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Final Performance Report

Lung Cancer in Uranium Miners: A Tissue Resource and Pilot Study

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Abstract

The project entitled, "Lung Cancer in Uranium Miners: A Tissue Resource and Pilot Study" incorporates two related research projects directed toward understanding respiratory carcinogenesis in radon-exposed former uranium miners. The first project involved a continuation of the tissue resource of lung cancer cases from former underground uranium miners and comparison cases from non-miners. The second project, entitled "The Uranium Miners' Health Study (UMHS)" was a pilot study for a proposed longitudinal study of respiratory carcinogenesis in former uranium miners. The objectives included facilitating the investigation of molecular changes in radon exposed lung cancer cases, developing methods for prospectively studying clinical, cytologic, cytogenetic, and molecular changes in the multi-event process of respiratory carcinogenesis, and assessing the feasibility of recruiting former uranium miners into a longitudinal study that collected multiple biological specimens.

Lung tissue for the tissue resource project was collected from five Albuquerque hospitals and from St. Mary's Hospital in Grand Junction, Colorado. A pilot study was conducted to determine whether blood collection, induced sputum, bronchial brushing, washings, and mucosal biopsies from participants at two of the hospitals could be included efficiently. A questionnaire was developed for the extended study and all protocols for specimen collection and tissue handling were completed. Resource utilization is in progress at ITRI and the methods have been developed to study molecular and cellular changes in exfoliated cells contained in sputum as well as susceptibility factors.

Introduction

The project entitled, "Lung Cancer in Uranium Miners: A Tissue Resource and Pilot Study" incorporates two related research projects directed toward understanding respiratory carcinogenesis in radon-exposed former uranium miners. The first project involved a continuation of the tissue resource of lung cancer cases from former underground uranium miners and comparison cases from non-miners. The second project, entitled "The Uranium Miners' Health Study (UMHS)" was a pilot study for a proposed longitudinal study of respiratory carcinogenesis in former uranium miners. The objectives included facilitating the investigation of molecular changes in radon exposed lung cancer cases, developing methods for prospectively studying clinical, cytologic, cytogenetic, and molecular changes in the multi-event process of respiratory carcinogenesis, and assessing the feasibility of recruiting former uranium miners into a longitudinal study that collected multiple biological specimens.

Funded on September 25, 1992, the extended project facilitated the development of the tissue resource, collection of tissues continued and a new protocol for collection of additional specimens including blood, induced sputum, and bronchoscopically obtained mucosal biopsies and washings was implemented. The principal tasks completed for the pilot investigation were to establish community networks, identify and recruit former uranium miners, identify study venues and resources in Albuquerque and Grants, New Mexico, strengthen the collaborative efforts between St. Mary's Hospital and the University of New Mexico, and develop and test interviewing and tissue handling protocols. As part of the pilot study, 89 former uranium miners participated in the clinical assessment and donated specimens. The sputum specimens have been used to develop methods while the blood specimens are currently being used to correlate susceptibility genotypes with molecular changes in the sputum.

Project Activities

Community cooperation: The investigators developed and maintained full medical community cooperation with the tissue collection protocol. Drs. Samet and Gilliland obtained support for the project from Drs. Arnolfo and Valdivia of the Grants clinic and other Grants area physicians involved in the care of former uranium miners and St. Mary's newly formed Cancer Research Center in Grand Junction, Colorado.

Tissue collection: Tissue samples from 127 cases were collected in Albuquerque and Grand Junction. An extended tissue collection protocol was implemented after gaining approval from the Institutional Review Board of the University of New Mexico School of Medicine and the Albuquerque Veteran's Administration Hospital. As part of the extended tissue collection protocol, blood, induced sputum, bronchial brushings, washings and biopsy specimens were collected on 89 former miners, including 10 individuals undergoing diagnostic bronchoscopy.

Tissue utilization: Twenty nine tissue specimens were analyzed by Dr. Lechner's group at ITRI and Dr. Harris's group at NCI. In addition, Dr. Lechner and Dr. Belinsky used sputum specimens to develop molecular techniques including FISH and promoter methylation that were used in the longitudinal study.

Pilot study for proposed longitudinal cohort study: Protocols were developed for collecting and handling specimens and conducting interviews. The Institutional Review Board of the University of New Mexico School of Medicine approved study protocols. A request to use the University of New Mexico's Clinical Research Center as the Albuquerque study venue was submitted and approved. The outreach program of Miner's Colfax Medical Center made their miner's screening van and

personnel available for screening in Grants. Recruitment materials were developed including a bilingual recruitment brochure, a toll-free telephone number for responses, and a telephone eligibility screening criteria sheet and script.

