodiments, the level of expression of anti-CRAB-PII antibody is detected in a serum sample isolated from a subject. In certain other embodiments,

the level of expression of anti-CRAB-PII antibody is detected using antibodies or fragments thereof. In particular embodiments, the antibodies or fragments thereof are operably linked to a detectable label such as an immunofluorescent label, radiolabel, and/or chemiluminescent label.

[0012] In some embodiments, the level of expression of CRAB-PII in the test fluid sample is at least 1.5 times greater than the level of expression of CRAB-PII in the control fluid sample. In other embodiments, the level of expression of CRAB-PII in the test fluid sample is at least 2 times greater than the level of expression of CRAB-PII in the control fluid sample. In still other embodiments, the level of expression of CRAB-PII in the test fluid sample is at least 4 times greater than the level of expression of CRAB-PII in the control fluid sample. In alternative embodiments, the level of expression of CRAB-PII in the test fluid sample is at least 6 times greater than the level of expression of CRAB-PII in the control fluid sample. In other embodiments, the level of expression of CRAB-PII in the test fluid sample is at least 8 times greater than the level of expression of CRAB-PII in the control fluid sample. In certain embodiments, the level of expression of CRAB-PII in the test fluid sample is at least 10 times greater than the level of expression of CRAB-PII in the control fluid sample. In some embodiments, the level of expression of CRAB-PII in the test fluid sample is at least 20 times greater than the level of expression of CRAB-PII in the control fluid sample.

[0013] In some embodiments, the test cell sample is obtained from a patient suffering from a metastasized ovarian neoplastic disease isolated from a tissue such as blood, bone marrow, spleen, lymph node, liver, thymus, kidney, brain, skin, gastrointestinal tract, eye, breast, and prostate. In other embodiments, the test cell sample is obtained from a patient suffering from an ovarian neoplasm such as ovarian carcinoma, ovarian epithelial adenocarcinoma, ovarian adenocarcinoma, sex cord-stromal carcinoma, endometrioid tumors, mucinous carcinoma, germ cell tumors, and clear cell tumors.

[0014] In some embodiments, the probe for detecting CRAB-PII is an anti-CRAB-PII antibody or binding fragment thereof. In other embodiments, the probe for detecting CRAB-PII is retinoic acid. In some embodiments, the probe detects CRAB-PII present in the test cell sample if the patient is suffering from neoplastic disease. In some embodiments, the probe is immobilized on a solid support.

[0015] In still another aspect, a method of diagnosing cancer in a subject is provided. The method comprises obtaining a test fluid sample (e.g., ovarian) and a control fluid sample from a non-neoplastic ovarian control sample. The method includes detecting a level of expression of CRAB-PII in the test fluid sample, and detecting a level of expression of CRAB-PII in the control fluid sample. The method further includes comparing the level of expression of CRAB-PII in the test fluid sample to the level of expression of CRAB-PII in the control fluid sample. Cancer is detected if the level of expression of CRAB-PII in the test fluid sample is greater than the level of expression of CRAB-PII in the control fluid sample.

[0016] In some embodiments, the method includes detecting the level of expression of CRAB-PII, which comprises isolating cellular cytoplasmic fractions from the test fluid sample and the control fluid sample, and separately detecting the level of expression of CRAB-PII in the cellular cytoplasmic fractions. In other embodiments, the method includes detecting the level of expression of CRAB-PII protein by contacting the test fluid sample and the control fluid sample with a protein binding agent selected from the group consisting of antibody and retinoic acid. In other embodiments, the method includes the level of protein binding agents bound to CRAB-PII protein is detected by a detectable label such as immunofluorescent label, radiolabel, and chemiluminescent label.

[0017] In some embodiments, the protein-binding agent is immobilized on a solid support. In certain embodiments, the level of expression of anti-CRAB-PII antibody is detected in a test fluid sample and a control fluid sample. In other embodiments, the level of expression of anti-CRAB-PII antibody is detected in a serum sample isolated from a subject. In still more embodiments, the level of expression of anti-CRAB-PII antibody is detected by antibodies or fragments thereof. In still further embodiments, the antibodies or fragments thereof are operably linked to a detectable label selected from the group consisting of a immunofluorescent label, radiolabel, and chemiluminescent label.

[0018] In other embodiments, the method involves the level of expression of CRAB-PII RNA is detected by contacting the test fluid and the non-neoplastic ovarian control fluid with a nucleic acid binding agent such as RNA, cDNA, cRNA, and RNA-DNA hybrids. In certain embodiments, the level of nucleic acid binding agent hybridized to CRAB-PII RNA is detected by a detectable label such as immunofluorescent label, radiolabel, and chemiluminescent label. In still other embodiments, the nucleic acid binding agent is immobilized on a solid support.

[0019] In some embodiments, the level of expression of CRAB-PII in the test fluid sample is 1.5 times greater than the level of expression of CRAB-PII in the control fluid sample. In other embodiments, the level of expression of CRAB-PII in the test fluid sample is 2 times greater than the level of expression of CRAB-PII in the control fluid sample. In still other embodiments, the level of expression of CRAB-PII in the test fluid sample is 4 times greater than the level of expression of CRAB-PII in the control fluid sample. In alternative embodiments, the level of expression of CRAB-PII in the test fluid sample is 6 times greater than the level of expression of CRAB-PII in the control fluid sample.

[0020] In other embodiments, the level of expression of CRAB-PII in the test fluid sample is 8 times greater than the level of expression of CRAB-PII in the control fluid sample. In certain embodiments, the level of expression of CRAB-PII in the test fluid sample is 10 times greater than the level of expression of CRAB-PII in the control fluid sample. In some embodiments, the level of expression of CRAB-PII in the test fluid sample is at least 20 times greater than the level of expression of CRAB-PII in the control fluid sample.

[0021] In some embodiments, the test fluid sample is from a patient suffering from a metastasized ovarian neoplastic disease isolated from a tissue such as blood, bone marrow, spleen, lymph node, liver, thymus, kidney, brain, skin, gastrointestinal tract, eye, breast, and prostate. In more embodiments, the test fluid sample is a patient suffering from an ovarian neoplasm such as ovarian carcinoma, ovarian epithelial adenocarcinoma, ovarian adenocarcinoma, sex cord-stromal carcinoma, endometrioid tumors, mucinous carcinoma, germ cell tumors, and clear cell tumors. In still other embodiments, the test cell sample is a cell such as blood cells, bone marrow cells, spleen cells, lymph node cells, liver cells, thy-

mus cells, kidney cells, brain cells, skin cells, gastrointestinal tract cells, eye cells, breast cells, prostate cells, uterine cells, and ovary cells.

[0022] In certain embodiments, the fluid sample is isolated from saliva, tears, urine, sweat, plasma, blood, or serum.

[0023] In another aspect a kit for diagnosing or detecting neoplasia is provided. The kit includes a probe for the detection of CRAB-PII.

[0024] In some embodiments, the probe for detecting CRAB-PII is an anti-CRAB-PII antibody or binding fragment thereof. In other embodiments, the probe for detecting CRAB-PII is retinoic acid. In some embodiments, the first probe detects CRAB-PII present in the test fluid sample if the patient is suffering from ovarian neoplastic disease. In still other embodiments, the second probe detects a marker present of the surface of the test cell if the patient is suffering from ovarian neoplastic disease. In some embodiments, the probe is immobilized on a solid support. In some embodiments, the CRAB-PII probe is a nucleic acid probe such as RNA, cDNA, cRNA, and RNA-DNA hybrids. In certain embodiments, the CRAB-PII probe is complementary to at least 20 a nucleotide sequence of a nucleic acid sequence consisting of SEQ ID NO: 1.

[0025] In other embodiments, the probe binds to an anti-CRAB-PII antibody. In particular embodiments, the probe is an antibody or fragment thereof operably linked to a detectable label.

BRIEF DESCRIPTION OF THE FIGURES

[0026] The foregoing and other objects of the present invention, the various features thereof, as well as the invention itself may be more fully understood from the following description, when read together with the accompanying drawings in which:

[0027] FIG. **1** is a photographic representation showing the results of 10 immunoblots on tumor samples isolated from breast cancer patients ("Tumors") and normal samples isolated from normal subjects ("Normal").

[0028] FIG. **2** is a graphic representation of nucleic acid microarray analyses of CRAB-PII RNA expression in samples from ovarian cancer patients (OVT), breast cancer patients (BrT), and lung cancer patients (LT) as well as normal, tissue-matched subjects (OVN, BrN, and LN).

[0029] FIG. **3** is a graphic representation showing scatter plots in which each dot represents the results of protein expression experiments (Numbered 1-10) on samples from normal subjects ("Normal") and breast cancer patients ("Tumor").

[0030] FIG. **4** is a graphic representation of a scatter plot showing the level of expression of CRAB-PII protein as compared to total protein isolated from malignant breast tumors obtained from patients ("Malignant") and isolated from benign breast tumors obtained from subjects ("Benign").

DETAILED DESCRIPTION OF THE INVENTION

[0031] Patent and scientific literature referred to herein establishes knowledge that is available to those of skill in the art. The issued US patents, allowed applications, published foreign applications, and references, including GenBank database sequences, that are cited herein are hereby incorporated by reference to the same extent as if each was specifically and individually indicated to be incorporated by reference.

1.1. General

[0032] Methods and kits are disclosed for diagnosing, detecting, or screening a test sample, such as a fluid sample, for tumorigenic potential and neoplastic characteristics such as aberrant growth. In addition, the methods and compositions allow for the improved clinical management of tumors by providing a method that detects the expression level of a gene or genes identified as markers for cancer.

[0033] Typically, a gene will affect the phenotype of the cell through its expression at the protein level. Mutations in the coding sequence of the gene can alter its protein product in such a way that the protein does not perform its intended function appropriately. Some mutations, however, affect the levels of protein expressed in the cell without altering the functionality of the protein, itself. Such mutations directly affect the phenotype of a cell by changing the delicate balance of protein expression in a cell. Therefore, an alteration in a gene's overall activity can be measured by determining the level of expression of the protein product of the gene in a cell.

[0034] Accordingly, one aspect provides a method for diagnosing cancer in a cell. The method utilizes protein-targeting agents to identify proteins, such as CRAB-PII, in a potentially cancerous cell sample or potentially cancerous serum or fluid sample. Increased levels of expression of particular protein markers in a cell or serum or fluid sample and a decreased expression level of other protein markers in a cell or serum or fluid sample indicate the presence of a neoplasm.

[0035] As used herein, "about" means a numeric value having a range of ±10% around the cited value. For example, a range of "about 1.5 times to about 2 times" includes the range "1.35 times to 2.2 times" as well as the range "1.65 times to 1.8 times," and all ranges in between.

[0036] As used herein, the term "greater than" means more than, such as when the level of expression for a particular marker in test sample is detectably more than the level of expression for the same marker in a control sample. In these circumstances, expression analyses are qualitatively determined. The level of expression for a marker can also be determined quantitatively in test and control samples. In quantitative studies, the level of expression for a marker in a test sample is greater than the level of expression for the same marker in a control sample when the level of expression in the test sample is quantifiably determined to be at least about 10% more than the level of expression in the control sample.

[0037] As used herein, the term "protein-targeting agent" means a molecule capable of binding or interacting with a protein or a portion of a protein. Such binding or interactions can include ionic bonds, van der Waals interactions, London forces, covalent bonds, and hydrogen bonds. The target protein can be bound in a receptor-binding pocket, on its surface, or any other portion of the protein that is accessible to binding or interactions with a molecule. Protein-targeting agents include, but are not limited to, proteins, peptides, ligands, peptidomimetic compounds, inhibitors, organic molecules, aptamers, or combinations thereof.

[0038] As used herein, the term "inhibitor" means a compound that prevents a biomolecule, e.g., a protein, nucleic acid, or ribozyme, from completing or initiating a reaction. An inhibitor can inhibit a reaction by competitive, uncompetitive, or non-competitive means. Exemplary inhibitors

include, but are not limited to, nucleic acids, proteins, small molecules, chemicals, peptides, peptidomimetic compounds, and analogs that mimic the binding site of an enzyme. In some embodiments, the inhibitor can be nucleic acid molecules including, but not limited to, siRNA that reduce the amount of functional protein in a cell.

