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(54) PLASMA AUTOANTIBODY BIOMARKERS FOR BASAL LIKE BREAST CANCER

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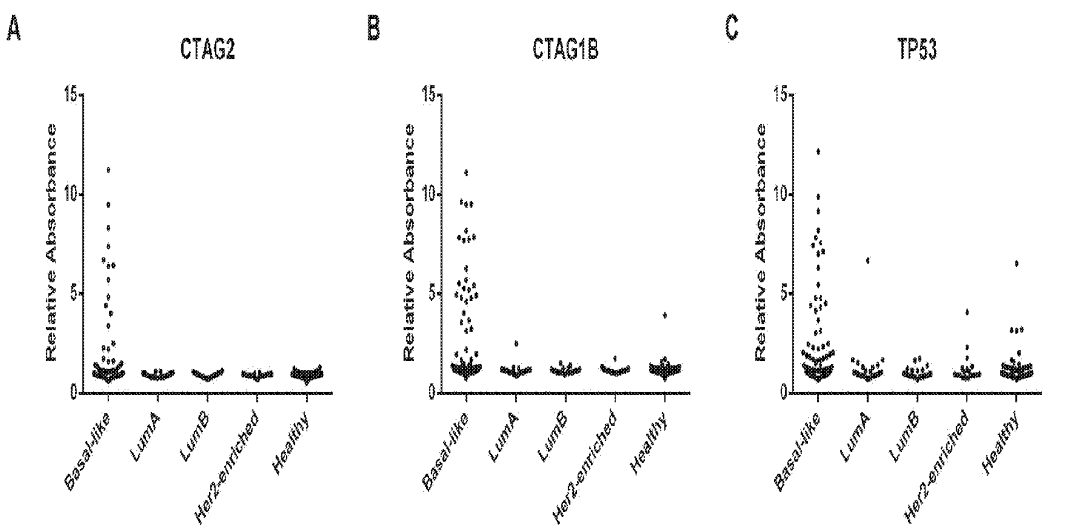
(57) **ABSTRACT**

Cancer patients make antibodies to tumor-derived proteins that are potential biomarkers for early detection. Twenty-eight antigens have been identified as potential biomarkers for the early detection of basal-like breast cancer (Tables 1, 2). Also, a 13-AAb classifier has been developed that differentiate patients with BLBC from healthy controls with 33% sensitivity at 98% specificity (Table 3).



