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Hanash et al.

(54) **LUNG CANCER DIAGNOSIS**

(76) Inventors: **Samir M. Hanash**, Mercer Island, WA (US); **Ji Qiu**, Chandler, AZ (US); **Sandra Faca**, Sao Paolo (BR)

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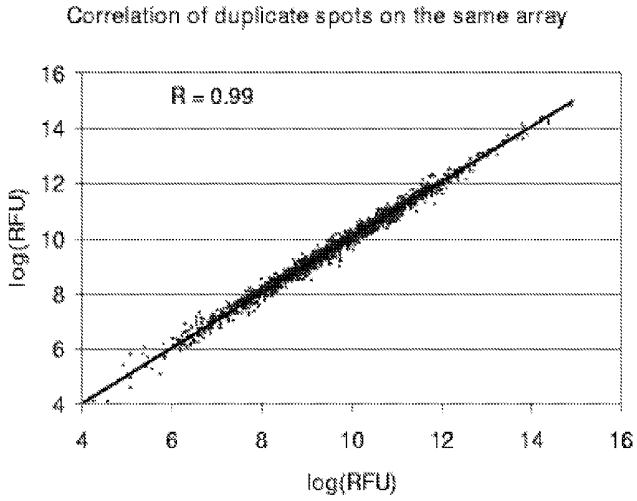
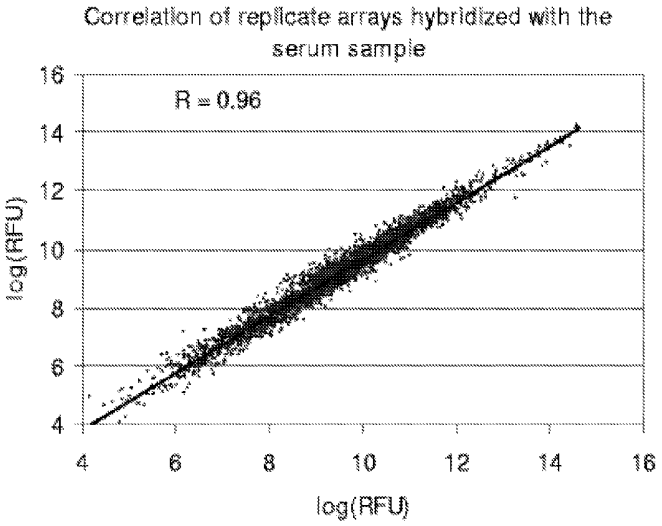
(52) **U.S. Cl.** **435/7.9**; 436/501

(57) **ABSTRACT**

Diagnosis of lung cancer in a subject before onset of symptoms is described herein (i.e., in a pre-diagnostic subject), by screening a biological fluid from the subject for the presence therein of autoantibodies that are specific for one or more pre-diagnostic lung cancer indicator proteins, including LAMR1, and optionally additionally or alternatively including annexin I and/or 14-3-3-theta and/or other pre-diagnostic lung cancer indicator proteins as presently disclosed, as the defined antigens. Related methods, including for monitoring immune reactivity against lung cancer indicator proteins in a lung cancer patient, typing lung cancer subjects or characterizing lung tumors, and application of the described proteomics approach for the identification of additional pre-diagnostic lung cancer indicator proteins, are also contemplated.





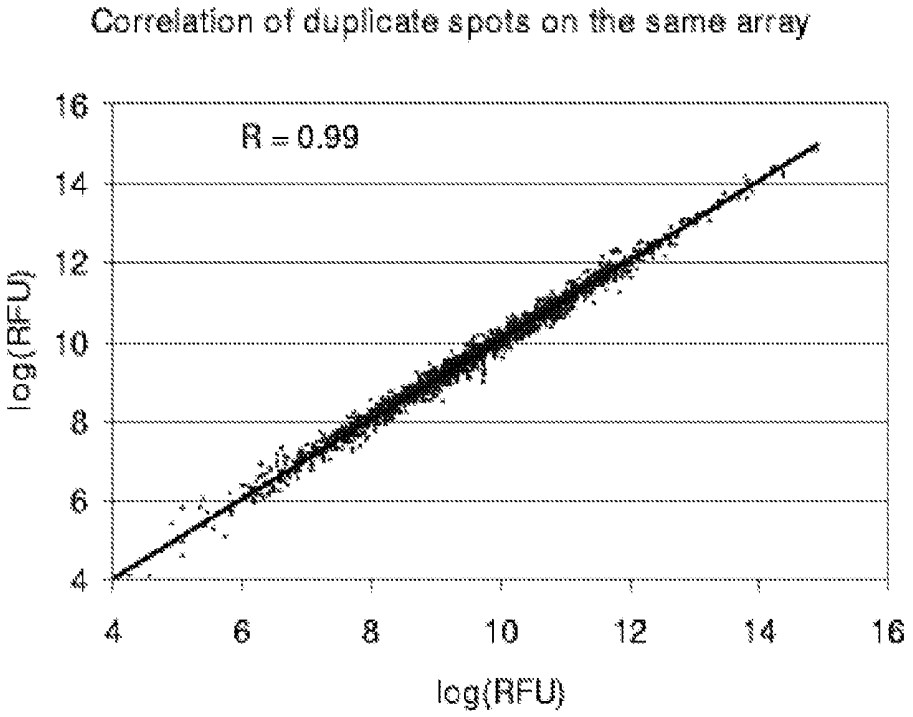

Correlation of duplicate spots on the same array
R = 0.99
log(RFU)
log(RFU)
16
14
12
10
8
6
4
4
6
8
10
12
14
16

FIG. 1A

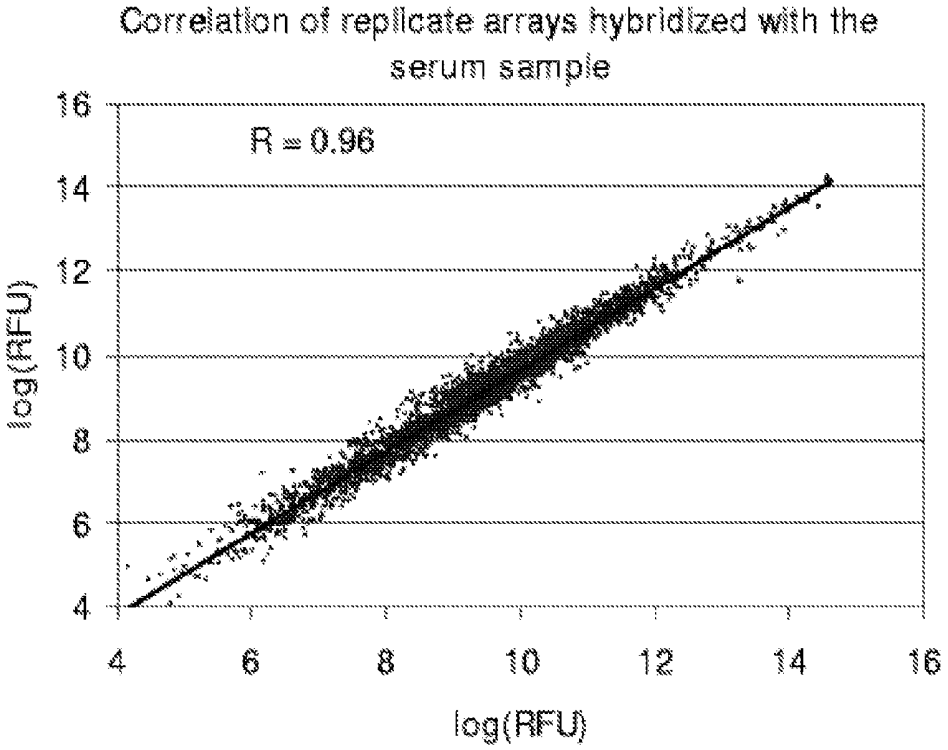

Correlation of replicate arrays hybridized with the serum sample
R = 0.96
log(RFU)
log(RFU)
16
14
12
10
8
6
4
4
6
8
10
12
14
16

FIG. 1B

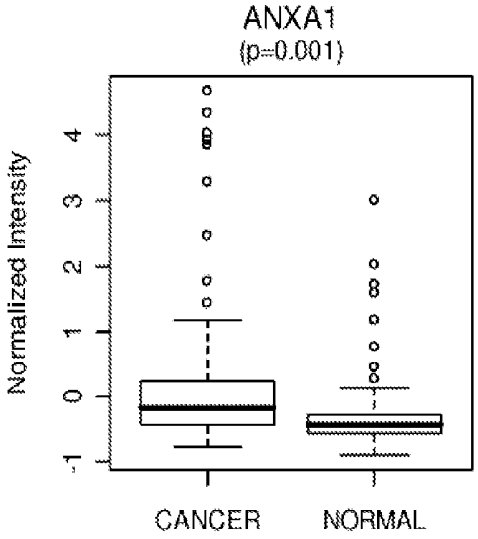


FIG. 2A

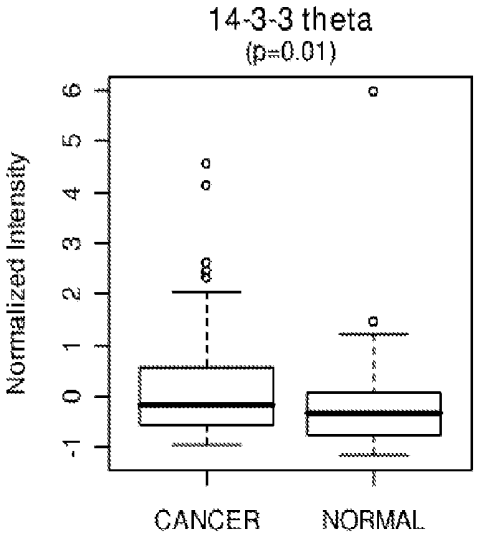


FIG. 2B

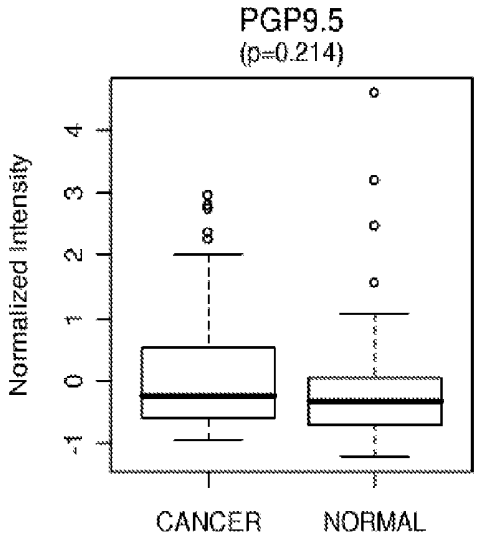


FIG. 2C

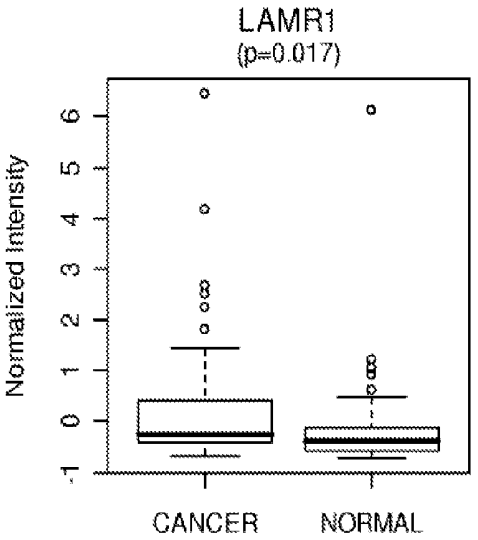


FIG. 2D

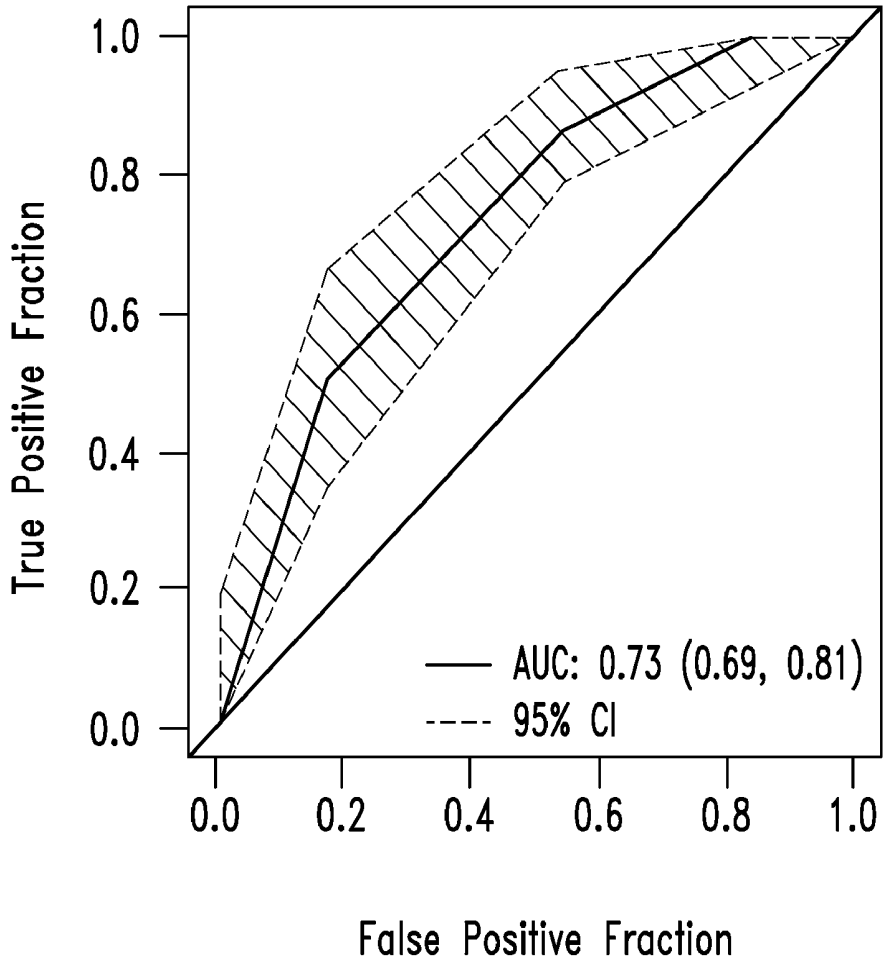

1.0
0.8
0.6
0.4
0.2
0.0
True Positive Fraction
AUC: 0.73 (0.69, 0.81)
95% CI
0.0
0.2
0.4
0.6
0.8
1.0
False Positive Fraction

FIG. 3

LUNG CANCER DIAGNOSIS

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of U.S. Provisional Application Ser. No. 61/095,269, filed Sep. 8, 2008.

STATEMENT OF GOVERNMENT INTEREST

[0002] This invention was made with government support under Grant No. UO1 CA084982, awarded by the National Cancer Institute/National Institutes of Health. The government has certain rights in this invention.

STATEMENT REGARDING SEQUENCE LISTING

[0003] The Sequence Listing associated with this application is provided in text format in lieu of a paper copy, and is hereby incorporated by reference into the specification. The name of the text file containing the Sequence Listing is 360056_404PC_SEQUENCE_LISTING.txt. The text file is 65 KB, was created on Aug. 27, 2009 and is being submitted electronically via EFS-Web to the U.S. PCT Receiving Office, concurrent with the filing of the specification.

BACKGROUND

[0004] 1. Technical Field

[0005] The presently disclosed invention embodiments relate to compositions and methods for the early detection of cancer. In particular, certain embodiments relate to compositions and methods for diagnosing lung cancer in patients otherwise exhibiting no signs of cancer, by detecting in patient samples the presence of autoantibodies that are specific for one or more lung cancer indicator proteins as described herein.

[0006] 2. Description of the Related Art

[0007] Lung cancer accounts for over 12% of all human cancers and close to 18% of cancer deaths, thus representing one of the most commonly occurring cancers in humans worldwide. (World Cancer Research Fund/American Institute for Cancer Research. Food, Nutrition and the Prevention of Cancer: A global perspective. Washington, D.C.: American Institute for Cancer Research, 2007.) Non-small cell lung cancer is most prevalent, including squamous cell carcinoma (~30-45% of all lung cancers), adenocarcinoma (~30-45%) and large cell carcinoma (~9%). Small-cell carcinoma represents approximately 10-15% of all lung cancers. In 2002, approximately 1.4 million lung cancer cases were reported. Id.

[0008] In its early stages, lung cancer produces no symptoms, thereby typically eluding detection until the disease has progressed to advanced stages that are associated with high mortality rates.

[0009] Currently available non-invasive approaches for screening and diagnosis of lung cancer include chest x-rays and other imaging methods such as computerized tomography (CT), for example, low-dose helical computed tomography. These approaches have not, however, been shown to reduce lung cancer mortality rates, and may generate false positive results that could lead to unnecessary and potentially harmful invasive procedures and/or therapeutic regimens. (Lung Cancer Screening PDQ® Summary, National Cancer Institute, National Institutes of Health, Bethesda, Md., 2008) Additionally or alternatively, cytological analysis of sputum samples may be performed in efforts to detect cancer cells, typically for patients presenting with productive cough, a symptom which may signify the presence of an advanced case of lung cancer.

[0010] Invasive procedures to obtain lung biopsy samples may also be performed in efforts to diagnose lung cancer, including procedures such as bronchoscopy, mediastinoscopy or imaging-guided needle biopsy. These procedures are typically practiced only in patients presenting with one or more potential signs or symptoms of advanced lung cancer (e.g., a mass or nodule appearing in x-ray of CT imaging studies), which may warrant the time and expense of such procedures and of the subsequent diagnostic work-up of the biopsy samples. Hence, these procedures are not amenable to routine screening for, or early detection of, lung cancer.

[0011] There is increasing evidence for a humoral immune response to cancer in humans, as demonstrated by the identification of autoantibodies against a number of intracellular and surface antigens in patients with various tumor types (Stocked et al., *J Exp Med* 187:1349-54, 1998; Tan E M, *J Clin Invest* 108:1411-5, 2001; Mintz et al., *Nat Biotechnol* 21:57-63, 2003; Gourevitch et al., *Br J Cancer* 72:934-8, 1995; Gure et al., *Cancer Res* 58:1034-41, 1998; Yamamoto et al., *Int J Cancer* 69:283-9, 1996; Dunn et al., *Nat Immunol* 3:991-8, 2002; Hanash, *Nat Biotechnol* 21:37-8, 2003; Old et al., *J Exp Med* 187:1163-7, 1998; Finn, *N Engl J Med* 353: 1288-90, 2005). Interest in the humoral response against tumor antigens relates in part to the potential screening and diagnostic utility of autoantibodies and their corresponding antigens.

[0012] A number of circulating autoantibodies in lung cancer have been identified by screening expression libraries with patient sera (Gure et al., *Cancer Res* 58:1034-41, 1998; Yamamoto et al., *Int J Cancer* 69:283-9, 1996; Diesinger et al., *Int J Cancer* 102:372-8, 2002; Gure et al., *Proc Natl Acad Sci USA* 97:4198-203, 2000; Ali Eldib et al., *Int J Cancer* 108:558-63, 2004; Yang et al., *J Proteome Res* 6:751-8, 2007). However, there remains a need to identify additional autoantibody targets to increase specificity and sensitivity.

[0013] Several proteomics methods are emerging as useful means for discovering autoantibody biomarkers (e.g., Hanash, *Nature* 422:226-32, 2003; Imafuku, Omenn and Hanash, *Dis Markers* 20:149-53, 2004; U.S. Pat. Nos. 6,645, 465; 7,202,045; 7,387,881). The merit of a proteomic approach is that it allows identification of autoantibodies to proteins that are directly derived from cancer cells or tumors and thus may uncover antigenicity associated with proteins as they occur in tumor cells, including proteins whose antigenicities have structural bases in their post-translational modification. Previous studies using two-dimensional gels of lung tumor cell lysates and Western blotting uncovered autoantibodies in lung cancer patient sera against annexin I, PGP9.5 and 14-3-3 theta proteins (Brichory et al., *Cancer Res* 61:7908-12, 2001; Brichory et al., *Proc Natl Acad Sci USA* 98:9824-9, 2001; Pereira-Faca et al., *Cancer Res* 67:12000-6, 2007).

[0014] More recently, a method has been implemented that utilized liquid-based procedures to separate intact proteins in tissue and tumor cell lysates (Wang and Hanash, *J Chromatogr B Analyt Technol Biomed Life Sci* 787:11-8, 2003; Faca et al., *J Proteome Res* 6:3558-65, 2007). Several hundreds of distinct protein-containing fractions were spotted onto microarrays, interrogated using various sources of sera, and quantitatively analyzed for bound antibodies. Anti-

PGP9.5 antibodies were successfully identified in sera of newly diagnosed lung cancer patients, and anti-UCHL3 antibodies were identified in colon cancer patient sera collected at the time of diagnosis, using this microarray approach (Nam et al., *Proteomics* 3:2108-15, 2003; Madoz-Gurpide et al., *Mol Cell Proteomics,* 2007), as well as autoantibodies in prostate cancer (Forrester et al., *Proteomics—Clinical Applications* 1:494-505, 2007), thus establishing the potential of natural protein microarrays to uncover antigens that induce an antibody response in cancer in a relatively high throughput approach.

[0015] Clearly there remains a significant unmet need for more and better compositions and methods for diagnosing lung cancer and for lung cancer screening, including additional biomarkers and conveniently practiced approaches that are capable of detecting lung cancer in a subject at an earlier point in the progression of the disease than is currently possible (e.g., in a pre-diagnostic subject), and preferably further including conveniently practiced methods that permit monitoring the severity of disease, progression of disease and/or disease responsiveness to a therapeutic course. Effective early diagnosis of lung cancer would reduce the tumor burden that is typically present at the inception of surgical, chemotherapeutic, immunotherapeutic, molecular therapeutic and/or radiation-based therapies, relative to the tumor burden presently confronting the clinician when current conventional diagnostics are relied upon, and could have a significant impact on lung cancer related mortality. The present invention thus addresses these needs and offers other related advantages.

BRIEF SUMMARY

[0016] In one embodiment, the present invention provides a method for diagnosing lung cancer in a pre-diagnostic subject, comprising (a) contacting (i) one or more antibodies from a biological fluid from the pre-diagnostic subject, and (ii) at least one isolated pre-diagnostic lung cancer indicator protein, under conditions and for a time sufficient for detecting specific binding of at least one antibody from the biological fluid to one or more of said pre-diagnostic lung cancer indicator proteins, and therefrom identifying presence of lung cancer in the pre-diagnostic subject.

[0017] In another embodiment, there is provided a screening method for lung cancer, comprising (a) contacting (i) one or more antibodies from a biological fluid from each subject of one or a plurality of subjects, and (ii) at least one isolated pre-diagnostic lung cancer indicator protein, under conditions and for a time sufficient for detecting specific binding of at least one antibody from the biological fluid to one or more of said pre-diagnostic lung cancer indicator proteins, wherein detection of specific binding indicates the subject has lung cancer, and thereby screening for lung cancer.

[0018] In another embodiment there is provided a method for diagnosing lung cancer in a pre-diagnostic subject, comprising (a) contacting (i) one or more antibodies from a biological fluid from the pre-diagnostic subject, and (ii) an isolated protein or polypeptide that comprises one or more antigenic epitopes of one or more pre-diagnostic lung cancer indicator proteins, under conditions and for a time sufficient for detecting specific binding of at least one antibody from the biological fluid to one or more of said antigenic epitopes, and therefrom identifying presence of lung cancer in the pre-diagnostic subject.

[0019] In another embodiment there is provided a screening method for lung cancer, comprising (a) contacting (i) one or more antibodies from a biological fluid from each subject of one or a plurality of subjects, and (ii) an isolated protein or polypeptide that comprises one or more antigenic epitopes of one or more pre-diagnostic lung cancer indicator proteins, under conditions and for a time sufficient for detecting specific binding of at least one antibody from the biological fluid to one or more of said antigenic epitopes, wherein detection of specific binding indicates the subject has lung cancer, and thereby screening for lung cancer.

[0020] In certain further embodiments of the above described methods, at least one of the one or more pre-diagnostic lung cancer indicator proteins comprises a LAMR1 protein, AKR1B10 protein [SEQ ID NO:11], GOT2 protein [SEQ ID NO:12], HNRPR protein [SEQ ID NO:13], PDIA3 protein [SEQ ID NO:14], NME2 protein [SEQ ID NO:15], RTN4 protein [SEQ ID NO:16], HI1FX protein [SEQ ID NO:17], G3BP protein [SEQ ID NO:18], HSPCA protein [SEQ ID NO:19], or ACTN4 protein [SEQ ID NO:20]. In certain still further embodiments the pre-diagnostic lung cancer indicator proteins further comprise at least one, two, three, four, five, six, seven, eight or more proteins selected from annexin I protein, 14-3-3 theta protein, AKR1B10 protein [SEQ ID NO:11], GOT2 protein [SEQ ID NO:12], HNRPR protein [SEQ ID NO:13], PDIA3 protein [SEQ ID NO:14], NME2 protein [SEQ ID NO:15], RTN4 protein [SEQ ID NO:16], HI1FX protein [SEQ ID NO:17], G3BP protein [SEQ ID NO:18], HSPCA protein [SEQ ID NO:19], and ACTN4 protein [SEQ ID NO:20].

