Detection of ras Gene Mutations in Human Lung Cancer: Comparison of Two Screening Assays Based on the Polymerase Chain Reaction

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We studied the prevalence of point mutations in ras oncogenes (K-ras and N-ras) in DNA from white blood cells and tumor tissue from 36 untreated patients with non-small-cell lung cancer, all of whom were smokers or ex-smokers. We observed somatic K-ras mutations in one-third of the lung carcinomas studied but no N-ras mutation. K-ras codon 12 mutations were found more frequently in adenocarcinomas than in the other histopathological subtypes studied. More than 60% (10/16) of the lung adenocarcinomas had a codon 12 mutation, most of which were G to T transversions. No mutation was found in white blood cell DNA. Two polymerase chain reaction screening methods, oligonucleotide hybridization and denaturing gradient gel electrophoresis (DGGE), were used to detect the mutations. The oligonucleotide method appears to be more sensitive than DGGE, but DGGE proved to be a reliable nonradioactive method for rapid screening of point mutations in genes relevant to carcinogenesis.

Introduction

Various genetic alterations are key events in the process leading to the development of cancer, both in experimental animals and in humans. Among the alterations most frequently associated with human malignancies are point mutations in the ras oncogenes, H-ras, K-ras, and N-ras (1,2), and in the proposed tumor-suppressor gene p53 (3–5), all of which are involved in the regulation of cell proliferation and differentiation (6). Involvement of mutually activated ras genes has been clearly demonstrated in several human cancers, including those of the pancreas, colorectum, and lung, as well as in animal tumors induced experimentally by exposure to carcinogens (1,7). Data accumulated during the last few years strongly suggest that these mutations in human tumors may originate from exposure to exogenous chemicals.

We have investigated the prevalence of point mutations in K-ras and N-ras genes of a set of lung cancer patients as part of a larger study to elucidate the role of exposure to asbestos and smoking in the etiology of lung cancer in Finland. The report by Anttila and her coworkers in this issue describes other results from the same collaborative effort (8).

We also sought a method that was simple and rapid enough to allow screening for point mutations in genes relevant to carcinogenesis in a large number of samples. The objective was to adopt and further develop a method suitable for detecting point mutations in oncogenes, tumor-suppressor genes, or genes involved in the metabolism of xenobiotics, as a new technique for biomonitoring. We used the well-established method of dot blotting and oligonucleotide hybridization (9) and compared the results with those obtained using denaturing gradient gel electrophoresis (DGGE) (10,11). Both methods are based on amplification of DNA by the polymerase chain reaction (PCR) in vitro (12).

Materials and Methods

DNA was isolated and extracted from white blood cells and lung tumor tissue from 36 lung cancer patients (34
male and 2 female patients, 35–79 years of age) by standard procedures. PCR amplification of the sequences studied in the oligohybridization assay (K-ras codons 12, 13, and 61; N-ras codons 12 and 61) was performed as described by Saiki et al. (13): 10–100 ng of genomic DNA in a total volume of 100 μL were amplified using 0.5 U of AmpliTaq polymerase (Cetus) and a DNA thermal cycler (Perkin-Elmer-Cetus), according to the procedures recommended by the manufacturers. The amplified DNA was spotted on nylon membranes and probed by 32P-end-labeled, mutation-specific oligonucleotides (Clontech Laboratories). Each set of 20-mer oligonucleotides covered the known activating point mutations of a particular codon.

DGGE was performed as described earlier (14,15) and an artificial GC-rich sequence was added onto one end of the amplified fragments using GC-clamped primers (11). The total length of the PCR products analyzed in DGGE varied from 150 to 175 bp.

Results

The distribution of the histopathological types of lung cancer investigated and the mutations found are presented in Table 1. All of the mutations detected in the 36 resected lung tumors by either method were K-ras mutations: no N-ras mutation was observed. The highest frequency of K-ras mutations was found in adenocarcinomas, (>60%; Table 1). The most prevalent type of mutation was a G to T mutation in codon 12 or 13 of K-ras; 6/16 adenocarcinomas (37.5%) carried this mutation. Mutations of other types were rare. All of these patients smoked for 21–46 years; two had, however, stopped smoking several years previously. Neither K-ras nor N-ras mutations were observed in white blood cell DNA. The results are reported in more detail elsewhere (K. Husgafvel-Pursiainen et al., in preparation).

Twelve mutations were detected by oligoprobing (Table 1), and nine of these were picked up by DGGE, with good separation (Fig. 1). An additional mutation was found by DGGE which remained undetected by oligohybridization. The nature of this mutation is as yet unknown, but we are working on its sequencing.

Discussion

We report here the detection of point mutations at the K-ras oncogene in more than 60% of human lung adenocarcinomas studied; similar results have been reported by others (16–19). The presence of K-ras mutations in these tumors has been shown to be a strong indication of a poor prognosis (20), and a G to T transversion is not only the most frequently observed type of mutation, but has been linked to smoking (18,21). We could not, however, demonstrate a correlation between the amount of smoking-related DNA adducts in peripheral lung tissue and the mutations observed in the tumor tissue of individual patients in preliminary 32P-postlabeling studies, even though the adduct levels in peripheral lung tissue were higher among smokers than among people who had given up smoking for several years (22). We have also observed

Table 1. Histological types of lung tumors studied and detection of K-ras mutations by two methods.

| Histological type         | No. of tumors | K-ras mutations | Oligoprobe assay | DGGE assay |
|---------------------------|---------------|-----------------|------------------|------------|
| Squamous-cell carcinoma   | 17            | 2 (12%)         | 2 (12%)          |            |
| Adenocarcinoma            | 16            | 10 (63%)        | 7 (44%)          |            |
| Small-cell carcinoma      | 3             | 0               | 0                | 0          |
| Total                     | 36            | 12 (33%)        | 9 (25%)          |            |

DGGE, denaturing gradient gel electrophoresis.
high levels of cytochrome P450IA in the lungs of patients with peripheral adenocarcinomas who smoked (23).

The oligonucleotide hybridization assay appears, on the basis of our results for the set of samples studied, to be somewhat more sensitive than DGGE for detecting K-ras mutations. We examined further the applicability of DGGE to the detection of ras gene mutations by constructing and running a set of plasmid DNAs carrying N-ras mutations as a positive control. Excellent separation was achieved (K. Kurvall et al., in preparation), so that DGGE appears to be very suitable for preliminary screening of large numbers of samples for point mutations: It is fast and requires no probing or handling of radioactive isotopes. DGGE and a modification involving a constant gradient (15), do not provide absolute identification of the mutations but, combined with either oligoprobing, as described here, or sequencing, it meets the primary screening criteria very well, as reported recently for p53, another important gene in carcinogenesis (24).

This manuscript was presented as a poster at the Conference on Biomonitoring and Susceptibility Markers in Human Cancer: Applications in Molecular Epidemiology and Risk Assessment that was held in Kailua-Kona, Hawaii, 26 October–1 November 1991.

We acknowledge the excellent technical assistance of Tsula Suitila and the linguistic assistance of our colleague, Anne Hand, BSc. The study was supported financially by the Finnish Environment Fund and the Nordic Fund for Technology and Industrial Development (P 88134) and the Swedish Work Environment Fund.

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