Mutagen Sensitivity as a Biological Marker of Lung Cancer Risk in African Americans

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Abstract

Cigarette smoking is the major determinant of lung cancer. However, only a fraction of smokers develops lung cancer; genetically determined susceptibility factors seem to play an important role also. Previous case-control studies have shown that in vitro bleomycin-induced mutagen sensitivity is an independent risk factor for head-and-neck cancers, and preliminary data suggest a similar association with lung cancer. However, these studies were almost exclusively performed on Caucasian populations. To test whether ethnic differences in cancer risk are due to differences in mutagen sensitivity, we are using the in vitro mutagen sensitivity assay to conduct a case-control study of mutagen sensitivity and lung cancer risk in low-risk (Mexican-American) and high-risk (African-American) groups. Here we report the results of our ongoing study of 209 African-Americans (90 cases and 119 controls) in the Houston-Galveston area. Mexican-American data will be reported separately as case accrual increases. Predictably, all measures of cigarette smoking status (including intensity, duration, tar content, depth of inhalation, and type of cigarette) were significant predictors of risk. In addition, 55.3% of the cases were mutagen sensitive (defined as >1 break/cell), compared with 24.6% of the controls, with an age-, sex-, and smoking-adjusted odds ratio (OR) of 3.7 (95% confidence limits = 1.4, 9.4). Of interest, higher risks were noted for former smokers (OR = 5.4) compared with current smokers (OR = 3.1) and especially for younger former smokers (<55 years). By histologic-specific analysis, mutagen sensitivity was significantly associated with risk for adenocarcinoma (OR = 4.8) and squamous cell carcinoma (OR = 8.5). Stratified analysis showed that there was an interaction between mutagen sensitivity and current and former smoking and heavy smoking (>20 pack-years) that appeared to be greater than multiplicative. These risk estimates are generally higher than those we reported for head-and-neck cancer in Caucasian populations.

Further research should focus on the cyto genetic and molecular evaluation of whether the break sites are random or occur at specific sites and on comparisons with DNA repair assay systems.

Introduction

Lung cancer accounts for about 25% of all cancers among black men and 13% of all cancers among black women (1). During 1987–1988, the age-adjusted lung cancer incidence rates/100,000 were 120 for black men, compared with 82.2 for white men. The comparable rates for black and white women were 39.8 and 40.2, respectively (2). While the patterns of cigarette smoking among United States blacks are well documented, they do not fully explain the ethnic risk differentials. Blacks exhibit a higher overall smoking prevalence than whites (3); however, survey data also document that they smoke fewer cigarettes per day. Sixty-four percent of black smokers smoke less than 1 pack/day, compared with 35% of white smokers (4). We have shown similar patterns among cancer patients referred to The University of Texas M. D. Anderson Cancer Center (5). Some but not all of the lung cancer excess is reduced by adjusting for census tract characteristics, including population density, educational level, and income, suggesting that the environmental correlates of being black are responsible for much but not all of the excess (6).

Therefore, although lung cancer is the paradigm of an environmentally induced disease, inherited predisposition is an important component of risk characterization. In addition to interindividual variation in the capacity for carcinogen metabolic activation or detoxification, DNA repair capacity can also modulate susceptibility. Hsu (7) has hypothesized interindividual differences in mutagen-induced chromatid breaks as another marker of cancer susceptibility. We previously demonstrated that in vitro mutagen sensitivity in peripheral lymphocyte cultures (measured by the number of bleomycin-induced chromatid breaks) was an independent risk factor for upper aerodigestive tract cancers after adjustment for tobacco and alcohol consumption (8, 9). Preliminary analyses suggested a similar effect for lung cancer (7).

However, these prior studies were conducted exclusively in Caucasian populations. The prevalence of mutagen sensitivity may vary by ethnic group; this might explain some of the ethnic differences in cancer risk. Accordingly, we designed and are conducting a case-control study of lung cancer in high-risk (African-Americans) and relatively low-risk (Mexican-Americans) subpopulations. We present here case-control data from this ongoing study evaluating mutagen sensitivity in risk of primary lung cancer in the 209 African-American cases and controls accrued thus far. Analyses of the Mexican-American cases await further case and control accrual.
Subjects and Methods

Newly diagnosed lung cancer patients who described themselves as being of African-American ancestry and who had not previously been treated with either radiotherapy or chemotherapy were recruited into the study from The M. D. Anderson Cancer Center; from county, community, and Veterans Administration hospitals in the Houston metropolitan area; and from Galveston, Texas. There were no age, histological, or stage restrictions but all cases were histologically confirmed. Controls were a convenience sample recruited from community centers, cancer-screening programs, churches, and employee groups by using a frequency-matching approach. The controls were matched to the cases by sex, ethnicity, and age (±5 years).

