A) FINAL PROGRESS REPORT

PI: Paul W. Brandt-Rauf, Dr.P.H., M.D., Sc.D.
Office of the Dean
School of Public Health
University of Illinois at Chicago
1603 West Taylor Street
Chicago, IL 60612
TEL: 312-996-5939
EMAIL: pwb1@uic.edu

Institution: Board of Trustees of the University of Illinois
304 AOBm, M/C 672
1737 West Polk Street
Chicago, IL 60612

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List of Terms and Abbreviations:

ELISA, enzyme-linked immunosorbent assay; KIF5A, kinesin 5A; KIF18A, kinesin 18A

Abstract

NIOSH recognizes the need for research for the development of methods for measuring the early markers of adverse health effects from workplace exposures. This is especially important for asbestos-related cancers because asbestos remains a major occupational health risk in several of the industries targeted in the National Occupational Research Agenda's Sectors Program, such as construction, manufacturing, mining, and transportation and utilities, and cancer remains a major disease category of concern in the NIOSH Cross-Sector Program; world-wide, it is estimated that more than a hundred thousand workers die each year from asbestos-related diseases, particularly cancer. In preliminary studies based on the proteomic analysis of a limited number of banked serum samples from a cohort of asbestosis cases, we had tentatively identified novel kinesin family protein biomarkers (KIF5A and KIF18A) that may be highly correlated with the subsequent development of cancer in these workers. The purpose of this proposal was to follow up on this finding in a systematic and quantitative fashion by using enzyme-linked immunosorbent assays (ELISAs) to analyze all of the 364 serum samples from all of the 110 workers in this cohort for levels of KIF5A and KIF18A. These levels will be correlated with the subsequent development of cancer in these workers. This research would be the first systematic study of kinesin proteins as potential serum biomarkers for cancer, and, if successful, would demonstrate that these kinesin biomarkers could be useful for identifying those individuals with asbestosis who are at the highest risk for developing cancer and who could then be selected for more aggressive and targeted preventive/therapeutic interventions, thus reducing the burden of asbestos-related malignancies.

Results for KIF5A in the worker samples showed that levels increased from 3.5 ng/ml in those cases with asbestosis that did not develop cancer to 3.9 ng/ml in those cases that did develop cancer, but this difference was not statistically significant. However, when compared to levels in normal, healthy individuals, the levels in both the workers who did not develop cancer and the workers who did develop cancer were statistically significantly elevated. Similarly, the percentages of KIF5A-positives (defined as the mean + 2SD of normals) increased from 34.2% in the workers who did not develop cancer to 48.4% in those that did, a statistically significant trend. These results remained statistically significant even after adjusting for differences in potential confounders, including asbestos exposure. Therefore, these results suggest that KIF5A levels may be a biomarker of disease progression following asbestos exposure. However, all other results, including for KIF18A, were negative or inconclusive.

Section 1

Highlights/Significant Findings:

This research has one significant finding. It identified KIF5A as a potential biomarker of asbestos exposure and asbestos-related disease occurrence and progression.

Translation of Findings:

The findings of this research can be translated for use in the early detection, treatment and prevention of occupational diseases related to asbestos exposure. Among asbestos workers at any given level of exposure, it is known that not all will develop disease (asbestosis or cancer). The newly identified KIF5A biomarker may be useful in identifying those workers who are at the greatest risk of disease from asbestos exposure. These workers could then be targeted for more aggressive interventions to prevent the occurrence or progression of disease.

Outcomes/Relevance/Impact:

The results of this research have significant potential outcomes which could impact workplace risk if used, as suggested above. In particular, the results could lead to better secondary prevention of occupational pneumoconiosis and cancer by identifying asbestos-exposed workers at the highest risk for subsequent development of disease who could be targeted for more aggressive interventions, thus reducing asbestos-related morbidity and mortality.

Section 2

Scientific Report

According to the National Occupational Research Agenda, one of NIOSH's primary research objectives is "identifying and investigating the relationships between hazardous working conditions and associated occupational disease", including the development of "methods for measuring early markers of adverse health effects". One area where recent advances in our understanding of these relationships have made this approach especially relevant is the study of asbestos carcinogenesis. In particular, some of our previous NIOSH-funded research had suggested that certain proteins may play an important role in asbestos carcinogenesis and may serve as useful early markers of cancer risk in asbestos-exposed workers. This is of great importance because asbestos remains a major occupational health risk in several of the industries targeted in the NORA Sectors Program, and cancer remains a major disease category of concern in NIOSH Cross-Sector Program. Therefore, any early marker of cancer risk from asbestos exposure could have a large impact on the amelioration of occupational disease by allowing the identification of individuals at the highest risk to be targeted for more aggressive intervention. Kinesin family proteins (KIFs) may be one such early marker of asbestos-related cancer risk.