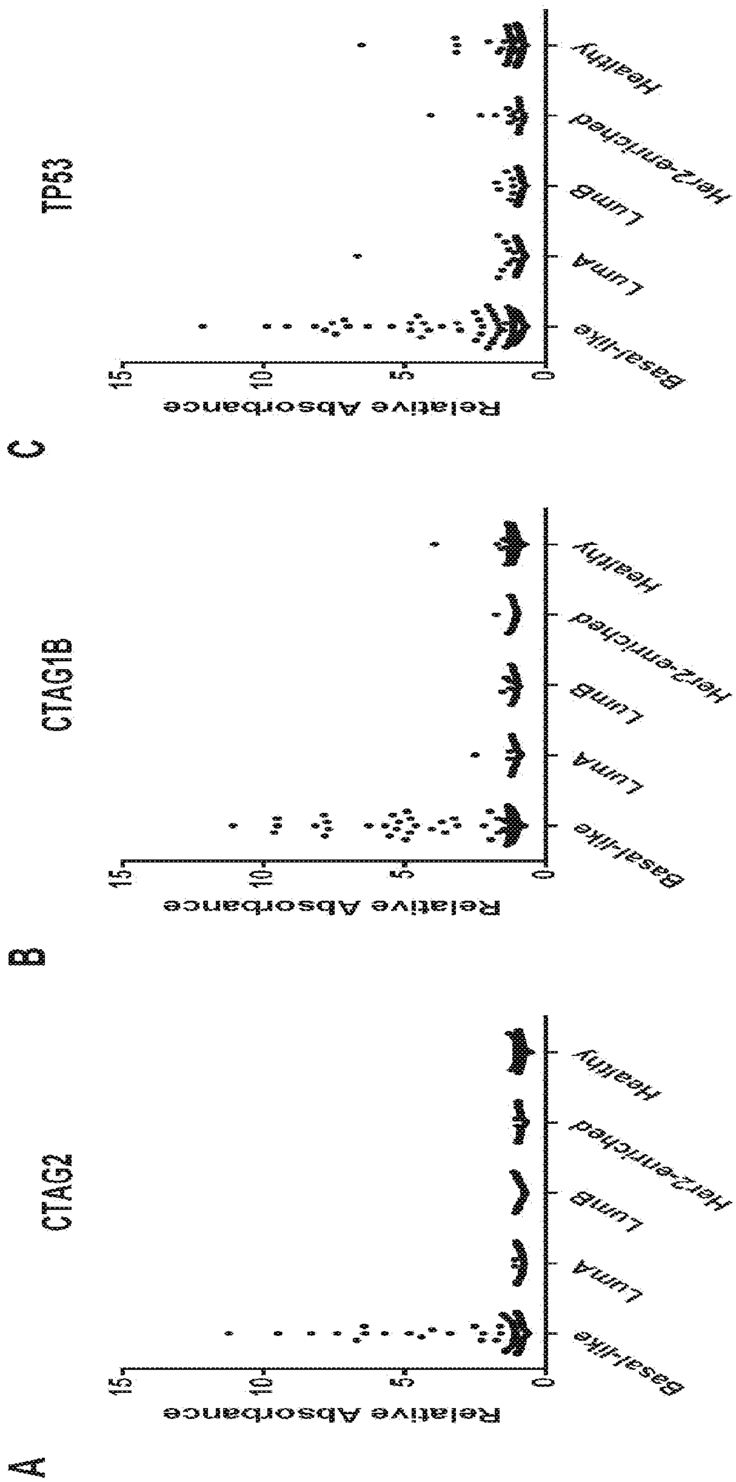

A
CTAG2
B
CTAG1B
C
TP53
Relative Absorbance
15
10
5
0
Basal-like
LumA
LumB
Her2-enriched
Healthy

FIG. 1

PLASMA AUTOANTIBODY BIOMARKERS FOR BASAL LIKE BREAST CANCER

CROSS REFERENCE

[0001] This application claims priority to U.S. provisional patent application 62/089,631 filed on Dec. 9, 2014, which is incorporated by reference herein in its entirety.

STATEMENT OF GOVERNMENT RIGHTS

[0002] This invention was made with government support under U01 CA117374 awarded by the National Institutes of Health. The government has certain rights in the invention.

TECHNICAL FIELD

[0003] The disclosure herein related to biomarkers for cancer and more particularly to autoantibody biomarkers for breast cancer.

BACKGROUND

[0004] Despite significant improvement in early detection by routine mammography, breast cancer remains a global challenge. Current screening mammography only detects 70% of breast cancers. Breast tumors associated with high breast density and highly proliferative cancers are frequently not detected by screening. Therefore, there is an urgent need for biomarkers that can detect potentially invasive breast cancer in their early stages.

[0005] Like other cancers, breast cancer is heterogeneous, comprising multiple molecular subtypes with unique characteristics of prognosis, response to treatment and risk of recurrence. This heterogeneity affects the biomarker discovery, requiring both larger sample sizes and different statistical approaches from traditional methods of evaluation. A biomarker (or panel) may perform well for one molecular subtype but not another.

[0006] Current screening mammography also has limitations in detecting the basal-like subtype cancers. The fraction of BLBC within all breast cancer cases detected by mammography is less than its natural frequency in the general population, indicating that BLBC was under-diagnosed by routine mammography and more frequently detected as interval breast cancer. Early detection of BLBC by screening mammography is compromised partly by its high proliferation rate and lack of suspicious features of regular breast malignancy, such as microcalcifications, irregular spiculated masses or pleomorphic microcalcifications. Moreover, BLBC is often present in women less than 50 years old, who are not recommended for routine mammogram by US Preventative Service Task Force mammogram guidelines.