After informed consent was obtained, a structured interview of approximately 45 min was conducted by trained interviewers/phlebotomists. Data were collected on sociodemographic characteristics, recent and prior tobacco use, other lifestyle habits, and family history of cancer. Blood was drawn into heparinized tubes for cytogenetic and molecular genetic analyses.

The methodology for the bleomycin assay was described in detail previously (10). Briefly, peripheral lymphocyte cultures were grown in RPMI 1640 supplemented with 15% fetal bovine serum. On the third day of incubation, the cultures were treated with 0.03 unit/ml bleomycin for 5 h. During the last hour, the cells were treated with 0.04 μg/ml Colcemid to accumulate arrested mitoses in metaphase before the cells were harvested for conventional air dried preparations. The number of breaks in 50 metaphases/sample was counted and expressed as the average number of breaks/cell. Only frank chromatid breaks or exchanges were recorded; chromatid gaps or attenuated regions were disregarded.

To test for significant associations between tobacco use and mutagen sensitivity, univariate ORs1 were calculated as estimates of the relative risks. Ninety-five% CLs were computed by the method of Cornfield (11). When numbers were small, the exact method was used. We tested for multiplicative interaction between mutagen sensitivity and smoking by simple stratified analysis. Logistic regression, calculated with the STATA program, was used to estimate risks, which was dichotomized at the breakpoint of 1 break/cell and was also analyzed as a continuous variable. All variables that were statistically significant in the bivariate analysis were included in the first logistical model. The final model reported here excluded variables and interaction terms that were not statistically significant in the preliminary model. CLs for the adjusted ORs were calculated by using the estimated logistic coefficient and the corresponding SE.

Results

This report is based on data from 90 African-American cases and 119 controls. Table 1 summarizes the distribution of select sociodemographic variables. Since we are using a frequency-matching approach for control selection and because the study is ongoing, exact age matching and sex matching of the controls to cases was not achieved. Although there was no overall difference in age between cases and controls, the male cases were an average of 5 years older (P = 0.005) and female cases were 11 years younger (P = 0.009) than their respective controls. We therefore adjusted for age in the multivariate analyses. The cases were significantly more likely than their controls (14.9 versus 2.4%, respectively) to have had only an elementary school education and less likely to have had a posthigh school education (23.0 versus 54.2%, respectively). These educational patterns did not result in any marked differences in the distribution of cases and controls by income categories. Fewer cases than controls (26.7 versus 33.7%, respectively) lived alone, and almost three times as many cases as controls lived in households of five or more occupants. This trend was statistically significant (P = 0.03).

Predictably, there were significant differences in smoking status between cases and controls (Table 2). Over 90% of the cases had ever been smokers, compared with about 62% of the controls. About 60% of the cases currently smoked, compared with 30.3% of the controls, resulting in a 3.2-fold elevated univariate risk of cancer associated with current smoking (95% CL = 4.9-35.7) and an OR of 7.4 (2.7 to 20.4) for former smoking. There were no differences in the mean age for starting smoking for the cases compared with the controls (18.5 versus 19.5 years, respectively) (P = 0.36). However, the means for lifetime pack-years of cigarette smoking were significantly higher for the cases (43.8) than for the controls (12.7; P < 0.0001; data not shown). We obtained similar results when we used the number of cigarettes smoked daily (23.4 for cases and 8.9 for controls). Risk also increased as the tar content of the cigarettes smoked increased, with an OR of 21.0 for smokers of cigarettes with the highest tar content (P < 0.0001). The highest risks were noted for smokers of cigarettes with the greatest nicotine content (OR = 25.2). Although depth of

