

SUMMARY

[0010] Using gene expression signatures in bronchial epithelium, we have defined distinct molecular subtypes of asthma. Surprisingly, supervised clustering of the data based on a set of genes whose expression was highly correlated to genes known to be upregulated by IL-4 or IL-13 stimulation revealed not one but two distinct clusters of asthma patients. Furthermore, analysis of these dichotomous subsets of asthmatics revealed significant associations between “IL-4/13 signature” status and serum total IgE levels, serum CEA levels, serum periostin levels, peripheral blood eosinophilia, (bronchoalveolar lavage) BAL eosinophilia, and responsiveness to inhaled corticosteroids (each $p < 0.05$ by Wilcoxon rank sum test).

[0011] Accordingly, the present ~~invention~~disclosure relates to methods of diagnosing a subpopulation of asthma patients comprising measuring the gene expression of any one or combination of genes selected from POSTN, CST1, CCL26, CLCA1, CST2, PRR4, SERPINB2, CEACAM5, iNOS, SERPINB4, CST4, PRB4, TPSD1, TPSG1, MFSD2, CPA3, GPR105, CDH26, GSN, C2ORF32, TRACH2000196 (TMEM71), DNAJC12, RGS13, SLC18A2, SERPINB10, SH3RF2, FCER1B, RUNX2, PTGS1, and ALOX15. In one embodiment, the gene expression is measured of any one or combination of genes selected from the group of consisting of POSTN, CST1, CST2, CCL26, CLCA1, PRR4, PRB4, SERPINB2, CEACAM5, iNOS, SERPINB4, CST4, and SERPINB10. According to one embodiment, the gene expression is measured by microarray. According to another embodiment, the gene expression is measured by observing protein expression levels of an aforementioned gene. According to another embodiment, the gene expression is considered elevated when compared to a healthy control if the relative mRNA level of the gene of interest is greater than 2.5 of the level of a control gene mRNA. According to another embodiment, the relative mRNA level of the gene of interest is greater than 3 fold, 5 fold, 10 fold, 15 fold 25 fold or 30 fold compared to a healthy control gene expression level. According to one embodiment, the gene expression is measured by a method selected from the group consisting of a PCR method, a microarray method or a immunoassay method. In one embodiment, the microarray method comprises the use of a microarray chip having one or more nucleic acid molecules that can hybridize under stringent conditions to a nucleic acid molecule encoding a gene mentioned above or having one or more polypeptides (such as peptides or antibodies) that can bind to one or more of the proteins encoded by the genes mentioned above. In one embodiment, the PCR method is qPCR. According to one

embodiment, the immunoassay method comprises the steps of binding an antibody to protein expressed from a gene mentioned above in a patient sample mentioned above and determining if the protein level from the patient sample is elevated. According to one embodiment, a control gene is a housekeeping gene selected from the group consisting of actin, GAPDH, GASB and GUSB.

[0011a] In one aspect, there is provided a method of diagnosing an asthma subtype in an asthma patient comprising measuring the gene expression of POSTN in a sample from the patient, wherein elevated expression levels of POSTN is indicative of the asthma subtype.

[0011b] In one aspect, there is provided a method of identifying an asthma subtype patient to be treated with an agent which is an immunoadhesin, a peptibody or an antibody that binds a target which is IL-9, IL-5, IL-13, IL-4, OX40L, TSLP, IL-25, IL-33, IgE, IL-9 receptor, IL-5 receptor, IL-4 receptor-alpha, IL-13 receptor-alpha1, IL-13 receptor-alpha2, OX40, TSLP-R, IL-7Ralpha (a co-receptor for TSLP), IL17RB (receptor for IL-25), ST2 (receptor for IL-33), CCR3, CCR4, CRTH2, FcepsilonRI or FcepsilonRII/CD23 (receptors for IgE) comprising measuring the POSTN level from a sample from a patient suffering from asthma and identifying the asthma patient having an elevated POSTN levels as a patient to be treated with the agent.

[0012] The present ~~invention~~disclosure provides a microarray chip comprising nucleic acid sequences encoding the following genes: POSTN, CST1, CST2, CCL26, CLCA1, PRR4, SERPINB2, CEACAM5, iNOS, SERPINB4, CST4, and SERPINB10 or fragments thereof.

The present ~~invention~~disclosure provides a microarray chip comprising nucleic acid sequences encoding the following genes: POSTN, CST1, CCL26, CLCA1, CST2, PRR4, SERPINB2, CEACAM5, iNOS, SERPINB4, CST4, PRB4, TPSD1, TPSG1, MFSD2, CPA3, GPR105, CDH26, GSN, C2ORF32, TRACH2000196 (TMEM71), DNAJC12, RGS13, SLC18A2, SERPINB10, SH3RF2, FCER1B, RUNX2, PTGS1, and ALOX1, or fragments thereof.