[0039] As used herein, the term "tumorigenic potential" means ability to give rise to either benign or malignant tumors. Tumorigenic potential may occur through genetic mechanisms such as mutation or through infection with vectors such as viruses and bacteria.

[0040] The term "cancer" refers herein to a disease condition in which a tissue or cells exhibit aberrant, uncontrolled growth and/or lack of contact inhibition. A cancer can be a single cell or a tumor composed of hyperplastic cells. In addition, cancers can be malignant and metastatic, spreading from an original tumor site to other tissues in the body. In contrast, some cancers are localized to a single tissue of the body.

[0041] As used herein, a "cancer cell" is a cell that shows aberrant cell growth, such as increased, uncontrolled cell proliferation and/or lack of contact inhibition. A cancer cell can be a hyperplastic cell, a cell from a cell line that shows a lack of contact inhibition when grown in vitro, or a cancer cell that is capable of metastasis in vivo. In addition, cancer cells include cells isolated from a tumor or tumors. As used herein, a "tumor" is a collection of cells that exhibit the characteristics of cancer cells. Non-limiting examples of cancer cells include melanoma, ovarian cancer, ovarian cancer, renal cancer, osteosarcoma, lung cancer, prostate cancer, sarcoma, leukemic retinoblastoma, hepatoma, myeloma, glioma, mesothelioma, carcinoma, leukemia, lymphoma, Hodgkin lymphoma, Non-Hodgkin lymphoma, promyelocytic leukemia, lymphoblastoma, and thymoma. Cancer cells are also located in the blood at other sites, and include, but are not limited to, lymphoma cells, melanoma cells, sarcoma cells, leukemia cells, retinoblastoma cells, hepatoma cells, renal cancer cells, osteosarcoma cells, myeloma cells, glioma cells, mesothelioma cells, and carcinoma cells.

[0042] Cancer cells may also have the ability to metastasize to other tissues in the body. Metastasis is the process by which a cancer cell is no longer confined to the tumor mass, and enters the blood stream, where it is transported to a second site. Upon entering the other tissue, the cancer cell gives rise to a second situs for the disease and can take on different characteristics from the original tumor. Nevertheless, the new tumor retains characteristics from the tissue from which it derives, allowing for clinical identification of the type of cancer no matter where in the body a cancer cell or group of cells metastasizes. The process of metastasis has been studied extensively and is known in the art (see, e.g., Hendrix et al. (2000) *Breast Cancer Res.* 2(6): 417-22).

[0043] In certain embodiments of the invention, the cancer cell sample is obtained from a metastasized tumor or group of cells. The metastasized cells may be isolated from tissues including, but not limited to, blood, bone marrow, lymph node, liver, thymus, kidney, brain, skin, gastrointestinal tract, breast, and prostate.

[0044] The term "protein markers" as used herein means any protein, peptide, polypeptides, group of peptides, polypeptides or proteins expressed from a gene, whether chromosomal, extrachromosomal, endogenous, or exogenous, which may produce a cancerous or non-cancerous phenotype in the cell or the organism.

[0045] As used herein, "gene" means any deoxyribonucleic acid sequence capable of being translated into a protein or peptide sequence. The gene is a DNA sequence that may be transcribed into an mRNA and then translated into a peptide or protein sequence. Extrachromosomal sources of nucleic acid sequences can include double-strand DNA viral genomes, single-stranded DNA viral genomes, double-stranded RNA viral genomes, single-stranded RNA viral genomes, bacterial DNA, mitochondrial genomic DNA, cDNA or any other foreign source of nucleic acid that is capable of generating a gene product.

[0046] Protein markers can have any structure or conformation, and can be in any location within a cell, including on the cell surface. Protein markers can also be secreted from the cell into an extracellular matrix or directly into the blood or other biological fluid. Protein markers can be a single polypeptide chain or peptide fragments of a polypeptide. Moreover, they can also be combinations of nucleic acids and polypeptides as in the case of a ribosome. Protein markers can have any secondary structure combination, any tertiary structure, and come in quaternary structures as well.

[0047] One useful protein marker used to identify a neoplastic disease is CRAB-PII protein. The primary and three-dimensional structure of CRAB-PII is known in the art (see, e.g., Wang et al. (1998) *Biochemistry* 37:12727-12736). Examples of CRAB-PII amino acid sequences include, but are not limited to, GenBank Accession Nos. P22935, P51673, P30370, AAA80225.1, P29373, and Q5PXY7.

[0048] As used herein, the term "test fluid sample" is a fluid that is obtained or isolated from a subject potentially suffering from a neoplastic disease. A fluid sample is isolated from urine, blood, lymph, pleural fluid, pus, marrow, cartilaginous fluid, saliva, seminal fluid, menstrual blood, and spinal fluid. Fluid samples can be isolated from tissues isolated from a subject. For instance, the tissues can be isolated from organs or tissues including, but not limited to, brain, kidney, blood, cartilage, lung, ovary, lymph nodes, salivary glands, breast, prostate, testes, uterus, skin, bone, and bone marrow. Fluid samples potentially include a neoplastic cell or group of cells. A test fluid sample can also be obtained from necrotic material isolated from a tumor or tumors. Such cell or group of cells may show aberrant cell growth, such as increased, uncontrolled cell proliferation and/or lack of contact inhibition. The test fluid sample can include, for example, a cancer cell that can be a hyperplastic cell, a cell from a cell line that shows a lack of contact inhibition when grown in vitro, or a cancer cell that is capable of metastasis in vivo.

[0049] As used herein, the term "test cell sample" refers to a cell, group of cells, or cells isolated from potentially cancerous tumor tissues. A test cell sample is one that potentially exhibits tumorigenic potential, metastatic potential, or aberrant growth in vivo or in vitro. A test cell sample can be isolated from tissues including, but not limited to, blood, bone marrow, spleen, lymph node, liver, thymus, kidney, brain, skin, gastrointestinal tract, eye, breast, and prostate.

[0050] As used herein, the term "non-neoplastic control cell sample" refers to a cell or group of cells that is exhibiting noncancerous normal characteristics for the particular cell type from which the cell or group of cells was isolated. A control cell sample does not exhibit tumorigenic potential, metastatic potential, or aberrant growth in vivo or in vitro. A control cell sample can be isolated from normal tissues in a subject that is not suffering from cancer. It may not be necessary to isolate a control cell sample each time a cell sample

is tested for cancer as long as the nucleic acids isolated from the normal control cell sample allow for probing against the focused microarray during the testing procedure.

[0051] In another aspect, the invention provides methods for diagnosing cancer in a test cell sample by detecting CRAB-PII protein using a dipstick assay, Western blots, and Enzyme-Linked Immunosorbent Assays ("ELISA's").

[0052] CRAB-PII can also be detected with different cancer markers using a protein microarray. The methods can be practiced using a microarray composed of capture probes affixed to a derivatized solid support such as, but not limited to, glass, nylon, metal alloy, or silicon. Non-limiting examples of derivatizing substances include aldehydes, gelatin-based substrates, epoxies, poly-lysine, amines and silanes. Techniques for applying these substances to solid surfaces are well known in the art. In useful embodiments, the solid support can be comprised of nylon.

[0053] As used herein, the term "capture probe" is intended to mean any agent capable of binding a gene product in a complex cell sample or fluid sample. Capture probes can be disposed on the derivatized solid support utilizing methods practiced by those of ordinary skill in the art through a process called "printing" (see, e.g., Schena et. al., (1995) *Science,* 270(5235): 467-470). The term "printing", as used herein, refers to the placement of spots onto the solid support in such close proximity as to allow a maximum number of spots to be disposed onto a solid support. The printing process can be carried out by, e.g., a robotic printer. The VersArray CHIP Writer Prosystem (BioRad Laboratories) using Stealth Micro Spotting Pins (Telechem International, Inc, Sunnyvale, Calif.) is a non-limiting example of a chip-printing device that can be used to produce a focused microarray for this aspect. The capture probes may be antibodies, fragments thereof, or any other molecules capable of binding a protein (herein termed "protein capture probes"). These probes may be attached to a solid support at predetermined positions.

[0054] The level of expression of CRAB-PII in the potentially cancerous test cell sample or potentially cancerous test fluid sample is compared to the level of expression of CRAB-PII in a non-neoplastic control cell or control fluid sample of the same tissue type. If the expression of CRAB-PII in the potentially cancerous cell or fluid sample is greater than the expression of CRAB-PII in the non-neoplastic control cell or fluid sample, then cancer is indicated. In some embodiments, the test cell or fluid sample is tumorigenic if the level of expression of CRAB-PII in the potentially cancerous cell or fluid sample is 1.5 times greater than the level of expression of CRAB-PII in the non-neoplastic control cell or fluid sample. In some embodiments, the test cell or fluid sample is tumorigenic if the level of expression of CRAB-PII in the potentially cancerous cell or fluid sample is at least 1.5 times greater than the level of expression of CRAB-PII in the non-neoplastic control cell or fluid sample. The test cell or fluid sample may be tumorigenic if the level of expression of CRAB-PII in the potentially cancerous cell or fluid sample is at least 2 times greater, at least 4 times greater, at least 6 times greater, between 8 and 12 times greater, at least 15 times greater, or at least 20 times greater than the level of expression of CRAB-PII in the non-neoplastic control cell or non-neoplastic fluid sample.

[0055] In embodiments in which test tissue and cell samples are used, cell samples can be isolated from human tumor tissues using means that are known in the art (see, e.g., Vara et al. (2005) *Biomaterials* 26(18):3987-93; Iyer et al. (1998) *J. Biol. Chem.* 273(5):2692-7). For example, the cell sample can be isolated from the ovary of a human patient with ovarian cancer. Ovarian cancer cells can be obtained from other tissues as well, as in the case of metastatic ovarian cancer. Non-limiting sites of ovarian cancer-derived metastases can include, but are not limited to, ovarian, bone, blood, lung, skin, brain, adipose tissue, muscle, gastrointestinal tissues, hepatic tissues, and kidney. Alternatively, the cell test or control cell sample can be obtained from a cell line. Cell lines can be obtained commercially from various sources (e.g., American Type Culture Collections, Mannassas, Va.). Alternatively, cell lines can be produced using techniques well known in the art.

[0056] In addition, the cell sample can be a cell line. Cancer cell lines can be created by one with skill in the art and are also available from common sources, such as the ATCC cell biology collections (American Type Culture Collections, Mannassas, Va.).

[0057] In certain embodiments, cancer in tissues that are of mixed cellular populations such as a mixture of cancer cells and normal cells is detected. In such cases, cancer cells can represent as little as 40% of the tissue isolated for the present invention to determine that the cell sample is tumorigenic. For example, the cell sample can be composed of 50% cancer cells for the present invention to detect tumorigenic potential. Cell samples composed of greater than 50% tumorigenic cells can also be used in the present invention. It should be noted that cell samples can be isolated from tissues that are less than 40% tumorigenic cells as long as the cell sample contains a portion of cells that are at least 40% tumorigenic.

[0058] In some embodiments, levels of expression of housekeeping proteins are used to normalize the signal obtained between patients. As used herein, the term "housekeeping proteins" refers to any protein that has relatively stable or steady expression at the protein level during the life of a cell. Housekeeping proteins can be protein markers that show little difference in expression between cancer cells and normal cells in a particular tissue type. Examples of housekeeping proteins are well known in the art, and include, but are not limited to, isocitrate lyase, acyltransferase, creatine kinase, TATA-binding protein, hypoxanthine phosphoribosyl transferase 1, and guanine nucleotide binding protein, beta polypeptide 2-like 1 (see, e.g., Pandey et al. (2004) *Bioinformatics* 20(17): 2904-2910). In addition, the housekeeping proteins are used to identify the proper signal level by which to compare the cell sample signals between proteins from different or independent experiments.