| Table 1 | Distribution of select demographic variables by case-control status |
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| Sex | N (%) | P |
| Cases (n = 90) | Controls (n = 119) |
| Male | 61 (67.8) | 80 (67.2) | 0.933 |
| Female | 29 (32.2) | 39 (32.8) | |
| Mean age (years ± SD) | 58.3 (11.2) | 57.9 (12.5) | 0.81 |
| Education (years) |  |
| ≤6 | 13 (14.9) | 2 (2.4) | 0.000 |
| 7-9 | 21 (24.1) | 7 (8.4) |  |
| 10-12 | 33 (37.9) | 29 (34.9) |  |
| 13+ | 20 (22.2) | 45 (54.2) |  |
| Income |  |
| <$5,000 | 16 (17.8) | 11 (14.5) | 0.146 |
| $5,000-$9,999 | 36 (40.0) | 23 (29.5) |  |
| $10,000-$19,999 | 16 (17.8) | 24 (30.8) |  |
| $20,000-$29,999 | 5 (5.6) | 11 (14.1) |  |
| $30,000-$39,999 | 11 (13.3) | 9 (11.5) |  |
| $40,000+ |  |  |
| Household size |  |
| Single | 23 (26.7) | 28 (33.7) | 0.032 |
| 2 | 21 (24.4) | 28 (33.7) |  |
| 3 | 20 (23.3) | 15 (18.1) |  |
| 4 | 9 (10.5) | 7 (8.4) |  |
| 5+ | 13 (15.1) | 5 (6.0) |  |

1 The abbreviations used are: OR, odds ratio; CL, confidence limit; SE, standard error.
inhaling was not measured precisely, we did analyze self-reported results and noticed increasing risk with increasing perceived depth of inhalation (OR = 13.1 for deepest inhalers). There was also a significant trend of increasing risk by increasing length of cigarette smoked. Using filtered (OR = 0.3) and menthol (OR = 0.4) cigarettes was significantly more prevalent among controls (90.6 and 62.1%, respectively) than cases (77.5 and 36.3%, respectively).

There was a 5.5-fold higher risk for those who stopped smoking after age 50 years compared with those who quit at a younger age. There were no significant differences between cases and controls in passive exposure to tobacco at home (data not shown). The overall OR for exposure at work was 2.6 (95% CI = 1.2-5.8; data not shown). There were few cases and controls who reported using tobacco products other than cigarettes. Although the ORs for chewing tobacco (2.7) and snuff dipping (1.9) were elevated, they were not statistically significant (data not shown).

Overall, 55.3% of the African-American cases had mutagen sensitivity scores greater than or equal to 1 break/cell, compared with 24.6% of the controls. The mean break/cell values were 1.24 for the male cases compared with 0.74 for male controls (P > 0.05), and the comparable values for females were 1.00 and 0.98 (Table 3). We also assessed the effect of cigarette smoking on the sensitivity profile of both cases and controls (Table 3). There were no significant differences in mean break/cell values by current or former smoking status, stratified by pack-year history, although values for cases were consistently higher than those for controls. There was no trend for increasing mutagen sensitivity to be expressed with more extensive exposure history. Furthermore, duration of cessation was unrelated to break/cell value.

By multivariate analysis, the overall OR for mutagen sensitivity, adjusted for age, sex, and years smoked was 3.7 (95% CI = 1.4-9.4) (Table 4). Mutagen sensitivity was significantly associated with risk for adenocarcinoma (OR = 4.8) and squamous-cell carcinoma (OR = 8.5). Risks associated with mutagen sensitivity were even higher for former smokers (pack-yr)
smokers (OR = 5.4) than current smokers (OR = 3.1). The data were also stratified by age at diagnosis <55 or ≥55 years (which was the median of the age distribution of the cases and controls). The risk estimate for younger patients who were former smokers was 16.5 (95% CI = 1.1-250.2) compared with 1.6 (95% CI = 0.4-5.9) for currently smoking younger patients. On the other hand, risks for older patients were 3.6 and 6.3, respectively.

We also performed stratified analysis using as the referent group nonsensitive, never smoking subjects (Table 5). The relative risk for former smoking subjects in the absence of mutagen sensitivity was 3.3; mutagen sensitivity in nonsmokers was estimated as 2.2. In the presence of both factors, the OR was 18.0. The respective relative risks for current smokers (OR = 9.2), mutagen sensitivity (OR = 2.2), and combined (OR = 28.6) followed the same pattern.

The data were stratified by pack-year at <20 or ≥20 pack-years (the 75th percentile of the smoking history of the controls and 25th percentile of the cases). A substantially higher combined OR was noted (55.0) for the heavier smokers. These data suggest that the joint effects of mutagen sensitivity and former smoking, current smoking, and heavy smoking are greater than multiplicative (18.0 versus 7.3; 28.6 versus 20.2; and 55.0 versus 27.7) although the interaction terms were not statistically significant in the logistical model (probably due to sample size).

**Discussion**

The existence of a proficient DNA repair process is one essential determinant of host susceptibility to carcinogenesis. The use of *in vitro* cytogenetic analyses to study genetic predisposition to cancer is gaining wider approval in formal hypothesis testing using classic epidemiological methodology (13). Using this technique to analyze common cancers and widely prevalent exposures may help us gain insight into the mechanisms of carcinogenesis.

