Cigarette Smoking and p53 Mutations in Lung Cancer and Bladder Cancer

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We report here a compilation of p53 mutation results from two smoking-related cancers, lung cancer and bladder cancer. The overall mutation frequencies reported for these two types of cancer were relatively similar—50% in lung cancer and 40% in bladder cancer. The compiled data from lung cancer and bladder cancer suggest an increasing proportion of patients with p53 mutations in nonsmokers, former smokers, and current smokers, in that order, in both cancer groups. Taken together, more than half (55% and 56% for lung cancer and bladder cancer, respectively) of the patients who continued smoking (CS), less than 40% (38% and 38%) of those who had stopped smoking before (≥1 or ≥5 years) clinical diagnosis (ES), and less than 30% (25% and 29%) of those who were nonsmokers (NS) had a p53 mutation. The differences seen in the mutation frequencies between the three smoking groups did not, however, reach statistical significance (lung cancer—CS vs ES: odds ratio [OR] = 2.0, 95% CI 0.7–5.4; CS vs NS: OR = 3.7, 95% CI 0.4–37; bladder cancer—CS vs ES: OR = 2.1, 95% CI 0.6–7.9; CS vs NS: OR = 3.1, 95% CI 0.7–13). Guanine to thymine transversions were the most common type in lung cancer followed by guanine to adenine transitions. In bladder cancer, on the contrary, G:C to A:T transitions at cytosine-guanine dinucleotide sites were the most frequently detected base substitutions. Analysis of the compiled p53 mutation data suggests that, in addition to lifetime cumulative exposure to cigarette smoke, also stopping smoking for years prior to clinical manifestation of the disease may affect the incidence of p53 mutations. The differences in the mutation profiles appear to support the view that the main genotoxic agents from cigarette smoke exposure may be different in bladder cancer as compared to lung cancer, as suggested previously by DNA adduct studies. — Environ Health Perspect 104(Suppl 3):553–556 (1996)

Key words: p53 mutation, lung cancer, bladder cancer, cigarette smoking, p53 mutation profile, environmental cancer

Introduction

The concept of molecular cancer epidemiology covers integration of data obtained using molecular biological methods (biomarkers) into studies on human cancer risk. The ultimate aim of this approach is improved resolution for cancer prevention. Mutations induced by a certain chemical exposure are applicable as representative markers or fingerprints of the agent (i.e., putative end points for biomarker studies).

A growing body of evidence suggests that mutations found in cancer-related target genes in clinically manifested neoplasms may, even if seen in such a late stage of the tumorigenesis process, reveal clues from the exposure(s) associated with the development of the disease (1–4).

Epidemiological studies have indicated that cancers of the lung and urinary bladder are both strongly associated with cigarette smoking. It is estimated that, in men, 80 to 90% of lung cancers and 40 to 50% of bladder cancers are attributable to tobacco smoking (5,6). In a recent study on squamous cell carcinoma of the head and neck, a high frequency of mutations of the p53 tumor suppressor gene were linked to tobacco smoking and alcohol use (7). The p53 mutation pattern has been demonstrated to be different in environmental cancers, including many tobacco-related cancers, as compared to other types of cancers (8).

We have recently completed analysis of the frequency and pattern of p53 mutation from patients with lung cancer, mainly of squamous cell carcinoma or adenocarcinoma type (9), and from patients with transitional cell carcinoma of the bladder (10). The majority of the patients were cigarette smokers. Despite the same main etiological background, it is generally postulated that the carcinogens/mutagens from tobacco smoke responsible for DNA damage in the two target organs are, at least in part, different. We therefore sought to receive more information on possible tissue specificity of smoking-related mutagenesis/carcinogenesis by contrasting molecular analysis of p53 gene from these two malignancies.

Subjects and Methods

One hundred and three lung tumors were studied from consecutive patients, mostly with non-small cell carcinoma (NSCLC), who attended Helsinki University Central Hospital for pulmonary resection. Demographic data, histopathology, and exposure data of the lung cancer patients, as well as details of the molecular analysis, were described earlier (9,11). Compilation of the p53 mutation data from the lung cancer patients who were current (n=78), former (n=21), stopped smoking ≥5 years prior to operation, or nonsmokers (n=4) were compared with that from patients with urinary bladder cancer. Paraffin-embedded formalin-fixed tumor samples from 55 patients with transitional cell carcinoma (TCC) of the bladder who had undergone surgery or biopsy at Turku University Central Hospital were analyzed for p53 mutation with the same methods (denaturing gradient gel electrophoresis [DGGE] and direct sequencing) as the lung tumor DNA samples (10). Based on the hospital records, 16 of the patients with TCC were current smokers, 21 were...
former smokers (stopped smoking ≥ 1 year prior to the operation), and 17 were non-smokers. For one case, no data on smoking were available. Details of study design, histopathology, patient exposure histories, and mutation analysis are given elsewhere (10).

