Identification of Serum Biomarkers for Lung Cancer Using Multiplexed Immunoassays

1 Sung Baek, 2 Elzbieta Izbicka, 3 David R. Campos, 4 Robert T. Streeper, 5 Armando Diaz III
1 JBNI Corporation, Bothell, WA, 2 CTRC Institute for Drug Development, San Antonio, TX.

Abstract

Background: Symptoms of lung cancer (LC) often do not appear until the disease is advanced; only 15% of LC cases are discovered while the tumor is in the early stages of development. Carcinogen exposure, asthma and smoking have been determined to be risk factors for the development of LC. Early detection of LC will likely have a major impact on the natural history of the disease and facilitate curative treatment. The objective of this study was to apply multiplexed immunoassays to identify a panel of biomarkers for early detection of LC.

Methods: Normal (NO) serum controls (n = 30) from healthy volunteers and lung cancer patients (n = 30) were acquired from a commercial vendor. Baseline (pre-treatment) serum specimens from individuals with AST (n = 28) and lung cancer risk (LCR; n = 72) were available from clinical trials of two novel agents that are being developed by JBNI Inc. for the respective indications. Serum levels of 59 cytokines, growth factors and biologically active peptides were quantified using multiplexed immunoassays using the Luminescence platform to identify biomarkers that are expressed in a significantly different manner in individuals with LC, LCR, or AST in comparison with NO subjects. Data were reduced using nearest neighbor cluster analysis with squared Euclidean distance to separate lung cancer into groups across analyses with inter-pathology comparisons determined using Student’s t test.

Results: Multiple analyses showed highly significant differences (p < 0.0002) between LC and healthy controls as single indices of pathologic state. In addition we were able to differentiate AST from NO (p < 0.0002) and LCR (p = 0.0001) using a panel of thirteen markers in various combinations. Using multiplexed assays we found significant differences in biomarker levels in sera separated by centrifugation, the supernatant was aliquoted into 96-well plates and frozen at -80 °C.

Conclusions: We have identified a group of markers having high inter-pathology discrimination power that are capable of reliably differentiating AST and LC from control specimens. This panel remains to be validated in a larger set of specimens but we are confident that these measures will produce clinical assays capable of reliably diagnosing lung pathology.

Materials and Methods

NO serum controls (n = 30) from healthy volunteers and from LC patients (n = 30) were acquired from a commercial vendor. Baseline (pre-treatment) serum specimens from individuals with AST (n = 28) and from LCR (n = 72) were acquired as part of a clinical trial of two novel agents for the treatment of asthma.

Serum levels of 59 cytokines, growth factors and biologically active peptides were quantified using multiplexed immunoassays using the Luminescence platform to identify biomarkers that are expressed in a significantly different manner in individuals with LC, LCR, or AST in comparison with NO subjects.

Samples were collected by venipuncture into sodium citrate Vacutainer tubes. Plasma was separated by centrifugation, the supernatant was aliquoted into 96-well plates and frozen at -80 °C.

Statistical analyses were accomplished by first breaking out data for each pathology for each marker measured into subgroups by univariate random seed & nearest neighbor cluster analysis.

The selected markers were extracted and grouped into combinatorial panels and subjected to further multivariable cluster analysis across pathologies followed by inter-subgroup t testing. Those markers found to present optimum combinations of characteristics were extracted, grouped together and again broken into clusters.

Comparative analyses of inter-pathology differences were accomplished by performing cluster analysis for identified sets of markers. Individual specimens contained within the generated subgroups were counted according to pathology by sub-group and the counts were used to calculate the discriminative capability of any given panel of markers.

Results

Figure 1. Univariate cluster patterns for 59 markers in lung cancer (A), normal (B) and asthma (C). Data are ranked by color with yellow, green, red and blue representing progressively higher intensities respectively.

Figure 2. Multivariate cluster analysis for 12 selected markers: IL-6, EGF, IL-8, IL-13, LIF, VEGF, MCP-1, MIP-1α, IL-10 and VEGF, having relatively poor inter-pathology differentiating power showing rank stratification of patient samples between lung cancer (A, E, normal (B, C) and asthma (D, F) patient groups. Statistical summary for inter-group comparisons for normal vs. lung cancer (A), normal vs. asthma (D) and asthma vs. lung cancer (E, F) and normal vs. asthma (I).

Figure 3. Multivariate cluster analysis for 13 selected markers* showing rank stratification of patient samples between lung cancer (A, E), normal (B, C) and asthma groups (D, F). Statistical summary for inter-group comparisons for normal vs. lung cancer (G), asthma vs. lung cancer (H) and normal vs. asthma (I). Histogram plots of attendance vs. class rank for normal vs. lung cancer (J), asthma vs. lung cancer (K) and normal vs. asthma (K).

* Patent pending

Summary

Given the wide intra- and inter-individual variance within a pathology for many markers, we adopted a multi-marker approach to pathology classification characterizing disease status using a fingerprint of molecular signatures designed to compensate for the wide variances encountered in single marker measurements.

We have identified several panels of markers that clearly and correctly classify the particular pathology present in the patient. The minimum predictive percentage observed was 76% for the comparison of LC to N specimens across 4 selected markers. We identified several combinations of markers capable of clearly differentiating N from LC with 86% probability and that further partition LC patients into at least 3 molecular signatures designed to compensate for the wide variances encountered in single marker measurements. During the course of these analyses we discovered that the LCR group, which is composed of samples from smokers, was diverse in composition to the point that inclusion of these specimens in the comparative analyses yielded no meaningful results (data not shown) and thus these specimens were excluded from further analysis.

Comparative analyses of inter-pathology differences were accomplished by performing cluster analysis for identified sets of markers. Individual specimens contained within the generated subgroups were counted according to pathology by sub-group and the counts were used to calculate the discriminative capability of any given panel of markers.

Research: JBNI Corporation