[0007] Considering the high frequency of disease in women younger than 50 years old, a potentially large population with BLBC does not benefit from present breast cancer screening and would benefit from a molecular test for the disease. Therefore, there is an urgent need for biomarkers that can detect potentially invasive basal like breast cancer in their early stages.

SUMMARY

[0008] We have identified 28 antigens as potential biomarkers for the early detection of basal like breast cancer. These biomarkers were selected out of 10,000 tumor antigens in a sequential screening study and yielded supporting evidence in a blinded validation study. These biomarkers should be useful components of diagnostic tests and personalized therapeutics for breast cancer.

[0009] All references cited throughout are hereby incorporated by reference herein.

DESCRIPTION OF DRAWINGS

[0010] FIG. **1** depicts dotplots of AAb responses in various breast cancer subtypes.

DETAILED DESCRIPTION

[0011] Notably, the sensitivity of a subtype-specific biomarker can never be higher than the prevalence of that subtype in the population where it is tested. The ability to find biomarkers for cancer detection with high sensitivities has likely been impaired by this effect. This is especially true for disease subtypes that are less common in the overall population. Basal like breast cancer is a breast cancer subtype with aggressive disease progression and poor prognosis. It overlaps significantly with triple negative breast cancer (TNBC), a clinical pathological subtype characterized by negative tissue staining of estrogen receptor (ER), progesterone receptor (PR), and the absence of human epidermal growth factor receptor 2 (HER2) amplification.

[0012] TNBC is associated with African American ethnicity, younger age, advanced stage at diagnosis and poorer outcomes. However, TNBC itself has been found to be molecularly heterogeneous in two recent studies. Among patients with TNBC, a core basal subtype with expression of epidermal growth factor receptor (EGFR) or basal cytokeratin 5/6 (CK5/6) characterized a group of patients with worse prognosis than the rest of the patients with TNBC. This five marker subtyping (ER−, PR−, HER2−, and either EGFR+ or CK5/6+) is highly correlated with BLBC defined by gene expression profiling.

[0013] Current screening mammography has limitations in detecting the basal-like subtype. The fraction of BLBC within all breast cancer cases detected by mammography is less than its natural frequency in the general population, indicating that BLBC was under-diagnosed by routine mammography and more frequently detected as interval breast cancer. Early detection of BLBC by screening mammography is compromised partly by its high proliferation rate and lack of suspicious features of regular breast malignancy, such as microcalcifications, irregular spiculated masses or pleomorphic microcalcifications.

[0014] Moreover, BLBC is often present in women less than 50 years old, who are not recommended for routine mammogram by US Preventative Service Task Force mammogram guidelines. Considering the high frequency of disease in women younger than 50 years old, a potentially large population with BLBC does not benefit from present breast cancer screening and would benefit from a molecular test for the disease.

[0015] In practical terms, molecular diagnostic tests for the early detection of cancer should be performed on readily accessible samples, like plasma, because they are likely to be performed on many individuals. The concentration of many cancer biomarkers in blood tends to be very low because it relies upon secretion by cancer cells which are few in number in the pre-clinical stage. Moreover, the biomarker gets diluted in a large volume of plasma volume and only a fraction of the secreted biomarker gets distributed to the plasma.

[0016] An alternative strategy is to exploit the ability of the immune system to detect the presence of tumor cells through the generation of autoantibodies. These responses of the adaptive immune system against target tumor antigens amplify the signals from the minute amount of tumor proteins released from cancer tissue.

[0017] We have previously conducted an autoantibody biomarker discovery for breast cancer on our nucleic acid programmable protein array (NAPPA) platform. NAPPA allows us to display thousands of freshly produced full length human proteins on glass slides without the need of protein purification and has been applied to the study of disease specific antibodies in diseases ranging from infectious to autoimmune to cancers. In that study, we took a three stage study design to identify autoantibody markers from 4988 human proteins.

