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FINAL PERFORMANCE REPORT

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Table of Contents

1. Significant Findings	3
2. Abstract	4
3. Report - New Method for Occupational Cancer Surveillance	5
A. Importance to Occupational Safety and Health	5
B. Objectives	5
C. Methodology	5
D. Findings	6
E. Conclusions	8
4. Publications	9

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1. Significant Findings

The significant findings of this research are as follows:

(a) A serum assay for oncogene-encoded proteins based on immunoblotting with monoclonal antibodies to oncogene protein sequences can be used to distinguish a percentage of cancer patients from normal, healthy controls. The most promising results were obtained by assaying for the ras oncogene encoded p21 protein which detected approximately half of the colon and lung cancer patients (compared to a positive rate of 5% or less in controls).

(b) Identification of the ras oncogene encoded p21 protein in serum can be used to detect a percentage of those individuals in cohorts of workers exposed to occupational carcinogens who will subsequently develop cancer. Thus, in a cohort of asbestos/silica exposed workers, serum ras p21 was detected in 39% of those who developed cancer (compared to a positive rate of 7% in those who did not; $p=0.012$), and the average lead time between serum detection and clinical diagnosis of disease ranged from 3 to 26 months (average=16.3 months).

2. Abstract

Biomarkers of response to exposure to occupational carcinogens may allow early detection of those individuals at risk for the development of occupational cancers. Since many occupational carcinogens appear to have an effect on oncogene activation in vitro, the detection of oncogene activation by assaying for the expression of oncogene encoded proteins in vivo via the detection of these proteins in serum may be a useful biomarker of response to exposure to occupational carcinogens. A serum immunoblotting assay based on monoclonal antibodies to oncogene protein sequences was used to investigate serum oncogene protein expression in cancer patients versus normal controls and in cohorts exposed to occupational carcinogens. Results in cancer patients indicate that this approach can be used to distinguish a percentage of individuals with common cancers (approximately 50% of cases of colon or lung cancer positive) from controls (less than 5% positive), and that these changes may be detectable early in the disease process at the level of pre-malignant lesions. Results in occupational cohorts exposed to carcinogens indicate that this approach can be used to distinguish a percentage of those individuals who subsequently develop cancer (39% of those who develop cancer were positive compared to 7% of those who did not develop cancer), and the average lead time between serum detection and clinical diagnosis was 16 months. These results suggest that immunoblotting detection of serum oncogene encoded proteins may be useful biomarkers of response for the early identification of workers at risk for the development of cancer due to their occupational carcinogen exposures.

3. Report - New Method for Occupational Cancer Surveillance

A. Importance to Occupational Safety and Health

An important goal of occupational health is the prevention of occupational cancers. A critical step toward that goal is the development of biomarkers of exposure and response to workplace carcinogens. Such biomarkers should allow identification of those individuals who are at an early stage of developing neoplastic disease so that the disease process can be aborted. This research demonstrates the utility of a newly developed biomarker, based on the detection of oncogene-encoded proteins in serum, in contributing to the early detection of biological response to workplace carcinogen exposure.

B. Objectives

The overall aim of this research was to develop monoclonal antibody immunoblotting assays for the detection of oncogene protein products in serum. The assay was applied to cancer patients and occupational cohorts at risk for the development of cancer due to workplace exposures to demonstrate the ability to detect early neoplastic changes in these groups. The approach was based on experimental evidence that many occupational carcinogen can produce cancer via a pathway that includes oncogene activation at a relatively early stage.

C. Methodology

The research consisted of two parts. The first part involved validation of the serum oncogene protein assay in cohorts of cancer patients and controls. Sensitivity, specificity, and reproducibility of the assay was examined, and

the seroprevalence of specific oncogene products among patients with various types and stages of cancer were examined. The second part involved examination of the value of the assay for early detection of those individuals who will develop cancer in occupational cohorts with carcinogen exposure and increased risk of malignancy in a nested case-control study based on banked serum specimens.

D. Findings

Results on cancer patients and controls indicate this assay to be highly sensitive (in the sub-nanogram range), specific (competitive inhibition with known protein completely blocks response), and reproducible (approximately 20% variability). Results in colon and lung cancer patients indicate that the most frequent cancer-associated abnormality occurs with the p21 ras oncogene protein. Thus, for example, positive results for p21 ras oncogene protein were found as follows in the colon cancer study: 2% of normal controls, 10% of small (<10mm) polyps, 30% of large (>10mm) polyps, 67% of carcinomas in situ, and 50% of early invasive (Duke's stage A) cancers. These results suggest that abnormalities of serum p21 are frequent in colonic carcinogenesis and may occur early in the process, so that serum p21 may be a useful early marker of response to carcinogenic insults. Similar positive results for p21 were found in lung cancer patients: 5% of normal controls versus 45% of cancer cases.

The most significant findings in occupational cohorts derive from studies of banked serum specimens from 46 asbestos and silica exposed workers, 18 of whom had subsequently developed

cancer (including 11 lung cancers and 2 pleural mesotheliomas). Positive serum p21 ras was detected in 7 of the 18 patients who developed cancer (39%, including 5 of the 11 lung cancers and both of the mesotheliomas) versus 2 of the 28 patients without cancer, a statistically significant difference ($p=0.012$). In addition, in these cases serum p21 ras positivity was found in the samples collected prior to the time of clinical diagnosis of disease (average=16.3 months, range=3-26 months prior to diagnosis), providing further confirmation that serum p21 may be a useful marker for early detection of neoplastic change, particularly in occupationally-exposed at-risk cohorts. An unexpected finding in this cohort was that growth factors (PDGF), although unrelated to cancer outcome, was detected significantly more frequently in advanced pneumoconiosis cases (ILO radiographic classification of 2/1 or greater) than in less advanced cases ($p=0.016$), and there was a tendency for these patients to have progression of their disease ($p=0.065$), suggesting that serum growth factors may be a marker for the development of severe and progressive pneumoconiosis. Further support for this premise was provided by the study of firefighters in which another serum growth factor (TGFB) was found more frequently in those firefighters with radiographic evidence of early asbestos-related disease (all radiographs were 1/1 or lower) than in those with normal radiographs. These findings suggest that further investigation of serum growth factor expression in pulmonary fibrotic disease may be worthwhile.

Based on these findings, several follow-up studies have been

initiated. For example, examination of serum p21 ras in environmentally exposed populations in Eastern Europe has found twice the rate of positivity in the exposed cohort as compared to unexposed controls. Examination of mutated serum p21 in vinyl chloride exposed workers indicate that individuals who develop hemangiosarcomas have detectable protein in their serum and tumor tissue, and a high proportion (50%) of individuals with long-term, high exposure also express the protein in their serum. Finally, this analytic approach has been expanded to include the detection of other important cancer related proteins in serum such as p185 erbB-2 and p53.

E. Conclusions

The results of this research indicate that the immunologic detection of serum oncogene proteins, in particular the ras oncogene encoded p21 protein, can be used to distinguish individuals with certain cancers from normal controls in a sensitive, specific, and reproducible fashion. Furthermore, when applied to the study of certain occupational cohorts with carcinogen exposure, such as asbestosis patients, serum p21 can identify a significant proportion of those individuals who develop cancer at a point in time prior to the clinical detection of disease. Thus, this approach may be useful for the early identification of workers at risk for the development of cancer due to their occupational carcinogen exposures.

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