[0059] Another aspect provides a method of diagnosing cancer in a fluid sample. In this method, expression of CRAB-PII in the fluid sample is measured. Expression levels for CRAB-PII can be determined using any techniques known in the art. Useful ways to determine such expression levels include, but not limited to, Western blot, protein microarrays, dipstick assays, and Enzyme-Linked Immunosorbent Assays ("ELISA") (see, e.g., U.S. Pat. Nos. 6,955,896; 6,087,012; 3,791,932; 3,850,752; and 4,034,074). Such examples are not intended to limit the potential means for determining the expression of a protein marker in a cell sample. Expression levels of markers in or by potentially cancerous cell samples and normal control cell samples can be compared using standard statistical techniques known to those of skill in the art (see, e.g., Ma et al., (2002) *Methods Mol. Biol.* 196:139-45).

[0060] The fluid sample can be isolated from a human patient by a physician and tested for expression of CRAB-PII

using a dipstick or any other method that relies on a solid support, solid state binding, change in color, or electric current. In addition, the cancer cell sample can be isolated from an organism that develops a tumor or cancer cells including, but not limited to, mouse, rat, horse, pig, guinea pig, or chinchilla. Cell samples can be stored for extended periods prior to testing or tested immediately upon isolation of the cell sample from the subject. Cell samples can be isolated by non-limiting methods such as surgical excision, aspiration from soft tissues such as adipose tissue or lymphatic tissue, biopsy, or removed from the blood. These methods are known to those of skill in the art.

[0061] In certain embodiments, the level of expression of anti-CRAB-PII antibodies in a fluid sample is detected. The level of expression of anti-CRAB-PII antibodies in a cell sample is detected using ELISA, western blot, and dot blot. The level of expression of anti-CRAB-PII antibodies can be detected using antibodies or fragments thereof, which are directed against anti-CRAB-PII antibodies. The level of expression of anti-CRAB-PII antibodies can be detected using antibody fragments (e.g., Fab, $F(ab)_2$, and Fv) or whole antibodies.

[0062] A normal or ovarian cancer cell sample can be isolated from a human patient by a physician and tested for expression of protein markers using a dipstick or any other method that relies on a solid support, solid state binding, change in color, or electric current. In addition, the cancer cell sample can be isolated from an organism that develops a tumor or cancer cells including, but not limited to mammals such as mouse, rat, horse, pig, guinea pig, or chinchilla. Cell samples can be isolated by non-limiting methods such as surgical excision, aspiration from soft tissues such as adipose tissue or lymphatic tissue, biopsy, or removed from the blood. These methods are known to those of skill in the art. Cell samples can be stored for extended periods prior to testing or tested immediately upon isolation of the cell sample from the subject.

1.2. Nucleic Acid Binding Agents

[0063] In another aspect, the method of detecting cancer includes detecting a level of expression of CRAB-PII RNA in a test fluid sample (i.e., neoplastic test fluid sample) and comparing the level of expression of CRAB-PII RNA detected in the test fluid sample to the level of expression of CRAB-PII RNA detected in the non-neoplastic control fluid sample. If the level of expression of CRAB-PII RNA is greater in the test fluid sample than in the non-neoplastic control fluid sample, then cancer is indicated.

[0064] In still another aspect, the method of detecting cancer includes detecting a level of expression of CRAB-PII RNA in a test cell sample (i.e., neoplastic test fluid sample) and comparing the level of expression of CRAB-PII RNA detected in the test cell sample to the level of expression of CRAB-PII RNA detected in the non-neoplastic control cell sample. If the level of expression of CRAB-PII RNA is greater in the test cell sample than in the non-neoplastic control cell sample, then cancer is indicated.

[0065] As used herein, "nucleic acid binding agent" means a nucleic acid capable of hybridizing with a particular target nucleic acid sequence. Nucleic acid binding agents include any structure that can hybridize with a target nucleic acid such as an mRNA. Nucleic acids can include, but are not limited to, DNA, RNA, RNA-DNA hybrids, siRNA, and aptamers. Moreover, any detectable labels can be used so long as the label does not affect the hybridizing of the nucleic acid with its targeting. Labels include, but are not limited to, fluorophores, chemical dyes, radiolabels, chemiluminescent compounds, colorimetric enzymatic reactions, chemiluminescent enzymatic reactions, magnetic compounds, and paramagnetic compounds.

[0066] Examples of CRAB-PII nucleic acid sequences detected in the present invention include, but are not limited to, GenBank Accession Nos. AR035503.1, AR035502.1, AR035501.1, U23407, M68867, L01528, AH001884, M87539, and M87538.

[0067] In certain embodiments, a focused microarray can be used to detect the levels of expression of CRAB-PII. The term "focused microarray" as used herein refers to a device that includes a solid support with capture probe(s) affixed to the surface of the solid support. In some embodiments, the focused microarray has nucleic acids attached to a solid support. The capture probes are directed to the diagnosis of a specific condition, e.g., chemotherapeutic drug resistance. Typically, the support consists of silicon, glass, nylon or metal alloy. Solid supports used for microarray production can be obtained commercially from, for example, Genetix Inc. (Boston, Mass.). Moreover, the support can be derivatized with a compound to improve nucleic acid association. Exemplary compounds that can be used to derivatize the support include aldehydes, poly-lysine, epoxy, silane containing compounds and amines. Derivatized slides can be obtained commercially from Telechem International (Sunnyvale, Calif.).

[0068] In the case of nucleic acid binding agents, nucleic acid sequences that are selected for detecting CRAB-PII expression may correspond to regions of low homology between genes, thereby limiting cross-hybridization to other sequences. Typically, this means that the sequences show a base-to-base identity of less than or equal to 30% with other known sequences within the organism being studied. Sequence identity determinations can be performed using the BLAST research program located at the NIH website (world wide web at ncbi.nlm.nih.gov/BLAST). Alternatively, the Needleman-Wunsch global alignment algorithm can be used to determine base homology between sequences (see Cheung et al., (2004) *FEMS Immunol. Med. Micorbiol.* 40(1): 1-9.). In addition, the Smith-Waterman local alignment can be used to determine a 30% or less homology between sequences (see Goddard et al., (2003) *J. Vector Ecol.* 28:184-9).

[0069] Expression levels for the CRAB-PII can be determined using techniques known in the art, such as, but not limited to, immunoblotting, quantitative RT-PCR, microarrays, RNA blotting, and two-dimensional gel-electrophoresis (see, e.g., Rehman et al. (2004) *Hum. Pathol.* 35(11):1385-91; Yang et al. (2004) *Mol. Biol. Rep.* 31(4):241-8). Such examples are not intended to limit the potential means for determining the expression of a gene marker in a breast cancer fluid sample.

1.3. Protein-Targeting Agents

[0070] Protein marker expression is used to identify tumorigenic potential. Protein markers, such as CRAB-PII, can be obtained by isolation from a cell sample, or a fluid sample, using any techniques available to one of ordinary skill in the art (see, e.g., Ausubel et. al., *Current Protocols in Molecular Biology*, Wiley and Sons, New York, N.Y., 1999). Isolation of protein markers, including CRAB-PII, from the potentially tumorigenic cell sample, or from a fluid sample obtained from a patient potentially suffering or suffering from neoplastic

disease, allows for the generation of target molecules, providing a means for determining the expression level of the protein markers in the potentially tumorigenic cell or fluid sample as described below. The protein markers, such as CRAB-PII, can be isolated from a tissue or fluid sample isolated from a human subject. The CRAB-PII and other protein markers can be isolated from a cytoplasmic fraction or a membrane fraction of the sample. Protein isolation techniques known in the art include, but are not limited to, column chromatography, spin column chromatography, and protein precipitation. CRAB-PII can be isolated using methods that are taught in, for example, Ausubel et al., *Current Protocols in Molecular Biology*, Vol. 1, John Wiley & Sons, Inc., (1993).

[0071] Protein-targeting agents are provided such as binding agents, e.g., antibodies or antigen binding fragments thereof. These embodiments are described in detail below. Other potential protein targeting agents include, but are not limited to, peptidomimetic compounds, peptides directed to the active sites of an enzyme, nucleic acids, nucleic acid aptamers.

[0072] Inhibitors can also be used as protein targeting agents to bind to protein markers. Useful inhibitors are compounds that bind to a target protein, and normally reduce the "effective activity" of the target protein in the cell or cell sample. Inhibitors include, but are not limited to, antibodies, antibody fragments such as "Fv," "F(ab')2," "F(ab)," "Dab" and single chains representing the reactive portion of an antibody ("SC-Mab"), peptides, peptidomimetic compounds, and small molecules (see, e.g., Lopez-Alemany et al. (2003) *Am. J. Hematol.* 72(4): 234-42; Miles et al. (1991) *Biochem.* 30(6): 1682-91). Inhibitors can perform their functions through a variety of means including, but not limited to, non-competitive, uncompetitive, and competitive mechanisms. For instance, the triosephosphate isomerase 1 inhibitor N-hydroxy-4-phosphono-butanamide has been described previously (see, e.g., Verlinde et al. (1989) *Protein Sci.* 1(12): 1578-84) and is useful.

[0073] Protein-targeting agents, including antibodies can also be conjugated to non-limiting materials such as magnetic compounds, paramagnetic compounds, proteins, nucleic acids, antibody fragments, or combinations thereof. Furthermore, antibodies can be disposed on an NPV membrane and placed into a dipstick. Antibodies can also be immobilized on a solid support at pre-determined positions such as in the case of a microarray. For instance, antibodies can be printed or cross-linked via their Fc regions to pre-derivatized surfaces of solid supports. In addition, antibodies can be cross-linked using bifunctional crosslinkers to a functionalized solid support. Such bifunctional crosslinking is well known in the art (see, e.g., U.S. Pat. Nos. 7,179,447; 7,183,373).

[0074] Crosslinking of proteins, such as antibodies, to a water-insoluble support matrix can be performed with bifunctional agents well known in the art including 1,1-bis (diazoacetyl)-2-phenylethane, glutaraldehyde, N-hydroxysuccinimide esters, for example, esters with 4-azidosalicylic acid, homobifunctional imidoesters, including disuccinimidyl esters such as 3,3'-dithiobis(succinimidylpropionate), and bifunctional maleimides such as bis-N-maleimido-1,8-octane. Bifunctional agents such as methyl-3-[(p-azidophenyl)dithio]propioimidate yield photoactivatable intermediates that are capable of forming crosslinks in the presence of light. Alternatively, reactive water-insoluble matrices such as cyanogen bromide-activated carbohydrates can be employed for protein immobilization.

[0075] Protein-targeting agents can be detectably labeled. As used herein, "detectably labeled" means that a targeting agent is operably linked to a moiety that is detectable. By "operably linked" is meant that the moiety is attached to the protein-targeting agent by either a covalent or non-covalent (e.g., ionic) bond. Methods for creating covalent bonds are known (see, e.g., Wong, S. S., *Chemistry of Protein Conjugation and Cross-Linking*, CRC Press 1991; Burkhart et al., *The Chemistry and Application of Amino Crosslinking Agents or Aminoplasts*, John Wiley & Sons Inc., New York City, N.Y., 1999).

[0076] Accordingly, a "detectable label" is a moiety that can be sensed. Such labels can be, without limitation, fluorophores (e.g., fluorescein (FITC), phycoerythrin, rhodamine), chemical dyes, or compounds that are radioactive, chemiluminescent, magnetic, paramagnetic, promagnetic, or enzymes that yield a product that may be colored, chemiluminescent, or magnetic. The signal is detectable by any suitable means, including spectroscopic, photochemical, biochemical, immunochemical, electrical, optical or chemical means. In certain cases, the signal is detectable by two or more means. In certain embodiments, protein targeting agents include fluorescent dyes, radiolabels, and chemiluminescent labels, which are examples that are not intended to limit the scope of the invention (see, e.g., Gruber et al. (2000) *Bioconjug. Chem.* 11(5): 696-704).