The kinesin superfamily currently includes 45 different proteins classified into a number of family groups. KIFs are a conserved class of microtubule-dependent molecular motor proteins that have ATPase activity and motion characteristics. The active movement of KIFs supports several critical cellular functions, such as mitosis, meiosis and the transport of macromolecules, for example, through axonal transport. Different KIFs may participate in different cellular functions, but KIF5s and KIF18s mainly participate in mitosis. In mitosis of

eukaryotic cells, KIFs participate in spindle formation, chromosome congression and alignment, and cytokinesis. To date, many studies have demonstrated that altered expression of KIFs is associated with the development and progression of various human cancers, including in the lung. Abnormal kinesis expression alters the equal distribution of genetic materials during cell mitosis because of chromosome hypercondensation, aberrant spindle formation, anaphase bridges, defective cytokinesis, aneuploidy and mitotic arrest. Of course, the resultant loss or gain of genetic material can lead to numerous defects in the daughter cells resulting in the promotion of carcinogenesis and/or the progression of aggressive behavior of the corresponding tumor cells. Finally it should be noted that asbestos has long been recognized to be able to induce significant mitotic aberrations leading to chromosomal instability that is associated with cancer; furthermore, in cell culture experiments, asbestos has been shown to induce these chromosomal effects by binding to proteins that regulate the cell cycle, cytoskeleton and the mitotic process. Thus, it was not a complete surprise that we identified KIF5A and KIF18A as potential biomarkers of asbestos-related cancer risk in our preliminary studies. These studies used a proteomic approach to biomarker discovery based on surface-enhanced laser desorption/ionization time-of-flight (SELDI-TOF) mass spectrometry, an approach which has shown promise for the discovery of new biomarkers for cancer risk. We applied this approach to the analysis of serum samples from asbestos workers with and without cancers. The results identified three protein peaks that could predict the development of cancer with good sensitivity and specificity. One minor peak did not correspond to any known protein. However, the other two protein peaks were identifiable as KIF5A and KIF18A.

The purpose of the current research was to follow-up on these proteomic results to further explore if these KIF proteins could indeed be useful biomarkers for predicting cancer risk from asbestos exposure. This was accomplished using enzyme-linked immunosorbent assays (ELISAs) to quantitate the levels of KIF5A and KIF18A in the banked serum samples from a cohort of asbestosis cases some of whom subsequently developed cancer and others that remained cancer-free. The results were only partially successful. First, the KIF5A levels did increase from 3.5 ng/ml in those cases with asbestosis that did not develop cancer to 3.9 ng/ml in those cases that did develop cancer; this difference was even greater for those cases that developed cancers most closely related to asbestos exposure (lung cancers and mesotheliomas where their value was 4.1 ng/ml) with the highest levels in the mesothelioma cases (5.5 ng/ml); but none of these differences were statistically significant. However, when compared to values in normal, healthy individuals, the results in both the workers that did not develop cancer and the workers that did develop cancer were statistically significantly elevated ($p<0.0001$), and there was a statistically significant trend for the values from normals to asbestosis cases without cancer to asbestosis cases with cancer ($p<0.0001$). Similarly, using a definition for an elevated KIF5A level as any value greater than the mean + 2SD of normal, healthy individuals, the percentage of KIF5A positives were 34.2% in the asbestosis cases without cancer to 48.8% in the asbestosis cases with cancer, again statistically significant differences ($p<0.0001$). These results remained statistically significant even after adjusting for differences in age, gender, smoking status, and asbestos exposure in logistic regression analyses ($p<0.0001$). Therefore, these results suggest that KIF5A levels may be a biomarker of disease progression following asbestos exposure, independent of the level of asbestos exposure itself. However, all other results were negative or inconclusive. In particular, in similar analyses, the results for KIF18A did not demonstrate any relationship to asbestos exposure or occurrence or progression of disease even in comparisons to the unexposed controls.

In conclusion, these results suggest that serum KIF5A levels (but not KIF18A levels) may be a biomarker of disease progression following asbestos exposure, independent of the

level of asbestos exposure itself or other risk factors. Further study of KIF5A in other, larger cohorts is warranted to confirm these findings.

Publications

Pending.

Inclusion of Gender and Minority Subjects

This research involved asbestos workers. Due to the nature of this workforce, which historically did not include women or other minorities, very few women and no other minorities could be included in this study.

Inclusion of Children

No children were included in this study due to the nature of the populations from which the subjects were drawn, namely adult workers.

Materials Available for Other Investigators

Not Applicable.