[0021] In certain other further embodiments the lung cancer is selected from (i) adenocarcinoma, (ii) squamous cell carcinoma, (iii) non-small cell lung cancer that is not (i) or (ii), and (iv) a lung cancer that can be defined based on one or more of causation and gene mutational status. In certain other further embodiments the subject or pre-diagnostic subject is at increased risk for developing lung cancer. In certain further embodiments the subject or pre-diagnostic subject has at least one indicator of increased risk for developing lung cancer that is selected from (i) a history of asbestos exposure, (ii) a history of smoking tobacco products, (iii) a history of radon gas exposure, (iv) a history of exposure to a source of ionizing radiation, (v) a history of recurrent lung inflammation, (vi) a history of tuberculosis, (vi) a history of silicosis, berylliosis or talc inhalation, (vii) a family history of lung cancer in genetically related individuals, (viii) a history of vitamin A deficiency or vitamin A excess, (ix) a history of smoking cannabis, and (x) exposure to toxic volatile substances or infectious agents.

[0022] In certain embodiments of the above described methods, the antibodies are isolated from the biological fluid prior to the step of contacting, and in certain other embodiments the antibodies are present in the biological fluid during the step of contacting. In certain embodiments the antibodies are autoantibodies. In certain embodiments of the above described methods, the biological fluid is selected from blood, serum, serosal fluid, plasma, lymph, urine, cerebrospinal fluid, saliva, a mucosal secretion, a vaginal secretion, ascites fluid, pleural fluid, pericardial fluid, peritoneal fluid, abdominal fluid, culture medium, conditioned culture medium and lavage fluid. In certain embodiments the biological fluid comprises serum.

[0023] In certain other embodiments of the above described methods, the pre-diagnostic indicator protein, or the isolated

protein or polypeptide that comprises one or more antigenic epitopes of a pre-diagnostic indicator protein, is selected from (i) a naturally occurring protein or polypeptide, (ii) a synthetic protein or polypeptide, (iii) a recombinant protein or polypeptide, and (iv) a fusion protein or polypeptide that comprises a fusion polypeptide domain fused to the pre-diagnostic indicator protein, or to the polypeptide that comprises one or more antigenic epitopes of the pre-diagnostic indicator protein.

[0024] In certain other embodiments of the above described methods, the pre-diagnostic indicator protein, or the isolated protein or polypeptide that comprises one or more antigenic epitopes of a pre-diagnostic indicator protein, is immobilized on a solid substrate. In certain further embodiments the immobilized pre-diagnostic indicator protein or the immobilized isolated protein or polypeptide that comprises one or more antigenic epitopes of a pre-diagnostic indicator protein, is immobilized by a covalent bond. In certain other further embodiments the immobilized pre-diagnostic indicator protein or the immobilized isolated protein or polypeptide that comprises one or more antigenic epitopes of a pre-diagnostic indicator protein, is non-covalently immobilized.

[0025] In certain other embodiments of the above described methods, detecting specific binding of the at least one antibody comprises detecting a signal that is selected from a fluorescent signal, a radiometric signal, an enzymatic signal and a spectrometric signal.

[0026] In certain other embodiments of the above described methods, the pre-diagnostic lung cancer indicator protein is selected from the group consisting of (i) a non-posttranslationally modified protein, (ii) a posttranslationally modified protein that is selected from a glycoprotein, a lipoprotein, a phosphoprotein, a proteolipid, a glypiated protein, a ubiquitinylated protein, a SUMOylated protein, a sulfated protein and a glycated protein, and (iii) a posttranslationally modified protein of (ii) in which one or more posttranslational modifications results in immunogenicity.

[0027] In another embodiment there is provided a method of monitoring lung cancer autoimmune reactivity in a lung cancer patient, comprising (a) contacting, after each of two or more timepoints, (i) one or more antibodies from a biological fluid that is taken from a subject at each of said timepoints, and (ii) a test antigen that is selected from the group consisting of (1) at least one isolated pre-diagnostic lung cancer indicator protein and (2) at least one isolated protein or polypeptide that comprises one or more antigenic epitopes of one or more pre-diagnostic lung cancer indicator proteins, under conditions and for a time sufficient for detecting specific binding of at least one antibody from the biological fluid to one or more of said pre-diagnostic lung cancer indicator proteins or antigenic epitopes thereof; and (b) comparing the specific binding that is detectable by antibodies from the biological fluid taken at each of said two or more timepoints, and thereby monitoring lung cancer autoimmune reactivity in the patient. In certain further embodiments, a first timepoint occurs before administration of a therapeutic agent to the patient and a second timepoint occurs after administration of the therapeutic agent to the patient.

[0028] These and other aspects of the invention will be evident upon reference to the following detailed description and attached drawings. All of the U.S. patents, U.S. patent application publications, U.S. patent applications, foreign patents, foreign patent applications and non-patent publications referred to in this specification and/or listed in the Application Data Sheet, are incorporated herein by reference in their entirety, as if each was incorporated individually. Aspects of the invention can be modified, if necessary, to employ concepts of the various patents, applications and publications to provide yet further embodiments of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0029] FIG. **1** shows reproducibility of natural protein microarrays. (FIG. **1**A), Quantitative reproducibility was assessed by hybridization of the same pooled sample on 6 different microarrays. (FIG. **1**B), Pearson correlations between replicate spots on each of the 6 different microarrays were all 0.99 and correlations between replicate microarrays were greater than 0.96. Representative scatter plots were presented.

[0030] FIG. **2** shows reactivity of annexin I (FIG. **2**A), 14-3-3 theta (FIG. **2**B), PGP9.5 (FIG. **2**C) and LAMR1 (FIG. **2**D) containing spotted fractions with all 85+85 CARET sera.

[0031] FIG. **3** shows combined ROC analysis of the LAMR1, annexin 1 and 14-3-3 theta fractions based on reactivity with all 85+85 CARET sera. The hatched 95% confidence band in the plot was estimated from 500 bootstraps.

DETAILED DESCRIPTION

[0032] The presently disclosed invention embodiments derive from the surprising discovery, in pre-diagnostic subjects, of autoantibodies that specifically react with one or more pre-diagnostic lung cancer indicator proteins as described herein. These embodiments offer unprecedented advantages associated with early detection of lung cancer in a subject at a time when the subject otherwise exhibits none of the previously recognized signs or symptoms of lung cancer. Accordingly, these and related embodiments will find uses in screening methods for lung cancer and in methods for diagnosing lung cancer in pre-diagnostic subjects, and in other related methods.

[0033] In particular, and as described in greater detail below, it has been found unexpectedly that in a biological fluid from a pre-diagnostic subject, for instance, a subject in whom lung cancer cannot be diagnosed by any other means, detection (i) of autoantibodies specific for a LAMR1 protein, (ii) of autoantibodies specific for a LAMR1 protein and autoantibodies specific for an annexin I protein, (iii) of autoantibodies specific for a LAMR1 protein and autoantibodies specific for a 14-3-3 theta protein, (iv) of autoantibodies specific for a LAMR1 protein and autoantibodies specific for an annexin I protein and autoantibodies specific for a 14-3-3 theta protein, or (v) of autoantibodies specific for at least one, two, three, four, five, six, seven, eight or more proteins selected from a LAMR1 protein, annexin I protein, 14-3-3 theta protein, AKR1B10 protein [SEQ ID NO:11], GOT2 protein [SEQ ID NO:12], HNRPR protein [SEQ ID NO:13], PDIA3 protein [SEQ ID NO:14], NME2 protein [SEQ ID NO:15], RTN4 protein [SEQ ID NO:16], HI1FX protein [SEQ ID NO:17], G3BP protein [SEQ ID NO:18], HSPCA protein [SEQ ID NO:19], and ACTN4 protein [SEQ ID NO:20], indicates the presence of lung cancer in the subject. Detection of such autoantibodies in pre-diagnostic subjects precedes the subsequent onset of lung cancer symptoms and/or the subsequent ability to diagnose lung cancer by other diagnostic means.

[0034] Without wishing to be bound by theory, it is believed according to the present disclosure that at a very early stage of

lung cancer, and in particular in lung cancer that cannot be detected in a subject by any previously available means for detecting lung cancer (e.g., imaging or biopsy, or by the appearance of clinical signs or symptoms), expression of one or more pre-diagnostic lung cancer indicator proteins, by lung cancer cells and/or by precancerous cells that are inexorably committed to progression into lung cancer cells and/or by other cells associated with such lung cancer or precancerous cells, elicits an immune response in the subject that results in the production of one or more detectable antibodies that specifically bind to the one or more pre-diagnostic lung cancer indicator proteins. Further according to non-limiting theory, these antibodies that specifically bind to one or more pre-diagnostic lung cancer indicator proteins are autoantibodies that are present in a biological fluid in the pre-diagnostic subject.

[0035] Accordingly, certain of the preferred embodiments disclosed herein contemplate a simple, non-invasive diagnostic assay whereby a biological fluid from a pre-diagnostic subject may be tested for the presence of antibodies (e.g., autoantibodies) that specifically bind to one or more pre-diagnostic lung cancer indicator proteins as provided herein, and/or that specifically bind to a polypeptide that comprises one or more antigenic epitopes of one or more pre-diagnostic lung cancer indicator proteins as provided herein. These and related embodiments provide screening and diagnostic methods for lung cancer that are inexpensive, readily amenable to screening a plurality of subjects such as in a high-throughput format, and that offer the advantage of lung cancer detection at an earlier stage in the onset and progression of disease than is afforded by any previously existing technology.

[0036] The methods described herein may therefore be used to diagnose lung cancer in a pre-diagnostic subject and/or to screen one or a plurality of subjects for lung cancer, which may include any cancer, tumor, neoplasia, malignancy or other cancer present in the lung, whether by virtue of having originated in the lung as a spontaneous or primary tumor, or by having metastasized, invaded, lodged or otherwise migrated to the lung from a different site. The lung cancer that is detected according to the presently disclosed methods may therefore be a non-small cell lung cancer such as squamous cell carcinoma, adenocarcinoma, large cell carcinoma or other non-small cell carcinoma, and may also be a small-cell carcinoma, but the invention is not intended to be so limited and also contemplates other lung cancers. For example, regardless of whether or not a particular lung cancer can be identified as being a non-small cell lung cancer or small-cell carcinoma, certain embodiments also contemplate a lung cancer that can additionally or alternatively be defined (e.g., typed, classified, characterized or otherwise identified according to art-accepted criteria) based on its causation and/or its gene mutational status. Thus, and as known in the art and discussed in certain of the publications cited herein, causal or causative factors such as exposure to environmental insults including but not limited to toxins, carcinogens, radiation, free radicals, infectious agents and/or other agents of cancer causation, and/or inherent or acquired genetic mutations at any one or more of a large number of known genetic loci in which particular mutations have been linked to cancer (e.g., p53), may underlie certain lung cancers that may but need not also be amenable to classification by other criteria.

[0037] Certain preferred embodiments contemplate a subject or biological source that is a human subject such as a pre-diagnostic subject, which includes a subject in whom lung cancer is not detectable by one or more art-accepted diagnostic methods for lung cancer that were in use prior the present disclosure. In certain embodiments, the herein described methods may be practiced using a biological fluid as provided herein from a patient that has been classified as being at risk for developing or acquiring lung cancer according to art-accepted clinical diagnostic criteria, and in certain embodiments the patient has been diagnosed as having lung cancer using previously described diagnostic criteria, which include criteria by which a given lung cancer may be typed as, e.g., small-cell or large-cell lung cancer, or as squamous cell carcinoma, adenocarcinoma, etc., such as the criteria of the U.S. National Cancer Institute (Bethesda, Md., USA) or as described in DeVita, Hellman, and Rosenberg's Cancer: *Principles and Practice of Oncology* (2008, Lippincott, Williams and Wilkins, Philadelphia/Ovid, New York); Pizzo and Poplack, *Principles and Practice of Pediatric Oncology* (Fourth edition, 2001, Lippincott, Williams and Wilkins, Philadelphia/Ovid, New York); and Vogelstein and Kinzler, *The Genetic Basis of Human Cancer* (Second edition, 2002, McGraw Hill Professional, New York). Certain embodiments contemplate a human subject that is known to be free of a risk for having, developing or acquiring cancer by such criteria.

[0038] In certain preferred embodiments of the invention, the subject or biological source may be suspected of having or being at risk for having a malignant condition, and in certain preferred embodiments of the invention the subject or biological source may be known to be free of a risk or presence of such disease. Certain embodiments contemplate performing the methods described herein using a biological fluid from a pre-diagnostic subject, e.g., a subject in whom lung cancer is not detectable by one or more art-accepted diagnostic methods for lung cancer that were in use prior the present disclosure.

[0039] Those familiar with the art will therefore appreciate that a subject, including a pre-diagnostic subject, from whom a biological fluid may be obtained in order to practice certain herein described screening and/or diagnostic methods, may be at increased (i.e., in a statistically significant manner relative to appropriate controls) risk for developing lung cancer, for example, even where no frank signs or symptoms of lung cancer are apparent. A subject having an increased risk for developing lung cancer typically exhibits one or more indicators of increased risk for developing lung cancer. These indicators of increased risk are known in the art and can be readily determined, and include but need not be limited to (i) a history of asbestos exposure, (ii) a history of smoking tobacco products including exposure to second-hand smoke, (iii) a history of radon gas exposure, (iv) a history of exposure to a source of ionizing radiation, (v) a history of recurrent lung inflammation, (vi) a history of tuberculosis, (vi) a history of silicosis, berylliosis or talc inhalation, (vii) a family history of lung cancer in genetically related individuals, (viii) a history of vitamin A deficiency or vitamin A excess, (ix) a history of smoking cannabis, and, (ix) a history of exposure to one or more toxic volatile substances and/or to one or more infectious agents.

[0040] For background on these and other indicators of increased risk for developing lung cancer, see, e.g., Alberg et al., *Chest* 2003, 123:21 S-49S; U.S. Department of Health and Human Services, Health Consequences of Smoking: A Report of the Surgeon General (2004); Institute of Medicine (IOM) National Cancer Policy Board. *Fulfilling the Potential of Cancer Prevention and Early Detection*, Curry S J, Byers

T, Hewitt M (eds), National Academies Press, Washington, D.C., 2003; National Institutes of Health/National Cancer Institute, *Smoking Tobacco control monograph 9: Cigars, health effects and trends*, NIH Publication No. 98-4302, Bethesda, Md., U.S. Department of Health and Human Services, 1998; Boffetta et al., *Journal of the National Cancer Institute* 1999, 91:697-701; National Research Council (NRC), Committee on Passive Smoking, *Environmental Tobacco Smoke: Measuring Exposures and Assessing Health Effects* (1986); U.S. Environmental Protection Agency, *Respiratory Health Effects of Passive Smoking*, (1992); International Agency for Research on Cancer (IARC), IARC Monographs on the Evaluation of Carcinogenic Risks to Humans and their Supplements, A complete list: *Involuntary Smoking*, Volume 83 (2002); International Agency for Research on Cancer (IARC), IARC Monographs on the Evaluation of Carcinogenic Risks to Humans and their Supplements, A complete list: *Overall Evaluations of Carcinogenicity: An Updating of IARC Monographs Volumes 1 to 42*. (1987); U.S. Department of Health and Human Services, Public Health Service, National Toxicology Program, *Report on Carcinogens, Eleventh Edition* (2004); International Agency for Research on Cancer (IARC), IARC Monographs on the Evaluation of Carcinogenic Risks to Humans and their Supplements, A complete list: *Some Metals and Metallic Compounds and Arsenic and Arsenic Compounds*, Volume 23 (1980); Etzel et al., *Cancer Research* 2003; 63:8531-8535; Brownson et al., *International Journal of Epidemiology* 1997; 26:256-263; Bromen et al., *American Journal of Epidemiology* 2000; 152:497-505; Mayne et al., *Cancer Epidemiology, Biomarkers & Prevention* 1999; 8:1065-1069; World Cancer Research Fund/American Institute for Cancer Research, Food, Nutrition and the Prevention of Cancer: A global perspective, Washington, D.C., American Institute for Cancer Research, 2007.

[0041] In preferred embodiments a biological fluid containing one or more antibodies is obtained from a subject (e.g., a pre-diagnostic subject) and contacted with at least one isolated pre-diagnostic lung cancer indicator protein, to detect the presence or absence in the biological fluid of an antibody that is capable of specifically binding to one or more of the pre-diagnostic lung cancer indicator proteins. Biological fluids are typically liquids at physiological temperatures and may include naturally occurring fluids present in, withdrawn from, expressed by or otherwise extracted from a subject or biological source (e.g., a human subject such as a pre-diagnostic subject or a patient, or a biological sample obtained directly or indirectly therefrom, such as a blood sample, biopsy specimen, tissue explant, organ culture or any other tissue or cell preparation from which an antibody-containing fluid can be prepared).

[0042] Certain biological fluids derive from particular tissues, organs or localized regions and certain other biological fluids may be more globally or systemically situated in a subject or biological source. Examples of biological fluids include blood, serum and serosal fluids, plasma, lymph, urine, cerebrospinal fluid, saliva, mucosal secretions of the secretory tissues and organs, vaginal secretions, ascites fluids such as those associated with non-solid tumors, fluids of the pleural, pericardial, peritoneal, abdominal and other body cavities, and the like. Biological fluids may also include liquid solutions contacted with a subject or biological source, for example, cell and organ culture medium including cell or organ conditioned medium, lavage fluids and the like. In certain highly preferred embodiments the biological sample is serum, and in certain other highly preferred embodiments the biological sample is plasma. In other preferred embodiments the biological sample is a cell-free liquid solution.

[0043] It is contemplated that in certain embodiments the antibodies are present in the biological fluid at the time of contacting with the pre-diagnostic lung cancer indicator protein(s), but the invention is not so limited and also contemplates embodiments in which the antibodies are isolated from the biological fluid prior to the step of contacting. The term "isolated" means that the material is removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally occurring antibody (e.g., autoantibody), polypeptide or polynucleotide present in a living subject (e.g., a pre-diagnostic subject or a patient) is not isolated, but the same antibody, polypeptide or polynucleotide, separated from some or all of the co-existing materials in the natural system, is isolated. Such antibodies, polypeptides or polynucleotides could be part of a composition, and still be isolated in that such composition is not part of its natural environment.

[0044] Isolation of antibodies may be achieved according to any of a wide variety of methodologies with which persons skilled in the art will be familiar, including biochemical and/or immunological methods. Suitable biochemical techniques may include differential precipitation (e.g., as a function of salt or other solute concentration, for example, ammonium sulfate or sodium sulfate or polyethylene glycol (PEG) or the like), gel filtration chromatography, ion exchange chromatography, affinity chromatography (e.g., using lectin affinity or Staphylococcal protein A/protein G or mimetic affinity), hydrophobic interaction chromatography, chromatofocusing or isoelectric focusing (IEF) including free-fluid recycling IEF, high performance liquid chromatography (HPLC) or any of a number of other biochemical techniques such as well known separation techniques. Suitable immunochemical techniques include, but need not be limited to, immunoaffinity chromatography, immunoprecipitation, solid phase immunoadsorption or other immunoaffinity methods. For these and other useful techniques, see, for example, Scopes, R. K., *Protein Purification: Principles and Practice,* 1987, Springer-Verlag, NY; Weir, D. M., *Handbook of Experimental Immunology,* 1986, Blackwell Scientific, Boston; and Hermanson, G. T. et al., *Immobilized Affinity Ligand Techniques,* 1992, Academic Press, Inc., California.

[0045] The term "antibodies" includes immunoglobulins such as polyclonal antibodies, monoclonal antibodies, fragments thereof such as $F(ab')_2$, and Fab fragments, as well as any naturally occurring immunoglobulin variable (V) region complementarity determining region (CDR)-containing binding partners (also including in certain embodiments antigen-binding CDR-containing T cell receptor polypeptides which are encoded by members of the immunoglobulin gene superfamily, whilst certain other embodiments expressly exclude such T cell-derived polypeptides), which are molecules that specifically bind a pre-diagnostic lung cancer indicator protein.

[0046] As described in greater detail below, particularly preferred embodiments relate to detection in a biological fluid from a pre-diagnostic subject of antibodies that are autoantibodies, i.e., antibodies that specifically recognize and bind to "self" antigenic epitopes that may also be found in the subject. Briefly, it is well accepted in the art that the immune system (e.g., adaptive immune system) is typically character-

ized as distinguishing foreign agents (or "non-self") agents from familiar or "self" components, such that foreign agents elicit immune responses while "self" components are ignored or tolerated. Exceptions to this paradigm arise, however, in the case of autoantibody generation, whereby a host immune system produces antibodies that react with "self" antigens. See, e.g., Theofilopoulos and Bona, *The Molecular Pathology of Autoimmune Diseases*, CRC Press, Boca Raton, Fla., 2002; Doria et al., *Handbook of Systemic Autoimmune Diseases* (Vols. 1-9), Elsevier, N.Y., 2004-2008; Roitt et al., *Immunology* (6^{th} Ed.), Mosby, N.Y., 2001, Ch. 26. Accordingly and as described herein, there are provided the present embodiments in which an autoantibody is detected in the lung cancer diagnostic methods, wherein the autoantibody detectably and specifically binds to a pre-diagnostic lung cancer indicator protein, which protein may be a "self" component in the pre-diagnostic subject.

[0047] Antibodies are defined to be "immunospecific" or to be capable of specifically binding if they bind a pre-diagnostic lung cancer indicator protein (or a polypeptide that comprises one or more antigenic epitopes of such a protein) with a K_a of greater than or equal to about 10^4 M^{-1}, preferably of greater than or equal to about 10^5 M^{-1}, more preferably of greater than or equal to about 10^6 M^{-1} and still more preferably of greater than or equal to about 10^7 M^{-1}.

[0048] Affinities of binding partners or antibodies can be readily determined using conventional techniques, for example those described by Scatchard et al., *Ann. N.Y. Acad. Sci.* 51:660 (1949), or by surface plasmon resonance (SPR) spectroscopy, or by any of a number of other known methods for identifying and characterizing antibodies or antibody-derived proteins that specifically interact with cognate antigens via recognition and binding of antigenic epitopes. For instance, to detect an antibody that specifically binds to a pre-diagnostic lung cancer indicator protein (or a polypeptide that comprises one or more antigenic epitopes of such a protein), there are a variety of assay formats, including but not limited to enzyme linked immunosorbent assay (ELISA), radioimmunoassay (RIA), immunofluorimetry, immunoprecipitation, equilibrium dialysis, immunodiffusion and other techniques. See, e.g., Harlow and Lane, *Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory,* 1988; Weir, D. M., *Handbook of Experimental Immunology,* 1986, Blackwell Scientific, Boston. See, e.g., Coligan et al. (Eds.), *Current Protocols in Immunology* (2007 John Wiley & Sons, NY); Harlow and Lane, *Antibodies: A Laboratory Manual* (1988 Cold Spring Harbor Press, Cold Spring Harbor, N.Y.); Harlow and Lane, *Using Antibodies* (1999 Cold Spring Harbor Press, Cold Spring Harbor, N.Y.). Therein can also be found parameters for designing immunoassay conditions, including conditions and incubation times that will be sufficient for detection of specific binding of an antibody to its cognate antigen.

Pre-Diagnostic Lung Cancer Indicator Protein

[0049] As described herein, the present embodiments relate to detecting the presence in a biological fluid from a pre-diagnostic subject of an antibody that specifically binds to one or more isolated pre-diagnostic lung cancer indicator proteins. A pre-diagnostic lung cancer indicator protein thus may include any one or more of a number of target antigens for which the presence in a biological sample from a pre-diagnostic subject of a specifically reactive cognate antibody indicates that the subject has lung cancer. As is well known in the immunological art, specifically-binding antibodies define antigenic epitopes on their cognate antigens, i.e., the molecular structure with which the antibody's antigen-combining site interacts. For example, an antigenic epitope of a pre-diagnostic lung cancer indicator protein may be defined by a region of protein primary structure (e.g., amino acid sequence), by protein secondary structure (e.g., a localized structure that is spatially defined such as may be formed by a common motif or repetitive domain, e.g, alpha-helix, beta-sheet, etc.), by protein tertiary structure (e.g., by three-dimensional structure that is created by protein folding or conformation) or by quarternary structure (e.g., by the interaction of two or more polypeptide subunits, as in a complex or oligomer). Antigenic epitopes may additionally or alternatively be defined in whole or in part by post-translational modifications to pre-diagnostic lung cancer indicator proteins, including by virtue of direct involvement of the post-translational modification in formation of the epitope structure and also including, e.g., conformational epitopes the presence of which may depend on the presence of a particular post-translational modification. Accordingly, certain embodiments described herein contemplate use of an intact pre-diagnostic lung cancer indicator, which may bear one, two, three, four, five, six or more antigenic epitopes that can be defined by antibody reactivities, while certain other embodiments contemplate use of an isolated protein or polypeptide that comprises one or more antigenic epitopes of one or more pre-diagnostic lung cancer indicator proteins.