[0077] For example, protein-targeting agents may be conjugated to Cy5/Cy3 fluorescent dyes. These dyes are frequently used in the art (see, e.g., Gruber et al. (2000) *Bioconjug. Chem.* 11(5): 696-704). The fluorescent labels can be selected from a variety of structural classes, including the non-limiting examples such as 1- and 2-aminonaphthalene, p,p'diaminostilbenes, pyrenes, quaternary phenanthridine salts, 9-aminoacridines, p,p'-diaminobenzophenone imines, anthracenes, oxacarbocyanine, marocyanine, 3-aminoequilenin, perylene, bisbenzoxazole, bis-p-oxazolyl benzene, 1,2-benzophenazin, retinol, bis-3-aminopridinium salts, hellebrigenin, tetracycline, sterophenol, benzimidazolyl phenylamine, 2-oxo-3-chromen, indole, xanthen, 7-hydroxycoumarin, phenoxazine, salicylate, strophanthidin, porphyrins, triarylmethanes, flavin, xanthene dyes (e.g., fluorescein and rhodamine dyes); cyanine dyes; 4,4-difluoro-4-bora-3a, 4a-diaza-s-indacene dyes and fluorescent proteins (e.g., green fluorescent protein, phycobiliprotein).

1.4. Antibodies for Detection of CRAB-PII

[0078] Aspects utilize monoclonal and polyclonal antibodies as protein targeting agents directed specifically against certain CRAB-PII protein, particularly CRAB-PII. In certain embodiments, CRAB-PII is used alone as a protein marker to diagnose cancer. Anti-CRAB-PII protein antibodies, both monoclonal and polyclonal, for use in the invention are available from several commercial sources (e.g., Santa Cruz Biotechnology, Santa Cruz, Calif.; and Biogenesis, Inc., Kingston, N.H.). CRAB-PII antibodies can be administered to a patient orally, subcutaneously, intramuscularly, intravenously, or interperitoneally for in vivo detection and/or imaging.

[0079] As used herein, the term "polyclonal antibodies" means a population of antibodies that can bind to multiple epitopes on an antigenic molecule. A polyclonal antibody is

specific to a particular epitope on an antigen, while the entire pool of polyclonal antibodies can recognize different epitopes. In addition, polyclonal antibodies developed against the same antigen can recognize the same epitope on an antigen, but with varying degrees of specificity. Polyclonal antibodies can be isolated from multiple organisms including, but not limited to, rabbit, goat, horse, mouse, rat, and primates. Polyclonal antibodies can also be purified from crude serums using techniques known in the art (see, e.g., Ausubel, et al., *Current Protocols in Molecular Biology*, Vol. 1, pp. 4.2.1-4.2.9, John Wiley & Sons, Inc., 1996).

[0080] The term "monoclonal antibody", as used herein, refers to an antibody obtained from a population of substantially homogenous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. By their nature, monoclonal antibody preparations are directed to a single specific determinant on the target. Novel monoclonal antibodies or fragments thereof mean in principle all immunoglobulin classes such as IgM, IgG, IgD, IgE, IgA, or their subclasses or mixtures thereof. Non-limiting examples of subclasses include the IgG subclasses IgG1, IgG2, IgG3, IgG2a, IgG2b, IgG3, or IgGM. The IgG subtypes IgG1/κ and IgG2b/κ are also included within the scope of the present invention. Antibodies can be obtained commercially from, e.g., BioMol International LP (Plymouth Meeting, Pa.), BD Biosciences Pharmingen (San Diego, Calif.), and Cell Sciences, Inc. (Canton, Mass.).

[0081] The monoclonal antibodies herein include hybrid and recombinant antibodies produced by splicing a variable (including hypervariable) domain of an anti-CRAB-PII protein antibody with a constant domain (e.g., "humanized" antibodies), or a light chain with a heavy chain, or a chain from one species with a chain from another species, or fusions with heterologous proteins, regardless of species of origin or immunoglobulin class or subclass designation, as well as antibody fragments (e.g., Fab, $F(ab)_2$, and Fv), so long as they exhibit the desired biological activity. (See, e.g., U.S. Pat. No. 4,816,567; Mage and Lamoyi, in *Monoclonal Antibody Production Techniques and Applications*, (Marcel Dekker, Inc., New York 1987, pp. 79-97). Thus, the modified "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention can be made by the hybridoma method (see, e.g., Kohler and Milstein (1975) *Nature* 256:495) or can be made by recombinant DNA methods (U.S. Pat. No. 4,816,567). The monoclonal antibodies can also be isolated from phage libraries generated using the techniques described in the art (see, e.g., McCafferty et al. (1990) *Nature* 348:552-554).

[0082] Alternative methods for producing antibodies can be used to obtain high affinity antibodies. Antibodies can be obtained from human sources such as serum. Additionally, monoclonal antibodies can be obtained from mouse-human heteromyeloma cell lines by techniques known in the art (see, e.g., Kozbor (1984) *J. Immunol.* 133, 3001; Boerner et al., (1991) *J. Immunol.* 147:86-95). Methods for the generation of human monoclonal antibodies using phage display, transgenic mouse technologies, and in vitro display technologies are known in the art and have been described previously (see, e.g., Osbourn et al. (2003) *Drug Discov. Today* 8: 845-51; Maynard and Georgiou (2000) *Ann. Rev. Biomed. Eng.* 2:339-76; U.S. Pat. Nos. 4,833,077; 5,811,524; 5,958,765; 6,413,771; and 6,537,809).

[0083] Aspects also utilize polyclonal antibodies for the detection of CRAB-PII. They can be prepared by known methods or commercially obtained.

1.5. Detection of Crab-PII from Biological Fluids

[0084] In another aspect, an assay is included for the detection of CRAB-PII protein using a protein-targeting agent to bind to the CRAB-PII protein. The CRAB-PII protein typically is a peptide, polypeptide, protein, glycoprotein, or protiolipid. The protein-targeting agent can comprise antigens and antibodies thereto; haptens and antibodies thereto; and hormones, ligands, vitamins, metabolites and pharmacological agents, and their receptors and binding substances. The protein-targeting agent may be an immunologically-active polypeptide or protein or molecular weight between 1,000 Daltons and 10,000,000 Daltons, such as an antibody or antigenic polypeptide or protein, or a hapten of molecular weight between 100 Daltons and 1,500 Daltons. Protein-targeting agents can bind to CRAB-PII protein that are obtained from biological fluids. As used herein, the term "biological fluids" means aqueous or semi-aqueous liquids isolated from an organism in which biological macromolecules may be identified or isolated. Biological fluids may be disposed internally as in the case of blood serum, bile, or cerebrospinal fluid. Biological fluids can be excreted as in the non-limiting cases of urine, saliva, sweat, tears, mucosal secretions, lacrimal secretions, seminal fluid, sperm, and sebaceous secretions.

[0085] For detection of markers in biological fluids, detection devices can be used that are in the form of a "dipstick." Such devices are known in the art, and have been applied to detecting CRAB-PII protein in serum and other biological fluids (see, e.g. U.S. Pat. No. 4,390,343). In some instances, a dipstick-type device can be comprised of analytical elements where protein-targeting agents, such as antibodies, inhibitors, organic molecules, peptidomimetic compounds, ligands, organic compounds, or combinations thereof, are incorporated into a gel. The gel can be comprised of non-limiting substances such as agarose, gelatin or PVP (see, e.g., U.S. Pat. No. 4,390,343). The gel can be contained within an analytical region for reaction with a protein marker.

[0086] The "dipstick" format (exemplified in U.S. Pat. Nos. 5,275,785, 5,504,013, 5,602,040, 5,622,871 and 5,656,503) typically consists of a strip of porous material having a biological fluid sample-receiving end, a reagent zone and a reaction zone. As used herein, the term "reagent zone" means the area within the dipstick in which the protein-targeting agent and the CRAB-PII protein in the biological sample come into contact. By the term "reaction zone", is meant the area within the dipstick in which an immobilized binding agent captures the protein-targeting agent/protein marker complex. As used herein, the term "binding agent" refers to any molecule or group of molecules that can bind, interact, or associate with a protein-targeting agent/protein marker complex.

[0087] In certain embodiments, the biological fluid sample is wicked along the assay device starting at the sample-receiving end and moving into the reagent zone. The protein marker(s) to be detected binds to a protein-targeting agent incorporated into the reagent zone, such as a labeled protein-targeting agent, to form a complex. For example, a labeled antibody can be the protein-targeting agent, which complexes

specifically with the protein marker. In other examples, the protein-targeting agent can be a receptor that binds to a protein marker in a receptor:ligand complex. In yet other examples, an inhibitor is used to bind to a protein marker, thereby forming a complex with the protein marker targeted by the particular inhibitor. In some examples, peptidomimetic compounds are used to bind to CRAB-PII protein to mimic the interaction of a protein marker with a normal peptide. In other examples, the protein-targeting agent can be an organic molecule capable of associating with the protein marker. In all cases, the protein-targeting agent has a label. The labeled protein-targeting agent-protein marker complex then migrates into the reaction zone, where the complex is captured by another specific binding partner firmly immobilized in the reaction zone. Retention of the labeled complex within the reaction zone thus results in a visible readout.

[0088] A number of different types of other useful assays that measure the presence of a protein market are well known in the art. One such assay is an immunoassay. Immunoassays may be homogeneous, i.e. performed in a single phase, or heterogeneous, where antigen or antibody is linked to an insoluble solid support upon which the assay is performed. Sandwich or competitive assays may be performed. The reaction steps may be performed simultaneously or sequentially. Threshold assays may be performed, where a predetermined amount of analyte is removed from the sample using a capture reagent before the assay is performed, and only analyte levels of above the specified concentration are detected. Assay formats include, but are not limited to, for example, assays performed in test tubes, wells or on immunochromatographic test strips, as well as dipstick, lateral flow or migratory format immunoassays.

[0089] A lateral flow test immunoassay device may be used in this aspect of the invention. In such devices, a membrane system forms a single fluid flow pathway along the test strip. The membrane system includes components that act as a solid support for immunoreactions. For example, porous or bibulous or absorbent materials can be placed on a strip such that they partially overlap, or a single material can be used, in order to conduct liquid along the strip. The membrane materials can be supported on a backing, such as a plastic backing. The test strip includes a glass fiber pad, a nitrocellulose strip and an absorbent cellulose paper strip supported on a plastic backing.

[0090] Antibodies that specifically bind with the target protein marker are immobilized on the solid support. The antibodies can be bound to the test strip by adsorption, ionic binding, van der Waals adsorption, electrostatic binding, or by covalent binding, by using a coupling agent, such as glutaraldehyde. For example, the antibodies can be applied to the conjugate pad and nitrocellulose strip using standard dispensing methods, such as a syringe pump, airbrush, ceramic piston pump or drop-on-demand dispenser. A volumetric ceramic piston pump dispenser can be used to stripe antibodies that bind the analyte of interest, including a labeled antibody conjugate, onto a glass fiber conjugate pad and a nitrocellulose strip.

[0091] The test strip can be treated, for example, with sugar to facilitate mobility along the test strip or with water-soluble non-immune animal proteins, such as albumins, including bovine (BSA), other animal proteins, water-soluble polyamino acids, or casein to block non-specific binding sites.

1.6. Cancer Diagnosis and Prediction Analysis

[0092] Cancer diagnoses can be performed by comparing the levels of expression of CRAB-PII in a potentially neoplastic cell sample to the levels of expression for a protein marker or a set of protein markers in a normal control cell sample of the same tissue type. Alternatively, the level of expression of CRAB-PII in a potentially cancerous cell sample is compared to a reference pool of CRAB-PII that represents the level of expression for CRAB-PII in a normal control population (herein termed "training set"). The training set also includes the data for a population that has a known tumor or class of tumors. This data represents the average level of expression that has been determined for the neoplastic cells isolated from the tumor or class of tumors. It also has data related to the average level of expression for CRAB-PII for normal cells of the same cell type within a population. In these embodiments, the algorithm compares newly generated expression data for CRAB-PII from a cell sample isolated from a patient containing potentially neoplastic cells to the levels of expression for CRAB-PII in the training set. The algorithm determines whether a cell sample is neoplastic or normal by aligning the level of expression for CRAB-PII with the appropriate group in the training set. In certain embodiments, software for performing the statistical manipulations described herein can be provided on a computer connected by data link to a data generating device, such as a microarray reader.