[0018] The goal for the discovery stage 1 was to eliminate non-reactive and uninformative (i.e. no difference between case and control) antigens and reduce the total number of antigens. The top 761 antigens were selected based on differential reactivity between cases and controls. The goal for the training stage II was to identify candidate autoantibody markers. The goal for the blinded validation stage III was to validate potential biomarkers. This yielded a panel of 28 markers showing sensitivities in the 10-30% range with specificities from 80-100%.

[0019] However, the sample cohort used in this study was a mixed population of predominantly women with ER+PR+ breast cancer. Therefore, the utility of these markers in subtypes like BLBC or Her2+ are likely limited, considering their relatively low percentage among breast cancer patients. Here, we aimed to identify BLBC specific autoantibodies by profiling humeral immune responses of BLBC patients against 10,000 human proteins.

[0020] One challenge associated with the use of "omics" technology to study a homogeneous cancer subtype is the requirement of a large number of samples to have sufficient power of analysis. To this end, our study was supported by the Polish Breast Cancer Study, in which over 2386 breast cancer patients were enrolled.

[0021] Paraffin embedded tissue samples were collected for immunohistochemical (IHC) analysis and disease classification. We successfully collected plasma samples from 145 patients classified to be basal-like subtype by either PAM50 gene signature based on mRNA expression profiling, or tissue IHC staining of ER, PR, HER2, EGFR and CK5/6. In addition, for each patient, we also collect plasma sample from an age, gender and location matched healthy donor. Here, we first profiled sero-reactivity against ~10,000 human proteins in 45 BLBC patients and 45 matched controls.

[0022] Candidate antigens were selected, assayed for their autoantibodies in BLBC using customized NAPPA and enzyme-linked immunosorbant assay (ELISA), and validated using an independent patient cohort in a blind fashion. See, for example, "Tracking humoral responses using self assembling protein microarrays," *Proteomics Clin Appl.* 2008 Oct. 2 (10-11):1518-27. A biomarker signature was also developed to discriminate basal-like tumors from age and location matched healthy individuals. We further evaluated the specificity of the panel of autoantibodies to basal-like tumors using a set of patients with other breast cancer subtypes.

[0023] The 28 antigens that we have identified as potential biomarkers for the early detection of basal like breast cancer are (Table 1, Table 2): P53 (TP53), NY-ESO-1 (CTAG1B), NY-ESO-2 (CTAG2), RNF216, PPHLN1, PIP4K2C, ZBTB16, TAS2R8, WBP2NL, DOK2, PSRC1, MN1, TRIM21, POU4F1, SSMEM1, LMO4, BCL2, KRT8, TSGA13, PVRL4, SNRK, DYRK3, RNF32, JUNB, KCNIP3, CCDC68, CSN3, TRAIP, which are available at the DNASU Plasmid Repository at the Bio Design Institute of the Arizona State University, Tempe, Ariz. In addition, we also developed a classifier to differentiate patients with BLBC from healthy controls with 33% sensitivity at 98% specificity (Table 3).

[0024] We designated samples as positive if they exceeded antigen-specific cutoffs for at least 2 of the 13 antigens. Antigen-specific cutoffs were set to achieve 98% classifier specificity by adjusting the specificity at the antigen level to 98.7%.

[0025] A point of novelty is the identification of the 28 antigens that are potential biomarkers for early detection of basal like breast cancer (Table 1, 2). Many of these 28 antigens have not been previously associated with basal like breast cancer. In addition, we also developed a classifier to differentiate patients with BLBC from healthy controls with 33% sensitivity at 98% specificity (Table 3).