[0050] A pre-diagnostic indicator protein thus may be (i) a naturally occurring protein or polypeptide, (ii) a synthetic protein or polypeptide, (iii) a recombinant protein or polypeptide, or (iv) a fusion protein or polypeptide that comprises a fusion polypeptide domain fused to the pre-diagnostic indicator protein, or to the polypeptide that comprises one or more antigenic epitopes of the pre-diagnostic indicator protein. A pre-diagnostic indicator protein may be one or more of a non-posttranslationally modified protein, a posttranslationally modified protein, for example, a glycoprotein, a lipoprotein, a phosphoprotein, a proteolipid, a glycosylphosphatidylinositol-modified ("glypiated") protein, a ubiquitinylated protein, a small ubiquitin-like modifier-modified ("SUMOylated", Hay, 2005 *Mol. Cell* 18:1-12) protein, a sulfated protein and a glycated protein, and may (including in certain preferred embodiments) also be a posttranslationally modified protein in which one or more of the posttranslational modifications results in immunogenicity. Criteria and methodologies for distinguishing among these and other classes of post-translationally modified proteins (or unmodified proteins) will be known to those familiar with the relevant art, as also will be methodologies for determining whether immunogenicity, including for example the formation of one or more particular antibody—(e.g., autoantibody) defined epitopes, resides in the structure formed by a posttranslational modification (e.g., Ahmed, *Principles and Reactions of Protein Extraction, Purification, and Characterization*, Taylor & Francis, NY, 2007; Scopes, R. K., *Protein Purification: Principles and Practice,* 1987, Springer-Verlag, NY; Coligan et al. (Eds.), *Current Protocols in Immunology* (2007 John Wiley & Sons, NY)).

[0051] Based on the disclosure herein and knowledge in the art concerning the making and testing of synthetic, recombinant and/or fusion polypeptides and proteins, it will also be appreciated that through routine methodologies non-naturally occurring polypeptides and proteins can be constructed

and immunologically (and/or structurally) probed for the presence of antibody-reactive epitopes. Exemplary methodologies may be found, for example, in e.g., Bonificano et al. (Eds.) *Current Protocols in Cell Biology,* 2007 John Wiley & Sons, NY; Ausubel et al. (Eds.) *Current Protocols in Molecular Biology,* 2007 John Wiley & Sons, NY; Coligan et al. (Eds.), *Current Protocols in Immunology,* 2007 John Wiley & Sons, NY; Robinson et al. (Eds), *Current Protocols in Cytometry,* 2007 John Wiley & Sons, NY. For instance, an antigenic epitope that can be specifically recognized by an autoantibody from a pre-diagnostic subject having lung cancer may be a truncated pre-diagnostic lung cancer indicator protein such as a functional fragment thereof, e.g., a portion of the protein that retains the antigenic epitope as can be readily determined by immunological cross-reactivity with the epitope-bearing, full length intact protein.

[0052] As also noted above, according to non-limiting theory the presence of lung cancer induces generation by the host immune system of antibodies reactive with the herein described pre-diagnostic lung cancer indicator protein(s), even in a pre-diagnostic subject, i.e., at a time when lung cancer is undetectable in the subject by any diagnostic means of the prior art, including in the absence of any signs or symptoms of lung cancer in the subject. Without wishing to be bound by theory, it is contemplated that certain pre-diagnostic lung cancer indicator protein-reactive antibodies (including in preferred embodiments autoantibodies) are generated by the pre-diagnostic subject's immune system in response to immune recognition of one or more pre-diagnostic lung cancer indicator proteins that may be expressed by lung cancer cells and/or by precancerous cells that are inexorably committed to progression into lung cancer cells and/or by other cells associated with such lung cancer or precancerous cells. The invention embodiments need not, however, be so limited, and also contemplate certain other pre-diagnostic lung cancer indicator protein-reactive antibodies (including in preferred embodiments autoantibodies) the production of which in the subject may be elicited by aberrant immune function, or serendipitously by cross-reactive antigens to give rise to heteroclitic antibodies (e.g., antibodies that react more strongly with antigens other than those used to elicit their production).

[0053] As described in greater detail below, certain particularly preferred embodiments relate to diagnostic and screening methods in which the pre-diagnostic lung cancer indicator protein is the laminin receptor precursor protein known as LAMR1, which may be any one of a set of related protein isoforms (including allelotypes) believed to be encoded by a common gene and identified in several different forms that differ in molecular weight, oligomeric state, post-translational modification, supramolecular associations, cell source and presumed function, as described below. For example, LAMR1 proteins include the 295 amino acid, 33 kDa laminin receptor precursor, and may also occur as a ribosomal 40S subunit-associated protein, and may also occur as an oncofetal antigen and/or as a eukaryotic cell prion receptor (e.g., accession numbers IP100411639.1; IP100413108.4; IP100553164.4; IP100790580.1; IP100793905.1; SEQ ID NOS:6-10).

[0054] In certain related embodiments one or both of the pre-diagnostic lung cancer indicator proteins Annexin 1 and 14-3-3 theta protein may also be used. Annexin proteins including annexin I may have variable posttranslationally added glycosylation patterns, which may contribute to immunogenicity of the resulting glycoproteins. Annexin 1 proteins thus include glycoforms and other isoforms (and further include allelotypes) of the cytoplasmic calcium-dependent phospholipid-binding annexin I member of the annexin family of conserved proteins that is widely expressed in eukaryotic cells (e.g., accession numbers IP100218918.5; IP100549413.2; IP100643231.1; SEQ ID NOS:1-3; see also, e.g., Brichory et al., 2001 *Proc. Nat. Acad. Sci. USA* 98:9824; U.S. Pat. No. 6,645,465).

[0055] 14-3-3 theta proteins include isoforms (and further include allelotypes) of the 14-3-3 theta gene products, which are embers of the widely expressed 14-3-3 protein family involved in signal transduction and cell cycle control; 14-3-3 theta proteins also include phosphorylated, acetylated, cleaved, and truncated variants (see, e.g., Pereira-Faca et al., 2007 *Canc. Res.* 67:12000). Exemplary sequences are set forth below (e.g., accession numbers IP100018146.1; IP100796727.1; SEQ ID NOS:4-5).

[0056] In certain related embodiments one, two, three, four, five, six, seven, eight or more of the pre-diagnostic lung cancer indicator proteins AKR1B10 protein [SEQ ID NO:11], GOT2 protein [SEQ ID NO:12], HNRPR protein [SEQ ID NO:13], PDIA3 protein [SEQ ID NO:14], NME2 protein [SEQ ID NO:15], RTN4 protein [SEQ ID NO:16], HI1FX protein [SEQ ID NO:17], G3BP protein [SEQ ID NO:18], HSPCA protein [SEQ ID NO:19], and ACTN4 protein [SEQ ID NO:20] may, additionally or alternatively, be used. (See, e.g., Table 5.)

[0057] As known in the art "similarity" between two polypeptides is determined by comparing the amino acid sequence and conserved amino acid substitutes thereto of the polypeptide to the sequence of a second polypeptide. Similarity between two polypeptide (or encoding polynucleotide) sequences, or even the percent identity, may be readily determined by comparing sequences using computer algorithms well known to those of ordinary skill in the art, such as the BLAST algorithm (Altschul, *J. Mol. Biol.* 219:555-565, 1991; Henikoff and Henikoff, *Proc. Natl. Acad. Sci. USA* 89:10915-10919, 1992), which is available at the NCBI website (http://www/ncbi.nlm.nih.gov/cgi-bin/BLAST). Default parameters may be used. Examples of other useful computer algorithms are those used in programs such as Align and FASTA, which may be accessed, for example, at the Genestream internet website of the Institut de Genetique Humaine, Montpellier, France (www2.igh.cnrs.fr/home.eng.html) and used with default parameters. Fragments or portions of the pre-diagnostic lung cancer indicator proteins or polypeptides derived therefrom may be employed in certain herein disclosed embodiments, which fragments or portions retain at least one antigenic epitope that is capable of being specifically recognized by an antibody from a biological fluid of a pre-diagnostic subject having lung cancer, and which may have at least 50, 60, 70, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99 percent identity to the herein disclosed pre-diagnostic lung cancer indicator protein sequences.

[0058] The methods to be practiced according to certain herein disclosed embodiments may involve any convenient format for interrogating a biological fluid, as provided herein, for the presence of one or more antibodies that are capable of specifically binding to a pre-diagnostic lung cancer indicator protein as provided herein. Alternatively, certain embodiments may involve any convenient format for interrogating an antibody-containing fraction that has been isolated from a biological fluid, as provided herein, for the presence of one or

more antibodies that are capable of specifically binding to a pre-diagnostic lung cancer indicator protein as provided herein.

[0059] As also noted above, any of a number of convenient formats may be employed for contacting one or more antibodies from a pre-diagnostic biological fluid with an isolated pre-diagnostic lung cancer indicator protein to detect specific antibody binding to the indicator protein, including but not limited to enzyme linked immunosorbent assay (ELISA), surface plasmon resonance (SPR) spectroscopy, western blot immunoassay, radioimmunoassay (RIA), immunofluorimetry, immunoprecipitation, equilibrium dialysis, immunodiffusion and other techniques. See, e.g., Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988; Weir, D. M., *Handbook of Experimental Immunology,* 1986, Blackwell Scientific, Boston. See, e.g., Coligan et al. (Eds.), *Current Protocols in Immunology* (2007 John Wiley & Sons, NY); Harlow and Lane, *Antibodies: A Laboratory Manual* (1988 Cold Spring Harbor Press, Cold Spring Harbor, N.Y.); Harlow and Lane, *Using Antibodies* (1999 Cold Spring Harbor Press, Cold Spring Harbor, N.Y.).

[0060] Briefly, for example by way of illustration and not limitation, one or more isolated pre-diagnostic lung cancer indicator proteins (or polypeptides comprising at least one antigenic epitope of a pre-diagnostic lung cancer indicator protein) may be immobilized on a solid-phase substrate, which may be non-covalent immobilization (e.g., by passive adsorption to the solid phase) or covalent immobilization (e.g., by chemically crosslinking the protein or polypeptide to the solid phase using any of a number of well known functional groups). The biological fluid from a pre-diagnostic subject is then contacted with the solid phase, under conditions and for a time sufficient for specific binding of at least one antibody from the fluid to be detected, which may typically include an incubation period to permit such binding followed by a washing step to remove any non-specifically binding components of the biological fluid. The specifically bound antibody may then be detected by any of a number of means for detecting, for example, by mass spectrometric shift if SPR is used, or as another example, by further contacting the solid phase with a detectably labeled secondary (or "second stage") antibody such as an anti-immunoglobulin (e.g., a rabbit anti-human immunoglobulin) followed, after a suitable incubation period and conditions with which the skilled artisan will be familiar, by another washing step to remove unbound secondary antibody, after which the detectable label may be detected and compared to one or more appropriate controls for purposes of determining whether an antibody that specifically binds to a pre-diagnostic lung cancer indicator protein is present. It will be appreciated that other assay techniques, formats and configurations may also be employed. As described herein, detection of an antibody, in a biological fluid from a pre-diagnostic subject, that specifically binds to a pre-diagnostic lung cancer indicator protein as provided herein, indicates that the pre-diagnostic subject has lung cancer.

[0061] Accordingly and in certain embodiments, detection of antibody binding to a pre-diagnostic lung cancer indicator protein (or to a polypeptide that comprises one or more antigenic epitopes therefrom) may comprise detection using a specific, detectably labeled secondary reagent (such as an anti-immunoglobulin antibody, or Staphylococcal protein A or protein G or an immunologically active fragment thereof or a mimetic thereof that specifically binds a human immunoglobulin constant region) that contains a detectable reporter moiety or label such as an enzyme, dye, radionuclide, luminescent group, fluorescent group or biotin, or the like. The amount of the detectably labeled secondary reagent that remains bound to the pre-diagnostic lung cancer indicator protein (or to the polypeptide that comprises one or more antigenic epitopes therefrom) is then determined using a method appropriate for the specific detectable reporter moiety or label. For radioactive groups, scintillation counting or autoradiographic methods are generally appropriate. Antibody-enzyme conjugates may be prepared using a variety of coupling techniques (for review see, e.g., Scouten, W. H., *Methods in Enzymology* 135:30-65, 1987). Spectroscopic methods may be used to detect dyes (including, for example, colorimetric products of enzyme reactions), luminescent groups and fluorescent groups. Biotin may be detected using avidin or streptavidin, coupled to a different reporter group (commonly a radioactive or fluorescent group or an enzyme). Enzyme reporter groups may generally be detected by the addition of substrate (generally for a specific period of time), followed by spectroscopic, spectrophotometric or other analysis of the reaction products.

[0062] In addition to providing embodiments that find uses as methods for diagnosing lung cancer and/or screening methods for lung cancer, e.g., convenient testing of biological fluids from a plurality of subjects such as a population of subjects that may be at increased risk for developing lung cancer, for instance, by virtue of having one or more indicators of increased risk for developing lung cancer as described above, other embodiments contemplated herein relate to a method of monitoring lung cancer autoimmune reactivity in a lung cancer patient. According to these and related embodiments, a subject that has already been diagnosed with lung cancer may exhibit qualitative and/or quantitative changes in the antibodies that are present in biological fluid at different points in time.

[0063] For instance, such changes from a first timepoint to a second timepoint may reflect progression of the disease. As another example, antibody reactivity against one or more pre-diagnostic lung cancer indicator proteins (or polypeptides comprising epitopes derived therefrom) may be tested at one or a plurality of timepoints before, during or after administration to the lung cancer patient of a therapeutic agent. Detection of autoantibody binding over time may therefore provide a means for monitoring the cancer status of the patient, such as indicating whether the patient is responding to therapy, and/or whether the patient may be in remission or may be relapsing. Determination of quantitative and qualitative changes over time in lung cancer indicator protein-reactive antibodies as disclosed herein is within the capability of the art, for instance, by using established immunochemical methodologies for assaying the amount of autoantibody in a patient's biological fluid, or the affinities of autoantibodies in the biological fluid, or a change in the isotypes of the autoantibodies, or a change in the antigen- and/or epitope specificities of the antibodies (e.g., autoantibodies) that are detected in the cancer patient's biological fluid.

[0064] Accordingly, there is provided in certain representative embodiments a method of monitoring lung cancer autoimmune reactivity in a lung cancer patient, comprising contacting, after each of two or more timepoints, (i) one or more antibodies from a biological fluid that is taken from a subject at each of said timepoints, and (ii) a test antigen that may be one or more isolated pre-diagnostic lung cancer indi-

cator proteins, or that may instead be one or more isolated proteins or polypeptides that comprise one or more antigenic epitopes of one or more pre-diagnostic lung cancer indicator proteins, under conditions and for a time sufficient for detecting specific binding of at least one antibody from the biological fluid to one or more of the pre-diagnostic lung cancer indicator proteins or antigenic epitopes thereof; and comparing the specific binding that is detectable by antibodies from the biological fluid taken at each of the two or more timepoints, and thereby monitoring lung cancer autoimmune reactivity in the patient. Two, three, four, five, six, seven, eight, nine, ten or more timepoints may be identified in order to practice these and related embodiments, as may be indicated for the appropriate monitoring of the lung cancer patient's autoimmune reactivity. For instance, a first timepoint may occur before administration of a therapeutic agent to the patient, and the second and subsequent timepoints may occur after administration of the therapeutic agent to the patient.

[0065] It is also contemplated according to certain other embodiments based on the disclosure made herein that the present compositions and methods may permit subtyping of a tumor in a subject (including but not limited to a pre-diagnostic subject), and may also permit subtyping of a plurality of patients, on the basis of the qualitative and/or quantitative autoantibody reactivity that can be detected against one or more of the pre-diagnostic lung cancer indicator proteins as provided herein. Thus, for example, accumulation of data obtained according to the herein disclosed methods may lead to more specific diagnostic information beyond the detection of the presence of lung cancer, such as the degree of progression or other qualitative and/or quantitative criteria by which the cancer can be characterized.

[0066] Additionally or alternatively, it is contemplated that detection of one or more autoantibodies specific for one or more of the pre-diagnostic lung cancer indicator proteins as provided herein may usefully define the histological subtype of one or more given lung cancers. For example, a lung cancer detected according to the present disclosure may be a primary tumor originating in the lung, or may instead be a lung cancer that results from metastasis to the lung of cancer cells originating in a distinct anatomical site, such as breast carcinoma, colorectal carcinoma, head and neck squamous cell carcinoma, gastrointestinal carcinoma, testicular cancer or any number of other potential primary tumors having distinct sites of origin. Information obtained through the practice of these and related embodiments may provide the clinician with important data for the formulation of an effective therapeutic strategy, given that tumors of different origins may respond differentially (or not at all) to different anti-cancer treatment regimens.

[0067] Application of the herein described proteomics approach for the identification of additional pre-diagnostic lung cancer indicator proteins, is also contemplated.

[0068] The following Examples are presented by way of illustration and not limitation.

EXAMPLES

[0069] In the present Examples, pre-diagnostic sera were utilized to determine if a set of antigens consisting of annexin I, PGP9.5 and 14-3-3 theta previously found to be associated with autoantibodies at the time of diagnosis discriminate between cases and control prior to onset of symptoms, and for discovery of additional antigens. Evidence is presented for the occurrence of autoantibodies against a novel antigen, LAMR1, in lung cancer, along with evidence for occurrence of autoantibodies against annexin I, 14-3-3 theta and LAMR1 in pre-diagnostic sera.

[0070] A high throughput platform for quantitative analysis of serum autoantibodies was applied to lung cancer for discovery of novel antigens, and for validation in pre-diagnostic sera of autoantibodies to antigens previously defined based on analysis of sera collected at the time of diagnosis.

[0071] Proteins from human lung adenocarcinoma cell line A549 lysates were subjected to extensive fractionation. The resulting 1824 fractions were spotted in duplicate on nitrocellulose coated slides. The microarrays produced were utilized in a blinded validation study to determine whether annexin I, PGP9.5, and 14-3-3 theta antigens previously found to be targets of autoantibodies in newly diagnosed subjects with lung cancer were associated with autoantibodies in sera collected at the pre-symptomatic stage and to determine whether additional antigens may be identified in pre-diagnostic sera. Individual sera collected from 85 subjects within a year prior to a diagnosis of lung cancer and 85 matched controls from the CARET cohort were hybridized to individual microarrays.

[0072] Evidence is presented herein for the occurrence in pre-diagnostic lung cancer sera of autoantibodies to annexin I, 14-3-3 theta, and a novel lung cancer antigen, LAMR1. Detectable appearance of these autoantibodies preceded onset of lung cancer symptoms and also preceded diagnosis of lung cancer by any other means. Diagnosis of lung cancer before onset of symptoms is thus described herein, by screening for autoantibodies specific for pre-diagnostic lung cancer indicator proteins as the defined antigens.

Example 1

Materials and Methods

Materials

[0073] Nitrocellulose-coated FAST slides were purchased from Whatman (Sanford, Me.). Alexa 647-labeled anti-human IgG and recombinant protein arrays were purchased from Invitrogen (Carlsbad, Calif.).

Serum Samples

[0074] Serum samples and controls were obtained following informed consent. Sera from newly diagnosed lung cancer patients and matched controls were collected through the Community Clinical Oncology Program at the University of Michigan. Pre-diagnostic blood samples from lung cancer patients and matched controls were randomly chosen in pairs from the CARET serum bank (Goodman et al., *J Natl Cancer Inst* 96:1743-50, 2004; Omenn et al., *N Engl J Med* 334:1150-5, 1996). The distribution of histology and time from blood draw to diagnosis for the 85 pre-diagnostic lung cancer cases are shown in Tables 1 and 2.

[0075] Natural Protein Microarray Production

[0076] 50 mg proteins from the human lung adenocarcinoma cell line A549 lysates were first separated by anion exchange HPLC, followed by reverse-phase chromatography as described previously (Wang and Hanash, J Chromatogr B Analyt Technol Biomed Life Sci 787:11-8, 2003). A total of 1,824 fractions were collected from the 2-dimensional separation. FR_XX_YY denotes the YYth fraction from the RP-HPLC of the XXth fraction from the AEX. Fractions were

lyophilized and re-suspended in 25 ul printing buffer (250 mM Tris-HCl, pH 6.8, 0.5% SDS, 25% Glycerol, 0.05% TritonX-100, 62.5 mM DTT). All 1,824 fractions, together with printing buffer as negative control and purified human IgG as positive controls, were printed in duplicate onto nitrocellulose-coated slides using a contact printer. These slides were designated as "full A549 natural protein microarrays." An A549 natural protein microarray containing selected fractions (targeted array) was produced in a similar way, whereby only selected fractions of interest (Fr_00_84, Fr_09_38, Fr 15_39 and Fr 15_46) were printed in duplicate on 16-pad FAST slides.

[0077] Detection of Autoantibodies in Serum Specimens

[0078] Serum samples were hybridized with protein microarrays using an indirect immunofluorescence protocol and local background subtracted median spot intensities for downstream statistical analysis were generated as described previously (Qiu et al., *J Proteome Res* 3:261-7, 2004).

[0079] Mass-Spectrometry Analysis

[0080] Samples were trypsin-digested and subjected to mass spectrometry analysis on an LTQ-Orbitrap as described previously (Faca et al., *J Proteome Res* 6:3558-65, 2007).

[0081] Statistical Analysis

[0082] Intensity data were linear normalized to make the 25th and 75th percentiles of the distribution of the intensities for each sample agree exactly with the average of the 25th and 75th percentiles of all samples by linear transformations. Linear normalized data were then standardized fraction-by-fraction. Each sample/fraction intensity was subtracted off the mean of the same fraction for all samples in the same printing batch and divided by the standard deviation of the fraction for all samples in the corresponding printing batch. A two-sample t-test was applied to each single fraction to compare the difference in mean intensity between cancers and controls.

[0083] The discriminatory capacity of selected fractions was also evaluated by the Receiver Operating Characteristic (ROC) curve. The area under the curve (AUC) was calculated which corresponds to the Mann-Whitney statistic. Parallel analyses using a generalized version of the "real boosting" algorithm with 10-fold cross-validation was also performed on the 1824 fractions to select the best combination of fraction(s) that can discriminate between cases and controls (Friedman et al., *Annals of Statistics* 28:337-407, 2000; Yasui et al., *Biostatistics* 4:449-63, 2003). The results were treated as part of a separate biomarker discovery process. Even if a promising fraction was identified as a previously-identified antigen associated with lung cancer, it was treated as an independent confirmation of the previous findings rather than as part of the validation study because that antigen had not been established for validation at the design stage of the study.

[0084] For combined analysis, the markers were integrated by a summation of the dichotomized markers, whereby each marker was dichotomized by its optimal cutoff point which corresponds to the minimum classification error rate. The 95% confidence interval (CI) band of the ROC curve was estimated from 500 bootstrap procedures. The combination rule was treated as a "discovered biomarker" and was regarded as amenable to further validation; however, it provided information on the complementarities of informative antigens.

Example 2

Results

[0085] Validation Study of Autoantibodies to Annexin I, PGP9.5, and 14-3-3 Theta in Pre-Diagnostic Sera

[0086] Annexin I, PGP9.5 and 14-3-3 theta were previously identified as inducing an autoantibody response in lung cancer, based on 2D Western analysis of sera from newly diagnosed subjects with lung cancer (Brichory et al., *Cancer Res* 61:7908-12, 2001; Brichory et al., *Proc Natl Acad Sci USA* 98:9824-9, 2001; Pereira-Faca et al., *Cancer Res* 67:12000-6, 2007). Natural protein microarrays were developed to screen tumor-derived proteins for antigens that induce autoantibodies, based on extensive protein fractionation followed by spotting of aliquots from individual fractions (Madoz-Gurpide et al., *Proteomics* 1:1279-87, 2001). Natural protein containing microarrays were utilized to investigate the occurrence of autoantibodies to annexin I, PGP9.5 and 14-3-3 theta reactivity in pre-diagnostic sera. Serum specimens from the CARET cohort, which consisted of subjects at increased risk for lung cancer followed longitudinally (Omenn et al., *N Engl J Med* 334:1150-5, 1996), were relied upon to investigate the occurrence of autoantibodies to lung cancer antigens within a year prior to diagnosis. Each case and control pair was matched for age at enrollment (5-year intervals), sex, intervention arm (active vitamins or placebo), exposure population (asbestos or heavy smoker), baseline smoking status (active or former), year of enrollment, and year of blood draw. Eighty-five pre-diagnostic lung cancer specimens and an equal number of matched controls were utilized for this study (Tables 1, 2).