[0093] Class prediction algorithms can be utilized to differentiate between the levels of expression of markers in a cell sample and the levels of expression of markers in a normal cell sample (Vapnik, *The Nature of Statistical Learning Theory*, Springer Publishing, 1995). Exemplary, non-limiting algorithms include, but are not limited to, compound covariate predictor, diagonal linear discriminant analysis, nearest neighbor predictor, nearest centroid predictor, and support vector machine predictor (Simon et al., *Design and Analysis of DNA Microarray Investigations: An Artificial Intelligence Milestone*., Springer Publishing, 2003). These statistical tests are well known in the art, and can be applied to ELISA or data generated using other protein expression determination techniques such as dot blotting, Western Blotting, and protein microarrays (see, e.g., U.S. Appln. No. 2005/0239079).

[0094] It should be recognized that statistical analysis of the levels of expression of protein markers in a cell sample to determine cancer state does not require a particular algorithm or set of particular algorithms. Any algorithm can be used in the present invention so long as it can discriminate between statistically significant and statistically insignificant differences in the levels of expression of protein markers in a cell sample as compared to the levels of expression of the same protein markers in a normal cell sample of the same tissue type.

[0095] In some embodiments, an increased level of expression of CRAB-PII in the potentially cancerous cell sample, or fluid sample, indicates that cancer cells exist in the cell sample. The algorithm makes the class prediction based upon the overall levels of expression found in the cell sample as compared to the levels of expression in the training set.

[0096] The type of analysis detailed above compares the level of expression for CRAB-PII in the cell sample to a training set containing reference pools of protein that are representative of a normal population and a neoplastic population. In certain embodiments, the training set can be obtained with kits that can be used to determine the level of expression of CRAB-PII in a patient cell sample. Alternatively, an investigator can generate new training sets using protein expression reference pools that can be obtained from

commercial sources such as Asterand, Inc. (Detroit, Mich.). Comparisons between the training sets and the cell samples are performed using standard statistical techniques that are well known in the art, and include, but are not limited to, the ArrayStat 1.0 program (Imaging Research, Inc., Brock University, St. Catherine's, Ontario, CA). Statistically significant increased levels of expression in the cell sample of CRAB-PII indicate that the cell sample contains a cancer cell or cells with tumorigenic potential. Also, standard statistical techniques such as the Student T test are well known in the art, and can be used to determine statistically significant differences in the levels of expression for CRAB-PII in a patient cell sample (see, e.g., Piedra et al. (1996) *Ped. Infect. Dis. J.* 15:1). In particular, the Student T test is used to identify statistically significant changes in expression using protein microarray analysis or ELISA analysis (see, e.g., Piedra et al. (1996) *Ped. Infect. Dis. J.* 15:1).

1.7. Protein Microarray

[0097] Protein microarrays can be prepared by methods disclosed in, e.g., U.S. Pat. Nos. 6,087,102, 6,139,831, and 6,087,103. In addition, protein-targeting agents conjugated to the surface of the protein microarray can be bound by detectably labeled protein markers isolated from a cell sample or a fluid sample. This method of detection can be termed "direct labeling" because the protein marker, which is the target, is labeled. In other embodiments, protein markers can be bound by protein-targeting agents, and then subsequently bound by a detectably labeled antibody specific for the protein marker. These methods are termed "indirect labeling" because the detectable label is associated with a secondary antibody or other protein-targeting agent. An overview of protein microarray technology in general can be found in Mitchell, *Nature Biotech.* (2002), 20:225-229, the contents of which are incorporated herein by reference.

1.8. Kits

[0098] Additionally, kits are provided for detecting neoplasms such as ovarian cancer in a cell or a fluid sample. The kits include targeting agents for the detection of CRAB-PII. A patient that potentially has a tumor or the potential to develop a tumor ("in need thereof") can be tested for the presence of a tumor or tumor potential by determining the level of expression of targeting agents in a cell or fluid sample derived from the patient.

[0099] The kit comprises labeled binding agents capable of detecting CRAB-PII in a biological sample, as well as means for determining the amount of CRAB-PII in the sample, and means for comparing the amount of CRAB-PII in the potentially cancerous sample with a standard (e.g., normal non-neoplastic control cells). The binding agents can be packaged in a suitable container. The kit can further comprise instructions for using the compounds or agents to detect CRAB-PII, as well as other neoplasm-associated markers. Such a kit can comprise, e.g., one or more antibodies, or fragments thereof as binding agents, that bind specifically to at least a portion of CRAB-PII.

[0100] The kit can also contain a probe for detection of housekeeping protein expression. These probes advantageously allow health care professionals to obtain an additional data point to determine whether a specific or general cancer treatment is working so CRAB-PII levels can be used to monitor the success of cancer treatment. The probes can be any binding agents such as labeled antibodies, or fragments thereof, specific for the housekeeping proteins. Alternatively or additionally, the probes can be inhibitors, peptidomimetic compounds, peptides and/or small molecules.

[0101] Data related to the levels of expression of the selected protein markers in normal tissues and neoplasms can be supplied in a kit or individually in the form of a pamphlet, document, floppy disk, or computer CD. The data can represent patient pools developed for a particular population (e.g., Caucasian, Asian, etc.) and is tailored to a particular cancer type. Such data can be distributed to clinicians for testing patients for the presence of a neoplasm such as an ovarian cancer. A clinician obtains the levels of expression for a protein marker or set of protein markers in a particular patient. The clinician then compares the expression information obtained from the patient to the levels of expression for the same protein marker or set of protein markers that had been determined previously for both normal control and cancer patient pools. A finding that the level of expression for the protein marker or the set of protein markers is similar to the normal patient pool data indicates that the cell sample obtained from the patient is not neoplastic. A finding that the level of expression for the protein marker or the set of protein markers is similar to the cancer patient pool data indicates that the cell sample obtained from the patient is neoplastic.

1.9. Testing

[0102] The diagnostic methods according to the invention were tested for their ability to diagnose cancer in test cell samples isolated from human subjects suffering from ovarian cancer, lung cancer, prostate cancer, hepatic cancer, pancreatic cancer, breast cancer, leukemia, sarcoma, melanoma, renal cancer, colon cancer, and osteosarchma.

[0103] The expression levels of CRAB-PII were analyzed for differential expression in ovarian samples by Western blotting and focused microarray. The testing and results are described in detail below in the Examples.

[0104] FIG. **1** shows the levels of expression detected for CRAB-PII in normal subjects and breast cancer patients. All individuals are designated with an OVXXX number. As shown in FIG. **1**, CRAB-PII expression is significantly increased in tumor samples as compared to normal samples. Therefore, CRAB-PII was a biomarker for breast cancer.

[0105] FIG. **2** shows the results of RNA expression experiments on normal samples isolated from normal subjects (OVN, BrN, and LN) and patient samples isolated from ovarian cancer patients (OVT), breast cancer patients (BrT), and lung cancer patients (LT). The cancer patients had increased levels of CRAB-PII, as identified by anti-CRAB-PII antibodies.

[0106] FIG. **3** shows that CRAB-PII is overexpressed in tumor tissues as compared to normal tissues in breast tissue. Each dot represents an individual sample. The results show that CRAB-PII is increased by approximately 3 times in breast tumor tissues as compared to normal breast tissues (FIG. **3**).

[0107] FIG. **4** shows the expression levels of CRAB-PII in patients suffering from malignant breast cancer and subjects having benign tumors. Each dot represents an individual sample. The results show that CRAB-PII appears to be expressed at higher levels in certain malignant samples as compared to tumor samples.

EXAMPLES

[0108] Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation,

numerous equivalents to the specific substances and procedures described herein. Such equivalents are intended to be encompassed in the scope of the claims that follow the examples below.

Example 1

Western Blot Analysis of Samples Isolated from Breast Cancer Patients and Normal Breast Subjects

1. Patient Samples and Normal Samples

[0109] Patient tissue samples were obtained from Asterand, Inc. (Detroit, Mich.), Clinomics Biosciences, Inc (Watervliet, N.Y.) and Biochain Institute, Inc. (Hayward, Calif.). The samples were isolated from normal breast and breast cancer samples, and were frozen into blocks of tissue. Protein cell extracts were then prepared from each block. Each patient included in the study was screened against the same normal total RNA pool in order to compare them together.

2. Western Blot Analysis of CRAB-PII in Breast Cancer and Breast Normal Samples

[0110] For breast cell samples, human tissues were homogenized using a Polytron PT10-35 (Brinkmann, Mississauga, Canada) for 30 seconds at speed setting of 4 in the presence of 300 μl of 10 mM HEPES-Tris, pH 7.4, 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholic acid, 0.1% SDS, 1 mM EDTA and a cocktail of protease inhibitors from Roche Corp. (Laval, Qc, Canada). 40 μg of proteins from human breast cancer patients and normal breast subjects were used in SDS-PAGE gels. Samples were mixed with Laemmli buffer (250 mM Tris-HCl, pH 8.0, 25% (v/v) b-mercaptoethanol, 50% (v/v) glycerol, 10% (w/v) SDS, 0.005% (w/v) bromophenol blue), heated for 5 mins. at 95° C. and resolved in 12% SDS-polyacrylamide gels (SDS-PAGE). Proteins were then electro-transferred onto Hybond-ECL nitrocellulose membranes (Amersham Biosciences, Baie d'Urfé, Canada) for 90 mins. at 100 volts at RT (RT). Membranes were blocked for 1 hr. at RT in blocking solution (PBS containing 5% fat-free dry milk). Membranes were washed with PBS and incubated with the primary anti-CRAB-PII polyclonal antibodies or monoclonal antibodies at the appropriate dilutions in blocking solution containing 0.02% sodium azide for 2 hrs. at RT. Antibodies were produced in house. PBS washing was performed, and the membranes were subsequently incubated for 1 hr. at RT with secondary anti-mouse, anti-rabbit or anti-goat antibodies labeled with horseradish peroxydase (Bio-Rad, Mississauga, Canada) diluted 1/3000 in PBS. Chemiluminescence detection was performed using the SuperSignal West Pico Chemiluminescent Substrate (Pierce, Rockford, Ill., USA) following the manufacturer's recommendations.

3. Results.

[0111] The results of expression analyses for CRAB-PII are shown in FIGS. **1** and **3**. CRAB-PII expression was significantly increased in tumor samples obtained from breast tumor patients as compared to normal samples isolated from normal subjects (FIG. **1**). Almost all normal subjects showed nearly undetectable levels, or very low levels, of CRAB-PII protein expression, while nearly 60% of samples obtained from breast cancer patients showed detectable levels of CRAB-PII (FIG. **1**). CRAB-PII protein expression was increased by 3 times in tumor tissues as compared to normal tissues (FIG. **3**). The scatter plot shows that the majority of individual tumor samples had higher levels of CRAB-PII expression as compared to the normal tissue samples.

Example 2

Preparation and Use of the Focused Microarray to Detect Crab-PII in Samples Obtained from Normal Breast Subjects and Breast Cancer Patients

[0112] 1. Total RNA Isolation and cDNA Labeling

[0113] Patient tissues samples were obtained from Asterand, Inc. (Detroit, Mich.), Clinomics Biosciences, Inc (Watervliet, N.Y.) and Biochain Institute, Inc. (Hayward, Calif.). Each patient included in the study was screened against the same normal total RNA pool in order to compare them together.

