Null Results in Brief

TP53 Mutation Spectrum in Lung Cancer Is Not Different in Women and Men

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Abstract

Whether women are more susceptible to lung cancer than men has been controversial. Several case-control studies suggested that women have greater risk of lung cancer compared with men at similar levels of cigarette smoking, whereas some large cohort studies failed to observe this association. Other studies indicated that lung cancer may have biological characteristics and mechanisms of carcinogenesis that are gender specific. Therefore, we hypothesized that women are more susceptible to the carcinogenic effects of tobacco smoke exposure, as evidenced by a higher frequency of G:C-to-T:A somatic mutations in tumors from women in comparison with men at similar levels of tobacco smoke exposure. To investigate our hypothesis, we examined the *TP53* mutational spectrum in a case-only (102 women and 201 men) series study where complete smoking information was available. A similar frequency and type of somatic *TP53* mutations were observed in women and men. In conclusion, our study indicates that the *TP53* mutation spectrum is similar in women and men. Our results are consistent with a recent large cohort study and summary of previous cohort studies, suggesting that women likely have equivalent susceptibility to lung cancer as men. (Cancer Epidemiol Biomarkers Prev 2005;14(4):1031–3)

Introduction

Whether women are more susceptible to lung cancer than men has been controversial. Several case control studies suggested that women have greater risk of lung cancer compared with men at similar levels of cigarette smoking, whereas some large cohort studies failed to observe this association (1-4). Other studies indicated lung cancer may have biological characteristics and mechanisms of carcinogenesis that are gender specific. Tissues from female lung cancer patients had higher levels of smoking-related DNA adducts and a higher frequency of G:C-to-T:A mutations in the *TP53* gene when compared with men with higher smoking levels (5-7). Therefore, we hypothesized that women are more susceptible to the carcinogenic effects of tobacco, as evidenced by a higher frequency of G:C-to-T:A somatic mutations in tumors from women in comparison with men at similar levels of tobacco smoke exposure. To investigate our hypothesis, we examined the *TP53* mutational spectrum in a case-only series study where complete smoking information was available.

Materials and Methods

Lung cancer patients were recruited at time of diagnosis at the University of Maryland, Baltimore Veteran Administration, Saint Agnes, North West Hospital Center, Sinai, and Union Memorial in Maryland with an Institutional Review Board approval. Smoking information, date of birth, self-reported race, and alcohol intake (ever/never) was obtained from interviews. Patients (n = 303, 102 women and 201 men) were selected in two pairwise matched sets. Men and women (n = 101) were pairwise matched by age (±5 years), race (African American or Caucasian), and surgery date (±5 years). Men and women (n = 99) were pairwise matched by age, race, surgery date, and smoking pack-years (±20 pack-years).

Exons 5 to 8 of *TP53* were sequenced using p53 GeneChip (Affymetrix, Santa Clara, CA), single-stranded conformation polymorphism, and manual sequencing of DNA from paraffin-embedded tissues from surgical resections as described (Supplemental Information; ref. 9). Differences in type of mutations in *TP53* were estimated by Fisher's exact tests when expected counts were ≤5, or χ^2 tests. Using two-sided tests, α of 0.05, and the frequency of G:C-to-T:A mutations in *TP53* in previous studies of 25% or 27% in men and 40% in women, our study had 89% or 78% power to detect a statistical difference (8, 5). Differences in pack-years of smoking were compared using t-tests. Conditional logistic regression was used to estimate odds ratios and 95% confidence intervals for *TP53* mutations (SAS Institute, Cary, NC) adjusted for race (African American or Caucasian), age (quintiles), surgery date (four equally spaced intervals of years), and smoking variables as indicated. Alcohol intake was not associated with *TP53* mutations and adjustment for intake did not alter results shown.

Results

Sixty-eight (22%) of the lung cancer patients were African American and 235 (78%) were Caucasian. The majority of lung cancer cases were current (172; 56%) and former

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Note: A.J. Marrogi and L.E. Mechanic contributed equally to this work.

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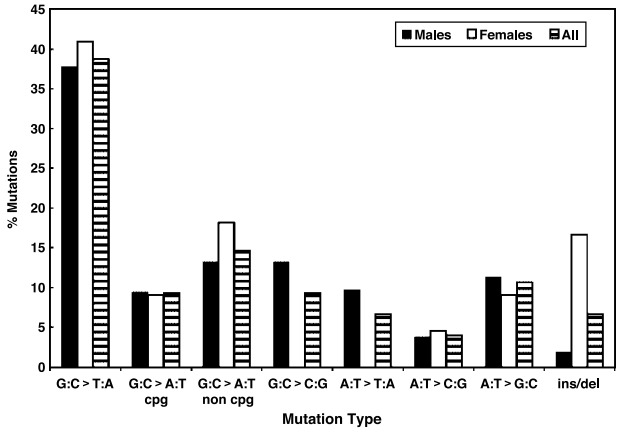

Figure 1. Distribution of somatic *TP53* mutation type associated with gender. Proportion of somatic *TP53* mutations classified according to type in males, females, and entire study population (*all*).