[0013] The present ~~invention~~disclosure provides a subpopulation of asthma patients to be treated with the therapeutic agents of this ~~invention~~disclosure, wherein the ratio of Muc5AC:MUC5B protein or mRNA levels in the airway epithelial cells of asthma patients is greater than 25.

[0014] The present ~~invention~~disclosure also relates to methods of diagnosing a subpopulation of asthma patients by taking single or combinations of measurements of systemic biomarkers selected from serum CEA levels, serum IgE levels, serum periostin levels, peripheral blood eosinophil counts and eosinophil percentages in bronchoalveolar lavage fluid (BAL). Systemic biomarkers typically are nongenetic biomarkers and are typically measured in samples obtained by noninvasive procedures, for example, but not limited to, collection of blood or blood components, e.g., serum or plasma. According to one embodiment, greater than 100IU/ml IgE levels and/or $0.14 \times 10^9/L$ eosinophils is predictive of a patient population to be treated with the therapeutic agents of this ~~invention~~disclosure.

[0015] The present ~~invention~~disclosure relates to methods of treating asthma comprising administering a therapeutic agent to a patient expressing elevated levels of any one or combination of the genes selected from POSTN, CST1, CCL26, CLCA1, CST2, PRR4, SERPINB2, CEACAM5, iNOS, SERPINB4, CST4, PRB4, TPSD1, TPSG1, MFSD2, CPA3, GPR105, CDH26, GSN, C2ORF32, TRACH2000196 (TMEM71), DNAJC12, RGS13, SLC18A2, SERPINB10, SH3RF2, FCER1B, RUNX2, PTGS1, ALOX15. According to one embodiment, the patient expresses elevated levels of any one or combination of genes selected from the group consisting of periostin, CST1, CST2, CCL26, CLCA1, PRR4,

SerpinB2, CEACAM5, iNOS, PRB4, SerpinB4, SerpinB10 and CST4. According to one embodiment, the patient to be treated is a mild-to-moderate, steroid-naïve (never treated with steroids) asthma patient. According to another embodiment, the patient to be treated is a moderate-to-severe, steroid-resistant (non-responsive to steroids) asthma patient. Such patients are treated with a therapeutically effective amount of a therapeutic agent. In one embodiment, the patient has asthma induced by the TH2 pathway.

[0016] According to one embodiment, the therapeutic agent is an anti-IL13/IL4 pathway inhibitor. According to another embodiment, the therapeutic agent targets the TH2 induced asthma pathway. Exemplary targets include, but are not limited to, cytokines or ligands such as: IL-9, IL-5, IL-13, IL-4, OX40L, TSLP, IL-25, IL-33 and IgE; and receptors such as: IL-9 receptor, IL-5 receptor, IL-4receptor alpha, IL-13receptoralpha1 and IL-13receptoralpha2, OX40, TSLP-R, IL-7Ralpha (a co-receptor for TSLP), IL17RB (receptor for IL-25), ST2 (receptor for IL-33), CCR3, CCR4, CRTH2, FcepsilonRI and FcepsilonRII/CD23 (receptors for IgE). Accordingly, a therapeutic agent according to this ~~invention~~disclosure includes an agent that can bind to the target above, such as a polypeptide(s) (e.g., an antibody, an immunoadhesin or a peptibody), an aptamer or a small molecule.

[0017] According to one embodiment, the therapeutic agent is an anti-IL13 antibody. According to another embodiment, the anti-IL-13 antibody comprises a VH sequence comprising SEQ ID NO: 193 and a VL sequence comprising SEQ ID NO:194. According to another embodiment, the anti-IL13 antibody comprises: (a) an HVR-L1 comprising amino acid sequence RASKSVDSYGNSFMH (SEQ ID NO:195); (b) an HVR-L2 comprising amino acid sequence LASNLES (SEQ ID NO:196); (c) an HVR-L3 comprising amino acid sequence QQNNEDPRT (SEQ ID NO: 197); (d) an HVR-H1 comprising amino acid sequence AYSVN (SEQ ID NO:198); (e) an HVR-H2 comprising amino acid sequence MIWGDGKIVYNSALKS (SEQ ID NO: 199); and (f) an HVR-H3 comprising amino acid sequence DGYYPYAMDN (SEQ ID NO: 200). According to another embodiment, the therapeutic agent is an anti-OX40 ligand (OX40L) antibody. According to another embodiment the therapeutic agent is an anti-IL13/anti-IL4 bispecific antibody. According to another embodiment, the therapeutic agent is an anti-IgE antibody. According to another embodiment, the therapeutic agent is an antibody directed against the membrane proximal M1' region of surface expressed IgE on B cells. According to another embodiment, the therapeutic agent is an inhaled corticosteroid. In certain embodiments, the inhaled

embodiment, the therapeutic agent is a bispecific antibody capable of binding both IL-17A and IL-17F. In one embodiment, the therapeutic agent is an anti-IL-17A/F antibody.