[0114] For breast cell samples, human tissues were homogenized using a Polytron PT10-35 (Brinkmann, Mississauga, Canada) for 30 seconds at speed setting of 4 in the presence of 300 μl of 10 mM HEPES-Tris, pH 7.4, 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholic acid, 0.1% SDS, 1 mM EDTA and a cocktail of protease inhibitors from Roche Corp. (Laval, Qc, Canada). Cell lysis, in the case of cell and tissue samples, and RNA extraction was done with the RNEasy kit, (#74104) (Qiagen, Inc., Valencia, Calif.) following the manufacturer's protocol. RNA was quantified by spectrophotometry using an Ultrospec 2000 spectrophotometer (Amersham-Biosciences, Corp., Piscataway, N.J.). RNA samples were dissolved in 10 mM Tris, pH 7.5 to determine the $A_{260/280}$ ratios. Samples with ratios between 1.9 and 2.3 were kept for probe preparation, while samples with ratios lower than 1.9 were discarded. RNA samples were dissolved in 1 μl DEPC—H_2O for total nucleic acid quantification. Total RNA from control and treated samples was dried by speed vacuum using a Heto Vacuum centrifuge system (KNF Neuberger, Inc., Trenton, N.J.) at varying time intervals. The total RNA was resuspended in 10 μl of DEPC—H_2O and stored at −20° C. until the labeling reaction.

[0115] First strand cDNA labeling was accomplished using 1-15 μg total RNA (depending on the cell lines to be tested) for the resistant and the sensitive cell lines separately. Total RNA was incubated with 4 ng control positive *Arabidopsis thaliana* RNA, 3 μg of Oligo $(dT)_{12-18}$ primer (#Y01212) (Invitrogen, Corp., Carlsbad, Calif.), 1 μg PdN6 random primer (Amersham, #272166-01) for 10 min. at 65° C., and immediately put on ice for 1 min. The mixture was then diluted in 5× First strand buffer (250 mM Tris-HCl, pH 8.3; 375 mM KCl; 15 mM $MgCl_2$) containing 0.1 M DTT, 0.5 μM dNTPs mix (dTTP, dGTP, dATP) (Invitrogen, #10297-018), 0.05 μM dCTP (Invitrogen, #10297-018), 5 μM Cy3-dCTP (#NEL 576) (NEN Life Science/Perkin Elmer, Boston, Mass.), 2.5 μM Cy5-dCTP (#NEL 577) (NEN Life Science/Perkin Elmer, Boston, Mass.) and 400 units SuperScript III RNAse H^- RT (Invitrogen, #I 8064-014). After incubating the reaction mixture for 5 min. at 25° C., the reaction mixture was incubated at 42° C. for 90 min. Finally, a total of 400 units of SuperScript II RNAse H^- RT (Invitrogen, #18064-014) were added and the reaction was incubated at 42° C. for another 90 min.

[0116] Digestion of the labeled cDNA with 5 units RNAse H (#M0297S) (NEB, Beverly, Mass.) and 40 units RNAse A (Amersham, #70194Y) was done at 37° C. for 30 min. The labeling probe was purified with the QIAquick PCR purification kit (Qiagen, Inc.) protocol with some modifications.

Briefly, the reaction volume was completed to 50 μl with DEPC—H_2O and 2.7 μl of 12 M NaOAc pH 5.2 was added. The reaction was diluted with 200 μl PB buffer, put on the purification column, spun 15 sec. at 10 000 g, followed by 3 washes of 500 μl PE buffer (15 sec.; 10 000 g) and eluted 2 times in 50 μl DEPC—H_2O total (1 min.; 10 000 g). Frequency of incorporation and amount of cDNA labeled produced were evaluated for both labeled dCTPs by spectrophotometer (Ultrospec 2000, Pharmacia Biotech) at $A_{260\,nm}$, $A_{550\,nm}$ and $A_{650\,nm}$. The labeling material was dry by speed vacuum (Heto Vacuum centrifuge system, LaboPort) and resuspended in 3.75 μl H_2O total for both Cy5 (resistant cell line) and Cy3 reactions (sensitive cell line).

2. Capture Probe Preparation

[0117] Capture probes, approximately 68 nucleotides in length, corresponding to targets of interest were designed using sequences showing less identity base to base (<30%) with other coding sequences (cds) submitted to NCBI bank. The comparisons between sequences were done by BLAST research (world wide web at.ncbi.nlm.nih.gov/BLAST). For BioChip ver1.0 and ver2.0, a basic melting point temperature at a salt concentration of 50 mM Na^+ (Tm) for each capture probe was calculated: the overall average was 76.97° C.+/−3.72° C. GC nucleotide content averaged 51.2%+/−9.4%. For the present invention, two negative controls (68 bp of the antisense cds of the BRCP and nucleophosmin targets) were synthesized.

[0118] The CRAB-PII nucleic acid capture probe targeted ACRABP-II (gi#6382069) 481-548 bp of cds.

[0119] The capture probe was synthesized by the BRI Institute (Biotechnology Research Institute, Clear Water Bay, Kowloon, Hong Kong, China) with the Expedilite™ Synthesizer at a coupling efficiency of over 99.5% (Applied Biosystems, Foster City, Calif.). The oligonucleotides were verified by polyacrylamide gel electrophoresis. Oligonucleotide quantification was done by spectrophotometry at $A_{260\,nm}$.

3. Printing of Capture Probes and Production of the Focused Microarray

[0120] Prior to printing of capture probes, different dilutions of *Arabidopsis thaliana* chlorophyll synthetase G4 DNA (undiluted solutions at 0.15 μg/μl and at 0.2 μg/μl; 1:2; 1:4; 1:8; 1:16) were printed on each grid as a positive control, and for normalization of results. Preparation of *Arabidopsis thaliana* control capture probes was performed as follows. Briefly, five micrograms of a Midi preparation using a HiSpeed™ Plasmid Midi kit (Qiagen, Inc.) of the *Arabidopsis thaliana* plasmid (gift of BRI) was digested with 40 units of Sac I enzyme (NEB) for 2 hr. at 37° C., purified with the QIAquick PCR purification kit (Qiagen,) and verified by 1% agarose migration. In vitro transcription of 2 μg Sac I digestion was performed in 10× transcription buffer (400 mM Tris-HCl, pH 8.0; 60 mM $MgCl_2$; 100 mM DTT; 20 mM Spermidin) containing 2 μl of 10 mM NTP mix (Invitrogen), 20 units RNAse OUT (Invitrogen, #10777-019) and 50 units T7 RNA polymerase (NEB) for approximately 2 hr. to 30 hr. at 37° C. The reaction was then treated with 2 units DNAse I (Invitrogen) in 10×DNAse buffer (200 mM Tris-HCl pH 8.4; 20 mM $MgCl_2$; 500 mM KCl) for 15 min. at 37° C. The RNA was cleaned with the RNEasy kit (Qiagen) and quantified by spectrophotometry using an Ultrospec 2000 (Amersham Biosciences, Corp. Piscataway, N.J.).

[0121] After the control capture probes were generated and printed, the capture probes complementary to marker genes from the cancer cell samples were printed at concentrations of 25 μM in 50% DMSO on CMT-GAPS II Slides (#40003) (Corning, 45 Nagog Park, Acton, Mass.) by the VersArray CHIP Writer Prosystems (BioRad Laboratories) with the Stealth Micro Spotting Pins (#SMP3) (Telechem International, Inc., Sunnyvale, Calif.). Each capture probe was printed in triplicate on duplicate grids. Buffer and Salmon Testis DNA (Sigma D-7656) were also printed for the BioChip analysis step. After printing was completed, the slides were dried overnight by incubation in the CHIP Writer chamber. Chips were then treated by UV (Stratagene, UV Stratalinker) at 600 mJoules and baked in an oven for 6-8 hr.

4. Quality Control of Focused Microarray

[0122] Prior to testing the invention on cancer cell samples, the focused microarray was tested at the BRI Institute (Kowloon Bay, Hong Kong). One slide for each printed batch was quality control tested using a terminal deoxynucleotidyl transferase (Tdt)-mediated nick end labeling assay protocol (see, e.g., Yeo et. al., (2004) *Clin. Cancer Res.* 10(24): 8687-96). Additionally, controls were performed to verify the specificity of the hybridization using three independent grids on the same focused microarray.

[0123] As a first quality control, a test was done by the BRI Institute on one slide for each batch printed with the following Tdt transferase protocol. Briefly, the slide was prehybridized in a Hybridization Chamber (#2551) (Corning, Inc., Life Sciences, 45 Nagog Park, Acton, Mass.) with 80 μl of preheated prehybridization buffer (5×SSC (750 mM NaCl; 75 mM sodium citrate); 0.1% SDS; 1% BSA (Sigma, #A-7888) at 37° C. for 30 min. Slides were washed in 0.1×SSC (15 mM NaCl; 1.5 mM sodium citrate) and air-dried. 50 μl of TdT reaction mixture [5×TdT buffer (125 mM Tris-HCl, pH 6.6, 1 M sodium cacodylate, 1.25 mg/ml BSA); 5 mM $CoCl_2$; 1 mM Cy3-dCTP (NEN Life Science, NEL 576); 50 units TdT enzyme (#27-0730-01) (Amersham BioSciences)], was added to the entire area of the BioChip. The slide was incubated in the Hybridization Chamber for 60 min. at 37° C. following by a first wash in 1×SSC (150 mM NaCl; 15 mM sodium citrate)/0.2% SDS (preheated at 37° C.) for 10 min., a second wash of 5 min. in 0.1×SCC (15 mM NaCl; 1.5 mM sodium citrate)/0.2% SDS at RT and finally a last wash of 5 min. at RT in 0.1×SSC (15 mM NaCl; 1.5 mM sodium citrate). The slide was scanned with the ScanArray™ Lite MicroArray Scanner (Packard BioSciences, Perkin Elmer, San Jose, Calif.).

[0124] As a second quality control step, the PARAGON™ DNA Microarray Quality Control Stain kit (Molecular Probes) was incubated with the microarray according to the manufacturer's recommendations.

5. Focused Microarray Hybridization with Labeled cDNA Probes

[0125] Focused microarray slides were pre-washed before the prehybridization step as follows. First, slides were washed for 20 min. at 42° C. in 2×SSC (300 mM NaCl; 30 mM sodium citrate)/0.2% SDS under agitation. The second wash was for 5 min. at RT in 0.2×SSC (30 mM NaCl, 3 mM Sodium citrate) under agitation, and then followed by a wash for 5 min. at RT in DEPC—H_2O with agitation. The slides were spin dried at 1000 g for 5 min. and prehybridized in Dig Easy Hyb Buffer (#1,603,558) (Roche Diagnostics Corporation, Indianapolis, Ind.) containing 400 μg Bovine Serum Albumin

(Roche, #711,454) at 42° C. in humid chamber for 3 hr. then washed 2 times in DEPC—H_2O, and once in Isopropanol (Sigma, 1-9516) and spun dry at 1000 g for 5 min.

[0126] To the mixed Cy5/Cy3 probe, 15 μg Baker tRNA (#109,495) (Roche Diagnostics Corp., Indianapolis, Ind.) and 1 μg Cot-1 DNA (Roche, #1,581,074) were added and the probe was incubated 5 min. at 95° C., put on ice for 1 min., and diluted with 14 μl Dig Easy Hyb buffer (Roche, #1,603, 558). After a 2 min. spin at 100 g, the probe was incubated at 42° C. for at least 5 min.

[0127] The three supergrids on the slide were separated by a Jet-Set Quick Dry TOP Coat 101 line (#FX268) (L'Oreal, Paris, FR). Each probe was added to its respective supergrid and covered by a preheated (42° C.) coverslip (Mandel, #S-104 84906). The slide was incubated at 42° C. in humid chamber for at least 15 hr.

[0128] The coverslips were removed by dipping in 1×SSC (150 mM NaCl; 15 mM sodium citrate)/0.2% SDS solution preheated at 50° C.). The slide was washed three times for 5 min. with agitation in 1×SSC (150 mM NaCl; 15 mM sodium citrate)/0.2% SDS solution preheated at 50° C.), and then washed three times with agitation in 0.1×SSC (15 mM NaCl; 1.5 mM sodium citrate)/0.2% SDS solution preheated at 37° C.). Finally, the slide was washed once in 0.1×SSC (15 mM NaCl; 1.5 mM sodium citrate) with agitation for 5 min. The slide was dipped several times in DEPC—H_2O and spun dry at 1000 g for 5 min.