[0087] A549 natural protein microarrays were prepared from 2-D separations of a batch of A549 cell lysates. Quantitative reproducibility of microarrays was assessed by replicate analysis. Reproducibility across microarrays was assessed by hybridization of the same sample on different microarrays. Reproducibility within slides was assessed by replicate spots on the same microarray. Correlation of replicate spot intensity measures in the same microarray was 0.99. (FIG. **1**A) Correlation of spot intensity measures between different microarrays hybridized with the same sera was 0.96 (FIG. **1**B). The median IgG reactivity for cancer samples, across the entire spotted array, was similar to that for normal controls (data not shown). However the number of fractions with significant p-values <0.05 with measures for cancer greater than for control was higher than the number of fractions for which control was greater than cancer. Of 1824 arrayed fractions, 68 fractions gave a p-value <0.05 with mean intensity for cancer greater than control, while there were only 16 fractions with a p-value <0.05 with mean intensity for controls greater than for cancer.

[0088] Annexin I was localized on the A549 natural protein microarrays in Fr_00_84. PGP9.5 was localized in Fr_09_38 and 14-3-3 theta was localized in Fr_15_46 based on reactivity with corresponding monoclonal antibodies. Following blinded analysis of all sera, the pre-diagnostic cases had significantly higher mean annexin I autoantibody levels than those of controls (t-test p-value=0.001) (FIG. **2**A). The p-value for 14-3-3 theta was also significant at 0.01 (FIG. **2**B). On the other hand, the average PGP9.5 autoantibody reactivity of pre-diagnostic cases was not significantly different from that of matched high-risk controls from CARET (p=0.2) (FIG. **2**C). These results were in concordance with prior results based on 2-D Western analysis of 18 of the 85 CARET pre-diagnostic sera used in the present study (Pereira-Faca et al, *Cancer Res* 67:12000-6, 2007).

[0089] Differential Reactivity to LAMR1 in Pre-Diagnostic Lung Cancer Sera

[0090] A boosting logistic regression method with leave-ten-percent-out for cross-validation was initially utilized to determine if additional arrayed proteins exhibited differential reactivity with pre-diagnostic cases relative to control sera. A spotted fraction (Fr_15_39), which was among the most informative in each of ten iterations of the model building procedure, yielded LAMR1 protein identification with high confidence by mass spectrometry based on mass spectral matching to 21 unique peptides in the LAMR1 sequence (65% coverage). The mean level of autoantibodies against LAMR1 in pre-diagnostic lung cancer sera was significantly higher than that in matched controls with a p-value of 0.017 (FIG. **2**D). The combined AUC for all three antigens, annexin I, 14-3-3 theta and LAMR1, in CARET pre-diagnostic sera vs. matched controls was 0.73 (FIG. **3**). The sensitivity and specificity at the optimal cutoff point was 51% and 82%, respectively.

[0091] CARET lung cancer cases represented three groups, adenocarcinoma (AC), squamous cell carcinoma (SCC), and other non-small cell lung cancer (NSCLC) (Table 1). An ANOVA test was performed to determine correlation between autoantibody levels against annexin I, PGP9.5, 14-3-3 theta, and LAMR1 and lung cancer subtype. Annexin I, PGP9.5 and LAMR1 did not show significant reactivity difference among the three subtypes. A significant p-value of 0.007 was obtained for 14-3-3 theta, with the AC and the SCC groups exhibiting lesser reactivity than other NSCLC. CARET cancer sera analyzed were collected within one year prior to diagnosis (Table 2). A possible relationship was investigated between autoantibody levels and the time from blood draw to diagnosis, by stratifying the samples into two groups, one group with cases collected between 0-6 months (inclusive) prior to diagnosis and the other group between 7-12 months (inclusive) prior to diagnosis. The mean reactivity for cases in the 0-6 group was higher than that for cases in the 7-12 group with t-test p-values of 0.05, 0.06 and 0.07 for PGP9.5, 14-3-3 theta and LAMR1 respectively. There was equivalent reactivity between the two groups for annexin 1 (Table 3). When comparing cases in each group against matched controls, the differences were also more significant in the 0-6 group than in the 7-12 group for PGP9.5, 14-3-3 theta and LAMR1 (Table 4).

[0092] Assessment of Autoantibody Reactivity to LAMR1 in Sera from Newly-Diagnosed Lung Cancer Subjects

[0093] The occurrence of autoantibodies to LAMR1 in sera from newly diagnosed subjects with lung cancer was determined using spotted microarrays containing purified recombinant LAMR1 that were reacted with sera from 45 newly diagnosed subjects with lung cancer and from an equal number of healthy controls that were matched for age and gender and time of blood collection. Increased LAMR1 reactivity among lung cancer sera relative to controls was observed based on two-sample t-test (p-value=0.024). Most of the lung cancer cases (32/45) had adenocarcinoma. A significant p-value of 0.03 was also obtained for the 32 subjects with adenocarcinoma relative to matched controls.

Discussion

[0094] The results indicated that autoantibodies against LAMR1, annexin I and 14-3-3 theta were significantly elevated in pre-clinical lung cancer patient sera compared with matched high risk controls that did not develop lung cancer during the period of follow-up. A combination of LAMR1, annexin 1 and 14-3-3 theta autoantibodies yielded an AUC of 0.73 in pre-clinical lung cancer sera. While autoantibodies to various cancer antigens have been reported in newly diagnosed lung cancer patient sera (Stockert et al., *J Exp Med* 187:1349-54, 1998; Gure et al., *Cancer Res* 58:1034-41, 1998; Yamamoto et al., *Int J Cancer* 69:283-9, 1996; Diesinger et al., *Int J Cancer* 102:372-8, 2002; Gure et al., *Proc Natl Acad Sci USA* 97:4198-203, 2000; Ali Eldib et al., *Int J Cancer* 108:558-63, 2004; Yang et al., *J Proteome Res* 6:751-8, 2007; Brichory et al., *Cancer Res* 61:7908-12, 2001; Brichory et al., *Proc Natl Acad Sci USA* 98:9824-9, 2001; He et al., *Cancer Sci* 98:1234-40, 2007; Tureci et al., *Cancer Lett* 236:64-71, 2006; Chang et al: *FEBS Lett* 579: 2873-7, 2005; Yagihashi et al: *Lung Cancer* 48:217-21, 2005; Matsumoto et al: *Int J Oncol* 19:1035-9, 2001; Fernandez-Madrid et al: *Clin Cancer Res* 5:1393-400, 1999; Lubin et al: *Nat Med* 1:701-2, 1995), a distinguishing feature of the present disclosure was the testing for occurrence of autoantibodies in pre-diagnostic sera and demonstration of significant autoantibody reactivity against LAMR1, annexin 1 and 14-3-3 theta. The sample size, 85 cases and an equal number of controls, and the characteristics of controls, heavy smokers or subjects who have been exposed to asbestos, were important features of the present study.

[0095] Autoantibodies against annexin I, PGP9.5 and 14-3-3 theta have previously been reported in newly diagnosed lung cancer patients (Brichory et al., *Cancer Res* 61:7908-12, 2001; Brichory et al., *Proc Natl Acad Sci USA* 98:9824-9, 2001). The data presented herein validated the occurrence of autoantibodies to annexin 1 and 14-3-3 theta, by demonstrating their presence also in pre-diagnostic lung cancer sera using natural protein arrays, yielding significant p-values of 0.001 and 0.01 respectively for differences with matched controls.

[0096] The present application discloses for the first time the occurrence of autoantibodies to LAMR1 in lung cancer.

[0097] The full length LAMR1 gene encodes a 33 kD precursor protein with 295 amino acids (Yow et al., *Proc Natl Acad Sci USA* 85:6394-8, 1988). Its precursor and post-translationally modified forms serve diverse biological functions in vivo. The 33 kD precursor protein dimerizes after acylation to form the mature 67LR (Buto et al., *J Cell Biochem* 69:244-51, 1998), which was initially purified using affinity chromatography on Sepharose® columns conjugated with laminin and designated as the 67 kD laminin receptor (67LR) (Rao et al., *Biochemistry* 28:7476-86, 1989; Lesot et al., *EMBO J* 2:861-865, 1983; Malinoff et al., *J Cell Biol* 96:1475-9, 1983).

[0098] The 33 kD precursor is an evolutionarily conserved ribosomal protein associated with the 40S subunit of the translational machinery (Auth et al., *Proc Natl Acad Sci USA* 89:4368-72, 1992; Ardini et al., *Mol Biol Evol* 15:1017-25, 1998). A 44 kD protein originally identified as an oncofetal antigen by Coggin et al. was subsequently found to be encoded by the same gene as 67LR (Coggin et al., *Anticancer Res* 19:5535-42, 1999; Coggin et al., *Immunol Today* 19:405-8, 1998). The precursor also serves as the receptor for the prion protein in eukaryotic cells (Rieger et al., *Nat Med* 3:1383-8, 1997).

[0099] 67LR plays a role in cancer invasion and metastasis, related to its high affinity to laminin, an important component of basement membrane (Wewer et al., *Cancer Res* 47:5691-8, 1987). Over-expression of 67LR has been observed in mela-

nomas, lymphomas and epithelial tumors (Menard et al., *Breast Cancer Res Treat* 52:137-45, 1998; Menard et al., *J Cell Biochem* 67:155-65, 1997; Cioce et al., *J Natl Cancer Inst* 83:29-36, 1991). Expression of 67LR correlates with poor prognosis in non-small cell lung cancer (Fontanini et al., *Clin Cancer Res* 3:227-31, 1997). There is evidence that the monomeric membrane-associated 44 kD OFA/iLRP (oncofetal antigen/immature laminin protein), but significantly, not 67LR, is immunogenic (Coggin et al., *Anticancer Res* 19:5535-42, 1999), consistent with the findings disclosed herein of autoantibodies in lung cancer. OFAs are expressed in fetal cells and a variety of cancers but not present in normal neonatal or adult tissues (Coggin et al., *Immunol Today* 19:405-8, 1998).

[0100] Immunization of adult hamsters with irradiated fetal hamster or mouse cells provides strong immunity to SV40-induced tumorigenesis (Ambrose et al., *Nature* 233:194-5, 1971; Coggin et al., *J Immunol* 107:526-33, 1971; Ambrose et al., *Nature* 233:321-4, 1971). OFA/iLRP was later identified as the protective antigen on the membrane of rodent and human fetal and tumor cells (Coggin et al., *Anticancer Res* 19:5535-42, 1999; Payne et al., *J Natl Cancer Inst* 75:527-44, 1985; Coggin et al., *Am J Pathol* 130:136-46, 1988; Gussack et al., *Cancer* 62:283-90, 1988). OFA/iLRP studies have largely focused on cellular immunity and its potential utility in T-cell based immunotherapy (Rohrer et al., *J Immunol* 152:754-64, 1994; Siegel et al., *J Immunol* 176:6935-44, 2006; Rohrer et al., *J Immunol* 154:2266-80, 1995; Holtl et al., *Clin Cancer Res* **8**:**3369**-76, 2002; Rohrer et al., *J Immunol* 155:5719-27, 1995; Rohrer et al., *J Immunol* 176:2844-56, 2006; Rohrer et al., *J Immunol* 162:6880-92, 1999).

[0101] Although a humoral immune response could be induced in mice immunized with recombinant OFA/iLRP (Rohrer et al., *Mod Asp Immunobiol* 1:191-5, 2001), the occurrence of autoantibodies against OFA/iLRP in human cancer patients has not previously been reported. Although OFA/iLRP is a glycosylated protein (Coggin et al., *Arch Otolaryngol Head Neck Surg* 119:1257-66, 1993), the data disclosed herein suggested that autoantibodies were not restricted to a glycan containing epitope in OFA/iLRP given the reactivity observed with recombinant LAMR1 in lung cancer patient sera. This observation was consistent with previous findings that bacterially expressed recombinant OFA/iLRP was competent in inducing CTL-mediated target cell lysis (Rohrer et al., *J Immunol* 176:2844-56, 2006). OFA/iLRP expression was found to precede clear histological evidence of malignant T cells or clinical lymphoma in irradiated mice that went on to develop T-cell lymphomas (Rohrer et al., *J Natl Cancer Inst* 84:602-9, 1992), consistent with an early immune response during tumorigenesis and the present demonstration of autoantibodies in pre-clinical (e.g., pre-diagnostic) sera.

[0102] Although in the present study, no significant difference in PGP9.5 reactivity was observed between pre-diagnostic cases as a group and controls (in contrast to prior findings based on analysis of sera collected at the time of diagnosis of lung cancer), this observation may be related to differences in patient and tumor characteristics or to the temporal pattern of PGP9.5 expression and/or immune response to PGP9.5 in lung cancer. In support of the latter is the finding of increased reactivity among subjects within six months from diagnosis, compared to subjects whose blood was collected 6-12 months prior to diagnosis. Nevertheless, autoantibodies to PGP9.5 may have diagnostic utility in symptomatic subjects in conjunction with an imaging modality.

[0103] Disclosed herein for the first time are data related to temporal changes of a humoral immune response to a set of tumor antigens in lung cancer for subjects whose blood was collected over a period ranging from the time of diagnosis to within a year prior to diagnosis as part of the CARET high risk cohort. PGP9.5, 14-3-3 theta and LAMR1 showed increases in reactivity in pre-diagnostic sera with higher reactivity at a time closer to diagnosis (0-6 months) relative to a time farther from diagnosis (7-12 months). Reactivity to annexin I did not show a relationship to time from diagnosis within the one-year time frame. This observation may indicate that an immune response to annexin I occurred earlier during the course of lung cancer development compared to PGP9.5, 14-3-3 theta and LAMR1. It has been demonstrated previously that the sugar moiety on annexin I was important to its antigenicity (Brichory et al., *Proc Natl Acad Sci USA* 98:9824-9, 2001). This posttranslational modification may have occurred early during tumor development. Alternatively a sugar-containing epitope may have been more immunogenic than a peptide backbone for yielding an early detectable immune response. Tumor antigens with different temporal reactive patterns may have different clinical utility for screening and diagnosis. These findings point to the value of pre-diagnostic sera in assessing the significance of autoreactivity to particular antigens.

[0104] While significant reactivity with pre-diagnostic sera was observed in this study for a small panel of antigenic proteins, a screening modality for lung cancer that includes testing for autoantibodies is also contemplated using additional antigenic targets to augment the sensitivity and specificity achieved in this study. An initial application of such a panel may be in conjunction with an imaging screening modality for subjects at an increased risk for lung cancer. Studies aimed at identifying and validating novel antigens are currently being undertaken through the National Cancer Institute Early Detection Research Network (http://edrn.nci.nih.gov) and through other efforts.

TABLE 1

DISTRIBUTION OF LUNG CANCER HISTOLOGICAL TYPES AMONG PRE-DIAGNOSTIC LUNG CANCER SERA.

	Number of subjects
Adenocarcinoma	32 (38%)
Squamous cell carcinoma	29 (34%)
Other non-small cell lung cancer	24 (28%)
Total	85

TABLE 2

TIME FROM BLOOD DRAW TO DIAGNOSIS FOR PRE-DIAGNOSTIC LUNG CANCER SERA.

Months	Number of subjects
0-3	26 (31%)
4-6	18 (21%)
7-9	19 (22%)
10-12	22 (26%)
Total	85

TABLE 3

REACTIVITY DIFFERENCES BETWEEN CASES IN THE 0-6 MONTH GROUP AND THE 7-12 MONTH GROUP.

Antigens	Blood_draw_to_diagnosis (month)	n	Mean reactivity	p*
Annexin I	0-6	44	0.20	0.72
	7-12	41	0.29	
PGP9.5	0-6	44	0.28	0.05
	7-12	41	−0.11	
LAMR1	0-6	44	0.40	0.07
	7-12	41	−0.05	
14-3-3 theta	0-6	44	0.40	0.06
	7-12	41	−0.03	

*Two-sample t test

TABLE 4

REACTIVITY DIFFERENCES BETWEEN CASES AND CONTROLS IN DIFFERENT GROUPS STRATIFIED BY BLOOD DRAW TIME TO DIAGNOSIS.

Antigens	Blood_draw_to_diagnosis (month)	Mean (SD) cancer	Mean (SD) normal	p*
Annexin I	0-6	0.20 (1.16)	−0.197 (0.80)	0.067
	7-12	0.29 (1.25)	−0.293 (0.49)	0.006
	all	0.24 (1.20)	−0.244 (0.67)	0.001
PGP9.5	0-6	0.28 (1.11)	−0.111 (1.06)	0.093
	7-12	−0.11 (0.64)	−0.078 (1.07)	0.886
	all	0.10 (0.93)	−0.095 (1.06)	0.214
LAMR1	0-6	0.40 (1.41)	−0.158 (0.49)	0.017
	7-12	−0.05 (0.65)	−0.207 (1.06)	0.414
	all	0.18 (1.13)	−0.182 (0.81)	0.017
14-3-3 theta	0-6	0.40 (1.13)	−0.254 (0.60)	0.001
	7-12	−0.028 (0.95)	−0.132 (1.13)	0.654
	all	0.20 (1.06)	−0.195 (0.89)	0.010

*Two-sample t test

Example 3

Exemplary Pre-Diagnostic Lung Cancer Indicator Proteins

[0105]

ANNEXIN 1
>ipi|IPI00218918|IPI00218918.5 ANNEXIN A1 (SEQ ID NO: 1):
MAMVSEFLKQAWFIENEEQEYVQTVKSSKGGPGSAVSPYPTFN

PSSDVAALHKAIMVKGVDEATIIDILTKRNNAQRQQIKAAYLQETGKPLDETLKK

ALTGHLEEVVLALLKTPAQFDADELRAAMKGLGTDEDTLIEILASRTNKEIRDIN

RVYREELKRDLAKDITSDTSGDFRNALLSLAKGDRSEDFGVNEDLADSDARAL

YEAGERRKGTDVNVFNTILTTRSYPQLRRVFQKYTKYSKHDMNKVLDLELKGD

IEKCLTAIVKCATSKPAFFAEKLHQAMKGVGTRHKALIRIMVSRSEIDMNDIKAF

YQKMYGISLCQAILDETKGDYEKILVALCGGN

>ipi|IPI00549413|IPI00549413.2 ANNEXIN A1 (SEQ ID NO: 2):
MNLILRYTFSKMAMVSEFLKQAWFIENEEQEYVQTVKSSKGGP

GSAVSPYPTFNPSSDVAALHKAIMVKGVDEATIIDILTKRNNAQRQQIKAAYLQE

TGKPLDETLKKALTGHLEEVVLALLKTPAQFDADELRAAMKGLGTDEDTLIEILA

SRTNKEIRDINRVYREELKRDLAKDITSDTSGDFRNALLSLAKGDRSEDFG

>ipi|IPI00643231|IPI00643231.1 ANNEXIN A1 (SEQ ID NO: 3):
MAMVSEFLKQAWFIENEEQEYVQTVKSSKGGPGSAVSPYPTFN

PSSDVAALHKAIMVKGVDEATIIDILTKRNNAQRQQIKAAYLQETGKPLDETLKK

ALTGHLEEVVLALLKTP

14-3-3 THETA
>ipi|IPI00018146|IPI00018146.1 14-3-3 PROTEIN THETA (SEQ ID NO: 4):
MEKTELIQKAKLAEQAERYDDMATCMKAVTEQGAELSNEERNL

LSVAYKNVVGGRRSAWRVISSIEQKTDTSDKKLQLIKDYREKVESELRSICTTV

LELLDKYLIANATNPESKVFYLKMKGDYFRYLAEVACGDDRKQTIDNSQGAYQ

EAFDISKKEMQPTHPIRLGLALNFSVFYYEILNNPELACTLAKTAFDEAIAELDTL

NEDSYKDSTLIMQLLRDNLTLWTSDSAGEECDAAEGAEN

-continued

>ipi|IPI00796727|IPI00796727.1 PUTATIVE UNCHARACTERIZED PROTEIN (SEQ ID NO: 5):
YWHAQMEKTELIQKAKLAEQAERYDDMATCMKAVTEQGAELSN

EERNLLSVAYKNVVGGRRSAWRVISSIEQKTDTSDKKLQLIKDYREKVESELRS

ICTTVLELLDKYLIANATNPESKVFYLKMKGDYFRYLAEVACGDDRKQTIDNSQ

GAY

LAMININ RECEPTOR 1 (LAMR1)
>ipi|IPI00411639|IPI00411639.1 LAMININ RECEPTOR-LIKE PROTEIN LAMRL5 (SEQ ID NO: 6):
MSGALDVLQMKEEDVLKFLAAGTHLGGTNLDFQMEQYIYKRKS

DGIYIINLKRTWEKLLLTARAIVAIENPADVSVISSRNTGQRAVLKFAAATGATPI

AGRFTPGTFTNQIQAAFREPRLLVVSDPRADHQPLTEASYVNLPTIALCNTDSP

LHYVDIAIPCNNKGTHSVGLMWWMLAREVLRMRGTISREHPWEVMPDLYFYR

DPEEIEKEEQAAAEKAMTREELQGEWTAPAPEFTATQPEVADWSEGVQVPSV

PIQQFPTEDWSTQRATEDWSAAPTAQATEWVGATTDWS

>ipi|IPI00413108|IPI00413108.4 33 KDA PROTEIN (SEQ ID NO: 7):
MSGALDVLQMKEEDVLKFLAAGTHLGGTNLDFQMEQYIYKRKS

DGIYIINLKRTWEKLLLAARAIVAIENPADVSVISSRNTGQVCGTVRAVLKFAAAT

GATPIAGRFTPGTFTNQIQAAFREPRLLWTDPRADHQPLTEASYVNLPTIALC

NTDSPLRYVDIAIPCNNKGAHSVGLMWWMLAREVLRMRGTISREHPWEVMP

DLYFYRDPEEIEKEEQAAAEKAVTKEEFQGEWTAPAPEFTATQPEVADWSEG

VQVPSVPIQQFPTEDWSAQPATEDWSAAPTAQATEWVGATTDWS

>ipi|IPI00553164|IPI00553164.4 40S RIBOSOMAL PROTEIN SA (SEQ ID NO: 8):
MSGALDVLQMKEEDVLKFLAAGTHLGGTNLDFQMEQYIYKRKS

DGIYIINLKRTWEKLLLAARAIVAIENPADVSVISSRNTGQRAVLKFAAATGATPI

AGRFTPGTFTNQIQAAFREPRLLVVTDPRADHQPLTEASYVNLPTIALCNTDSP

LRYVDIAIPCNNKGAHSVGLMWWMLAREVLRMRGTISREHPWEVMPDLYFYR

DPEEIEKEEQAAAEKAVTKEEFQGEWTAPAPEFTATQPEVADWSEGVQVPSV

PIQQFPTEDWSAQPATEDWSAAPTAQATEWVGATTDWS

>ipi|IPI00790580|IPI00790580.1 16 KDA PROTEIN (SEQ ID NO: 9):
MSGALDVLQMKEEDVLKFLAAGTHLGGTNLDFQMEQYIYKRKS

DGIYIINLKRTWEKLLLAARAIVAIENPADVSVISSRNTGQRAVLKFAAATGATPI

AGRFTPGTFTNQIQAAFREPRLLVVTDPRADHQPLTEASYVNLPTIAL

>ipi|IPI00793905|IPI00793905.1 24 KDA PROTEIN (SEQ ID NO: 10):
MSGALDVLQMKEEDVLKFLAAGTHLGGTNLDFQMEQYIYKRKS

DGIYIINLKRTWEKLLLAARAIVAIENPADVSVISSRNTGQGAHSVGLMWWMLA

REVLRMRGTISREHPWEVMPDLYFYRDPEEIEKEEQAAAEKAVTKEEFQGEW

TAPAPEFTATQPEVADWSEGVQVPSVPIQQFPTEDWSAQPATEDWSAAPTA

QATEWVGATTDWS

Example 4

Identification of Additional Pre-Diagnostic Lung Cancer Indicator Proteins

[0106] Using the methodologies described above in Examples 1 and 2, additional pre-diagnostic lung cancer indicator proteins were identified on the basis of detection of specifically binding autoantibodies in 30 pre-diagnostic lung cancer sera, using 30 matched normal sera as controls. The results are summarized in Table 5.

