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Mutagen Sensitivity as a Marker of Cancer Susceptibility

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Abstract Modulation of environmental exposures by host genetic factors may explain interindividual variation in susceptibility to carcinogenesis. One determinant of susceptibility is mutagen sensitivity measured by the frequency of bleomycin-induced breaks in an in vitro lymphocyte assay. Mutagen sensitivity is a significant predictor of aerodigestive tract cancer risk. *In this case-control study of lung-cancer susceptibility markers,* 54% of 132 lung-cancer cases had mutagen-sensitivity scores greater than or equal to 1 break/cell, compared with only 22% of 232 controls. The mean breaks/cell value (±SE) for the 88 African-American cases was 1.11 (±0.60), compared with 0.82 (±0.49) for the 121 controls ($P < 0.001$). For the 44 Mexican-American cases and 111 controls, the comparable values were 1.11 (±0.52) and 0.76 (±0.38), respectively. The overall odds ratio (OR) for mutagen sensitivity (dichotomized at ≥ 1 break/cell), after adjusting for ethnicity and smoking status, was 3.62 (95% confidence limits [CL] = 2.2, 5.9). For current smokers the adjusted risk associated with mutagen sensitivity was 2.52 (1.2, 5.3). For former smokers, the comparable OR (95% CL) was 6.19 (2.7, 14.1). The risk estimate for those under 61 years of age was 4.85 (2.3, 10.4), compared with 2.85 (1.5, 5.6) for older subjects. The risk also appeared to be higher for lighter smokers (<20 cigarettes daily) than heavier smokers (ORs = 5.72 and 3.20, respectively). The ethnicity-adjusted ORs by quartile of breaks/cell were 1.0, 1.40, 2.46, and 4.80; the trend test was significant at $P < 0.001$. The joint effects of mutagen sensitivity and former smoking, current smoking, or heavy smoking were greater than additive, although the interaction terms were not statistically significant in the logistic model. Mutagen sensitivity may therefore be a useful member of a panel of susceptibility markers for defining high-risk subgroups for chemoprevention trials. J. Cell. Biochem: 25S:80–84. © 1997 Wiley-Liss, Inc.

Key words: bleomycin-induced chromosome breakage; cancer susceptibility; lung cancer; minority populations; smoking

INTRODUCTION

Lung cancer is the paradigm of an environmentally induced disease. Eighty-seven percent of lung cancers are attributed to tobacco exposure, and the relative risk of lung cancer for current smokers compared with those who have never smoked is up to 20-fold greater [1]. However, only a fraction of exposed individuals will develop neoplastic lesions. Genetically determined modulation of environmental exposures is an attractive mechanism to explain the variation in host susceptibility [2]. Iannuzzi and Miller maintain that "no organ system is more dependent on the interaction of the environment and heredity than the lungs" [3].

A correlation between high spontaneous chromosome fragility (indicative of high mutation rates) and increased risk of cancer has been found in several chromosome-breakage syndromes [4]. For example, hypersensitivity to ionizing radiation and to bleomycin (a radiomimetic agent) is a hallmark of ataxia telangiectasia [5]. Hsu and coworkers have suggested that inherited susceptibility to chromosome breakage varies along a continuum with these syndromes at the extreme end [6].

As an indirect measure of constitutional susceptibility to environmental carcinogens, Hsu and colleagues developed a mutagen sensitivity assay based on the quantification of bleomycin-induced chromatid breaks in cultured lymphocytes [7,8]. We and other investigators, using a molecular epidemiologic approach, have found that lymphocyte analysis of individuals can be

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used to identify those at higher risk of developing cancer [9–12].

We previously reported that in vitro bleomycin-induced mutagen sensitivity (analyzed either as a continuous or a dichotomous variable) was an independent risk factor for upper aerodigestive-tract cancer [9,10] and a significant predictor of multiple primary cancer risk after an initial head and neck cancer [11,12]. We also reported data from an ongoing case-control study of lung cancer in African-American [13] and Mexican-American populations [14] showing that mutagen sensitivity was significantly associated with lung-cancer risk for each ethnic group. In this paper, we present updated and aggregated data.

MATERIALS AND METHODS

Newly diagnosed, previously untreated lung cancer patients of African-American or Mexican-American ancestry were recruited into the study from The University of Texas M.D. Anderson Cancer Center and from county and community hospitals in the Houston metropolitan area; and from Galveston, Texas. There were no age, histologic, or stage restrictions, but all cases were histologically confirmed. The 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, and other lifestyle habits.

The methodology for the bleomycin assay was described in detail previously [6]. For each sample, a minimum of 50 well-spread metaphases per sample were read under a 100× dry objective to determine the frequency of spontaneous aberrations. Fifty metaphases were also counted for the number of induced chromosome breaks and the results averaged to the number of breaks/cell. Gaps or attenuated regions were disregarded.