6. Scanning and Statistical Analysis

[0129] The slides were scanned with a ScanArray™ Lite MicroArray Scanner (Packard BioSciences, Perkin Elmer, San Jose, Calif.) and the analysis was performed with a QuantArray® Microarray Analysis software version 3.0 (Packard BioSciences, Perkin Elmer, San Jose, Calif.).

[0130] The QuantArray® data results were analyzed according to the following procedures. All analysis of the results was performed with the spot background subtracted values for Cy5 and Cy3. Spots with lower signal ratio to noise lower than 1.5 were discarded. Normalization of the ratios with the spike positive control (*Arabidopsis thaliana*) was done to have a ratio equal to one for that control on each slide. Slides were discarded on which the negative and/or positive controls did not work. Also, slides were discarded with high background and with different mean no offset correction (ArrayStat software). Mean for each target was calculated with at least six different experiments (including two reciprocal labeling reactions), each experiment using different total RNA preparations. Statistical analysis was accomplished with the ArrayStat 1.0 (Imaging Research Inc., Brock University, St. Catherine's, Ontario, CA). A log transformation of the ratio data is followed by a Student T test for two independent conditions using a proportional model without offsets at a $p<0.05$ threshold. Significant increases (ratio Cy5/Cy3 higher than 1.5) or decreases (ratio Cy5/Cy3 lower than 0.5) were considered to be significant if the p value was lower than 0.05.

7. Results.

[0131] CRAB-PII mRNA expression correlated with CRAB-PII protein expression. Increased levels of CRAB-PII mRNA were detected in tumor samples (BrT) obtained patients suffering from breast cancer as compared to normal subjects (BrN) (FIG. **2**). Tumor samples from patients suffering from breast cancer had between 5 and 6 times higher levels of CRAB-PII RNA expression than normal subjects (FIG. **2**).

Example 3

Western Blot Analysis of Samples Isolated from Lung Cancer Patients and Normal Lung Subjects

1. Patient Samples and Normal Samples

[0132] Patient lung tissues and pleural fluid samples are obtained from Asterand, Inc. (Detroit, Mich.), Clinomics Biosciences, Inc (Watervliet, N.Y.) and Biochain Institute, Inc. (Hayward, Calif.). Each patient included in the study is screened against the same normal total RNA pool in order to compare them together.

2. Western Blot Analysis of CRAB-PII in Lung Cancer and Lung Normal Samples

[0133] Fluid samples are prepared as described in Example 1. Lung tissue samples are prepared as described in Example 1.

3. Results.

[0134] The results of expression analyses for the protein markers is that CRAB-PII expression is significantly increased in cell and fluid samples obtained from lung tumor patients as compared to cell and fluid samples isolated from normal subjects. All normal subjects show nearly undetectable levels, or low levels, of CRAB-PII protein expression, while several samples obtained from lung cancer patients show detectable levels, or increased levels of CRAB-PII expression as compared to control samples.

Example 4

Preparation and Use of the Focused Microarray to Detect Crab-PII in Samples Obtained From Normal Lung Subjects and Lung Cancer Patients

[0135] 1. Total RNA Isolation and cDNA Labeling

[0136] Patient lung tissue samples and pleural fluid samples were obtained from Asterand, Inc. (Detroit, Mich.), Clinomics Biosciences, Inc (Watervliet, N.Y.) and Biochain Institute, Inc. (Hayward, Calif.). Each patient included in the study was screened against the same normal total RNA pool in order to compare them together.

[0137] Fluid samples were prepared as described in Example 2. Lung tissue samples were homogenized as described in Example 2.

3. Results.

[0138] CRAB-PII mRNA expression correlates with CRAB-PII protein expression. Increased levels of CRAB-PII mRNA were detected in samples obtained patients suffering from lung cancer (LT) as compared to normal subjects (LN) (FIG. **2**). Samples from patients suffering from lung cancer showed up two times higher levels of CRAB-PII RNA expression than normal subjects (FIG. **2**).

Example 5

Western Blot Analysis of Samples Isolated from Ovarian Cancer Patients and Normal Ovarian Subjects

1. Patient Samples and Normal Samples

[0139] Patient ovarian tissues and pleural fluid samples are obtained from Asterand, Inc. (Detroit, Mich.), Clinomics

Biosciences, Inc (Watervliet, N.Y.) and Biochain Institute, Inc. (Hayward, Calif.). Each patient included in the study is screened against the same normal total RNA pool in order to compare them together.

2. Western Blot Analysis of CRAB-PII in Ovarian Cancer and Ovarian Normal Samples

[0140] Fluid samples are prepared as described in Example 1. Ovarian tissue samples are prepared as described in Example 1.

3. Results.

[0141] The results of expression analyses for the protein markers is that CRAB-PII expression is significantly increased in cell and fluid samples obtained from ovarian tumor patients as compared to cell and fluid samples isolated from normal subjects. All normal subjects show nearly undetectable levels, or low levels, of CRAB-PII protein expression, while several samples obtained from lung cancer patients show detectable levels, or increased levels of CRAB-PII expression as compared to control samples.

Example 6

Preparation and Use of the Focused Microarray to Detect Crab-PII in Samples Obtained from Normal Ovarian Subjects and Ovarian Cancer Patients

[0142] 1. Total RNA Isolation and cDNA Labeling

[0143] Patient ovarian tissue samples and pleural fluid samples were obtained from Asterand, Inc. (Detroit, Mich.), Clinomics Biosciences, Inc (Watervliet, N.Y.) and Biochain Institute, Inc. (Hayward, Calif.). Each patient included in the study was screened against the same normal total RNA pool in order to compare them together.

[0144] Fluid samples were prepared as described in Example 2. Ovarian tissue samples were homogenized as described in Example 2.

3. Results.

[0145] CRAB-PII mRNA expression correlates with CRAB-PII protein expression. Increased levels of CRAB-PII mRNA were detected in samples obtained patients suffering from ovarian cancer (OVT) as compared to normal subjects (OVN) (FIG. **2**). Samples from patients suffering from ovarian cancer showed between 7 and 10 times higher levels of CRAB-PII RNA expression than normal subjects (FIG. **2**).

Example 7

Western Blot Analysis of Samples Isolated from Leukemia Patients and Normal Subjects

1. Patient Samples and Normal Samples

[0146] Patient marrow tissues and blood are obtained from Asterand, Inc. (Detroit, Mich.), Clinomics Biosciences, Inc (Watervliet, N.Y.) and Biochain Institute, Inc. (Hayward, Calif.). Each patient included in the study is screened against the same normal total RNA pool in order to compare them together.

2. Western Blot Analysis of CRAB-PII in Leukemia and Normal Samples

[0147] Blood samples are prepared by isolating blood from leukemia patients. The blood samples are fractioned initially to isolate remove red-blood cells. The remaining samples containing all white blood cell are further fractionated by FACS sorting based on size defractions and/or using surface specific monoclonal antibodies. Purified cells are then lysed in lysis buffer as in the above examples. Quantified cell lysates from leukemia samples and normal blood cells are then resolved on SDS-PAGE and prepared for Western blotting to probe for CRAB-PII.

3. Results.

[0148] The results of expression analyses for the protein markers is that CRAB-PII expression is significantly increased in cell and fluid samples obtained from tumor patients as compared to cell and fluid samples isolated from normal subjects. All normal subjects show nearly undetectable levels, or low levels, of CRAB-PII protein expression, while several samples obtained from lung cancer patients show detectable levels, or increased levels of CRAB-PII expression as compared to control samples.

Example 8

Preparation and Use of the Focused Microarray to Detect Crab-PII in Samples Obtained From Normal Subjects and Leukemia Patients

[0149] 1. Total RNA Isolation and cDNA Labeling

[0150] Patient marrow tissues and blood are obtained from Asterand, Inc. (Detroit, Mich.), Clinomics Biosciences, Inc (Watervliet, N.Y.) and Biochain Institute, Inc. (Hayward, Calif.). Each patient included in the study is screened against the same normal total RNA pool in order to compare them together.

[0151] Blood samples are prepared as described in Example 7. For leukemia tissue samples, human marrow tissues are homogenized and prepared for analysis following procedures described in Example 1.

[0152] First strand cDNA labeling, cDNA digestion, capture probe preparation and focused microarray preparation are accomplished using procedures described in Example 2. In addition, quality control and focused microarray hybridization are performed according to procedures described in Example 2. The QuantArray® data results are analyzed according to the procedures described above in Example 2(6).

3. Results.

[0153] CRAB-PII mRNA expression correlates with CRAB-PII protein expression. Increased levels of CRAB-PII mRNA are detected in cell and fluid samples obtained patients suffering from leukemia as compared to normal subjects. Cell and fluid samples from patients suffering from leukemia had higher levels of CRAB-PII mRNA expression than normal subjects.

Example 9

Western Blot Analysis of Samples Isolated from Colon Patients and Normal Subjects

1. Patient Samples and Normal Samples

[0154] Patient tissues and fluid samples are obtained from Asterand, Inc. (Detroit, Mich.), Clinomics Biosciences, Inc (Watervliet, N.Y.) and Biochain Institute, Inc. (Hayward,

Calif.). Each patient included in the study is screened against the same normal total RNA pool in order to compare them together.

2. Western Blot Analysis of CRAB-PII in Colon and Normal Samples

[0155] Samples are prepared as described in Example 1. Western blot analysis is then performed as detailed in Example 1.

[0156] The results of expression analyses for the protein markers is that CRAB-PII expression is significantly increased in cell and fluid samples obtained from tumor patients as compared to cell and fluid samples isolated from normal subjects. All normal subjects show nearly undetectable levels, or low levels, of CRAB-PII protein expression, while several samples obtained from lung cancer patients show detectable levels, or increased levels of CRAB-PII expression as compared to control samples.

Example 10

Preparation and Use of the Focused Microarray to Detect Crab-PII in Samples Obtained from Normal Subjects and Colon Patients

[0157] 1. Total RNA Isolation and cDNA Labeling

[0158] Patient tissue and fluid samples are obtained from Asterand, Inc. (Detroit, Mich.), Clinomics Biosciences, Inc (Watervliet, N.Y.) and Biochain Institute, Inc. (Hayward, Calif.). Each patient included in the study is screened against the same normal total RNA pool in order to compare them together.

[0159] For colon tissue samples, human tissues are homogenized using the procedure described in Example 2. RNA is isolated and prepared according to procedures described in Example 2.

[0160] First strand cDNA labeling, cDNA digestion, capture probe preparation and focused microarray preparation are accomplished using procedures described in Example 2. In addition, quality control and focused microarray hybridization are performed according to procedures described in Example 2. The QuantArray® data results are analyzed according to the procedures described above in Example 2(6).

2. Results.

[0161] CRAB-PII mRNA expression correlates with CRAB-PII protein expression. Increased levels of CRAB-PII mRNA are detected in cell and fluid samples obtained patients suffering from colon as compared to normal subjects. Cell and fluid samples from patients suffering from colon had higher levels of CRAB-PII mRNA expression than normal subjects.

Example 11

Western Blot Analysis of Samples Isolated from Prostate Patients and Normal Subjects

1. Patient Samples and Normal Samples

[0162] Patient tissues and fluid samples are obtained from Asterand, Inc. (Detroit, Mich.), Clinomics Biosciences, Inc (Watervliet, N.Y.) and Biochain Institute, Inc. (Hayward, Calif.). Each patient included in the study is screened against the same normal total RNA pool in order to compare them together.

2. Western Blot Analysis of CRAB-PII in Prostate and Normal Samples

[0163] Samples are prepared as described in Example 1. Western blot analysis is then performed as detailed in Example 1.