TABLE 5

ADDITIONAL PRE-DIAGNOSTIC LUNG CANCER INDICATOR PROTEINS

Gene	Uniprot_id	p-value for M2 statistics	sensitivity at 95% specificity	Description	SEQ ID NO:
AKR1B10	O60218	0.000085	0.47	Aldo-keto reductase family 1, member B10	11
GOT2	P00505	0.000216	0.43	Glutamic-oxaloacetic transaminase 2	12
HNRPR	O43390	0.001233	0.37	Heterogeneous nuclear ribonucleoprotein r	13
PDIA3	P30101	0.001233	0.37	Protein disulfide-isomerase a3	14
NME2	P22392	0.001233	0.37	Nonmetastatic cells 2, protein expressed in	15
RTN4	Q9NQC3	0.026157	0.23	Reticulon-4	16
HI1FX	Q92522	0.026157	0.23	H1 histone family, member x	17
G3BP	Q13283	0.051395	0.20	Ras-GTPase-activating-protein SH3-domain Binding Protein 1	18
HSPCA	P07900	0.051395	0.20	Heat shock 90 kda protein 1, alpha	19
ACTN4	O43707	0.051395	0.20	Actinin, alpha 4	20

SEQ ID NO: 11
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FIEQGINVCLCQSYAKNMGLYGERVGAFTMVCKDADEAKRVESQLKILIRPMY
SNPPLNGARIAAAILNTPDLRKQWLQEVKGMADRIIGMRTQLVSNLKKEGSTH
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SEQ ID NO: 13
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EFYQDTYGQQWK
SEQ ID NO: 14
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DGEEAGAYDGPRTADGIVSHLKKQAGPASVPLRTEEEFKKFISDKDASIVGFF
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FEDKTVAYTEQKMTSGKIKKFIQENIFGICPHMTEDNKDLIQGKDLLIAYYDVDY
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GEIPVVAIRTAKGEKFVMQEEFSRDGKALERFLQDYFDGNLKRYLKSEPIPESN
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REATN PPVIQEEKPKKKKKAQEDL

TABLE 5-continued

ADDITIONAL PRE-DIAGNOSTIC LUNG CANCER INDICATOR PROTEINS

Gene	Uniprot_id	p-value for M2 statistics	sensitivity at 95% specificity	Description	SEQ ID NO:

SEQ ID NO: 15
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DFCIQVGRNIIHGSDSVKSAEKEISLWFKPEELVDYKSCAHDWVYE
SEQ ID NO: 16
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EVLERKPAAGLSAAPVPTAPAAGAPLMDFGNDFVPPAPRGPLPAAPPVAPER
QPSWDPSPVSSTVPAPSPLSAAAVSPSKLPEDDEPPARPPPPPPASVSPQAE
PVWTPPAPAPAAPPSTPAAPKRRGSSGSVDETLFALPAASEPVIRSSAENMDL
KEQPGNTISAGQEDFPSVLLETAASLPSLSPLSAASFKEHEYLGNLSTVLPTEG
TLQENVSEASKEVSEKAKTLLIDRDLTEFSELEYSEMGSSFSVSPKAESAVIVA
NPREEIIVKNKDEEEKLVSNNILHNQQELPTALTKLVKEDEVVSSEKAKDSFNE
KRVAVEAPMREEYADFKPFERVWEVKDSKEDSDMLAAGGKIESNLESKVDKK
CFADSLEQTNHEKDSESSNDDTSFPSTPEGIKDRSGAYITCAPFNPAATESIAT
NIFPLLGDPTSENKTDEKKIEEKKAQIVTEKNTSTKTSNPFLVAAQDSETDYVTT
DNLTKVTEEVVANMPEGLTPDLVQEACESELNEVTGTKIAYETKMDLVQTSEV
MQESLYPAAQLCPSFEESEATPSPVLPDIVMEAPLNSAVPSAGASVIQPSSSPL
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SDDSIPDVPQKQDETVMLVKESLTETSFESMIEYENKEKLSALPPEGGKPYLES
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TFSDSSPIEIIDEFPTLISSKTDSFSKLAREYTDLEVSHKSEIANAPDGAGSLPCT
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PKVLVKEAEKKLPSDTEKEDRSPSAIFSAELSKTSVVDLLYWRDIKKTGVVFGA
SLFLLLSLTVFSIVSVTAYIALALLSVTISFRIYKGVIQAIQKSDEGHPFRAYLESE
VAISEELVQKYSNSALGHVNCTIKELRRLFLVDDLVDSLKFAVLMWVFTYVGAL
FNGLTLLILALISLFSVPVIYERHQAQIDHYLGLANKNVKDAMAKIQAKIPGLKRK
AE
SEQ ID NO: 17
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GTGANGSFKLNRKKLEGGGERRGAPAAATAPAPTAHKAKKAAPGAAGSRRA
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PKGRK
SEQ ID NO: 18
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PPSGAVPVTGIPPHVVKVPASQPRPESKPESQIPPQRPQRDQRVREQRINIPP
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SEQ ID NO: 19
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YLNFIRGVVDSEDLPLNISREMLQQSKILKVIRKNLVKKCLELFTELAEDKENYK
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DTSAAVTEEMPPLEGDDDTSRMEEVD
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SVEETSAKEGLLLWCQRKTAPYKNVNVQNFHISWKDGLAFNALIHRHRPELIE
YDKLRKDDPVTNLNNAFEVAEKYLDIPKMLDAEDIVNTARPDEKAIMTYVSSFY
HAFSGAQKAETAANRICKVLAVNQENEHLMEDYEKLASDLLEWIRRTIPWLED
RVPQKTIQEMQQKLEDFRDYRRVHKPPKVQEKCQLEINFNTLQTKLRLSNRPA
FMPSEGKMVSDINNGWQHLEQAEKGYEEWLLNEIRRLERLDHLAEKFRQKAS
IHEAWTDGKEAMLKHRDYETATLSDIKALIRKHEAFESDLAAHQDRVEQIAAIA
QELNELDYYDSHNVNTRCQKICDQWDALGSLTHSRREALEKTEKQLEAIDQLH

TABLE 5-continued

ADDITIONAL PRE-DIAGNOSTIC LUNG CANCER INDICATOR PROTEINS

Gene	Uniprot_id	p-value for M2 statistics	sensitivity at 95% specificity	Description	SEQ ID NO:

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EQSKQQSNEHLRRQFASQANVVGPWIQTKMEEIGRISIEMNGTLEDQLSHLKQ
YERSIVDYKPNLDLLEQQHQLIQEALIFDNKHTNYTMEHIRVGWEQLLTTIARTI
NEVENQILTRDAKGISQEQMQEFRASFNHFDKDHGGALGPEEFKACLISLGYD
VENDRQGEAEFNRIMSLVDPNHSGLVTFQAFIDFMSRETTDTDTADQVIASFK
VLAGDKNFITAEELRRELPPDQAEYCIARMAPYQGPDAVPGALDYKSFSTALY
GESDL

REFERENCES

[0107] 1. Stockert E, Jager E, Chen Y T, et al: A survey of the humoral immune response of cancer patients to a panel of human tumor antigens. J Exp Med 187:1349-54, 1998

[0108] 2. Tan EM: Autoantibodies as reporters identifying aberrant cellular mechanisms in tumorigenesis. J Clin Invest 108:1411-5, 2001

[0109] 3. Mintz P J, Kim J, Do K A, et al: Fingerprinting the circulating repertoire of antibodies from cancer patients. Nat Biotechnol 21:57-63, 2003

[0110] 4. Gourevitch M M, von Mensdorff-Pouilly S, Litvinov S V, et al: Polymorphic epithelial mucin (MUC-1)-containing circulating immune complexes in carcinoma patients. Br J Cancer 72:934-8, 1995

[0111] 5. Gure A O, Altorki N K, Stockert E, et al: Human lung cancer antigens recognized by autologous antibodies: definition of a novel cDNA derived from the tumor suppressor gene locus on chromosome 3p21.3. Cancer Res 58:1034-41, 1998

[0112] 6. Yamamoto A, Shimizu E, Ogura T, et al: Detection of auto-antibodies against L-myc oncogene products in sera from lung cancer patients. Int J Cancer 69:283-9, 1996

[0113] 7. Dunn G P, Bruce A T, Ikeda H, et al: Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol 3:991-8, 2002

[0114] 8. Hanash S: Harnessing immunity for cancer marker discovery. Nat Biotechnol 21:37-8, 2003

[0115] 9. Old L J, Chen Y T: New paths in human cancer serology. J Exp Med 187:1163-7, 1998

[0116] 10. Finn O J: Immune response as a biomarker for cancer detection and a lot more. N Engl J Med 353:1288-90, 2005

[0117] 11. Diesinger I, Bauer C, Brass N, et al: Toward a more complete recognition of immunoreactive antigens in squamous cell lung carcinoma. Int J Cancer 102:372-8, 2002

[0118] 12. Gure A O, Stocked E, Scanlan M J, et al: Serological identification of embryonic neural proteins as highly immunogenic tumor antigens in small cell lung cancer. Proc Natl Acad Sci USA 97:4198-203, 2000

[0119] 13. Ali Eldib A M, Ono T, Shimono M, et al: Immunoscreening of a cDNA library from a lung cancer cell line using autologous patient serum: Identification of XAGE-1b as a dominant antigen and its immunogenicity in lung adenocarcinoma. Int J Cancer 108:558-63, 2004

[0120] 14. Yang F, Xiao Z Q, Zhang X Z, et al: Identification of tumor antigens in human lung squamous carcinoma by serological proteome analysis. J Proteome Res 6:751-8, 2007

[0121] 15. Hanash S: Disease proteomics. Nature 422:226-32, 2003

[0122] 16. Imafuku Y, Omenn G S, Hanash S: Proteomics approaches to identify tumor antigen directed autoantibodies as cancer biomarkers. Dis Markers 20:149-53, 2004

[0123] 17. Brichory F, Beer D, Le Naour F, et al: Proteomics-based identification of protein gene product 9.5 as a tumor antigen that induces a humoral immune response in lung cancer. Cancer Res 61:7908-12, 2001

[0124] 18. Brichory F M, Misek D E, Yim A M, et al: An immune response manifested by the common occurrence of annexins I and II autoantibodies and high circulating levels of IL-6 in lung cancer. Proc Natl Acad Sci USA 98:9824-9, 2001

[0125] 19. Pereira-Faca S R, Kuick R, Purays E, et al: Identification of 14-3-3 theta as an antigen that induces a humoral response in lung cancer. Cancer Res 67:12000-6, 2007

[0126] 20. Wang H, Hanash S: Multi-dimensional liquid phase based separations in proteomics. J Chromatogr B Analyt Technol Biomed Life Sci 787:11-8, 2003

[0127] 21. Faca V, Pitteri S J, Newcomb L, et al: Contribution of protein fractionation to depth of analysis of the serum and plasma proteomes. J Proteome Res 6:3558-65, 2007

[0128] 22. Nam M J, Madoz-Gurpide J, Wang H, et al: Molecular profiling of the immune response in colon cancer using protein microarrays: occurrence of autoantibodies to ubiquitin C-terminal hydrolase L3. Proteomics 3:2108-15, 2003

[0129] 23. Madoz-Gurpide J, Kuick R, Wang H, et al: Integral protein microarrays for the identification of lung cancer antigens in sera that induce a humoral immune response. Mol Cell Proteomics, 2007

[0130] 24. Forrester S, Qiu J, L. M, et al: An experimental strategy for quantitative analysis of the humoral immune response to prostate cancer antigens using natural protein microarrays. Proteomics—Clinical Applications 1:494-505, 2007

[0131] 25. Goodman G E, Thornquist M D, Balmes J, et al: The Beta-Carotene and Retinol Efficacy Trial: incidence of lung cancer and cardiovascular disease mortality during 6-year follow-up after stopping beta-carotene and retinol supplements. J Natl Cancer Inst 96:1743-50, 2004

[0132] 26. Omenn G S, Goodman G E, Thornquist M D, et al: Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular disease. N Engl J Med 334:1150-5, 1996

[0133] 27. Qiu J, Madoz-Gurpide J, Misek D E, et al: Development of natural protein microarrays for diagnosing cancer based on an antibody response to tumor antigens. J Proteome Res 3:261-7, 2004

[0134] 28. Friedman J, Hastie T, Tibshirani R: Additive Logistic Regression: A Statistical View of Boosting. Annals of Statistics 28:337-407, 2000

[0135] 29. Yasui Y, Pepe M, Thompson M L, et al: A data-analytic strategy for protein biomarker discovery: profiling of high-dimensional proteomic data for cancer detection. Biostatistics 4:449-63, 2003

[0136] 30. Madoz-Gurpide J, Wang H, Misek D E, et al: Protein based microarrays: a tool for probing the proteome of cancer cells and tissues. Proteomics 1:1279-87, 2001

[0137] 31. He P, Naka T, Serada S, et al: Proteomics-based identification of alpha-enolase as a tumor antigen in non-small lung cancer. Cancer Sci 98:1234-40, 2007

[0138] 32. Tureci O, Mack U, Luxemburger U, et al: Humoral immune responses of lung cancer patients against tumor antigen NY-ESO-1. Cancer Lett 236:64-71, 2006

[0139] 33. Chang J W, Lee S H, Jeong J Y, et al: Peroxiredoxin-I is an autoimmunogenic tumor antigen in non-small cell lung cancer. FEBS Lett 579:2873-7, 2005

[0140] 34. Yagihashi A, Asanuma K, Kobayashi D, et al: Detection of autoantibodies to livin and survivin in Sera from lung cancer patients. Lung Cancer 48:217-21, 2005

[0141] 35. Matsumoto S, Teramoto H, Nakamoto M, et al: Presence of antibodies against retinoblastoma tumor suppressor protein in patients with lung cancer. Int J Oncol 19:1035-9, 2001

[0142] 36. Fernandez-Madrid F, VandeVord P J, Yang X, et al: Antinuclear antibodies as potential markers of lung cancer. Clin Cancer Res 5:1393-400, 1999

[0143] 37. Lubin R, Zalcman G, Bouchet L, et al: Serum p53 antibodies as early markers of lung cancer. Nat Med 1:701-2, 1995

[0144] 38. Yow H K, Wong J M, Chen H S, et al: Increased mRNA expression of a laminin-binding protein in human colon carcinoma: complete sequence of a full-length cDNA encoding the protein. Proc Natl Acad Sci USA 85:6394-8, 1988

[0145] 39. Buto S, Tagliabue E, Ardini E, et al: Formation of the 67-kDa laminin receptor by acylation of the precursor. J Cell Biochem 69:244-51, 1998

[0146] 40. Rao C N, Castronovo V, Schmitt M C, et al: Evidence for a precursor of the high-affinity metastasis-associated murine laminin receptor. Biochemistry 28:7476-86, 1989

[0147] 41. Lesot H, Kuhl U, Mark K V: Isolation of a laminin-binding protein from muscle cell membranes. Embo J 2:861-865, 1983

[0148] 42. Malinoff H L, Wicha M S: Isolation of a cell surface receptor protein for laminin from murine fibrosarcoma cells. J Cell Biol 96:1475-9, 1983

[0149] 43. Auth D, Brawerman G: A 33-kDa polypeptide with homology to the laminin receptor: component of translation machinery. Proc Natl Acad Sci USA 89:4368-72, 1992

[0150] 44. Ardini E, Pesole G, Tagliabue E, et al: The 67-kDa laminin receptor originated from a ribosomal protein that acquired a dual function during evolution. Mol Biol Evol 15:1017-25, 1998

[0151] 45. Coggin J H, Jr., Barsoum A L, Rohrer J W: 37 kiloDalton oncofetal antigen protein and immature laminin receptor protein are identical, universal T-cell inducing immunogens on primary rodent and human cancers. Anticancer Res 19:5535-42, 1999

[0152] 46. Coggin J H, Jr., Barsoum A L, Rohrer J W: Tumors express both unique TSTA and crossprotective 44 kDa oncofetal antigen. Immunol Today 19:405-8, 1998

[0153] 47. Rieger R, Edenhofer F, Lasmezas C I, et al: The human 37-kDa laminin receptor precursor interacts with the prion protein in eukaryotic cells. Nat Med 3:1383-8, 1997

[0154] 48. Wewer U M, Taraboletti G, Sobel M E, et al: Role of laminin receptor in tumor cell migration. Cancer Res 47:5691-8, 1987

[0155] 49. Menard S, Tagliabue E, Colnaghi M I: The 67 kDa laminin receptor as a prognostic factor in human cancer. Breast Cancer Res Treat 52:137-45, 1998

[0156] 50. Menard S, Castronovo V, Tagliabue E, et al: New insights into the metastasis-associated 67 kD laminin receptor. J Cell Biochem 67:155-65, 1997

[0157] 51. Cioce V, Castronovo V, Shmookler B M, et al: Increased expression of the laminin receptor in human colon cancer. J Natl Cancer Inst 83:29-36, 1991

[0158] 52. Fontanini G, Vignati S, Chine S, et al: 67-Kilodalton laminin receptor expression correlates with worse prognostic indicators in non-small cell lung carcinomas. Clin Cancer Res 3:227-31, 1997

[0159] 53. Ambrose K R, Anderson N G, Coggin J H: Interruption of SV40 oncogenesis with human foetal antigen. Nature 233:194-5, 1971

[0160] 54. Coggin J H, Jr., Ambrose K R, Bellomy B B, et al: Tumor immunity in hamsters immunized with fetal tissues. J Immunol 107:526-33, 1971

[0161] 55. Ambrose K R, Anderson N G, Coggin J H, Jr.: Cytostatic antibody and SV40 tumour immunity in hamsters. Nature 233:321-4, 1971

[0162] 56. Payne W J, Jr., Coggin J H, Jr.: Mouse monoclonal antibody to embryonic antigen: development, cross-reactivity with rodent and human tumors, and preliminary polypeptide characterization. J Natl Cancer Inst 75:527-44, 1985

[0163] 57. Coggin J H, Jr., Rohrer S D, Leinbach E D, et al: Radiation-induced lymphoblastic lymphomas/leukemias and sarcomas of mice express conserved, immunogenic 44-kilodalton oncofetal antigen. Am J Pathol 130:136-46, 1988

[0164] 58. Gussack G S, Rohrer S D, Hester R B, et al: Human squamous cell carcinoma lines express oncofetal 44-kD polypeptide defined by monoclonal antibody to mouse fetus. Cancer 62:283-90, 1988

[0165] 59. Rohrer J W, Rohrer S D, Barsoum A, et al: Differential recognition of murine tumor-associated oncofetal transplantation antigen and individually specific tumor transplantation antigens by syngeneic cloned BALB/c and RFM mouse T cells. J Immunol 152:754-64, 1994

[0166] 60. Siegel S, Wagner A, Friedrichs B, et al: Identification of HLA-A*0201-presented T cell epitopes derived

from the oncofetal antigen-immature laminin receptor protein in patients with hematological malignancies. J Immunol 176:6935-44, 2006

[0167] 61. Rohrer J W, Culpepper C, Barsoum A L, et al: Characterization of RFM mouse T lymphocyte anti-oncofetal antigen immunity in apparent tumor-free, long-term survivors of sublethal X-irradiation by limiting dilution T lymphocyte cloning. J Immunol 154:2266-80, 1995

[0168] 62. Holtl L, Zelle-Rieser C, Gander H, et al: Immunotherapy of metastatic renal cell carcinoma with tumor lysate-pulsed autologous dendritic cells. Clin Cancer Res 8:3369-76, 2002

[0169] 63. Rohrer J W, Coggin J H, Jr.: CD8 T cell clones inhibit antitumor T cell function by secreting IL-10. J Immunol 155:5719-27, 1995

[0170] 64. Rohrer J W, Barsoum A L, Coggin J H, Jr.: Identification of oncofetal antigen/immature laminin receptor protein epitopes that activate BALB/c mouse OFA/iLRP-specific effector and regulatory T cell clones. J Immunol 176:2844-56, 2006

[0171] 65. Rohrer J W, Barsoum A L, Dyess D L, et al: Human breast carcinoma patients develop clonable oncofetal antigen-specific effector and regulatory T lymphocytes. J Immunol 162:6880-92, 1999

[0172] 66. Rohrer J, Barsoum A, Coggin J: The Development of a New Universal Tumor Rejection Antigen Expressed on Human and Rodent Cancers for Vacccination, Prevention of Cancer, and Anti-Tumor Therapy. Mod Asp Immunobiol 1:191-5, 2001

[0173] 67. Coggin J H, Jr., Rohrer S D, Hester R D, et al: 44-kd oncofetal transplantation antigen in rodent and human fetal cells. Implications of recrudescence in human and rodent cancers. Arch Otolaryngol Head Neck Surg 119:1257-66, 1993

[0174] 68. Rohrer S D, Sarli R N, Barsoum A L, et al: Expression of 44-kilodalton oncofetal antigen as a premalignancy marker in X irradiation-induced murine T-cell lymphoma. J Natl Cancer Inst 84:602-9, 1992

[0175] The various embodiments described above can be combined to provide further embodiments. All of the U.S. patents, U.S. patent application publications, U.S. patent applications, foreign patents, foreign patent applications and non-patent publications referred to in this specification and/or listed in the Application Data Sheet are incorporated herein by reference, in their entirety. Aspects of the embodiments can be modified, if necessary to employ concepts of the various patents, applications and publications to provide yet further embodiments.

[0176] These and other changes can be made to the embodiments in light of the above-detailed description. In general, in the following claims, the terms used should not be construed to limit the claims to the specific embodiments disclosed in the specification and the claims, but should be construed to include all possible embodiments along with the full scope of equivalents to which such claims are entitled. Accordingly, the claims are not limited by the disclosure.

SEQUENCE LISTING

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Gly Ser Ala Val Ser Pro Tyr Pro Thr Phe Asn Pro Ser Ser Asp Val
        35                  40                  45

Ala Ala Leu His Lys Ala Ile Met Val Lys Gly Val Asp Glu Ala Thr
    50                  55                  60

Ile Ile Asp Ile Leu Thr Lys Arg Asn Asn Ala Gln Arg Gln Gln Ile
65                  70                  75                  80

Lys Ala Ala Tyr Leu Gln Glu Thr Gly Lys Pro Leu Asp Glu Thr Leu
                85                  90                  95

Lys Lys Ala Leu Thr Gly His Leu Glu Glu Val Val Leu Ala Leu Leu
            100                 105                 110

Lys Thr Pro Ala Gln Phe Asp Ala Asp Glu Leu Arg Ala Ala Met Lys
        115                 120                 125

Gly Leu Gly Thr Asp Glu Asp Thr Leu Ile Glu Ile Leu Ala Ser Arg
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Thr Asn Lys Glu Ile Arg Asp Ile Asn Arg Val Tyr Arg Glu Glu Leu
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            180                 185                 190

Gly Val Asn Glu Asp Leu Ala Asp Ser Asp Ala Arg Ala Leu Tyr Glu
        195                 200                 205

Ala Gly Glu Arg Arg Lys Gly Thr Asp Val Asn Val Phe Asn Thr Ile
    210                 215                 220

Leu Thr Thr Arg Ser Tyr Pro Gln Leu Arg Arg Val Phe Gln Lys Tyr
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Thr Lys Tyr Ser Lys His Asp Met Asn Lys Val Leu Asp Leu Glu Leu
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Lys Gly Asp Ile Glu Lys Cys Leu Thr Ala Ile Val Lys Cys Ala Thr
            260                 265                 270

Ser Lys Pro Ala Phe Phe Ala Glu Lys Leu His Gln Ala Met Lys Gly
        275                 280                 285

Val Gly Thr Arg His Lys Ala Leu Ile Arg Ile Met Val Ser Arg Ser
    290                 295                 300

Glu Ile Asp Met Asn Asp Ile Lys Ala Phe Tyr Gln Lys Met Tyr Gly
305                 310                 315                 320

Ile Ser Leu Cys Gln Ala Ile Leu Asp Glu Thr Lys Gly Asp Tyr Glu
                325                 330                 335

Lys Ile Leu Val Ala Leu Cys Gly Gly Asn
            340                 345


<210> SEQ ID NO 2
<211> LENGTH: 204
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 2

Met Asn Leu Ile Leu Arg Tyr Thr Phe Ser Lys Met Ala Met Val Ser
1               5                   10                  15

Glu Phe Leu Lys Gln Ala Trp Phe Ile Glu Asn Glu Glu Gln Glu Tyr
            20                  25                  30

Val Gln Thr Val Lys Ser Ser Lys Gly Gly Pro Gly Ser Ala Val Ser
        35                  40                  45

Pro Tyr Pro Thr Phe Asn Pro Ser Ser Asp Val Ala Ala Leu His Lys
    50                  55                  60

Ala Ile Met Val Lys Gly Val Asp Glu Ala Thr Ile Ile Asp Ile Leu
65                  70                  75                  80

Thr Lys Arg Asn Asn Ala Gln Arg Gln Gln Ile Lys Ala Ala Tyr Leu
                85                  90                  95

Gln Glu Thr Gly Lys Pro Leu Asp Glu Thr Leu Lys Lys Ala Leu Thr
            100                 105                 110

Gly His Leu Glu Glu Val Val Leu Ala Leu Leu Lys Thr Pro Ala Gln
        115                 120                 125

Phe Asp Ala Asp Glu Leu Arg Ala Ala Met Lys Gly Leu Gly Thr Asp
    130                 135                 140

Glu Asp Thr Leu Ile Glu Ile Leu Ala Ser Arg Thr Asn Lys Glu Ile
145                 150                 155                 160
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Arg Asp Ile Asn Arg Val Tyr Arg Glu Glu Leu Lys Arg Asp Leu Ala
                165                 170                 175