TABLE 1

Training and Validation Statistics for Potential basal-like breast cancer Biomarkers

	Training (Cohort1&2: basal, n = 95; healthy, n = 95)			Validation (Cohort3: basal, n = 50; healthy, n = 50)	
Antigen	sensitivity	specificity	cutoffs	sensitivity	specificity
CTAG1B	0.213	0.979	1.606	0.200	1.000
CTAG2	0.191	0.979	1.149	0.180	0.960
TRIM21	0.158	0.979	1.208	0.140	0.860
RNF216	0.110	0.978	1.369	0.043	0.956
MN1	0.105	0.979	1.311	0.060	0.920
PIP4K2C	0.105	0.979	1.200	0.020	1.000
TP53	0.084	0.979	3.171	0.200	1.000
ZBTB16	0.084	0.979	1.393	0.040	0.980
TRAIP	0.074	0.979	2.682	0.040	0.980
DOK2	0.074	0.979	1.164	0.060	1.000
CSN3	0.063	0.979	1.955	0.060	0.980
PPHLN1	0.063	0.979	3.394	0.080	1.000
TAS2R8	0.063	0.979	1.064	0.080	0.940
SSMEM1	0.063	0.979	1.562	0.060	0.960
DYRK3	0.063	0.979	1.462	0.040	0.940
KRT8	0.053	0.979	1.645	0.060	0.960
LMO4	0.053	0.979	1.199	0.020	0.980
WBP2NL	0.053	0.979	1.991	0.060	0.980
JUNB	0.042	0.979	1.165	0.020	0.960
TSGA13	0.042	0.979	1.313	0.020	0.980
PVRL4	0.042	0.979	0.899	0.020	0.920
CCDC68	0.042	0.979	2.438	0.000	0.940
BCL2	0.042	0.979	1.160	0.000	1.000
SNRK	0.032	0.979	4.127	0.020	0.960
PSRC1	0.032	0.979	1.372	0.120	0.960
KCNIP3	0.032	0.979	0.973	0.000	0.960
POU4F1	0.032	0.979	0.992	0.080	0.940
RNF32	0.021	0.979	1.445	0.040	0.980

TABLE 2

Performance of 28 antigens in all subtypes of breast cancer.

	Sensitivity				
Antigen	basal-like	luminal A	luminal B	Her2-enriched	Specificity
CTAG1B	0.208	0.033	0.045	0.056	0.979
CTAG2	0.188	0.000	0.000	0.000	0.979
TP53	0.124	0.033	0.000	0.056	0.979
RNF216	0.088	0.133	0.095	0.000	0.978
PPHLN1	0.083	0.100	0.182	0.000	0.979
PIP4K2C	0.076	0.100	0.091	0.111	0.979
ZBTB16	0.069	0.000	0.000	0.000	0.979
TAS2R8	0.069	0.000	0.000	0.056	0.979
WBP2NL	0.069	0.100	0.091	0.000	0.979
DOK2	0.069	0.133	0.091	0.056	0.979
PSRC1	0.063	0.033	0.045	0.056	0.979
MN1	0.062	0.100	0.000	0.056	0.979
TRAIP	0.062	0.067	0.045	0.000	0.979
CSN3	0.062	0.100	0.182	0.000	0.979
TRIM21	0.055	0.033	0.000	0.056	0.979
POU4F1	0.048	0.033	0.000	0.222	0.979
SSMEM1	0.048	0.033	0.136	0.000	0.979
LMO4	0.041	0.033	0.000	0.056	0.979
BCL2	0.041	0.033	0.045	0.000	0.979
KRT8	0.034	0.033	0.000	0.056	0.979
TSGA13	0.034	0.000	0.000	0.056	0.979
PVRL4	0.034	0.000	0.000	0.000	0.979
SNRK	0.028	0.000	0.045	0.000	0.979
DYRK3	0.028	0.033	0.045	0.000	0.979
RNF32	0.028	0.033	0.000	0.056	0.979
JUNB	0.021	0.000	0.000	0.000	0.979
KCNIP3	0.014	0.033	0.000	0.000	0.979
CCDC68	0.007	0.000	0.000	0.000	0.979

TABLE 3

Cutoffs for 13-AAb classifier

Antigen	Cutoff
CTAG1B	1.606
CTAG2	1.176
TP53	3.171
RNF216	1.459
PPHLN1	3.448
PIP4K2C	1.201
ZBTB16	1.925
TAS2R8	1.178
WBP2NL	2.120
DOK2	1.164
PSRC1	1.461
MN1	1.687
TRIM21	5.509

[0026] An example of how a patient sample would be handled to detect and diagnose basal-like breast cancer using one or more of the discovered biomarkers in a kit with a suitable detection agent is as follows.