[0164] The results of expression analyses for the protein markers is that CRAB-PII expression is significantly increased in cell and fluid samples obtained from tumor patients as compared to cell and fluid samples isolated from normal subjects. All normal subjects show nearly undetectable levels, or low levels, of CRAB-PII protein expression, while several samples obtained from lung cancer patients show detectable levels, or increased levels of CRAB-PII expression as compared to control samples.

Example 12

Preparation and Use of the Focused Microarray to Detect Crab-PII in Samples Obtained from Normal Subjects and Prostate Patients

[0165] 1. Total RNA Isolation and cDNA Labeling

[0166] Patient tissue and fluid samples are obtained from Asterand, Inc. (Detroit, Mich.), Clinomics Biosciences, Inc (Watervliet, N.Y.) and Biochain Institute, Inc. (Hayward, Calif.). Each patient included in the study is screened against the same normal total RNA pool in order to compare them together.

[0167] For prostate tissue samples, human tissues are homogenized using the procedure described in Example 2. RNA is isolated and prepared according to procedures described in Example 2.

[0168] First strand cDNA labeling, cDNA digestion, capture probe preparation and focused microarray preparation are accomplished using procedures described in Example 2. In addition, quality control and focused microarray hybridization are performed according to procedures described in Example 2. The QuantArray® data results are analyzed according to the procedures described above in Example 2(6).

2. Results.

[0169] CRAB-PII mRNA expression correlates with CRAB-PII protein expression. Increased levels of CRAB-PII mRNA are detected in cell and fluid samples obtained patients suffering from prostate as compared to normal subjects. Cell and fluid samples from patients suffering from prostate had higher levels of CRAB-PII mRNA expression than normal subjects.

EQUIVALENTS

[0170] Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, numerous equivalents to the specific compositions and procedures described herein. Such equivalents are considered to be within the scope of this invention, and are covered by the following claims.

I claim:

1. A method for detecting a neoplasm comprising

a) obtaining a potentially neoplastic test cell sample and a non-neoplastic control cell sample;

b) detecting a level of CRAB-PII expression in the test cell sample;

c) detecting a level of CRAB-PII expression in the control cell sample;

d) comparing the level of CRAB-PII expression in the test cell sample to the level of

CRAB-PII expression in the control cell sample, and

wherein the test cell sample is neoplastic if the level of CRAB-PII expression in the test cell sample is greater than the level of CRAB-PII expression in the control cell sample.

2. The method of claim **1**, wherein detecting the level of expression of CRAB-PII comprises isolating a cellular cytoplasmic fraction from the test cell sample and from the control cell sample, and then separately detecting the level of expression of CRAB-PII in these cellular cytoplasmic fractions.

3. The method of claim **1**, wherein the level of expression of CRAB-PII protein is detected by contacting the test cell sample and the control cell sample with a CRAB-PII-specific protein binding agent selected from the group consisting of an antibody, CRAB-PII binding portions of an antibody and retinoic acid.

4. The method of claim **3**, wherein the level of protein binding agents bound to CRAB-PII protein is detected by a detectable label selected from the group consisting of immunofluorescent label, a radiolabel, and a chemiluminescent label.

5. The method of claim **3**, wherein the protein binding agent is immobilized on a solid support.

6. The method of claim **1**, wherein the level of expression of anti-CRAB-PII antibody is detected in a test cell sample and a control cell sample.

7. The method of claim **6**, wherein the level of expression of anti-CRAB-PII antibody is detected in a serum sample isolated from a subject.

8. The method of claim **6**, wherein the level of expression of anti-CRAB-PII antibody is detected using antibodies or fragments thereof.

9. The method of claim **8**, wherein the antibodies or fragments thereof are operably linked to a detectable label selected from the group consisting of a immunofluorescent label, radiolabel, and chemiluminescent label.

10. The method of claim **1**, wherein the level of expression of CRAB-PII RNA is detected by contacting the test cell sample and the control cell sample with a nucleic acid binding agent selected from the group consisting of RNA, cDNA, cRNA, and RNA-DNA hybrids.

11. The method of claim **10**, wherein the level of nucleic acid binding agent hybridized to CRAB-PII RNA is detected using a detectable label operably linked to the binding agent, the binding agent being selected from the group consisting of an immunofluorescent label, a radiolabel, and a chemiluminescent label.

12. The method of claim **10**, wherein the nucleic acid binding agent is immobilized on a solid support.

13. The method of claim **1**, wherein the level of expression of CRAB-PII in the test cell sample is at least 1.5 times greater than the level of expression of CRAB-PII in the control cell sample.

14. The method of claim **1**, wherein the level of expression of CRAB-PII in the test cell sample is at least 2 times greater than the level of expression of CRAB-PII in the control cell sample.

15. The method of claim **1**, wherein the level of expression of CRAB-PII in the test cell sample is at least 4 times greater than the level of expression of CRAB-PII in the control cell sample.

16. The method of claim **1**, wherein the level of expression of CRAB-PII in the test cell sample is at least 6 times greater than the level of expression of CRAB-PII in the control cell sample.

17. The method of claim **1**, wherein the level of expression of CRAB-PII in the test cell sample is at least 8 times greater than the level of expression of CRAB-PII in the control cell sample.

18. The method of claim **1**, wherein the level of expression of CRAB-PII in the test cell sample is at least 10 times greater than the level of expression of CRAB-PII in the control cell sample.

19. The method of claim **1**, wherein the level of expression of CRAB-PII in the test cell sample is at least 20 times greater than the level of expression of CRAB-PII in the control cell sample.

20. The method of claim **1**, wherein the test cell sample is isolated from a tissue of a patient suffering from a metastasized ovarian neoplastic disease, the tissue being selected from the group consisting of blood, bone marrow, spleen, lymph node, liver, thymus, kidney, brain, skin, gastrointestinal tract, eye, breast, and prostate.

21. The method of claim **1**, wherein the test cell sample is isolated from a patient suffering from an ovarian neoplasm selected from the group consisting of ovarian carcinoma, ovarian epithelial adenocarcinoma, ovarian adenocarcinoma, sex cord-stromal carcinoma, endometrioid tumors, mucinous carcinoma, germ cell tumors, and clear cell tumors.

22. A method for diagnosing cancer in a subject comprising:

a) obtaining a potentially neoplastic test fluid sample from a subject and a non-neoplastic control fluid sample;

b) detecting a level of CRAB-PII expression in the test fluid sample;

c) detecting a level of CRAB-PII expression in the control fluid sample; and

d) comparing the level of CRAB-PII expression in the test fluid sample to the level of

CRAB-PII expression in the control fluid sample, and

wherein cancer is diagnosed if the level of CRAB-PII expression in the test fluid sample is greater than the level of CRAB-PII expression in the control fluid sample.

23. The method of claim **22**, wherein detecting the level of CRAB-PII expression comprises isolating cellular cytoplasmic fractions from the test fluid sample and the control fluid sample, and separately detecting the level of CRAB-PII expression in the cellular cytoplasmic fractions.

24. The method of claim **22**, wherein the levels of CRAB-PII expression protein are detected by contacting the test fluid sample and the control fluid sample with a protein binding agent selected from the group consisting of antibody and retinoic acid.

25. The method of claim **24**, wherein the level of protein binding agents bound to CRAB-PII protein is detected with detectable label selected from the group consisting of an immunofluorescent label, a radiolabel, and a chemiluminescent label.

26. The method of claim **24**, wherein the protein binding agent is immobilized on a solid support.

27. The method of claim 22, wherein the level of expression of anti-CRAB-PII antibody is detected in a test fluid sample and a control fluid sample.

28. The method of claim 27, wherein the level of expression of anti-CRAB-PII antibody is detected in a serum sample isolated from a subject.

29. The method of claim 28, wherein the level of expression of anti-CRAB-PII antibody is detected by antibodies or fragments thereof.

30. The method of claim 29, wherein the antibodies or fragments thereof are operably linked to a detectable label selected from the group consisting of a immunofluorescent label, radiolabel, and chemiluminescent label.

31. The method of claim 22, wherein the level of CRAB-PII RNA expression is detected by contacting the test fluid and the non-neoplastic fluid control fluid with a nucleic acid binding agent selected from the group consisting of RNA, cDNA, cRNA, and RNA-DNA hybrids.

32. The method of claim 31, wherein the level of nucleic acid binding agent hybridized to CRAB-PII RNA is detected by a detectable label selected from the group consisting of immunofluorescent label, radiolabel, and chemiluminescent label.

33. The method of claim 31, wherein the nucleic acid binding agent is immobilized on a solid support.

34. The method of claim 22, wherein the level of expression of CRAB-PII in the test fluid sample is about 1.5 times greater than the level of expression of CRAB-PII in the control fluid sample.

35. The method of claim 22, wherein the level of expression of CRAB-PII in the test fluid sample is about 2 times greater than the level of expression of CRAB-PII in the control fluid sample.

36. The method of claim 22, wherein the level of expression of CRAB-PII in the test fluid sample is about 4 times greater than the level of expression of CRAB-PII in the control fluid sample.

37. The method of claim 22, wherein the level of expression of CRAB-PII in the test fluid sample is about 6 times greater than the level of expression of CRAB-PII in the control fluid sample.

38. The method of claim 22, wherein the level of expression of CRAB-PII in the test fluid sample is about 8 times greater than the level of expression of CRAB-PII in the control fluid sample.

39. The method of claim 22, wherein the level of expression of CRAB-PII in the test fluid sample is about 10 times greater than the level of expression of CRAB-PII in the control fluid sample.

40. The method of claim 22, wherein the level of expression of CRAB-PII in the test fluid sample is at least 20 times greater than the level of expression of CRAB-PII in the control fluid sample.

41. The method of claim 22, wherein the test fluid sample is from a patient suffering from a metastasized neoplastic disease isolated from a tissue selected from the group consisting of blood, bone marrow, spleen, lymph node, liver, thymus, kidney, brain, skin, gastrointestinal tract, eye, breast, and prostate.

42. The method of claim 22, wherein the test fluid sample is from a patient suffering from an ovarian neoplasm selected from the group consisting of ovarian carcinoma, ovarian epithelial adenocarcinoma, ovarian adenocarcinoma, sex cord-stromal carcinoma, endometrioid tumors, mucinous carcinoma, germ cell tumors, and clear cell tumors.

43. A kit for diagnosing or detecting neoplasia, comprising a probe for the detection of CRAB-PII.

44. The kit of claim 43, wherein the probe for detecting CRAB-PII is an anti-CRAB-PII antibody or CRAB-PII binding fragment thereof.

45. The kit of claim 43, wherein the probe for detecting CRAB-PII is retinoic acid.

46. The kit of claim 43, wherein the probe detects CRAB-PII present in the test cell if it is neoplastic.

47. The kit of claim 43, wherein the CRAB-PII probe is a nucleic acid probe selected from the group consisting of RNA, cDNA, cRNA, and RNA-DNA hybrids.

48. The kit of claim 47, wherein the CRAB-PII probe is complementary to at least a 20 nucleotide sequence of a nucleic acid sequence consisting of SEQ ID NO:1.

49. The kit of claim 43, wherein the probe binds to an anti-CRAB-PII antibody.

50. The kit of claim 49, wherein the probe is an antibody or fragment thereof operably linked to a detectable label.

* * * * *

专利名称(译)	Crab-PII针对肿瘤疾病的定向诊断		
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[标]申请(专利权)人(译)	GEORGES ELIAS BONNEAU安妮玛丽		
申请(专利权)人(译)	GEORGES ELIAS BONNEAU安妮 - 玛丽·		
当前申请(专利权)人(译)	GEORGES ELIAS BONNEAU安妮 - 玛丽·		
[标]发明人	GEORGES ELIAS BONNEAU ANNE MARIE		
发明人	GEORGES, ELIAS BONNEAU, ANNE-MARIE		
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摘要(译)

公开了通过检测测试细胞样品或流体样品中CRAB-PII的表达水平与对照细胞中CRAB-PII的表达水平相比来诊断测试细胞样品或流体样品中的癌症的方法。从正常受试者中分离的样品或液体样品。

FIG 1