Lys Asp Ile Thr Ser Asp Thr Ser Gly Asp Phe Arg Asn Ala Leu Leu
            180                 185                 190

Ser Leu Ala Lys Gly Asp Arg Ser Glu Asp Phe Gly
        195                 200


<210> SEQ ID NO 3
<211> LENGTH: 115
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 3

Met Ala Met Val Ser Glu Phe Leu Lys Gln Ala Trp Phe Ile Glu Asn
1               5                   10                  15

Glu Glu Gln Glu Tyr Val Gln Thr Val Lys Ser Ser Lys Gly Gly Pro
            20                  25                  30

Gly Ser Ala Val Ser Pro Tyr Pro Thr Phe Asn Pro Ser Ser Asp Val
        35                  40                  45

Ala Ala Leu His Lys Ala Ile Met Val Lys Gly Val Asp Glu Ala Thr
    50                  55                  60

Ile Ile Asp Ile Leu Thr Lys Arg Asn Asn Ala Gln Arg Gln Gln Ile
65                  70                  75                  80

Lys Ala Ala Tyr Leu Gln Glu Thr Gly Lys Pro Leu Asp Glu Thr Leu
                85                  90                  95

Lys Lys Ala Leu Thr Gly His Leu Glu Glu Val Val Leu Ala Leu Leu
            100                 105                 110

Lys Thr Pro
        115


<210> SEQ ID NO 4
<211> LENGTH: 245
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 4

Met Glu Lys Thr Glu Leu Ile Gln Lys Ala Lys Leu Ala Glu Gln Ala
1               5                   10                  15

Glu Arg Tyr Asp Asp Met Ala Thr Cys Met Lys Ala Val Thr Glu Gln
            20                  25                  30

Gly Ala Glu Leu Ser Asn Glu Glu Arg Asn Leu Leu Ser Val Ala Tyr
        35                  40                  45

Lys Asn Val Val Gly Gly Arg Arg Ser Ala Trp Arg Val Ile Ser Ser
    50                  55                  60

Ile Glu Gln Lys Thr Asp Thr Ser Asp Lys Lys Leu Gln Leu Ile Lys
65                  70                  75                  80

Asp Tyr Arg Glu Lys Val Glu Ser Glu Leu Arg Ser Ile Cys Thr Thr
                85                  90                  95

Val Leu Glu Leu Leu Asp Lys Tyr Leu Ile Ala Asn Ala Thr Asn Pro
            100                 105                 110

Glu Ser Lys Val Phe Tyr Leu Lys Met Lys Gly Asp Tyr Phe Arg Tyr
        115                 120                 125

Leu Ala Glu Val Ala Cys Gly Asp Asp Arg Lys Gln Thr Ile Asp Asn
    130                 135                 140
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Ser Gln Gly Ala Tyr Gln Glu Ala Phe Asp Ile Ser Lys Lys Glu Met
145                 150                 155                 160

Gln Pro Thr His Pro Ile Arg Leu Gly Leu Ala Leu Asn Phe Ser Val
                165                 170                 175

Phe Tyr Tyr Glu Ile Leu Asn Asn Pro Glu Leu Ala Cys Thr Leu Ala
            180                 185                 190

Lys Thr Ala Phe Asp Glu Ala Ile Ala Glu Leu Asp Thr Leu Asn Glu
        195                 200                 205

Asp Ser Tyr Lys Asp Ser Thr Leu Ile Met Gln Leu Leu Arg Asp Asn
    210                 215                 220

Leu Thr Leu Trp Thr Ser Asp Ser Ala Gly Glu Glu Cys Asp Ala Ala
225                 230                 235                 240

Glu Gly Ala Glu Asn
                245


<210> SEQ ID NO 5
<211> LENGTH: 154
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 5

Tyr Trp His Ala Gln Met Glu Lys Thr Glu Leu Ile Gln Lys Ala Lys
1               5                   10                  15

Leu Ala Glu Gln Ala Glu Arg Tyr Asp Asp Met Ala Thr Cys Met Lys
            20                  25                  30

Ala Val Thr Glu Gln Gly Ala Glu Leu Ser Asn Glu Glu Arg Asn Leu
        35                  40                  45

Leu Ser Val Ala Tyr Lys Asn Val Val Gly Gly Arg Arg Ser Ala Trp
    50                  55                  60

Arg Val Ile Ser Ser Ile Glu Gln Lys Thr Asp Thr Ser Asp Lys Lys
65                  70                  75                  80

Leu Gln Leu Ile Lys Asp Tyr Arg Glu Lys Val Glu Ser Glu Leu Arg
                85                  90                  95

Ser Ile Cys Thr Thr Val Leu Glu Leu Leu Asp Lys Tyr Leu Ile Ala
            100                 105                 110

Asn Ala Thr Asn Pro Glu Ser Lys Val Phe Tyr Leu Lys Met Lys Gly
        115                 120                 125

Asp Tyr Phe Arg Tyr Leu Ala Glu Val Ala Cys Gly Asp Asp Arg Lys
    130                 135                 140

Gln Thr Ile Asp Asn Ser Gln Gly Ala Tyr
145                 150


<210> SEQ ID NO 6
<211> LENGTH: 295
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 6

Met Ser Gly Ala Leu Asp Val Leu Gln Met Lys Glu Glu Asp Val Leu
1               5                   10                  15

Lys Phe Leu Ala Ala Gly Thr His Leu Gly Gly Thr Asn Leu Asp Phe
            20                  25                  30

Gln Met Glu Gln Tyr Ile Tyr Lys Arg Lys Ser Asp Gly Ile Tyr Ile
        35                  40                  45
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Ile Asn Leu Lys Arg Thr Trp Glu Lys Leu Leu Leu Thr Ala Arg Ala
    50                  55                  60

Ile Val Ala Ile Glu Asn Pro Ala Asp Val Ser Val Ile Ser Ser Arg
65                  70                  75                  80

Asn Thr Gly Gln Arg Ala Val Leu Lys Phe Ala Ala Ala Thr Gly Ala
                85                  90                  95

Thr Pro Ile Ala Gly Arg Phe Thr Pro Gly Thr Phe Thr Asn Gln Ile
            100                 105                 110

Gln Ala Ala Phe Arg Glu Pro Arg Leu Leu Val Val Ser Asp Pro Arg
        115                 120                 125

Ala Asp His Gln Pro Leu Thr Glu Ala Ser Tyr Val Asn Leu Pro Thr
    130                 135                 140

Ile Ala Leu Cys Asn Thr Asp Ser Pro Leu His Tyr Val Asp Ile Ala
145                 150                 155                 160

Ile Pro Cys Asn Asn Lys Gly Thr His Ser Val Gly Leu Met Trp Trp
                165                 170                 175

Met Leu Ala Arg Glu Val Leu Arg Met Arg Gly Thr Ile Ser Arg Glu
            180                 185                 190

His Pro Trp Glu Val Met Pro Asp Leu Tyr Phe Tyr Arg Asp Pro Glu
        195                 200                 205

Glu Ile Glu Lys Glu Glu Gln Ala Ala Ala Glu Lys Ala Met Thr Arg
    210                 215                 220

Glu Glu Leu Gln Gly Glu Trp Thr Ala Pro Ala Pro Glu Phe Thr Ala
225                 230                 235                 240

Thr Gln Pro Glu Val Ala Asp Trp Ser Glu Gly Val Gln Val Pro Ser
                245                 250                 255

Val Pro Ile Gln Gln Phe Pro Thr Glu Asp Trp Ser Thr Gln Arg Ala
            260                 265                 270

Thr Glu Asp Trp Ser Ala Ala Pro Thr Ala Gln Ala Thr Glu Trp Val
        275                 280                 285

Gly Ala Thr Thr Asp Trp Ser
    290                 295


<210> SEQ ID NO 7
<211> LENGTH: 300
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 7

Met Ser Gly Ala Leu Asp Val Leu Gln Met Lys Glu Glu Asp Val Leu
1               5                   10                  15

Lys Phe Leu Ala Ala Gly Thr His Leu Gly Gly Thr Asn Leu Asp Phe
            20                  25                  30

Gln Met Glu Gln Tyr Ile Tyr Lys Arg Lys Ser Asp Gly Ile Tyr Ile
        35                  40                  45

Ile Asn Leu Lys Arg Thr Trp Glu Lys Leu Leu Leu Ala Ala Arg Ala
    50                  55                  60

Ile Val Ala Ile Glu Asn Pro Ala Asp Val Ser Val Ile Ser Ser Arg
65                  70                  75                  80

Asn Thr Gly Gln Val Cys Gly Thr Val Arg Ala Val Leu Lys Phe Ala
                85                  90                  95

Ala Ala Thr Gly Ala Thr Pro Ile Ala Gly Arg Phe Thr Pro Gly Thr
            100                 105                 110
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Phe Thr Asn Gln Ile Gln Ala Ala Phe Arg Glu Pro Arg Leu Leu Val
        115                 120                 125

Val Thr Asp Pro Arg Ala Asp His Gln Pro Leu Thr Glu Ala Ser Tyr
    130                 135                 140

Val Asn Leu Pro Thr Ile Ala Leu Cys Asn Thr Asp Ser Pro Leu Arg
145                 150                 155                 160

Tyr Val Asp Ile Ala Ile Pro Cys Asn Asn Lys Gly Ala His Ser Val
                165                 170                 175

Gly Leu Met Trp Trp Met Leu Ala Arg Glu Val Leu Arg Met Arg Gly
            180                 185                 190

Thr Ile Ser Arg Glu His Pro Trp Glu Val Met Pro Asp Leu Tyr Phe
        195                 200                 205

Tyr Arg Asp Pro Glu Glu Ile Glu Lys Glu Glu Gln Ala Ala Ala Glu
    210                 215                 220

Lys Ala Val Thr Lys Glu Glu Phe Gln Gly Glu Trp Thr Ala Pro Ala
225                 230                 235                 240

Pro Glu Phe Thr Ala Thr Gln Pro Glu Val Ala Asp Trp Ser Glu Gly
                245                 250                 255

Val Gln Val Pro Ser Val Pro Ile Gln Gln Phe Pro Thr Glu Asp Trp
            260                 265                 270

Ser Ala Gln Pro Ala Thr Glu Asp Trp Ser Ala Ala Pro Thr Ala Gln
        275                 280                 285

Ala Thr Glu Trp Val Gly Ala Thr Thr Asp Trp Ser
    290                 295                 300


<210> SEQ ID NO 8
<211> LENGTH: 295
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 8

Met Ser Gly Ala Leu Asp Val Leu Gln Met Lys Glu Glu Asp Val Leu
1               5                   10                  15

Lys Phe Leu Ala Ala Gly Thr His Leu Gly Gly Thr Asn Leu Asp Phe
            20                  25                  30

Gln Met Glu Gln Tyr Ile Tyr Lys Arg Lys Ser Asp Gly Ile Tyr Ile
        35                  40                  45

Ile Asn Leu Lys Arg Thr Trp Glu Lys Leu Leu Leu Ala Ala Arg Ala
    50                  55                  60

Ile Val Ala Ile Glu Asn Pro Ala Asp Val Ser Val Ile Ser Ser Arg
65                  70                  75                  80

Asn Thr Gly Gln Arg Ala Val Leu Lys Phe Ala Ala Ala Thr Gly Ala
                85                  90                  95

Thr Pro Ile Ala Gly Arg Phe Thr Pro Gly Thr Phe Thr Asn Gln Ile
            100                 105                 110

Gln Ala Ala Phe Arg Glu Pro Arg Leu Leu Val Val Thr Asp Pro Arg
        115                 120                 125

Ala Asp His Gln Pro Leu Thr Glu Ala Ser Tyr Val Asn Leu Pro Thr
    130                 135                 140

Ile Ala Leu Cys Asn Thr Asp Ser Pro Leu Arg Tyr Val Asp Ile Ala
145                 150                 155                 160

Ile Pro Cys Asn Asn Lys Gly Ala His Ser Val Gly Leu Met Trp Trp
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                165                 170                 175

Met Leu Ala Arg Glu Val Leu Arg Met Arg Gly Thr Ile Ser Arg Glu
            180                 185                 190

His Pro Trp Glu Val Met Pro Asp Leu Tyr Phe Tyr Arg Asp Pro Glu
        195                 200                 205

Glu Ile Glu Lys Glu Glu Gln Ala Ala Ala Glu Lys Ala Val Thr Lys
    210                 215                 220

Glu Glu Phe Gln Gly Glu Trp Thr Ala Pro Ala Pro Glu Phe Thr Ala
225                 230                 235                 240

Thr Gln Pro Glu Val Ala Asp Trp Ser Glu Gly Val Gln Val Pro Ser
                245                 250                 255

Val Pro Ile Gln Gln Phe Pro Thr Glu Asp Trp Ser Ala Gln Pro Ala
            260                 265                 270

Thr Glu Asp Trp Ser Ala Ala Pro Thr Ala Gln Ala Thr Glu Trp Val
        275                 280                 285

Gly Ala Thr Thr Asp Trp Ser
    290                 295


<210> SEQ ID NO 9
<211> LENGTH: 147
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 9

Met Ser Gly Ala Leu Asp Val Leu Gln Met Lys Glu Glu Asp Val Leu
1               5                   10                  15

Lys Phe Leu Ala Ala Gly Thr His Leu Gly Gly Thr Asn Leu Asp Phe
            20                  25                  30

Gln Met Glu Gln Tyr Ile Tyr Lys Arg Lys Ser Asp Gly Ile Tyr Ile
        35                  40                  45

Ile Asn Leu Lys Arg Thr Trp Glu Lys Leu Leu Leu Ala Ala Arg Ala
    50                  55                  60

Ile Val Ala Ile Glu Asn Pro Ala Asp Val Ser Val Ile Ser Ser Arg
65                  70                  75                  80

Asn Thr Gly Gln Arg Ala Val Leu Lys Phe Ala Ala Ala Thr Gly Ala
                85                  90                  95

Thr Pro Ile Ala Gly Arg Phe Thr Pro Gly Thr Phe Thr Asn Gln Ile
            100                 105                 110

Gln Ala Ala Phe Arg Glu Pro Arg Leu Leu Val Val Thr Asp Pro Arg
        115                 120                 125

Ala Asp His Gln Pro Leu Thr Glu Ala Ser Tyr Val Asn Leu Pro Thr
    130                 135                 140

Ile Ala Leu
145


<210> SEQ ID NO 10
<211> LENGTH: 213
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 10

Met Ser Gly Ala Leu Asp Val Leu Gln Met Lys Glu Glu Asp Val Leu
1               5                   10                  15

Lys Phe Leu Ala Ala Gly Thr His Leu Gly Gly Thr Asn Leu Asp Phe
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            20                  25                  30

Gln Met Glu Gln Tyr Ile Tyr Lys Arg Lys Ser Asp Gly Ile Tyr Ile
        35                  40                  45

Ile Asn Leu Lys Arg Thr Trp Glu Lys Leu Leu Leu Ala Ala Arg Ala
    50                  55                  60

Ile Val Ala Ile Glu Asn Pro Ala Asp Val Ser Val Ile Ser Ser Arg
65                  70                  75                  80

Asn Thr Gly Gln Gly Ala His Ser Val Gly Leu Met Trp Trp Met Leu
                85                  90                  95

Ala Arg Glu Val Leu Arg Met Arg Gly Thr Ile Ser Arg Glu His Pro
            100                 105                 110

Trp Glu Val Met Pro Asp Leu Tyr Phe Tyr Arg Asp Pro Glu Glu Ile
        115                 120                 125

Glu Lys Glu Glu Gln Ala Ala Ala Glu Lys Ala Val Thr Lys Glu Glu
    130                 135                 140

Phe Gln Gly Glu Trp Thr Ala Pro Ala Pro Glu Phe Thr Ala Thr Gln
145                 150                 155                 160

Pro Glu Val Ala Asp Trp Ser Glu Gly Val Gln Val Pro Ser Val Pro
                165                 170                 175

Ile Gln Gln Phe Pro Thr Glu Asp Trp Ser Ala Gln Pro Ala Thr Glu
            180                 185                 190

Asp Trp Ser Ala Ala Pro Thr Ala Gln Ala Thr Glu Trp Val Gly Ala
        195                 200                 205

Thr Thr Asp Trp Ser
    210


<210> SEQ ID NO 11
<211> LENGTH: 316
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 11

Met Ala Thr Phe Val Glu Leu Ser Thr Lys Ala Lys Met Pro Ile Val
1               5                   10                  15

Gly Leu Gly Thr Trp Lys Ser Pro Leu Gly Lys Val Lys Glu Ala Val
            20                  25                  30

Lys Val Ala Ile Asp Ala Gly Tyr Arg His Ile Asp Cys Ala Tyr Val
        35                  40                  45

Tyr Gln Asn Glu His Glu Val Gly Glu Ala Ile Gln Glu Lys Ile Gln
    50                  55                  60

Glu Lys Ala Val Lys Arg Glu Asp Leu Phe Ile Val Ser Lys Leu Trp
65                  70                  75                  80

Pro Thr Phe Phe Glu Arg Pro Leu Val Arg Lys Ala Phe Glu Lys Thr
                85                  90                  95

Leu Lys Asp Leu Lys Leu Ser Tyr Leu Asp Val Tyr Leu Ile His Trp
            100                 105                 110

Pro Gln Gly Phe Lys Ser Gly Asp Asp Leu Phe Pro Lys Asp Asp Lys
        115                 120                 125

Gly Asn Ala Ile Gly Gly Lys Ala Thr Phe Leu Asp Ala Trp Glu Ala
    130                 135                 140

Met Glu Glu Leu Val Asp Glu Gly Leu Val Lys Ala Leu Gly Val Ser
145                 150                 155                 160
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Asn Phe Ser His Phe Gln Ile Glu Lys Leu Leu Asn Lys Pro Gly Leu
                165                 170                 175

Lys Tyr Lys Pro Val Thr Asn Gln Val Glu Cys His Pro Tyr Leu Thr
            180                 185                 190

Gln Glu Lys Leu Ile Gln Tyr Cys His Ser Lys Gly Ile Thr Val Thr
        195                 200                 205

Ala Tyr Ser Pro Leu Gly Ser Pro Asp Arg Pro Trp Ala Lys Pro Glu
    210                 215                 220

Asp Pro Ser Leu Leu Glu Asp Pro Lys Ile Lys Glu Ile Ala Ala Lys
225                 230                 235                 240

His Lys Lys Thr Ala Ala Gln Val Leu Ile Arg Phe His Ile Gln Arg
                245                 250                 255

Asn Val Ile Val Ile Pro Lys Ser Val Thr Pro Ala Arg Ile Val Glu
            260                 265                 270

Asn Ile Gln Val Phe Asp Phe Lys Leu Ser Asp Glu Glu Met Ala Thr
        275                 280                 285

Ile Leu Ser Phe Asn Arg Asn Trp Arg Ala Cys Asn Val Leu Gln Ser
    290                 295                 300

Ser His Leu Glu Asp Tyr Pro Phe Asp Ala Glu Tyr
305                 310                 315


<210> SEQ ID NO 12
<211> LENGTH: 430
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 12

Met Ala Leu Leu His Ser Gly Arg Val Leu Pro Gly Ile Ala Ala Ala
1               5                   10                  15

Phe His Pro Gly Leu Ala Ala Ala Ala Ser Ala Arg Ala Ser Ser Trp
            20                  25                  30

Trp Thr His Val Glu Met Gly Pro Pro Asp Pro Ile Leu Gly Val Thr
        35                  40                  45

Glu Ala Phe Lys Arg Asp Thr Asn Ser Lys Lys Met Asn Leu Gly Val
    50                  55                  60

Gly Ala Tyr Arg Asp Asp Asn Gly Lys Pro Tyr Val Leu Pro Ser Val
65                  70                  75                  80

Arg Lys Ala Glu Ala Gln Ile Ala Ala Lys Asn Leu Asp Lys Glu Tyr
                85                  90                  95

Leu Pro Ile Gly Gly Leu Ala Glu Phe Cys Lys Ala Ser Ala Glu Leu
            100                 105                 110

Ala Leu Gly Glu Asn Ser Glu Val Leu Lys Ser Gly Arg Phe Val Thr
        115                 120                 125

Val Gln Thr Ile Ser Gly Thr Gly Ala Leu Arg Ile Gly Ala Ser Phe
    130                 135                 140

Leu Gln Arg Phe Phe Lys Phe Ser Arg Asp Val Phe Leu Pro Lys Pro
145                 150                 155                 160

Thr Trp Gly Asn His Thr Pro Ile Phe Arg Asp Ala Gly Met Gln Leu
                165                 170                 175

Gln Gly Tyr Arg Tyr Tyr Asp Pro Lys Thr Cys Gly Phe Asp Phe Thr
            180                 185                 190

Gly Ala Val Glu Asp Ile Ser Lys Ile Pro Glu Gln Ser Val Leu Leu
        195                 200                 205
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Leu His Ala Cys Ala His Asn Pro Thr Gly Val Asp Pro Arg Pro Glu
    210                 215                 220

Gln Trp Lys Glu Ile Ala Thr Val Val Lys Lys Arg Asn Leu Phe Ala
225                 230                 235                 240

Phe Phe Asp Met Ala Tyr Gln Gly Phe Ala Ser Gly Asp Gly Asp Lys
                245                 250                 255

Asp Ala Trp Ala Val Arg His Phe Ile Glu Gln Gly Ile Asn Val Cys
            260                 265                 270

Leu Cys Gln Ser Tyr Ala Lys Asn Met Gly Leu Tyr Gly Glu Arg Val
        275                 280                 285

Gly Ala Phe Thr Met Val Cys Lys Asp Ala Asp Glu Ala Lys Arg Val
    290                 295                 300

Glu Ser Gln Leu Lys Ile Leu Ile Arg Pro Met Tyr Ser Asn Pro Pro
305                 310                 315                 320

Leu Asn Gly Ala Arg Ile Ala Ala Ala Ile Leu Asn Thr Pro Asp Leu
                325                 330                 335

Arg Lys Gln Trp Leu Gln Glu Val Lys Gly Met Ala Asp Arg Ile Ile
            340                 345                 350

Gly Met Arg Thr Gln Leu Val Ser Asn Leu Lys Lys Glu Gly Ser Thr
        355                 360                 365

His Asn Trp Gln His Ile Thr Asp Gln Ile Gly Met Phe Cys Phe Thr
    370                 375                 380

Gly Leu Lys Pro Glu Gln Val Glu Arg Leu Ile Lys Glu Phe Ser Ile
385                 390                 395                 400

Tyr Met Thr Lys Asp Gly Arg Ile Ser Val Ala Gly Val Thr Ser Ser
                405                 410                 415

Asn Val Gly Tyr Leu Ala His Ala Ile His Gln Val Thr Lys
            420                 425                 430


<210> SEQ ID NO 13
<211> LENGTH: 633
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 13

Met Ala Asn Gln Val Asn Gly Asn Ala Val Gln Leu Lys Glu Glu Glu
1               5                   10                  15

Glu Pro Met Asp Thr Ser Ser Val Thr His Thr Glu His Tyr Lys Thr
            20                  25                  30

Leu Ile Glu Ala Gly Leu Pro Gln Lys Val Ala Glu Arg Leu Asp Glu
        35                  40                  45

Ile Phe Gln Thr Gly Leu Val Ala Tyr Val Asp Leu Asp Glu Arg Ala
    50                  55                  60

Ile Asp Ala Leu Arg Glu Phe Asn Glu Glu Gly Ala Leu Ser Val Leu
65                  70                  75                  80

Gln Gln Phe Lys Glu Ser Asp Leu Ser His Val Gln Asn Lys Ser Ala
                85                  90                  95

Phe Leu Cys Gly Val Met Lys Thr Tyr Arg Gln Arg Glu Lys Gln Gly
            100                 105                 110

Ser Lys Val Gln Glu Ser Thr Lys Gly Pro Asp Glu Ala Lys Ile Lys
        115                 120                 125

Ala Leu Leu Glu Arg Thr Gly Tyr Thr Leu Asp Val Thr Thr Gly Gln
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    130                 135                 140

Arg Lys Tyr Gly Gly Pro Pro Pro Asp Ser Val Tyr Ser Gly Val Gln
145                 150                 155                 160

Pro Gly Ile Gly Thr Glu Val Phe Val Gly Lys Ile Pro Arg Asp Leu
                165                 170                 175

Tyr Glu Asp Glu Leu Val Pro Leu Phe Glu Lys Ala Gly Pro Ile Trp
            180                 185                 190

Asp Leu Arg Leu Met Met Asp Pro Leu Ser Gly Gln Asn Arg Gly Tyr
        195                 200                 205

Ala Phe Ile Thr Phe Cys Gly Lys Glu Ala Ala Gln Glu Ala Val Lys
    210                 215                 220