Results

The overall mutation frequency was 50% among the lung cancer patients and 40% among the bladder cancer patients (9,10). When the patients were divided into groups of nonsmokers, former smokers, and current smokers according to their smoking status, a clear gradient in the mutation frequency was observed, with the highest amount of mutations in the patients who had continued smoking (Figure 1).

In lung cancer, one out of four nonsmokers (25%, NS), 8 of 21 (38%) former smokers (ES) and 43 of 78 (55%) current smokers (CS) had a p53 mutation in the tumor tissue. The differences in the mutation frequencies among the three smoking groups did not, however, reach statistical significance (CS vs ES; odds ratio [OR] = 2.0, 95% CI 0.7 – 5.4, p = 0.13; CS vs NS; OR = 3.7, 95% CI 0.4 – 37, p = 0.25). The lung cancer patients considered as former smokers were those who had stopped smoking 5 years or more prior to surgery. They had a mean exposure history of cigarette smoking of 40 pack-years (SD = 26) and had refrained from smoking for 17 years (mean; SD = 11). The mean age at which these former smokers had stopped smoking was 51 years (SD = 12). The patients who were currently smoking had been exposed to cigarette smoke on average for 44 pack-years (mean, SD = 20).

Interestingly, the prevalence of mutations found among the bladder cancer patients is very similar to lung cancer: 5 out of 17 nonsmokers (29%), 8 of 21 ex-smokers (38%), and 9 of 16 (56%) current smokers had at least one p53 mutation (10). The differences between the groups were statistically nonsignificant (CS vs ES: OR = 2.1, 95% CI, 0.6 – 7.9, p = 0.22; CS vs NS: OR = 3.1, 95% CI, 0.7 – 13, p = 0.11). For the bladder cancer patients, the smoking status was given by the patient at the time of diagnosis (stopped smoking 1 year or more before diagnosis) without pack-year details; these data were obtained from the hospital records.

Figure 2 shows the comparison of the types of base substitutions between these two malignancies. Guanine to thymine transversions were, as expected, the most common type of base substitution (33%) seen in lung cancer, followed by guanine to adenine transitions (31%) (9). In bladder cancer, G:C to A:T transitions were the most frequently detected base substitutions, and the second most frequent alteration was A:T to C:G (10). In this set of bladder tumors, no G:C to T:A transversions were seen. The majority (5/7) of the G:C to A:T mutations detected in bladder cancer were at CpG sites (10), whereas in lung cancer only one-fourth (3/12) of G:C to A:T transitions had occurred at cytosine–guanine dinucleotide sites (Figure 2).

Discussion

Here we report compiled results of p53 mutation analysis from two cancers, lung cancer (9) and bladder cancer (10), with environmental etiology. The overall mutation frequencies are relatively similar, although they are somewhat higher in lung cancer (50%) than in bladder cancer (40%); both of these frequencies are comparable to those reported in the literature (8,12,13). The main recorded source of exposure is cigarette smoking, although occupational exposure to asbestos has been documented for a subset of patients in both cancer groups (10,11). Occupational exposure to asbestos, however, has not been found to be associated with p53 mutations in bladder cancer (10), and the preliminary data from lung cancer also appear to be negative (Husgafvel-Pursiainen et al., in preparation). Earlier, we observed a link that remained statistically nonsignificant between occupational exposure to asbestos and mutations in the K-ras gene in lung adenocarcinoma (14).