In the pilot phase, 89 miners from New Mexico were entered into the study protocol, 45 in Albuquerque and 44 in Grants. Ten volunteers underwent bronchoscopy based on clinical indications and exposure status. The Grand Junction investigators continued follow-up of a group of former miners and developed their own grant-supported program. Data from the pilot phase were used to support several grant applications and indicate that a longitudinal study of former uranium miners is feasible. We plan to submit an application for funding of a longitudinal cohort.

Uranium Miners' Health Study

The Uranium Miners' Health Study (UMHS) Pilot study determined the feasibility and developed methods for a cohort of uranium miners for molecular epidemiology studies. We successfully recruited and medically screened 89 former uranium miners in the Grants and Albuquerque, New Mexico areas. High-risk miners were recruited with one mailed solicitation. Miners received the mailing if they were New Mexico residents and if they had been seen at anytime through the Miners' Colfax Medical Center outreach program, a statewide screening and medical care program for miners. Miners responded to the mailing by calling a toll-free number. A single mailing of approximately 400 letters resulted in an enrollment of 120 volunteers, some of whom were placed on a waiting list. A staff member interviewed the miners and determined eligibility based on the calendar year when underground work began and cigarette smoking history. In this pilot study, most subjects were current or former smokers with a lengthy history of underground work. Eligible miners received appointments for screening in Grants or in Albuquerque at the University of New Mexico's Clinical Research Center. Miners completed a structured interview that covered mining, occupation, smoking, family history, and other lung cancer risk factors and also included a standard respiratory symptoms questionnaire. Participants had a chest x-ray, pulmonary function testing (spirometry), and sputum induction. A second set of three-day sputum samples was collected by mail approximately one year later. Blood samples were collected, separated into serum, red cells, and buffy coat and frozen. Sputum was fixed for cytology and molecular assays using CytoLyt. Chest x-rays were clinically evaluated for abnormalities including lung cancer and pneumoconiosis and were read for the presence of infiltrates by two "B-readers" certified by the National Institute for Occupational Safety and Health (NIOSH) in compliance with the 1980 protocol of

the International Labor Office. Databases for study administration and data entry were developed and a manuscript is in preparation.

Sputum cytology interpretation: We found that collection of sputum by mail is feasible. We then collaborated with Dr. Geno Saccomanno on sputum cytology interpretation and sent 100 sputum samples to Grand Junction for analysis. We found that marked and severe dysplasia were uncommon.

Detection of p53 protein in sputum: IHC analysis of the p53 protein relies on the nuclear localization and increased stability of mutant p53 protein for the identification of cells carrying altered p53. The ability to detect aberrant p53 protein in sputum from patients with squamous cell carcinoma was evaluated in a blind study. Squamous cell carcinomas containing p53 mutations identified by sequencing were selected for this study. To date, 18 paired samples (tumor and sputum) have been examined. Sputum specimens were collected from one day to one month prior to tumor biopsy. A method was developed to double-stain sputum samples for cytokeratin and p53 to identify epithelial cells expressing mutant p53 protein and eliminate artificial staining of nonepithelial cells by the p53 antibody. Immunoreactive p53 protein was detected in 17 of 18 tumors examined, and concordance with sputum was observed in 11 cases. In four cases where discordance was observed, cellularity of the sputum preparation was greatly reduced. These data were collected prior to implementing current procedures for collecting induced sputum. These preliminary data indicate that techniques have been developed to double-stain epithelial cells within sputum for p53 protein and cytokeratin and that dysfunctional p53 protein present within the tumor can also be detected in cells exfoliated from the respiratory tract. The cleanup of sputum in conjunction with the collection of induced sputum should increase the sensitivity for detection of immunoreactive p53. The results will be included in a manuscript on the cross sectional portion of the study.

Improved exposure assessment using lead-210 (210Pb) bioassay: In collaboration with Dr. Ray Guilmette, at the Inhalation Toxicology Research Institute, we conducted a pilot study of 210Pb skull counting in 20 former miners.

Annual WLM Estimates: Overall, the working histories obtained by interview agreed almost exactly with information in the New Mexico Tumor Registry (NMTR) database. The earliest year of work in underground uranium mines was 1950 and the latest year was 1990 when the last operating mine in New Mexico closed (San Mateo mine operated by Chevron USA). The median first year of exposure was 1957. The median last year of work in underground uranium mines was 1982, when the price of uranium dropped dramatically and the mines began to close. The mean number of years of work in underground uranium mines was 22 years (median = 21 years).