smokers (122; 40%). Nine patients were nonsmokers (3%). Most of the lung tumors were non–small cell lung carcinomas (50% adenocarcinomas, 16% adenosquamous carcinomas, and 30% squamous cell carcinomas). A few cases were neuroendocrine tumors (carcinoids and large cell and small cell carcinomas). The majority of cases were stage I tumors (63%), 19% were stage II, and 17% were stages III to IV. *TP53* mutations were detected in tumors from 25% of patients. The distribution of type of *TP53* mutation was similar in men and women (Fig. 1; $P = 0.117$). Odds of *TP53* mutation (odds ratio, 0.78; 95% confidence interval, 0.43-1.43) or G:C-to-T:A mutation in *TP53* (odds ratio, 0.90; 95% confidence interval, 0.43-1.43) remained similar in women compared with men when controlling for smoking dose, duration, or pack-years, although pack-years of smoking in women was lower than men ($P = 0.026$). The percentage of *TP53* mutations that were G:C-to-T:A mutations in women (41%) was slightly higher than in men (38%), but this increase was not statistically significant ($P = 0.614$). *TP53* mutation frequency or G:C-to-T:A mutations in *TP53* was similar in women and men when controlling for histologic type (adenocarcinoma, adenosquamous carcinoma, squamous cell carcinoma) and frequencies of G:C-to-T:A mutations were similar in women and men in the major histologic types. Women had a higher proportion of insertion/deletion mutations detected in *TP53* compared with men ($P = 0.044$).

Discussion

The type and frequency of *TP53* mutations was similar in tumors from women and men. The difference in G:C-to-T:A mutation frequency in women versus men was not statistically significant even when adjusting for smoking dose, duration, pack-years, time since quitting smoking, or histologic type. In addition, the proportion of G:C-to-T:A mutations in the IARC R9 database was similar in men (26.9%) and women (27.8%; ref. 10). Consistent with these results, in previous studies that reported a higher frequency of G:C-to-T:A mutations in women, gender differences were not statistically significant (8, 5). Women smoked fewer cigarettes than men in these studies. In our study, no statistical difference in the frequency of G:C-to-T:A mutations with gender was detected at high or low smoking levels. A few studies noted that women who smoked had a higher frequency of polycyclic aromatic hydrocarbon-DNA adducts in lung tissue compared with smoking men, after adjustment for smoking (6, 7). In these studies, gender differences in DNA adduct levels was limited to smokers, whereas in nonsmokers, a trend towards higher level of adducts was observed in men. Moreover, in a recent study, where women and men reported smoking similar amounts, more polycyclic aromatic hydrocarbons were found in lung tissue from men than women (11).

In a recent review of the IARC p53 mutation database (R6), differences between the *TP53* mutation spectrum associated with smoking were reported to be stronger in women than men, suggesting that women were more susceptible to cigarette smoking–induced *TP53* mutations (12). In this analysis, nonsmoking versus smoking women and nonsmoking versus smoking men were compared and a larger difference in the frequency of G:C-to-T:A mutations associated with smoking was observed in women than men. However, the difference between males and females likely represents differences between female smokers and nonsmokers, because there are few male nonsmokers in the database (127 women, 38 men, R9 version IARC, using exclusion criteria outlined, ref. 12). Our study was composed predominantly of smokers and the mutation frequency was similar in male and female smokers. These results are consistent with the comparison of male and female smokers in the IARC database; the frequency of G:C-to-T:A mutations was similar (34% in women, 26% in men, $P = 0.080$, IARC R9). Importantly, a strength of our analysis is that it was done in a well-defined study population with complete smoking histories.

One limitation of our analysis is the frequency of *TP53* mutations detected was low. The lower than expected frequency of *TP53* mutations observed may be due to assay sensitivity. GeneChip Assay and manual sequencing fail to detect a proportion of *TP53* mutations (13). The DNA for *TP53* mutation determination in our study was obtained from archival paraffin-embedded tissues. These samples, particularly older samples, did not amplify well, reflecting difficulties with DNA extraction and 10-exon multiplex PCR required for successful GeneChip analysis. It is possible that some of the cases defined as wild-type for *TP53* contained *TP53* mutations. A sensitivity analysis was done defining discordant samples (samples defined as mutated by GeneChip with a score above 13 but wild-type by manual sequencing) as mutated (if no other mutations were detected at other exons from the same person; Supplemental Methods). In this analysis, gender was not associated with G:C-to-T:A *TP53* mutation frequency. Another possible explanation for the low mutation frequency is the majority of tissue samples were obtained from stage I tumors where the *TP53* mutation frequency is lower than later stages (14). Importantly, overall trends in the mutation frequency of *TP53* in our study are consistent with previous reports. The distribution of type of somatic *TP53* mutations observed was similar to the IARC R9 database ($P = 0.761$) and we observed an increased frequency of *TP53* mutations in squamous cell carcinomas (34%) compared with adenocarcinomas (16%), as reported (14, 15).

In conclusion, our study indicates that the *TP53* mutation spectrum is similar in women and men. Our results support the notion that the biological characteristics of lung cancer is similar in men and women. This notion may be consistent with a recent large cohort study and a summary of previous cohort studies, suggesting that women likely have equivalent susceptibility to lung cancer as men (16).

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