To test for significant associations between tobacco use and mutagen sensitivity, univariate odds ratios (ORs) were calculated as estimates of the relative risks. Ninety-five percent confidence limits (CLs) were computed by the method of Cornfield [15]. When the 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, that were adjusted for multiple factors [16]. Mutagen sensitivity was dichotomized at the level of 1 break/cell and also analyzed as a continuous variable. All variables that were statistically significant in the bivariate analysis were included in the first logistic model. The final model reported here excluded variables and interaction terms that were not statistically significant in the preliminary model. The CLs for the adjusted ORs were calculated by using the estimated logistic coefficient and the corresponding standard error.

RESULTS

This report is based on data from 132 cases (88 African American and 44 Mexican American) and 232 controls (121 African American and 111 Mexican American) for whom both questionnaire and mutagen-sensitivity data were available. Table I summarizes the distribution of select sociodemographic variables. There were no significant differences between cases and controls in terms of sex, age, or years of education (data not shown). Only 7.6% of the cases had never smoked, compared with 41.3% of the controls. Predictably, smoking status was a key determinant of lung-cancer risk; the ORs for former and current smokers were 6.1 (CL = 3.4, 11.2) and 7.2 (CL = 4.0, 13.0), respectively (data not shown). The cases were also significantly heavier smokers in terms of number of cigarettes smoked per day. Over three-quarters of the cases smoked a pack or more per day compared with 35% of the controls (Table I).

As we had hypothesized a priori, mutagen sensitivity (defined as $\geq$ 1 break/cell ± SE) was a significant predictor of lung cancer risk. For African Americans, the mean breaks/cell value for cases was 1.11 (±0.60) compared to 0.82 (±0.49) for the controls ($P < 0.001$). For Mexican Americans, the comparable values were 1.11 (±0.52) and 0.76 (±0.38), respectively. Overall, 53.8% of the lung-cancer cases had mutagen sensitivity scores greater than or equal to 1 break/cell, compared with 22.4% of the controls (Table I).

TABLE I. Distribution of Select Host Characteristics by Case-Control Status

	Number (%)		
	Cases (N = 132)	Controls (N = 232)	*P* value
Sex			
Male	94 (71.2)	158 (68.1)	
Female	38 (28.8)	74 (31.9)	0.537
Ethnicity			
African American	88 (66.7)	121 (52.2)	
Mexican American	44 (33.3)	111 (47.8)	0.007
Smoking status			
Never	10 (7.6)	96 (41.3)	
Former	54 (40.9)	60 (25.9)	
Current	68 (51.5)	76 (32.8)	<0.001
Number of cigarettes/day current and former smokers			
<20	27 (22.1)	88 (64.7)	
≥20	95 (77.9)	48 (35.3)	<0.001
Mutagen sensitivity (breaks/cell)			
<1	61 (46.2)	180 (77.6)	
≥1	71 (53.8)	52 (22.4)	<0.001

TABLE II. Multivariate Analyses of Mutagen Sensitivity

Variable	OR	95% CL	
Age (years)[a]			
≤61	4.85	2.3	10.4
62+	2.85	1.5	5.6
Smoking status[b]			
Current	2.52	1.2	5.3
Former	6.19	2.7	14.1
Number of cigarettes/day[b]			
<20	5.72	2.2	15.0
≥20	3.20	1.5	6.9
Overall[a]	3.62	2.2	5.9

[a]adjusted for race and smoking status
[b]adjusted for race

The overall OR for mutagen sensitivity (dichotomized at ≥ 1 break/cell), after adjusting for ethnicity and smoking status was 3.62 (95% CL = 2.2, 5.9, Table II). The data were dichotomized at 61 years of age (the median of the age distribution of the cases and controls). The risk estimate for younger patients was 4.85 (2.3, 10.4) compared with 2.85 (1.5, 5.6) for older patients. The risk estimate in current smokers was 2.52 (1.2, 5.3). For former smokers, the OR was even higher, 6.19 (2.7, 14.1). Lighter smokers (< one pack per day) appeared to be at higher risk (OR = 5.72) than heavier smokers (3.20). The highest risks associated with mutagen sensitivity were for squamous cell carcinoma (OR = 8.5) and adenocarcinomas (OR = 4.8) (data not shown).

We also assessed the effect of cigarette smoking on the sensitivity profile of both cases and controls. There were no significant differences in mean breaks/cell values by current or former smoking status, stratified by pack-year history, although the values for cases were consistently higher than those for controls. There was no trend for increasing mutagen sensitivity with more extensive exposure history. Furthermore, duration of smoking cessation was unrelated to breaks/cell value. When the subjects were categorized into quartiles of breaks/cell values, with 0.50 breaks/cell as the referent category, there was a dose-response relationship between lung-cancer risk and mutagen sensitivity. The ORs stratified by quartile of induced breaks were 1.0, 1.40 (0.6, 3.3), 2.46 (1.1, 5.4), and 4.80 (2.3, 10.0), respectively. The trend test by chi-squared analysis was significant ($P <$ 0.001).