The induction and quantification of chromatid breaks after *in vitro* exposure to mutagens is a promising biomarker of genetic susceptibility. Recent work has demonstrated that the sensitivity phenotype differs by cancer site and by mutagenic exposure. For example, head-and-neck cancer patients express sensitivity to bleomycin, a radiomimetic agent, but not to 4-nitroquinoline-1-oxide, an UV light-mimetic agent (14). The reverse is true of melanoma patients. The reproducibility of the assay has been tested in two independent laboratories (7, 15). Both groups have shown that the patterns appeared stable over time. Cloos et al. (15) demonstrated low mean intrapersonal variation (0.08) compared with a mean interindividual variation of 0.35, confirming the earlier work of Hsu (7).

The risk estimates for mutagen sensitivity and lung cancer in African-Americans are even higher than those noted with our two previous case-control studies of head-and-neck cancer (8, 9), a disease that can be considered a model for the study of lung cancer. In both cancers, there were elevated risks associated with mutagen sensitivity after adjusting for potential confounding, and there was evidence of interaction between cigarette smoking and mutagen sensitivity in the risk estimations. The fact that higher risks were noted for former smokers (particularly for those who were younger at diagnosis) is of great interest. It has been demonstrated that lung cancer patients with susceptible genotypes for CYP1A1 have lower cigarette-dose exposures than those who are nonsusceptible (16). One might also predict that susceptible individuals have an earlier age at cancer onset.

Our two previous case-control studies (8, 9) included mostly Caucasians, for whom the prevalence of mutagen sensitivity (defined as ≥1 break/cell) among the controls was about 18% compared with 25% of the African-American controls in this study. We had predicted a priori that African-Americans, who share a disproportionate burden of cancer, with higher incidence rates, earlier age at onset, and poorer survival than Caucasians, might have a higher prevalence of mutagen sensitivity. We are in the process of genotyping our population for metabolic polymorphisms to extend our research of genetic predisposition in ethnic subgroups.

This case-control study was designed specifically to study differences in genetic susceptibility to lung cancer. We incorporated a number of features to increase its epidemiological rigor. Attention was given to the selection of an ethnically homogeneous population. For example, only black Americans of African ancestry are eligible for inclusion. Although case identification is not population based, only incident cases from metropolitan hospitals in the Houston and Galveston areas are included. We used a pretested instrument with standardized validated questions. The interviewers were specially trained in cultural sensitivity and interviewing techniques. It was obviously not possible to blind them as to case-control status, but the cytogenetic assays were performed on coded samples by two of us (T. C. H., X. W.).

There are limitations inherent in the assay and its application to case-control research. It might be argued that expression of chromosomal damage in peripheral lymphocytes does not reflect cytogenetic changes in the target tissue and that mutagen sensitivity may be an effect rather than a cause of the cancer. However, we (17) and others (18) have shown that patient characteristics, including smoking, age, sex, and tumor stage, have no effect on mutagen sensitivity. We have selected only newly diagnosed cases to minimize any misclassification resulting from therapeutic interventions or the disease process. Ideally, a prospective study would provide the strongest basis for validating this assay as a marker of cancer susceptibility. In a recently published cohort study of 3182 workers ex-
posed occupationally to mutagenic agents and studied for chromosomal aberrations at baseline, there was a statistically significant linear trend in cancer risk with increasing number of aberrations. The risk estimate in the highest stratum was 2.1 (19). We have also prospectively demonstrated that baseline mutagen sensitivity in patients with initial head-and-neck cancer is a predictor of risk of subsequent cancer development (17, 20).

The in vitro mutagen sensitivity assay only measures aberrations or breaks in metaphase chromosomes; more subtle modifications such as point mutations on inter- and intrastrand cross-links cannot be detected by this system. Thus, using functional DNA repair assay systems is desirable. For example, the host cell reactivation assay measures reactivated reporter gene expression (overall repair) (21). Moreover, we need to establish whether the induced chromatid breaks are randomly distributed or are occurring at specific breakage locations. The chromosome rearrangements observed in the peripheral blood lymphocytes of subjects exposed in vitro to a clastogenic agent might be evidence of a targeted mutagenesis. A similar targeted mutagenesis might occur in the lung tissue. We are in the process of exploring these hypotheses in paired lung and lymphocyte samples in our lung cancer cases.

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**References**


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