Identification of tumor antigens directed antibodies

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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. We have implemented a proteomic strategy to identify tumor antigens that induce a humoral immune response in lung cancer based on the analysis of tumor cell proteins. Chromatographically fractionated protein extracts from 3 lung cancer cell lines were subjected to Western blotting and hybridization with individual sera to determine serum antibody binding. Two sets of sera were initially investigated. One set consisted of sera from 19 newly diagnosed subjects with lung adenocarcinoma and 19 matched controls. A second independent set consisted of sera from 26 newly diagnosed subjects with lung adenocarcinoma and 24 controls matched for age, gender and smoking history. One protein that exhibited significant reactivity with both sets of cancer sera ($p= 0.0008$) was confidently identified by mass spectrometry as 14-3-3 theta. Remarkably, significant autoantibody reactivity against 14-3-3 theta was also observed in an analysis of a third set consisting of 18 pre-diagnostic lung cancer sera collected as part of the Beta-Carotene and Retinol Efficacy Trial (CARET) cohort study, relative to 19 matched controls ($p= 0.0042$). A panel of 3 proteins consisting of 14-3-3 theta identified in this study, plus annexin 1 and PGP 9.5 proteins previously identified as associated with autoantibodies in lung cancer, gave a sensitivity of 55% at 95% specificity in discriminating lung cancer at the preclinical stage from matched controls. A high throughput platform based on protein microarray for quantitative analysis of serum autoantibodies was used for validation. The microarrays produced were utilized in a blinded validation study to determine whether annexin I, PGP9.5, and 14-3-3 theta antigens are associated with autoantibodies in sera collected at the pre-symptomatic stage. 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. We present evidence for the occurrence in lung cancer sera of autoantibodies to annexin I, 14-3-3 theta, and a novel lung cancer antigen, LAMR1, which precede onset of symptoms and diagnosis.