We performed stratified analysis using as the referent group nonsensitive subjects who had never smoked. The combined risk for mutagen sensitivity and ever smoking (OR = 28.07, CL = 10.4, 76.1) was greater than the additive effects of smoking (OR = 8.07, CL = 3.1, 21.1) and mutagen sensitivity (OR = 4.68, CL = 1.2, 18.3).

We also studied the location of the bleomycin-induced chromatid breaks in primary blood cultures of 75 randomly selected cases of lung cancer and 78 controls frequency matched to the cases for age, sex, and ethnicity [17]. The frequency of induced chromatid breaks and the locations of the breaks were determined in Q-banded preparations. After adjustment for their length, the larger chromosomes had more breaks than smaller chromosomes in both cases and controls. The cases had significantly more breaks on chromosomes 4 and 5 than the controls did, with ORs of 4.9 (95% CL = 2.0, 11.7) and 3.9 (95% CL = 1.6, 9.3), respectively. There also appeared to be a dose-response relationship with breaks on chromosomes 4 and 5. The risk estimates were 1.0, 3.3, 15.3, and 26.6 ($P <$ 0.001), respectively, for increasing quartiles of number of chromosome 5 breaks. There was a similar pattern for breaks on chromosome 4.

DISCUSSION

In vitro chromosomal analyses have been frequently used to study individual sensitivity to genotoxicity and cancer risk and are gaining wider approval in formal hypothesis-testing studies using classic epidemiologic methodology [18]. In a recently published cohort study of 3,182 workers occupationally exposed to mutagenic agents and studied for chromosomal aberrations at entry into the study, Hagmar et al. [19] reported a statistically significant increase in cancer risk (relative risk = 2.1) in the highest stratum of aberrations. Studies such as this confirm the value of using chromosomal aberrations in peripheral lymphocytes as a marker of cancer risk.

The fact that we noted higher risks for former smokers, lighter smokers, and those who were younger at diagnosis is of interest. One might predict that susceptible individuals have an earlier age at cancer onset and less carcinogenic exposure than do nonsusceptible people. It has been demonstrated that lung-cancer patients with susceptible CYP1A1 genotypes have lower cigarette dose-exposures than those who are nonsusceptible and that individuals with the susceptible genotype were at remarkably high risk at a low dose level of cigarette smoking [20]. The genetic difference in risk tends to be reduced at high dose levels at which the environmental influence may overpower genetic predisposition.

Interindividual variation in response to genotoxic exposures is complex and may be mediated by a number of mechanisms, including variability in carcinogen metabolism and in DNA repair capacity. The underlying mechanism for mutagen sensitivity associated with cancer proneness probably does not only reflect altered DNA repair. Pandita and Hittelman [21] suggested that the mutagen-sensitive phenotype may also involve an inherent chromatin alteration that leads to an increased efficiency of translation of DNA damage into chromosome damage after mutagen exposure. They also argue that if chromatin alterations do play such a role, future strategies for chemopreventive interventions should include agents that stabilize chromatin structure.

There are limitations inherent in the mutagen-sensitivity 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 cancer. However, we [12] and others [22] have shown that patient characteristics such as smoking, age, sex, and tumor stage have no effect on mutagen sensitivity. We examined only newly diagnosed cases to minimize any misclassification resulting from therapeutic intervention or the disease process. A prospective study would provide the strongest basis for validating this assay as a measure of cancer susceptibility.

The in vitro mutagen sensitivity assay only measures aberrations or breaks in metaphase chromosomes; more subtle modifications such as point mutations or interstrand and intrastrand cross-links cannot be detected by this system. Nevertheless, we need to further evaluate our finding that the induced chromatid breaks are not randomly distributed but occur at specific locations. We found nonrandom breaks at chromosomal regions that harbor clusters of putative tumor suppressor genes (5q21 and 5q31), oncogenes (2q14), and growth-factor genes (4q25 and 5q31). However, identification of breaks in specific regions near critical genes at the cytogenetic level is rather a crude measurement of gene involvement and should be more definitively examined by using fluorescence in situ hybridization techniques with specific chromosome probes and cosmid clones. The chromosome rearrangements observed in the peripheral blood lymphocytes exposed in vitro to a clastogenic agent might be evidence of targeted mutagenesis. Similar targeted mutagenesis may also occur in lung tissue. We are in the process of exploring these hypotheses in paired lung and lymphocyte samples from our lung-cancer cases.

In summary, the induction of chromosome breaks after in vitro exposure to mutagens is a promising measure of genetic susceptibility. Furthermore, that particular chromosome loci are more susceptible than others to mutagenic damage is an intriguing finding that requires further investigation at the molecular level. It is unlikely that a single genetic marker will be sufficiently predictive of risk. Mutagen sensitivity may be one of a panel of susceptibility markers useful in defining high-risk subgroups for chemoprevention trials.

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