[0019a] In one aspect, there is provided an agent which is an immunoadhesin, a peptibody or an antibody that binds a target which is IL-9, IL-5, IL-13, IL-4, OX40L, TSLP, IL-25, IL-33, IgE, IL-9 receptor, IL-5 receptor, IL-4 receptor-alpha, IL-13 receptor-alpha1, IL-13 receptor-alpha2, OX40, TSLP-R, IL-7Ralpha (a co-receptor for TSLP), IL17RB (receptor for IL-25), ST2 (receptor for IL-33), CCR3, CCR4, CRTH2, FcepsilonRI or FcepsilonRII/CD23 (receptors for IgE), for use in a method of treatment of asthma in a patient, wherein the patient has been determined to express elevated expression levels of POSTN gene.

[0019b] In one aspect, there is provided the use of an agent which is an immunoadhesin, a peptibody or an antibody that binds a target which is IL-9, IL-5, IL-13, IL-4, OX40L, TSLP, IL-25, IL-33, IgE, IL-9 receptor, IL-5 receptor, IL-4 receptor-alpha, IL-13 receptor-alpha1, IL-13 receptor-alpha2, OX40, TSLP-R, IL-7Ralpha (a co-receptor for TSLP), IL17RB (receptor for IL-25), ST2 (receptor for IL-33), CCR3, CCR4, CRTH2, FcepsilonRI or FcepsilonRII/CD23 (receptors for IgE), in the preparation of a medicament for the treatment of a patient having asthma, wherein the patient has been determined to express elevated expression levels of POSTN gene.

[0020] The present invention disclosure provides a kit for diagnosing an asthma subtype in a patient comprising (1) one or more nucleic acid molecules that hybridize with a gene, wherein the gene is selected from the group of consisting of POSTN, CST1, CST2, CCL26, CLCA1, PRR4, PRB4, SERPINB2, CEACAM5, iNOS, SERPINB4, CST4, and SERPINB10 and (2) instructions for measuring the expression levels of the gene from an asthma patient sample, wherein the elevated expression levels of any one, combination or all of said genes is indicative of the asthma subtype. According to one embodiment, the kit further comprises a gene selected from the group consisting of: PRB4, TPSD1, TPSG1, MFSD2, CPA3, GPR105, CDH26, GSN, C2ORF32, TRACH2000196 (TMEM71), DNAJC12, RGS13, SLC18A2, SH3RF2, FCER1B, RUNX2, PTGS1, and ALOX15. In one further embodiment, the gene expression level is measured by assaying for mRNA levels. In another further embodiment, the assay comprises a PCR method or the use of a microarray chip. In yet a further embodiment, the PCR method is qPCR. In one embodiment, the mRNA levels of the gene of interest relative to a control gene mRNA level greater than 2.5 fold is indicative of the asthma subtype.

[0021] The ~~invention~~disclosure provides a kit for diagnosing an asthma subtype in a patient comprising (1) one or more protein molecules that bind to a protein selected from the group of consisting of POSTN, CST1, CST2, CCL26, CLCA1, PRR4, PRB4, SERPINB2, CEACAM5, iNOS, SERPINB4, CST4, and SERPINB10 and (2) instructions for measuring the expression levels of the protein from a patient sample, wherein the elevated expression levels of any one, combination or all of said proteins is indicative of the asthma subtype. In one embodiment, the kit further comprises a protein molecule that binds to a protein selected from the group consisting of: PRB4, TPSD1, TPSG1, MFSD2, CPA3, GPR105, CDH26, GSN, C2ORF32, TRACH2000196 (TMEM71), DNAJC12, RGS13, SLC18A2, SH3RF2, FCER1B, RUNX2, PTGS1, and ALOX15. In one embodiment the protein molecule is a antibody, a peptide or a peptibody. In a further embodiment, the kit comprises a microarray chip comprising the protein molecule(s).

[0022] The present ~~invention~~disclosure provides a kit for diagnosing an asthma subtype in a patient comprising instructions for measuring any one of the biomarkers from a patient sample selected from the group consisting of: serum total IgE levels, serum CEA levels, serum periostin levels, peripheral blood eosinophils and bronchoalveolar lavage (BAL) eosinophils,

wherein elevated levels of CEA, serum periostin, peripheral blood eosinophils and bronchoalveolar lavage (BAL) eosinophils. According to one embodiment, the kit provides instructions wherein an IgE level greater than 100IU/ml is indicative of the asthma subtype. According to another embodiment, the kit provides instruction, wherein a peripheral blood eosinophil level greater than $0.14 \times 10^9/L$ is indicative of the asthma subtype.