[0027] A patient's plasma sample is obtained, and then subsequently tested for autoantibody responses against the proposed protein panels. Briefly, protein targets are produced either freshly in situ or purified ahead of time, and immobilized on solid surface. A plasma sample is then incubated with these protein targets. Labeled secondary antibody that can recognize human immunoglobulins are used for the signal read out. Accordingly, data such as that shown in FIG. **1** can be obtained and used to detect and/or diagnose basal-like breast cancer.

[0028] Various changes in the details and components that have been described may be made by those skilled in the art within the principles and scope of the invention herein described in the specification and defined in the appended claims. Therefore, while the present invention has been shown and described herein in what is believed to be the most practical and preferred embodiments, it is recognized that departures can be made therefrom within the scope of the invention, which is not to be limited to the details disclosed herein but is to be accorded the full scope of the claims so as to embrace any and all equivalent processes and products.

What is claimed is:

1. A method for detection of basal-like breast cancer in a patient sample harboring one or more antigens, comprising:

detecting a tumor antigen biomarker in said patient sample, wherein the biomarker is selected from the antigens: P53 (TP53), NY-ESO-1 (CTAG1B), NY-ESO-2 (CTAG2), RNF216, PPHLN1, PIP4K2C, ZBTB16, TAS2R8, WBP2NL, DOK2, PSRC1, MN1, TRIM21, POU4F1, SSMEM1, LMO4, BCL2, KRT8, TSGA13, PVRL4, SNRK, DYRK3, RNF32, JUNB, KCNIP3, CCDC68, CSN3, TRAIP.

2. The method of claim **1**, wherein said detecting comprises utilizing an enzyme-linked immunosorbant assay (ELISA).

3. The method of claim **1**, further comprising detecting at least two of said tumor antigen biomarkers and comparing a level of detection to predetermined sensitivity and specificity threshold to determine if basal-like breast cancer is indicated.

4. A basal-like breast cancer diagnostic test kit, comprising an antibody against a tumor antigen biomarker selected from the group consisting of: P53 (TP53), NY-ESO-1 (CTAG1B), NY-ESO-2 (CTAG2), RNF216, PPHLN1, PIP4K2C, ZBTB16, TAS2R8, WBP2NL, DOK2, PSRC1, MN1, TRIM21, POU4F1, SSMEM1, LMO4, BCL2, KRT8, TSGA13, PVRL4, SNRK, DYRK3, RNF32, JUNB, KCNIP3, CCDC68, CSN3, TRAIP, and a suitable detection agent.

5. The kit of claim **4**, wherein an antibody against each of said tumor antigen biomarkers is present in said kit.

6. A method for diagnosing basal-like breast cancer from a patient sample harboring one or more antigens, comprising:

a) detecting a tumor antigen biomarker in said patient sample, wherein the biomarker is selected from the antigens: P53 (TP53), NY-ESO-1 (CTAG1B), NY-ESO-2 (CTAG2), RNF216, PPHLN1, PIP4K2C, ZBTB16, TAS2R8, WBP2NL, DOK2, PSRC1, MN1, TRIM21, POU4F1, SSMEM1, LMO4, BCL2, KRT8, TSGA13, PVRL4, SNRK, DYRK3, RNF32, JUNB, KCNIP3, CCDC68, CSN3, TRAIP; and

b) comparing a detection result of step a) with a control.

* * * * *