A Novel Panel of Serum Biomarkers Distinguishes Asthma from Non-Small Cell Lung Cancer

Abstract

Purpose: Asthma affects an increasing proportion of the population and may develop as an allergic lung disorder. Frequent misdiagnosis of asthma and lack of early detection tests for lung cancer might be mitigated by better diagnostic tests. We used multiplexed immunoassays and mass spectrometry to identify serum biomarkers capable of distinguishing asthma from non-small cell lung cancer, the most common type of lung cancer.

Methods: Sera from normal subjects (NO, n = 300), patients with asthma (AST, n=100), and non-small cell lung cancer (LC, n = 134) were acquired from commercial vendors. Levels of 50 cytokines, chemokines, and growth factors were quantified using multiplexed immunoassays. For biomarker discovery, sera were digested with trypsin and analyzed by liquid chromatography electrospray ionization MS (LC ESI MS/MS). Proteins were identified using Mascot search software. Validation of select biomarkers identified by MS was achieved by immunodetection of target proteins in serum specimens. Data were reduced using inter-pathology comparisons with statistical significance determined using Student's t-test.

Results: Multiplexed immunoassays identified 29 analytes with significantly (t < 0.01) inter-pathology differences. 16 of these were highly significant (t < 0.0001). We found a subset of biomarkers with unexpectedly large inter-gender differences and distinct gender-specific expression patterns. Serum levels of interleukin-13 and small inducible cytokine (I-TAC) showed the greatest difference between AST, LC, and NO sera. MS analysis data identified 11 differentially expressed proteins that included 3 putative yet unnamed proteins identified by gene sequencing and 1 known protein product corresponding to chromosome X open reading frame 38. The presence of select proteins discovered by MS (syntaxin 11, cAMP-regulated phosphoprotein corresponding to chromosome X open reading frame 38, and arginase) was confirmed and quantified by immunoblotting.

Conclusion: We have identified a group of serum biomarkers having high inter-pathology discrimination power that are capable of differentiating AST and LC from normal controls and that may potentially be used in diagnostic tests for early detection of lung diseases. Our experimental strategy is widely applicable to discovery and validation of biomarkers for diverse human diseases, response to a therapy or pre-selection of patients for clinical trials.

Introduction

There is no simple, reliable method of diagnosing pathologies of human lung tissues early in their development. Furthermore, there is not a blood test available today which can diagnose a pathology for many marks, hence a multi-marker approach to pathology classification characterizing disease status using a fingerprint of molecular signatures designed to accommodate the variations encountered in single marker measurements. We applied two different analytical methods to identify panels of biomarkers that straitly and correctly classify the physiologic state of a subject with regard to non-small cell lung cancer and asthma.

Bibliography


Conclusions

We have identified several panels of markers that straitly and correctly classify the physiologic state of a subject with regard to non-small cell lung cancer and asthma. Our findings support a multi-marker strategy for the diagnosis of non-small cell lung cancer by measuring expression levels of groups of biomarkers and facilitate early detection of the disease through simple blood tests, characterize disease progression and to differentiate among diverse human conditions.

Figure 1: Scatter plots of MMP-1 expression in subsets of individual specimens from men and women with non-small lung cancer (LC), asthma (AST), and normal controls (NO).

Figure 2: Relative levels of biomarkers in non-small cell lung cancer (LC), asthma (AST) and normal controls (NO). The biomarkers (see Table 1) are not separated by gender. The data are shown as percentage values in the respective reference groups; NO for AST and LC or LC for AST. The upregulated and downregulated biomarkers are shown as outbound and inbound spikes, respectively.

Table 1. Comparison of t values for differential biomarker expression levels in non-small lung cancer (LC), asthma (AST), and normal controls (NO).

Table 2. Mass spectral identification of differentially expressed proteins in sera from patients with non-small lung cancer and asthma in comparison with normal controls. The presence of highlighted proteins was further confirmed by immunodetection.

Table 3. Mass spectral identification of differentially expressed proteins in sera from subjects with non-small lung cancer and asthma in comparison with normal controls. The presence of highlighted proteins was further confirmed by immunodetection.

Table 4. Global biomarker diversity in men and women subjects across three types of specimens; normal controls (NO), asthma (AST), and non-small lung cancer (LC) assessed by k-nearest neighbor analysis. The percentages of biomarkers are tabulated in the order of increasing significance and indicated by colors from yellow to brown.