[0023] The present ~~invention~~disclosure provides a kit for diagnosing an asthma subtype in a patient comprising instructions for measuring the ratio of Muc5AC:MUC5B mRNA or protein from a sample of an asthma patient, wherein a ratio greater than 25 is indicative of the asthma subtype. In one embodiment, the sample is obtained from an epithelial brushing. In another embodiment, the sample comprises airway epithelial cells. In one embodiment, the kit provides a nucleic acid molecule that hybridizes under stringent conditions with Muc5AC and a nucleic acid molecule that hybridizes under stringent conditions with MUC5B. In one embodiment, the kit provides a protein molecule that binds to Muc5AC and a protein molecule that binds to MUC5B. In one embodiment, the protein molecule is an antibody.

[0023a] In one aspect, there is provided a kit for diagnosing an asthma subtype in an asthma patient comprising:

(1) one or more nucleic acid molecules that hybridize with a gene or one or more protein molecules that bind to a protein, wherein the gene or protein is POSTN and optionally one or more genes or proteins selected from the group of consisting of CST1, CST2, CCL26, CLCA1, PRR4, PRB4, SERPINB2, CEACAM5, iNOS, SERPINB4, CST4, and SERPINB10;

(2) instructions for measuring the expression levels of the genes or proteins from a patient sample, wherein the elevated expression levels of any one, combination or all of said genes or proteins is indicative of the asthma subtype; and

(3) an agent which is an immunoadhesin, a peptibody or an antibody that binds a target, wherein the target is IL-9, IL-5, IL-13, IL-4, OX40L, TSLP, IL-25, IL-33, IgE, IL-9 receptor, IL-5 receptor, IL-4 receptor-alpha, IL-13 receptor-alpha1, IL-13 receptor-alpha2, OX40, TSLP-R, IL-7Ralpha (a co-receptor for TSLP), IL17RB (receptor for IL-25), ST2 (receptor for IL-33), CCR3, CCR4, CRTH2, FcepsilonRI or FcepsilonRII/CD23 (receptors for IgE) .

[0023b] In one aspect, there is provided a kit for diagnosing an asthma subtype from serum sample in an asthma patient comprising:

- (1) one or more nucleic acid molecules that hybridize with a gene or one or more protein molecules that bind to a protein, wherein the gene or protein is POSTN and optionally one or more genes or proteins selected from the group of consisting of CST1, CST2, CCL26, CLCA1, PRR4, PRB4, SERPINB2, CEACAM5, iNOS, SERPINB4, CST4, and SERPINB10; and
- (2) instructions for measuring the expression levels of the genes or proteins from a patient sample, wherein the elevated expression levels of any one, combination or all of said genes or proteins is indicative of the asthma subtype.

BRIEF DESCRIPTION OF THE DRAWINGS

[0024] Figure 1 shows gene expression levels in airway epithelium as described in Examples 1 and 2. (A) Relative expression levels of periostin (left panel), CLCA1 (middle panel), and serpinB2 (right panel) in healthy controls (N=27) and in asthmatics (N= 42) are shown. Normalized fluorescence units are indicated on the left axis of each plot. (B) Two-way comparisons of expression levels of periostin and CLCA1 (left panel), periostin and serpinB2 (middle panel), and CLCA1 and serpinB2 (right panel) in 42 asthmatics are shown. Spearman's rank order correlation (ρ) and p-values are indicated in each panel. (C) Gene expression microarray analysis for healthy controls and asthmatics identifying expression levels of periostin and co-regulated genes; IL-4/13 signature high cluster (cluster 1); IL-4/13 signature low cluster (cluster 2); healthy controls. (D) Heatmap depicting unsupervised hierarchical clustering (Euclidean complete) of periostin, CLCA1, and serpinB2 expression levels in bronchial epithelium across all subjects at baseline. (E) Mean ($\pm$ SEM) expression levels of IL-4, IL-5, and IL-13 in bronchial biopsy homogenates obtained contemporaneously with bronchial brushings from a subset of subjects depicted in Figures 1A-D (cluster 1: 18 "IL-13 high" asthmatics; cluster 2: 16 healthy controls and 14 "IL-13 low" asthmatics). Two-way correlations across all subjects between IL-4, IL-5, and IL-13 indicated at right (Spearman's rank order correlation, ρ , and p-values).