In the present study, p53 mutations have been found to be associated with the tobacco-exposure status of the patients with lung cancer and bladder cancer. Despite the gradient observed for both cancer types, the associations are not statistically significant, probably due to small numbers. The compiled data from the two groups indicate that, on average, more than half of the patients who continued smoking, less than 40% of those who had stopped smoking before clinical diagnosis, and less than 30% of those who were nonsmokers had a p53 mutation. A previous study on lung adenocarcinoma found an increasing proportion of patients with p53 overexpression in current smokers versus former smokers versus nonsmokers (56% vs 35% vs 0%) (15). These observations of a decreased frequency of p53 alterations in former smokers and nonsmokers, as contrasted to smokers, appear to be consistent with the epidemiological data pointing to a clear beneficial effect of stopping smoking, as seen in cancer mortality for most primary sites including the lung and bladder (16).

A positive association with smoking (cancer deaths) has been recently confirmed, as well as the association of smoking with cancers of the mouth, pharynx, and larynx (16). A study on the squamous cell carcinoma of the head and neck found an overall mutation frequency of 42% and a significant dependence of the incidence of p53 mutations on the use of tobacco and alcohol (7). In that study, 58% of the patients who smoked cigarettes and used alcohol, 33% of the patients who smoked but abstained from alcohol, and 17% of the patients who neither smoked nor drank alcohol had p53 mutations. The head and neck cancer patients classified as smokers were those who had smoked (at least 20 pack-years) during the 20 years preceding their treatment for the cancer.

In lung cancer, earlier studies have suggested a link between cigarette smoking
and p53 mutations or nuclear overexpression (17–20). We failed to see a statistically significant difference in the frequency of mutations between lung cancer patients with more than 40 pack-years of cigarette smoking and those with 40 pack-years or less overall, when stopping smoking was not taken into account (9). The difficulty in finding a clear dose dependence between smoking and lung cancer may, in part, be explained by the fact that the majority of lung cancers arise in heavy to moderate smokers, and consequently, tumors from lifetime nonsmokers are rarely available for molecular analysis. The few nonsmoker cases reported at this time show an overall level of mutations comparable to, but a base substitution pattern deviant from, that in smokers (21). Nuclear overexpression or mutations have been found in 40 to 45% of bladder tumors from smokers and at a lower level (30–35%) among ex-smokers and nonsmokers in many (12,22) but not all (23) studies.

It is well known that the patterns of mutations observed in lung cancer and some other smoking-related cancers differ from those in cancers with different etiological backgrounds (8,24). The specific base substitution profiles analyzed in this study for the two cancers appear to reveal differences. The clearest differences were seen in G:C to T:A transversions and G:C to A:T transitions. Guanine to thymine transversions were missing in bladder cancer but were the most common type in lung cancer. Secondly, G:C to A:T transitions were the most frequent type seen in bladder cancer and typically occurred at cytosine–guanine dinucleotide (CpG) sites; this has also been reported in other studies (13,25). It is postulated that G to A or C to T transitions at CpG sites can result from various spontaneous cellular processes or endogenous mutagens (8). This could imply a more prominent role for such endogenous mechanisms in bladder carcinogenesis as compared to lung carcinogenesis or a greater contribution to p53 mutagenesis of tobacco smoke constituents capable of preferentially inducing this type of base substitutions in bladder tissue than in lung tissue of smokers. In lung cancer, G:C to A:T transitions have been more frequent and G:C to T:A transversions less frequent in nonsmokers than in smokers (21). It has been proposed, that, for bladder cancer related to chronic urinary infection with *Schistosoma haematobium*, an excess of transitions at CpG sites of the p53 gene is induced by a mechanism involving production of nitric oxide provoked by inflammation (26).

In this study, we analyzed tumor tissue samples with similar molecular methods from two sets of cancer patients. Therefore, it is possible that the observed mutation profiles reflect differences in the types of predominant mutations rather than just methodological variation in the molecular analyses. In bladder cancer, however, the number of mutations available for the comparison was relatively small (10). Literature data on bladder cancer seem, however, to support our findings (13).

In summary, the present analysis of the compiled p53 mutation data, as well as data from other studies, appears to support the view that the main genotoxic agents from cigarette smoke may be different in bladder cancer than in lung cancer, as suggested previously by DNA adduct studies (27,28). The analysis indicates an increasing occurrence of p53 mutations in nonsmokers, former smokers, and current smokers, in that order, in both these sets of cancer patients. The differences in the mutation frequencies are not, however, statistically significant. This suggests that, in addition to lifetime cumulative exposure to cigarette smoke estimated as pack-years, stopping smoking for years prior to clinical manifestation of the disease may affect the incidence of p53 mutations.

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