Leu Cys Asp Ser Tyr Glu Ile Arg Pro Gly Lys His Leu Gly Val Cys
225                 230                 235                 240

Ile Ser Val Ala Asn Asn Arg Leu Phe Val Gly Ser Ile Pro Lys Asn
                245                 250                 255

Lys Thr Lys Glu Asn Ile Leu Glu Glu Phe Ser Lys Val Thr Glu Gly
            260                 265                 270

Leu Val Asp Val Ile Leu Tyr His Gln Pro Asp Asp Lys Lys Lys Asn
        275                 280                 285

Arg Gly Phe Cys Phe Leu Glu Tyr Glu Asp His Lys Ser Ala Ala Gln
    290                 295                 300

Ala Arg Arg Arg Leu Met Ser Gly Lys Val Lys Val Trp Gly Asn Val
305                 310                 315                 320

Val Thr Val Glu Trp Ala Asp Pro Val Glu Glu Pro Asp Pro Glu Val
                325                 330                 335

Met Ala Lys Val Lys Val Leu Phe Val Arg Asn Leu Ala Thr Thr Val
            340                 345                 350

Thr Glu Glu Ile Leu Glu Lys Ser Phe Ser Glu Phe Gly Lys Leu Glu
        355                 360                 365

Arg Val Lys Lys Leu Lys Asp Tyr Ala Phe Val His Phe Glu Asp Arg
    370                 375                 380

Gly Ala Ala Val Lys Ala Met Asp Glu Met Asn Gly Lys Glu Ile Glu
385                 390                 395                 400

Gly Glu Glu Ile Glu Ile Val Leu Ala Lys Pro Pro Asp Lys Lys Arg
                405                 410                 415

Lys Glu Arg Gln Ala Ala Arg Gln Ala Ser Arg Ser Thr Ala Tyr Glu
            420                 425                 430

Asp Tyr Tyr Tyr His Pro Pro Pro Arg Met Pro Pro Pro Ile Arg Gly
        435                 440                 445

Arg Gly Arg Gly Gly Gly Arg Gly Gly Tyr Gly Tyr Pro Pro Asp Tyr
    450                 455                 460

Tyr Gly Tyr Glu Asp Tyr Tyr Asp Asp Tyr Tyr Gly Tyr Asp Tyr His
465                 470                 475                 480

Asp Tyr Arg Gly Gly Tyr Glu Asp Pro Tyr Tyr Gly Tyr Asp Asp Gly
                485                 490                 495

Tyr Ala Val Arg Gly Arg Gly Gly Gly Arg Gly Gly Arg Gly Ala Pro
            500                 505                 510

Pro Pro Pro Arg Gly Arg Gly Ala Pro Pro Pro Arg Gly Arg Ala Gly
        515                 520                 525

Tyr Ser Gln Arg Gly Ala Pro Leu Gly Pro Pro Arg Gly Ser Arg Gly
    530                 535                 540
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Gly Arg Gly Gly Pro Ala Gln Gln Gln Arg Gly Arg Gly Ser Arg Gly
545                 550                 555                 560

Ser Arg Gly Asn Arg Gly Gly Asn Val Gly Gly Lys Arg Lys Ala Asp
                565                 570                 575

Gly Tyr Asn Gln Pro Asp Ser Lys Arg Arg Gln Thr Asn Asn Gln Gln
            580                 585                 590

Asn Trp Gly Ser Gln Pro Ile Ala Gln Gln Pro Leu Gln Gln Gly Gly
        595                 600                 605

Asp Tyr Ser Gly Asn Tyr Gly Tyr Asn Asn Asp Asn Gln Glu Phe Tyr
    610                 615                 620

Gln Asp Thr Tyr Gly Gln Gln Trp Lys
625                 630


<210> SEQ ID NO 14
<211> LENGTH: 505
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 14

Met Arg Leu Arg Arg Leu Ala Leu Phe Pro Gly Val Ala Leu Leu Leu
1               5                   10                  15

Ala Ala Ala Arg Leu Ala Ala Ala Ser Asp Val Leu Glu Leu Thr Asp
            20                  25                  30

Asp Asn Phe Glu Ser Arg Ile Ser Asp Thr Gly Ser Ala Gly Leu Met
        35                  40                  45

Leu Val Glu Phe Phe Ala Pro Trp Cys Gly His Cys Lys Arg Leu Ala
    50                  55                  60

Pro Glu Tyr Glu Ala Ala Ala Thr Arg Leu Lys Gly Ile Val Pro Leu
65                  70                  75                  80

Ala Lys Val Asp Cys Thr Ala Asn Thr Asn Thr Cys Asn Lys Tyr Gly
                85                  90                  95

Val Ser Gly Tyr Pro Thr Leu Lys Ile Phe Arg Asp Gly Glu Glu Ala
            100                 105                 110

Gly Ala Tyr Asp Gly Pro Arg Thr Ala Asp Gly Ile Val Ser His Leu
        115                 120                 125

Lys Lys Gln Ala Gly Pro Ala Ser Val Pro Leu Arg Thr Glu Glu Glu
    130                 135                 140

Phe Lys Lys Phe Ile Ser Asp Lys Asp Ala Ser Ile Val Gly Phe Phe
145                 150                 155                 160

Asp Asp Ser Phe Ser Glu Ala His Ser Glu Phe Leu Lys Ala Ala Ser
                165                 170                 175

Asn Leu Arg Asp Asn Tyr Arg Phe Ala His Thr Asn Val Glu Ser Leu
            180                 185                 190

Val Asn Glu Tyr Asp Asp Asn Gly Glu Gly Ile Ile Leu Phe Arg Pro
        195                 200                 205

Ser His Leu Thr Asn Lys Phe Glu Asp Lys Thr Val Ala Tyr Thr Glu
    210                 215                 220

Gln Lys Met Thr Ser Gly Lys Ile Lys Lys Phe Ile Gln Glu Asn Ile
225                 230                 235                 240

Phe Gly Ile Cys Pro His Met Thr Glu Asp Asn Lys Asp Leu Ile Gln
                245                 250                 255

Gly Lys Asp Leu Leu Ile Ala Tyr Tyr Asp Val Asp Tyr Glu Lys Asn
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           260                 265                 270

Ala Lys Gly Ser Asn Tyr Trp Arg Asn Arg Val Met Met Val Ala Lys
        275                 280                 285

Lys Phe Leu Asp Ala Gly His Lys Leu Asn Phe Ala Val Ala Ser Arg
    290                 295                 300

Lys Thr Phe Ser His Glu Leu Ser Asp Phe Gly Leu Glu Ser Thr Ala
305                 310                 315                 320

Gly Glu Ile Pro Val Val Ala Ile Arg Thr Ala Lys Gly Glu Lys Phe
                325                 330                 335

Val Met Gln Glu Glu Phe Ser Arg Asp Gly Lys Ala Leu Glu Arg Phe
            340                 345                 350

Leu Gln Asp Tyr Phe Asp Gly Asn Leu Lys Arg Tyr Leu Lys Ser Glu
        355                 360                 365

Pro Ile Pro Glu Ser Asn Asp Gly Pro Val Lys Val Val Val Ala Glu
    370                 375                 380

Asn Phe Asp Glu Ile Val Asn Asn Glu Asn Lys Asp Val Leu Ile Glu
385                 390                 395                 400

Phe Tyr Ala Pro Trp Cys Gly His Cys Lys Asn Leu Glu Pro Lys Tyr
                405                 410                 415

Lys Glu Leu Gly Glu Lys Leu Ser Lys Asp Pro Asn Ile Val Ile Ala
            420                 425                 430

Lys Met Asp Ala Thr Ala Asn Asp Val Pro Ser Pro Tyr Glu Val Arg
        435                 440                 445

Gly Phe Pro Thr Ile Tyr Phe Ser Pro Ala Asn Lys Lys Leu Asn Pro
    450                 455                 460

Lys Lys Tyr Glu Gly Gly Arg Glu Leu Ser Asp Phe Ile Ser Tyr Leu
465                 470                 475                 480

Gln Arg Glu Ala Thr Asn Pro Pro Val Ile Gln Glu Glu Lys Pro Lys
                485                 490                 495

Lys Lys Lys Lys Ala Gln Glu Asp Leu
            500                 505


<210> SEQ ID NO 15
<211> LENGTH: 152
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 15

Met Ala Asn Leu Glu Arg Thr Phe Ile Ala Ile Lys Pro Asp Gly Val
1               5                   10                  15

Gln Arg Gly Leu Val Gly Glu Ile Ile Lys Arg Phe Glu Gln Lys Gly
            20                  25                  30

Phe Arg Leu Val Ala Met Lys Phe Leu Arg Ala Ser Glu Glu His Leu
        35                  40                  45

Lys Gln His Tyr Ile Asp Leu Lys Asp Arg Pro Phe Phe Pro Gly Leu
    50                  55                  60

Val Lys Tyr Met Asn Ser Gly Pro Val Val Ala Met Val Trp Glu Gly
65                  70                  75                  80

Leu Asn Val Val Lys Thr Gly Arg Val Met Leu Gly Glu Thr Asn Pro
                85                  90                  95

Ala Asp Ser Lys Pro Gly Thr Ile Arg Gly Asp Phe Cys Ile Gln Val
            100                 105                 110
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Gly Arg Asn Ile Ile His Gly Ser Asp Ser Val Lys Ser Ala Glu Lys
        115                 120                 125

Glu Ile Ser Leu Trp Phe Lys Pro Glu Glu Leu Val Asp Tyr Lys Ser
    130                 135                 140

Cys Ala His Asp Trp Val Tyr Glu
145                 150


<210> SEQ ID NO 16
<211> LENGTH: 1192
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 16

Met Glu Asp Leu Asp Gln Ser Pro Leu Val Ser Ser Ser Asp Ser Pro
1               5                   10                  15

Pro Arg Pro Gln Pro Ala Phe Lys Tyr Gln Phe Val Arg Glu Pro Glu
            20                  25                  30

Asp Glu Glu Glu Glu Glu Glu Glu Glu Glu Glu Asp Glu Asp Glu Asp
        35                  40                  45

Leu Glu Glu Leu Glu Val Leu Glu Arg Lys Pro Ala Ala Gly Leu Ser
    50                  55                  60

Ala Ala Pro Val Pro Thr Ala Pro Ala Ala Gly Ala Pro Leu Met Asp
65                  70                  75                  80

Phe Gly Asn Asp Phe Val Pro Pro Ala Pro Arg Gly Pro Leu Pro Ala
                85                  90                  95

Ala Pro Pro Val Ala Pro Glu Arg Gln Pro Ser Trp Asp Pro Ser Pro
            100                 105                 110

Val Ser Ser Thr Val Pro Ala Pro Ser Pro Leu Ser Ala Ala Ala Val
        115                 120                 125

Ser Pro Ser Lys Leu Pro Glu Asp Asp Glu Pro Pro Ala Arg Pro Pro
    130                 135                 140

Pro Pro Pro Pro Ala Ser Val Ser Pro Gln Ala Glu Pro Val Trp Thr
145                 150                 155                 160

Pro Pro Ala Pro Ala Pro Ala Ala Pro Pro Ser Thr Pro Ala Ala Pro
                165                 170                 175

Lys Arg Arg Gly Ser Ser Gly Ser Val Asp Glu Thr Leu Phe Ala Leu
            180                 185                 190

Pro Ala Ala Ser Glu Pro Val Ile Arg Ser Ser Ala Glu Asn Met Asp
        195                 200                 205

Leu Lys Glu Gln Pro Gly Asn Thr Ile Ser Ala Gly Gln Glu Asp Phe
    210                 215                 220

Pro Ser Val Leu Leu Glu Thr Ala Ala Ser Leu Pro Ser Leu Ser Pro
225                 230                 235                 240

Leu Ser Ala Ala Ser Phe Lys Glu His Glu Tyr Leu Gly Asn Leu Ser
                245                 250                 255

Thr Val Leu Pro Thr Glu Gly Thr Leu Gln Glu Asn Val Ser Glu Ala
            260                 265                 270

Ser Lys Glu Val Ser Glu Lys Ala Lys Thr Leu Leu Ile Asp Arg Asp
        275                 280                 285

Leu Thr Glu Phe Ser Glu Leu Glu Tyr Ser Glu Met Gly Ser Ser Phe
    290                 295                 300

Ser Val Ser Pro Lys Ala Glu Ser Ala Val Ile Val Ala Asn Pro Arg
305                 310                 315                 320
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Glu Glu Ile Ile Val Lys Asn Lys Asp Glu Glu Glu Lys Leu Val Ser
                325                 330                 335

Asn Asn Ile Leu His Asn Gln Gln Glu Leu Pro Thr Ala Leu Thr Lys
            340                 345                 350

Leu Val Lys Glu Asp Glu Val Val Ser Ser Glu Lys Ala Lys Asp Ser
        355                 360                 365

Phe Asn Glu Lys Arg Val Ala Val Glu Ala Pro Met Arg Glu Glu Tyr
    370                 375                 380

Ala Asp Phe Lys Pro Phe Glu Arg Val Trp Glu Val Lys Asp Ser Lys
385                 390                 395                 400

Glu Asp Ser Asp Met Leu Ala Ala Gly Gly Lys Ile Glu Ser Asn Leu
                405                 410                 415

Glu Ser Lys Val Asp Lys Lys Cys Phe Ala Asp Ser Leu Glu Gln Thr
            420                 425                 430

Asn His Glu Lys Asp Ser Glu Ser Ser Asn Asp Asp Thr Ser Phe Pro
        435                 440                 445

Ser Thr Pro Glu Gly Ile Lys Asp Arg Ser Gly Ala Tyr Ile Thr Cys
    450                 455                 460

Ala Pro Phe Asn Pro Ala Ala Thr Glu Ser Ile Ala Thr Asn Ile Phe
465                 470                 475                 480

Pro Leu Leu Gly Asp Pro Thr Ser Glu Asn Lys Thr Asp Glu Lys Lys
                485                 490                 495

Ile Glu Glu Lys Lys Ala Gln Ile Val Thr Glu Lys Asn Thr Ser Thr
            500                 505                 510

Lys Thr Ser Asn Pro Phe Leu Val Ala Ala Gln Asp Ser Glu Thr Asp
        515                 520                 525

Tyr Val Thr Thr Asp Asn Leu Thr Lys Val Thr Glu Glu Val Val Ala
    530                 535                 540

Asn Met Pro Glu Gly Leu Thr Pro Asp Leu Val Gln Glu Ala Cys Glu
545                 550                 555                 560

Ser Glu Leu Asn Glu Val Thr Gly Thr Lys Ile Ala Tyr Glu Thr Lys
                565                 570                 575

Met Asp Leu Val Gln Thr Ser Glu Val Met Gln Glu Ser Leu Tyr Pro
            580                 585                 590

Ala Ala Gln Leu Cys Pro Ser Phe Glu Glu Ser Glu Ala Thr Pro Ser
        595                 600                 605

Pro Val Leu Pro Asp Ile Val Met Glu Ala Pro Leu Asn Ser Ala Val
    610                 615                 620

Pro Ser Ala Gly Ala Ser Val Ile Gln Pro Ser Ser Ser Pro Leu Glu
625                 630                 635                 640

Ala Ser Ser Val Asn Tyr Glu Ser Ile Lys His Glu Pro Glu Asn Pro
                645                 650                 655

Pro Pro Tyr Glu Glu Ala Met Ser Val Ser Leu Lys Lys Val Ser Gly
            660                 665                 670

Ile Lys Glu Glu Ile Lys Glu Pro Glu Asn Ile Asn Ala Ala Leu Gln
        675                 680                 685

Glu Thr Glu Ala Pro Tyr Ile Ser Ile Ala Cys Asp Leu Ile Lys Glu
    690                 695                 700

Thr Lys Leu Ser Ala Glu Pro Ala Pro Asp Phe Ser Asp Tyr Ser Glu
705                 710                 715                 720
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Met Ala Lys Val Glu Gln Pro Val Pro Asp His Ser Glu Leu Val Glu
                725                 730                 735

Asp Ser Ser Pro Asp Ser Glu Pro Val Asp Leu Phe Ser Asp Asp Ser
            740                 745                 750

Ile Pro Asp Val Pro Gln Lys Gln Asp Glu Thr Val Met Leu Val Lys
        755                 760                 765

Glu Ser Leu Thr Glu Thr Ser Phe Glu Ser Met Ile Glu Tyr Glu Asn
    770                 775                 780

Lys Glu Lys Leu Ser Ala Leu Pro Pro Glu Gly Gly Lys Pro Tyr Leu
785                 790                 795                 800

Glu Ser Phe Lys Leu Ser Leu Asp Asn Thr Lys Asp Thr Leu Leu Pro
                805                 810                 815

Asp Glu Val Ser Thr Leu Ser Lys Lys Glu Lys Ile Pro Leu Gln Met
            820                 825                 830

Glu Glu Leu Ser Thr Ala Val Tyr Ser Asn Asp Asp Leu Phe Ile Ser
        835                 840                 845

Lys Glu Ala Gln Ile Arg Glu Thr Glu Thr Phe Ser Asp Ser Ser Pro
    850                 855                 860

Ile Glu Ile Ile Asp Glu Phe Pro Thr Leu Ile Ser Ser Lys Thr Asp
865                 870                 875                 880

Ser Phe Ser Lys Leu Ala Arg Glu Tyr Thr Asp Leu Glu Val Ser His
                885                 890                 895

Lys Ser Glu Ile Ala Asn Ala Pro Asp Gly Ala Gly Ser Leu Pro Cys
            900                 905                 910

Thr Glu Leu Pro His Asp Leu Ser Leu Lys Asn Ile Gln Pro Lys Val
        915                 920                 925

Glu Glu Lys Ile Ser Phe Ser Asp Asp Phe Ser Lys Asn Gly Ser Ala
    930                 935                 940

Thr Ser Lys Val Leu Leu Leu Pro Pro Asp Val Ser Ala Leu Ala Thr
945                 950                 955                 960

Gln Ala Glu Ile Glu Ser Ile Val Lys Pro Lys Val Leu Val Lys Glu
                965                 970                 975

Ala Glu Lys Lys Leu Pro Ser Asp Thr Glu Lys Glu Asp Arg Ser Pro
            980                 985                 990

Ser Ala Ile Phe Ser Ala Glu Leu Ser Lys Thr Ser Val Val Asp Leu
        995                 1000                1005

Leu Tyr Trp Arg Asp Ile Lys Lys Thr Gly Val Val Phe Gly Ala Ser
    1010                1015                1020

Leu Phe Leu Leu Leu Ser Leu Thr Val Phe Ser Ile Val Ser Val Thr
1025                1030                1035                1040

Ala Tyr Ile Ala Leu Ala Leu Leu Ser Val Thr Ile Ser Phe Arg Ile
                1045                1050                1055

Tyr Lys Gly Val Ile Gln Ala Ile Gln Lys Ser Asp Glu Gly His Pro
            1060                1065                1070

Phe Arg Ala Tyr Leu Glu Ser Glu Val Ala Ile Ser Glu Glu Leu Val
        1075                1080                1085

Gln Lys Tyr Ser Asn Ser Ala Leu Gly His Val Asn Cys Thr Ile Lys
    1090                1095                1100

Glu Leu Arg Arg Leu Phe Leu Val Asp Asp Leu Val Asp Ser Leu Lys
1105                1110                1115                1120

Phe Ala Val Leu Met Trp Val Phe Thr Tyr Val Gly Ala Leu Phe Asn
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               1125                1130                1135

Gly Leu Thr Leu Leu Ile Leu Ala Leu Ile Ser Leu Phe Ser Val Pro
            1140                1145                1150

Val Ile Tyr Glu Arg His Gln Ala Gln Ile Asp His Tyr Leu Gly Leu
        1155                1160                1165

Ala Asn Lys Asn Val Lys Asp Ala Met Ala Lys Ile Gln Ala Lys Ile
    1170                1175                1180

Pro Gly Leu Lys Arg Lys Ala Glu
1185                1190


<210> SEQ ID NO 17
<211> LENGTH: 213
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 17

Met Ser Val Glu Leu Glu Glu Ala Leu Pro Val Thr Thr Ala Glu Gly
1               5                   10                  15

Met Ala Lys Lys Val Thr Lys Ala Gly Gly Ser Ala Ala Leu Ser Pro
            20                  25                  30

Ser Lys Lys Arg Lys Asn Ser Lys Lys Lys Asn Gln Pro Gly Lys Tyr
        35                  40                  45

Ser Gln Leu Val Val Glu Thr Ile Arg Arg Leu Gly Glu Arg Asn Gly
    50                  55                  60

Ser Ser Leu Ala Lys Ile Tyr Thr Glu Ala Lys Lys Val Pro Trp Phe
65                  70                  75                  80

Asp Gln Gln Asn Gly Arg Thr Tyr Leu Lys Tyr Ser Ile Lys Ala Leu
                85                  90                  95

Val Gln Asn Asp Thr Leu Leu Gln Val Lys Gly Thr Gly Ala Asn Gly
            100                 105                 110

Ser Phe Lys Leu Asn Arg Lys Lys Leu Glu Gly Gly Gly Glu Arg Arg
        115                 120                 125

Gly Ala Pro Ala Ala Ala Thr Ala Pro Ala Pro Thr Ala His Lys Ala
    130                 135                 140

Lys Lys Ala Ala Pro Gly Ala Ala Gly Ser Arg Arg Ala Asp Lys Lys
145                 150                 155                 160

Pro Ala Arg Gly Gln Lys Pro Glu Gln Arg Ser His Lys Lys Gly Ala
                165                 170                 175

Gly Ala Lys Lys Asp Lys Gly Gly Lys Ala Lys Lys Thr Ala Ala Ala
            180                 185                 190

Gly Gly Lys Lys Val Lys Lys Ala Ala Lys Pro Ser Val Pro Lys Val
        195                 200                 205

Pro Lys Gly Arg Lys
    210


<210> SEQ ID NO 18
<211> LENGTH: 466
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 18

Met Val Met Glu Lys Pro Ser Pro Leu Leu Val Gly Arg Glu Phe Val
1               5                   10                  15

Arg Gln Tyr Tyr Thr Leu Leu Asn Gln Ala Pro Asp Met Leu His Arg
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           20                  25                  30

Phe Tyr Gly Lys Asn Ser Ser Tyr Val His Gly Gly Leu Asp Ser Asn
        35                  40                  45

Gly Lys Pro Ala Asp Ala Val Tyr Gly Gln Lys Glu Ile His Arg Lys
    50                  55                  60

Val Met Ser Gln Asn Phe Thr Asn Cys His Thr Lys Ile Arg His Val
65                  70                  75                  80

Asp Ala His Ala Thr Leu Asn Asp Gly Val Val Val Gln Val Met Gly
                85                  90                  95

Leu Leu Ser Asn Asn Asn Gln Ala Leu Arg Arg Phe Met Gln Thr Phe
            100                 105                 110

Val Leu Ala Pro Glu Gly Ser Val Ala Asn Lys Phe Tyr Val His Asn
        115                 120                 125

Asp Ile Phe Arg Tyr Gln Asp Glu Val Phe Gly Gly Phe Val Thr Glu
    130                 135                 140

Pro Gln Glu Glu Ser Glu Glu Glu Val Glu Glu Pro Glu Glu Arg Gln
145                 150                 155                 160

Gln Thr Pro Glu Val Val Pro Asp Asp Ser Gly Thr Phe Tyr Asp Gln
                165                 170                 175

Ala Val Val Ser Asn Asp Met Glu Glu His Leu Glu Glu Pro Val Ala
            180                 185                 190

Glu Pro Glu Pro Asp Pro Glu Pro Glu Pro Glu Gln Glu Pro Val Ser
        195                 200                 205

Glu Ile Gln Glu Glu Lys Pro Glu Pro Val Leu Glu Glu Thr Ala Pro
    210                 215                 220

Glu Asp Ala Gln Lys Ser Ser Ser Pro Ala Pro Ala Asp Ile Ala Gln
225                 230                 235                 240

Thr Val Gln Glu Asp Leu Arg Thr Phe Ser Trp Ala Ser Val Thr Ser
                245                 250                 255

Lys Asn Leu Pro Pro Ser Gly Ala Val Pro Val Thr Gly Ile Pro Pro
            260                 265                 270

His Val Val Lys Val Pro Ala Ser Gln Pro Arg Pro Glu Ser Lys Pro
        275                 280                 285

Glu Ser Gln Ile Pro Pro Gln Arg Pro Gln Arg Asp Gln Arg Val Arg
    290                 295                 300

Glu Gln Arg Ile Asn Ile Pro Pro Gln Arg Gly Pro Arg Pro Ile Arg
305                 310                 315                 320

Glu Ala Gly Glu Gln Gly Asp Ile Glu Pro Arg Arg Met Val Arg His
                325                 330                 335

Pro Asp Ser His Gln Leu Phe Ile Gly Asn Leu Pro His Glu Val Asp
            340                 345                 350