Lifetime WLM exposures ranged from 20 to 2,605: The mean lifetime exposure was 761 WLM with a standard deviation of 575. The median level of exposure was 596 WLM and the interquartile range was 465 to 708 WLM. For the purposes of this study, missing data were estimated by linear interpolation between adjacent years where information was available (e.g., the years 1958, 1959, 1960 for Subject 7) and in one case, by assigning an average value derived from State Mining Inspector records (i.e., the years 1955, 1956, and 1957 for Subject 20).

Age, Ethnicity, and Respiratory Health of Subjects: The ages of subjects ranged from 47 to 85 years, with a mean of 66 years and median of 65 years. Twelve Anglo (non-Hispanic whites) and eight Hispanic whites participated. All but one subject reported that they had never been diagnosed with chronic obstructive lung disease, silicosis, coal worker's pneumonconiosis, or other dust-related lung disease. One subject had a single episode of coughing up blood during the past year, and he is under follow-up with St. Mary's Hospital in Denver and Dr. Gino Saccomano.

Smoking: The sample included two subjects who were current smokers, 13 former smokers, and five subjects who had never smoked. Among smokers and former smokers the average level of consumption was one-half to one pack per day.

Diet: Dietary calcium was assessed by interviews conducted by two senior nutritionists from UNM Clinical Nutrition. Intake of major sources of calcium and supplements was assessed for three decades corresponding to 1965, 1980, and 1995. Intake was assessed using an interview validated against seven-day diet records on other New Mexican populations. Calcium intakes were moderately correlated across these time intervals. Mean calcium intake decreased across this time period, consistent with aging and changes in diet as children leave the household. The current mean calcium intake for this sample of 20 men was 80 percent of the reported U.S. mean intake.

While the primary purpose of this pilot study was to demonstrate the feasibility of measuring ^{210}Pb levels in men whose largest exposures to Rn progeny occurred approximately 30 years ago, another important aspect was the successful demonstration that these former uranium miners could be located and recruited for measurement. Although this pilot study was limited to 20 subjects and those with the highest exposures, our experience indicates that the database maintained by NMTR contains accurate exposure histories. Furthermore, it is feasible to collect data on smoking history and dietary intakes of calcium for use in biokinetic modeling and backward estimation of lung exposure to Rn progeny.

Female uranium miners study: We collaborated with Drs. Gary Madsen and Susan Dawson from Utah State University to study the experience of female miners. We identified 800 women who were employed in the mining industry, obtained current contact information, and interviewed approximately 50 women. We found that women reported a high number of symptoms that they thought were related to

uranium mining. The results will be presented at an international conference and a manuscript is in the final stages of submission.

Trisomy 7: In collaboration with scientists at ITRI, bronchial cells obtained at bronchoscopy were cultured and then assessed for genetic changes commonly found in lung cancer. We found that cells from normal bronchial mucosa of uranium miners and some smokers had genetic alterations, including trisomy 7. We published two manuscripts describing our Trisomy 7 findings and another manuscript is in press describing Trisomy 20 results. Methods to examine molecular changes in sputum have been developed, and a manuscript is in press describing the p53 method. In addition, methods for assays of 9p23 LOH in sputum have been refined and are being used in our newly funded case-control study. We have also described gene promoter methylation changes in the epithelium and a manuscript is in preparation.

CONCLUSIONS

The need for research in the area of respiratory carcinogenesis is evident. Lung cancer remains a pressing worldwide public health problem and indoor radon is now recognized as the second leading cause of lung cancer in the United States. Techniques of molecular and cellular biology will provide new markers for describing the longitudinal process of carcinogenesis and for screening. Further research is needed to explore the role of error in the exposure-response relationship, to identify intermediate markers for use in risk assessment and for the primary prevention of lung cancer. Establishing a large cohort of former miners would provide the opportunity to validate intermediate markers and conduct clinical prevention trials.

FUTURE PLANS

We have completed the specific aims that were presented in the original application. Although the awardees have moved to Johns Hopkins University and the University of Southern California, they continue to actively study uranium.

Publication of the results from the multiple projects supported in part, by this grant are in progress. Newly funded projects will continue including a case-control study of molecular markers of risk and molecular markers in lung adenocarcinomas. An additional grant application has been submitted for improved exposure assessment and to establish a long-term longitudinal follow-up study of 2000 former miners.

We anticipate that the expanding collaborative group track record and ongoing funded work in respiratory carcinogenesis will be highly productive.

Manuscripts

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