Lys Ser Glu Leu Lys Asp Phe Phe Gln Ser Tyr Gly Asn Val Val Glu
        355                 360                 365

Leu Arg Ile Asn Ser Gly Gly Lys Leu Pro Asn Phe Gly Phe Val Val
    370                 375                 380

Phe Asp Asp Ser Glu Pro Val Gln Lys Val Leu Ser Asn Arg Pro Ile
385                 390                 395                 400

Met Phe Arg Gly Glu Val Arg Leu Asn Val Glu Glu Lys Lys Thr Arg
                405                 410                 415

Ala Ala Arg Glu Gly Asp Arg Arg Asp Asn Arg Leu Arg Gly Pro Gly
            420                 425                 430
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Gly Pro Arg Gly Gly Leu Gly Gly Gly Met Arg Gly Pro Pro Arg Gly
        435                 440                 445

Gly Met Val Gln Lys Pro Gly Phe Gly Val Gly Arg Gly Leu Ala Pro
    450                 455                 460

Arg Gln
465


<210> SEQ ID NO 19
<211> LENGTH: 732
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 19

Met Pro Glu Glu Thr Gln Thr Gln Asp Gln Pro Met Glu Glu Glu Glu
1               5                   10                  15

Val Glu Thr Phe Ala Phe Gln Ala Glu Ile Ala Gln Leu Met Ser Leu
            20                  25                  30

Ile Ile Asn Thr Phe Tyr Ser Asn Lys Glu Ile Phe Leu Arg Glu Leu
        35                  40                  45

Ile Ser Asn Ser Ser Asp Ala Leu Asp Lys Ile Arg Tyr Glu Ser Leu
    50                  55                  60

Thr Asp Pro Ser Lys Leu Asp Ser Gly Lys Glu Leu His Ile Asn Leu
65                  70                  75                  80

Ile Pro Asn Lys Gln Asp Arg Thr Leu Thr Ile Val Asp Thr Gly Ile
                85                  90                  95

Gly Met Thr Lys Ala Asp Leu Ile Asn Asn Leu Gly Thr Ile Ala Lys
            100                 105                 110

Ser Gly Thr Lys Ala Phe Met Glu Ala Leu Gln Ala Gly Ala Asp Ile
        115                 120                 125

Ser Met Ile Gly Gln Phe Gly Val Gly Phe Tyr Ser Ala Tyr Leu Val
    130                 135                 140

Ala Glu Lys Val Thr Val Ile Thr Lys His Asn Asp Asp Glu Gln Tyr
145                 150                 155                 160

Ala Trp Glu Ser Ser Ala Gly Gly Ser Phe Thr Val Arg Thr Asp Thr
                165                 170                 175

Gly Glu Pro Met Gly Arg Gly Thr Lys Val Ile Leu His Leu Lys Glu
            180                 185                 190

Asp Gln Thr Glu Tyr Leu Glu Glu Arg Arg Ile Lys Glu Ile Val Lys
        195                 200                 205

Lys His Ser Gln Phe Ile Gly Tyr Pro Ile Thr Leu Phe Val Glu Lys
    210                 215                 220

Glu Arg Asp Lys Glu Val Ser Asp Asp Glu Ala Glu Glu Lys Glu Asp
225                 230                 235                 240

Lys Glu Glu Glu Lys Glu Lys Glu Glu Lys Glu Ser Glu Asp Lys Pro
                245                 250                 255

Glu Ile Glu Asp Val Gly Ser Asp Glu Glu Glu Glu Lys Lys Asp Gly
            260                 265                 270

Asp Lys Lys Lys Lys Lys Lys Ile Lys Glu Lys Tyr Ile Asp Gln Glu
        275                 280                 285

Glu Leu Asn Lys Thr Lys Pro Ile Trp Thr Arg Asn Pro Asp Asp Ile
    290                 295                 300

Thr Asn Glu Glu Tyr Gly Glu Phe Tyr Lys Ser Leu Thr Asn Asp Trp
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305                 310                 315                 320

Glu Asp His Leu Ala Val Lys His Phe Ser Val Glu Gly Gln Leu Glu
                325                 330                 335

Phe Arg Ala Leu Leu Phe Val Pro Arg Arg Ala Pro Phe Asp Leu Phe
            340                 345                 350

Glu Asn Arg Lys Lys Lys Asn Asn Ile Lys Leu Tyr Val Arg Arg Val
        355                 360                 365

Phe Ile Met Asp Asn Cys Glu Glu Leu Ile Pro Glu Tyr Leu Asn Phe
    370                 375                 380

Ile Arg Gly Val Val Asp Ser Glu Asp Leu Pro Leu Asn Ile Ser Arg
385                 390                 395                 400

Glu Met Leu Gln Gln Ser Lys Ile Leu Lys Val Ile Arg Lys Asn Leu
                405                 410                 415

Val Lys Lys Cys Leu Glu Leu Phe Thr Glu Leu Ala Glu Asp Lys Glu
            420                 425                 430

Asn Tyr Lys Lys Phe Tyr Glu Gln Phe Ser Lys Asn Ile Lys Leu Gly
        435                 440                 445

Ile His Glu Asp Ser Gln Asn Arg Lys Lys Leu Ser Glu Leu Leu Arg
    450                 455                 460

Tyr Tyr Thr Ser Ala Ser Gly Asp Glu Met Val Ser Leu Lys Asp Tyr
465                 470                 475                 480

Cys Thr Arg Met Lys Glu Asn Gln Lys His Ile Tyr Tyr Ile Thr Gly
                485                 490                 495

Glu Thr Lys Asp Gln Val Ala Asn Ser Ala Phe Val Glu Arg Leu Arg
            500                 505                 510

Lys His Gly Leu Glu Val Ile Tyr Met Ile Glu Pro Ile Asp Glu Tyr
        515                 520                 525

Cys Val Gln Gln Leu Lys Glu Phe Glu Gly Lys Thr Leu Val Ser Val
    530                 535                 540

Thr Lys Glu Gly Leu Glu Leu Pro Glu Asp Glu Glu Glu Lys Lys Lys
545                 550                 555                 560

Gln Glu Glu Lys Lys Thr Lys Phe Glu Asn Leu Cys Lys Ile Met Lys
                565                 570                 575

Asp Ile Leu Glu Lys Lys Val Glu Lys Val Val Val Ser Asn Arg Leu
            580                 585                 590

Val Thr Ser Pro Cys Cys Ile Val Thr Ser Thr Tyr Gly Trp Thr Ala
        595                 600                 605

Asn Met Glu Arg Ile Met Lys Ala Gln Ala Leu Arg Asp Asn Ser Thr
    610                 615                 620

Met Gly Tyr Met Ala Ala Lys Lys His Leu Glu Ile Asn Pro Asp His
625                 630                 635                 640

Ser Ile Ile Glu Thr Leu Arg Gln Lys Ala Glu Ala Asp Lys Asn Asp
                645                 650                 655

Lys Ser Val Lys Asp Leu Val Ile Leu Leu Tyr Glu Thr Ala Leu Leu
            660                 665                 670

Ser Ser Gly Phe Ser Leu Glu Asp Pro Gln Thr His Ala Asn Arg Ile
        675                 680                 685

Tyr Arg Met Ile Lys Leu Gly Leu Gly Ile Asp Glu Asp Asp Pro Thr
    690                 695                 700

Ala Asp Asp Thr Ser Ala Ala Val Thr Glu Glu Met Pro Pro Leu Glu
705                 710                 715                 720
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Gly Asp Asp Asp Thr Ser Arg Met Glu Glu Val Asp
                725                 730


<210> SEQ ID NO 20
<211> LENGTH: 911
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 20

Met Val Asp Tyr His Ala Ala Asn Gln Ser Tyr Gln Tyr Gly Pro Ser
1               5                   10                  15

Ser Ala Gly Asn Gly Ala Gly Gly Gly Gly Ser Met Gly Asp Tyr Met
            20                  25                  30

Ala Gln Glu Asp Asp Trp Asp Arg Asp Leu Leu Leu Asp Pro Ala Trp
        35                  40                  45

Glu Lys Gln Gln Arg Lys Thr Phe Thr Ala Trp Cys Asn Ser His Leu
    50                  55                  60

Arg Lys Ala Gly Thr Gln Ile Glu Asn Ile Asp Glu Asp Phe Arg Asp
65                  70                  75                  80

Gly Leu Lys Leu Met Leu Leu Leu Glu Val Ile Ser Gly Glu Arg Leu
                85                  90                  95

Pro Lys Pro Glu Arg Gly Lys Met Arg Val His Lys Ile Asn Asn Val
            100                 105                 110

Asn Lys Ala Leu Asp Phe Ile Ala Ser Lys Gly Val Lys Leu Val Ser
        115                 120                 125

Ile Gly Ala Glu Glu Ile Val Asp Gly Asn Ala Lys Met Thr Leu Gly
    130                 135                 140

Met Ile Trp Thr Ile Ile Leu Arg Phe Ala Ile Gln Asp Ile Ser Val
145                 150                 155                 160

Glu Glu Thr Ser Ala Lys Glu Gly Leu Leu Leu Trp Cys Gln Arg Lys
                165                 170                 175

Thr Ala Pro Tyr Lys Asn Val Asn Val Gln Asn Phe His Ile Ser Trp
            180                 185                 190

Lys Asp Gly Leu Ala Phe Asn Ala Leu Ile His Arg His Arg Pro Glu
        195                 200                 205

Leu Ile Glu Tyr Asp Lys Leu Arg Lys Asp Asp Pro Val Thr Asn Leu
    210                 215                 220

Asn Asn Ala Phe Glu Val Ala Glu Lys Tyr Leu Asp Ile Pro Lys Met
225                 230                 235                 240

Leu Asp Ala Glu Asp Ile Val Asn Thr Ala Arg Pro Asp Glu Lys Ala
                245                 250                 255

Ile Met Thr Tyr Val Ser Ser Phe Tyr His Ala Phe Ser Gly Ala Gln
            260                 265                 270

Lys Ala Glu Thr Ala Ala Asn Arg Ile Cys Lys Val Leu Ala Val Asn
        275                 280                 285

Gln Glu Asn Glu His Leu Met Glu Asp Tyr Glu Lys Leu Ala Ser Asp
    290                 295                 300

Leu Leu Glu Trp Ile Arg Arg Thr Ile Pro Trp Leu Glu Asp Arg Val
305                 310                 315                 320

Pro Gln Lys Thr Ile Gln Glu Met Gln Gln Lys Leu Glu Asp Phe Arg
                325                 330                 335

Asp Tyr Arg Arg Val His Lys Pro Pro Lys Val Gln Glu Lys Cys Gln
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           340                 345                 350

Leu Glu Ile Asn Phe Asn Thr Leu Gln Thr Lys Leu Arg Leu Ser Asn
        355                 360                 365

Arg Pro Ala Phe Met Pro Ser Glu Gly Lys Met Val Ser Asp Ile Asn
    370                 375                 380

Asn Gly Trp Gln His Leu Glu Gln Ala Glu Lys Gly Tyr Glu Glu Trp
385                 390                 395                 400

Leu Leu Asn Glu Ile Arg Arg Leu Glu Arg Leu Asp His Leu Ala Glu
                405                 410                 415

Lys Phe Arg Gln Lys Ala Ser Ile His Glu Ala Trp Thr Asp Gly Lys
            420                 425                 430

Glu Ala Met Leu Lys His Arg Asp Tyr Glu Thr Ala Thr Leu Ser Asp
        435                 440                 445

Ile Lys Ala Leu Ile Arg Lys His Glu Ala Phe Glu Ser Asp Leu Ala
    450                 455                 460

Ala His Gln Asp Arg Val Glu Gln Ile Ala Ala Ile Ala Gln Glu Leu
465                 470                 475                 480

Asn Glu Leu Asp Tyr Tyr Asp Ser His Asn Val Asn Thr Arg Cys Gln
                485                 490                 495

Lys Ile Cys Asp Gln Trp Asp Ala Leu Gly Ser Leu Thr His Ser Arg
            500                 505                 510

Arg Glu Ala Leu Glu Lys Thr Glu Lys Gln Leu Glu Ala Ile Asp Gln
        515                 520                 525

Leu His Leu Glu Tyr Ala Lys Arg Ala Ala Pro Phe Asn Asn Trp Met
    530                 535                 540

Glu Ser Ala Met Glu Asp Leu Gln Asp Met Phe Ile Val His Thr Ile
545                 550                 555                 560

Glu Glu Ile Glu Gly Leu Ile Ser Ala His Asp Gln Phe Lys Ser Thr
                565                 570                 575

Leu Pro Asp Ala Asp Arg Glu Arg Glu Ala Ile Leu Ala Ile His Lys
            580                 585                 590

Glu Ala Gln Arg Ile Ala Glu Ser Asn His Ile Lys Leu Ser Gly Ser
        595                 600                 605

Asn Pro Tyr Thr Thr Val Thr Pro Gln Ile Ile Asn Ser Lys Trp Glu
    610                 615                 620

Lys Val Gln Gln Leu Val Pro Lys Arg Asp His Ala Leu Leu Glu Glu
625                 630                 635                 640

Gln Ser Lys Gln Gln Ser Asn Glu His Leu Arg Arg Gln Phe Ala Ser
                645                 650                 655

Gln Ala Asn Val Val Gly Pro Trp Ile Gln Thr Lys Met Glu Glu Ile
            660                 665                 670

Gly Arg Ile Ser Ile Glu Met Asn Gly Thr Leu Glu Asp Gln Leu Ser
        675                 680                 685

His Leu Lys Gln Tyr Glu Arg Ser Ile Val Asp Tyr Lys Pro Asn Leu
    690                 695                 700

Asp Leu Leu Glu Gln Gln His Gln Leu Ile Gln Glu Ala Leu Ile Phe
705                 710                 715                 720

Asp Asn Lys His Thr Asn Tyr Thr Met Glu His Ile Arg Val Gly Trp
                725                 730                 735

Glu Gln Leu Leu Thr Thr Ile Ala Arg Thr Ile Asn Glu Val Glu Asn
            740                 745                 750
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```
Gln Ile Leu Thr Arg Asp Ala Lys Gly Ile Ser Gln Glu Gln Met Gln
        755                 760                 765

Glu Phe Arg Ala Ser Phe Asn His Phe Asp Lys Asp His Gly Gly Ala
    770                 775                 780

Leu Gly Pro Glu Glu Phe Lys Ala Cys Leu Ile Ser Leu Gly Tyr Asp
785                 790                 795                 800

Val Glu Asn Asp Arg Gln Gly Glu Ala Glu Phe Asn Arg Ile Met Ser
                805                 810                 815

Leu Val Asp Pro Asn His Ser Gly Leu Val Thr Phe Gln Ala Phe Ile
            820                 825                 830

Asp Phe Met Ser Arg Glu Thr Thr Asp Thr Asp Thr Ala Asp Gln Val
        835                 840                 845

Ile Ala Ser Phe Lys Val Leu Ala Gly Asp Lys Asn Phe Ile Thr Ala
    850                 855                 860

Glu Glu Leu Arg Arg Glu Leu Pro Pro Asp Gln Ala Glu Tyr Cys Ile
865                 870                 875                 880

Ala Arg Met Ala Pro Tyr Gln Gly Pro Asp Ala Val Pro Gly Ala Leu
                885                 890                 895

Asp Tyr Lys Ser Phe Ser Thr Ala Leu Tyr Gly Glu Ser Asp Leu
            900                 905                 910
```

What is claimed is:

1. A method for diagnosing lung cancer in a pre-diagnostic subject, comprising:

(a) contacting (i) one or more antibodies from a biological fluid from the pre-diagnostic subject, and (ii) at least one isolated pre-diagnostic lung cancer indicator protein, under conditions and for a time sufficient for detecting specific binding of at least one antibody from the biological fluid to one or more of said pre-diagnostic lung cancer indicator proteins, and therefrom identifying presence of lung cancer in the pre-diagnostic subject.

2. A screening method for lung cancer, comprising:

(a) contacting (i) one or more antibodies from a biological fluid from each subject of one or a plurality of subjects, and (ii) at least one isolated pre-diagnostic lung cancer indicator protein, under conditions and for a time sufficient for detecting specific binding of at least one antibody from the biological fluid to one or more of said pre-diagnostic lung cancer indicator proteins, wherein detection of specific binding indicates the subject has lung cancer, and thereby screening for lung cancer.

3. A method for diagnosing lung cancer in a pre-diagnostic subject, comprising:

(a) contacting (i) one or more antibodies from a biological fluid from the pre-diagnostic subject, and (ii) an isolated protein or polypeptide that comprises one or more antigenic epitopes of one or more pre-diagnostic lung cancer indicator proteins, under conditions and for a time sufficient for detecting specific binding of at least one antibody from the biological fluid to one or more of said antigenic epitopes, and therefrom identifying presence of lung cancer in the pre-diagnostic subject.

4. A screening method for lung cancer, comprising:

(a) contacting (i) one or more antibodies from a biological fluid from each subject of one or a plurality of subjects, and (ii) an isolated protein or polypeptide that comprises one or more antigenic epitopes of one or more pre-diagnostic lung cancer indicator proteins, under conditions and for a time sufficient for detecting specific binding of at least one antibody from the biological fluid to one or more of said antigenic epitopes, wherein detection of specific binding indicates the subject has lung cancer, and thereby screening for lung cancer.

5. The method of claim **1** wherein at least one of the one or more pre-diagnostic lung cancer indicator proteins comprises a LAMR1 protein.

6. The method of claim **5** wherein the pre-diagnostic lung cancer indicator proteins further comprise at least one protein selected from the group consisting of:

(a) annexin I protein

(b) 14-3-3 theta protein

7. The method of claim **1** wherein the lung cancer is selected from the group consisting of (i) adenocarcinoma, (ii) squamous cell carcinoma, (iii) non-small cell lung cancer that is not (i) or (ii), and (iv) lung cancer that can be defined based on one or more of causation and gene mutational status.

8. The method of claim **6** wherein the lung cancer is selected from the group consisting of (i) adenocarcinoma, (ii) squamous cell carcinoma, (iii) non-small cell lung cancer that is not (i) or (ii), and (iv) lung cancer that can be defined based on one or more of causation and gene mutational status.

9. The method of claim **1** wherein the subject or pre-diagnostic subject is at increased risk for developing lung cancer.

10. The method of claim **9** wherein the subject or pre-diagnostic subject has at least one indicator of increased risk for developing lung cancer that is selected from the group consisting of (i) a history of asbestos exposure, (ii) a history of smoking tobacco products, (iii) a history of radon gas exposure, (iv) a history of exposure to a source of ionizing

radiation, (v) a history of recurrent lung inflammation, (vi) a history of tuberculosis, (vi) a history of silicosis, berylliosis or talc inhalation, (vii) a family history of lung cancer in genetically related individuals, (viii) a history of vitamin A deficiency or vitamin A excess, (ix) a history of smoking cannabis, and (x) exposure to toxic volatile substances or infectious agents.

11. The method of claim **1** wherein the antibodies are isolated from the biological fluid prior to the step of contacting.

12. The method of claim **1** wherein the antibodies are present in the biological fluid during the step of contacting.

13. The method of claim **1** wherein the antibodies are autoantibodies.

14. The method of claim **1** wherein the biological fluid is selected from the group consisting of blood, serum, serosal fluid, plasma, lymph, urine, cerebrospinal fluid, saliva, a mucosal secretion, a vaginal secretion, ascites fluid, pleural fluid, pericardial fluid, peritoneal fluid, abdominal fluid, culture medium, conditioned culture medium and lavage fluid.

15. The method of claim **1** wherein the biological fluid comprises serum.

16. The method of claim **1** wherein the pre-diagnostic indicator protein, or the isolated protein or polypeptide that comprises one or more antigenic epitopes of a pre-diagnostic indicator protein, is selected from the group consisting of:

(i) a naturally occurring protein or polypeptide,

(ii) a synthetic protein or polypeptide,

(iii) a recombinant protein or polypeptide, and

(iv) a fusion protein or polypeptide that comprises a fusion polypeptide domain fused to the pre-diagnostic indicator protein, or to the polypeptide that comprises one or more antigenic epitopes of the pre-diagnostic indicator protein.

17. The method of claim **1** wherein the pre-diagnostic indicator protein, or the isolated protein or polypeptide that comprises one or more antigenic epitopes of a pre-diagnostic indicator protein, is immobilized on a solid substrate.

18. The method of claim **17** wherein the immobilized pre-diagnostic indicator protein or the immobilized isolated protein or polypeptide that comprises one or more antigenic epitopes of a pre-diagnostic indicator protein, is immobilized by a covalent bond.

19. The method of claim **17** wherein the immobilized pre-diagnostic indicator protein or the immobilized isolated protein or polypeptide that comprises one or more antigenic epitopes of a pre-diagnostic indicator protein, is non-covalently immobilized.

20. The method of claim **1** wherein detecting specific binding of the at least one antibody comprises detecting a signal that is selected from the group consisting of a fluorescent signal, a radiometric signal, an enzymatic signal and a spectrometric signal.

21. The method of claim **1** wherein the pre-diagnostic lung cancer indicator protein is selected from the group consisting of (i) a non-posttranslationally modified protein, (ii) a post-translationally modified protein that is selected from a glycoprotein, a lipoprotein, a phosphoprotein, a proteolipid, a glypiated protein, a ubiquitinylated protein, a SUMOylated protein, a sulfated protein and a glycated protein, and (iii) a posttranslationally modified protein of (ii) in which one or more posttranslational modifications result in immunogenicity.

22. The method of claim **1** wherein at least one of the one or more pre-diagnostic lung cancer indicator proteins comprises a protein that is selected from the group consisting of (a) AKR1B10 protein [SEQ ID NO:11], (b) GOT2 protein [SEQ ID NO:12], (c) HNRPR protein [SEQ ID NO:13], (d) PDIA3 protein [SEQ ID NO:14], (e) NME2 protein [SEQ ID NO:15], (f) RTN4 protein [SEQ ID NO:16], (g) HI1FX protein [SEQ ID NO:17], (h) G3BP protein [SEQ ID NO:18], (i) HSPCA protein [SEQ ID NO:19], and (j) ACTN4 protein [SEQ ID NO:20].

23. The method of claim **5** wherein the pre-diagnostic lung cancer indicator proteins further comprise at least one protein that is selected from the group consisting of (a) AKR1B10 protein [SEQ ID NO:11], (b) GOT2 protein [SEQ ID NO:12], (c) HNRPR protein [SEQ ID NO:13], (d) PDIA3 protein [SEQ ID NO:14], (e) NME2 protein [SEQ ID NO:15], (f) RTN4 protein [SEQ ID NO:16], (g) HI1FX protein [SEQ ID NO:17], (h) G3BP protein [SEQ ID NO:18], (i) HSPCA protein [SEQ ID NO:19], and (j) ACTN4 protein [SEQ ID NO:20].

24. A method of monitoring lung cancer autoimmune reactivity in a lung cancer patient, comprising:

(a) contacting, after each of two or more timepoints,

(i) one or more antibodies from a biological fluid that is taken from a subject at each of said timepoints, and

(ii) a test antigen that is selected from the group consisting of (1) at least one isolated pre-diagnostic lung cancer indicator protein and (2) at least one isolated protein or polypeptide that comprises one or more antigenic epitopes of one or more pre-diagnostic lung cancer indicator proteins, under conditions and for a time sufficient for detecting specific binding of at least one antibody from the biological fluid to one or more of said pre-diagnostic lung cancer indicator proteins or antigenic epitopes thereof; and

(b) comparing the specific binding that is detectable by antibodies from the biological fluid taken at each of said two or more timepoints, and thereby monitoring lung cancer autoimmune reactivity in the patient.

25. The method of claim **24** wherein a first timepoint occurs before administration of a therapeutic agent to the patient and a second timepoint occurs after administration of the therapeutic agent to the patient.

* * * * *

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[标]申请(专利权)人(译)	HANASH SAMIR中号 秋姬 FACA SANDRA		
申请(专利权)人(译)	HANASH SAMIR M. 秋姬 FACA SANDRA		
当前申请(专利权)人(译)	HANASH SAMIR M. 秋姬 FACA SANDRA		
[标]发明人	HANASH SAMIR M QIU JI FACA SANDRA		
发明人	HANASH, SAMIR M. QIU, JI FACA, SANDRA		
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摘要(译)

本文描述了在症状发作之前受试者中肺癌的诊断（即，在预诊断受试者中），通过筛选来自受试者的生物流体，其中存在对一种或多种预诊断肺具有特异性的自身抗体。癌症指示蛋白，包括LAMR1，和任选地另外或可选地包括膜联蛋白I和/或14-3-3-θ和/或如本文公开的其他诊断前肺癌指示蛋白，作为确定的抗原。相关方法，包括监测肺癌患者对肺癌指示蛋白的免疫反应性，分型肺癌受试者或表征肺肿瘤，以及应用所述蛋白质组学方法鉴定其他预诊断肺癌指示蛋白，也是可以预期的。