Detection of genomic instability in lung cancer tissues by random amplified polymorphic DNA analysis

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Genomic instability resulting in multiple mutations is believed to be a driving force in the carcinogenic process. In this study, the random amplified polymorphic DNA (RAPD) technique, a simple PCR-based DNA polymorphism assay system, was used for detecting genomic instability in lung cancer tissues. DNAs from 20 lung cancer (18 non-small cell lung cancers and two small cell lung cancers) and their corresponding normal tissues were amplified individually by RAPD with seven different 10-base arbitrary primers. PCR products from RAPD were electrophoretically separated in agarose gels and banding profiles were visualized by ethidium bromide staining. The ability to detect genomic instability in 20 cancer tissues by each single primer ranged from 15 to 75%. DNA changes were detected by at least one primer in 19 (95%) cancer tissues. These results seem to indicate that genomic rearrangement is associated with lung carcinogenesis and that RAPD analysis is useful for the detection of genomic instability in lung cancer tissues.

Introduction

Recent systematic analysis of genetic changes in the development of colon cancer demonstrated that multiple mutations are required for the evolution of normal cells into cancer cells (1). There is increasing evidence that multiple mutations are also responsible for the development of other cancers (2). Since the number of mutations that are reported for human cancers appears to be greater than the spontaneous mutation rate, cancer cells must exhibit a mutator phenotype (3). This mutator phenotype is likely to be responsible for the genomic instability found in cancer tissues. The mutator phenotype, such as defective mismatch repair, is known to cause microsatellite instability, which is associated with hereditary non-polyposis colorectal cancer and has been found in certain cases of sporadic cancers including endometrial and gastric cancers (see 4 for references). Recently, many studies have been carried out to determine the relationship between microsatellite instability and other cancers (5–8). However, information on genomic instability in human lung cancer is still very limited and varying results have been reported regarding the association between microsatellite instability and human lung cancer. Available data indicate that the detection of microsatellite instability in lung cancer tissues ranges from 45 to 76% (6,8,9) for small cell lung cancer (SCLC*) and only from 2 to 34% (5,6,10–12) for non-small cell lung cancer (NSCLC). The varying results may be due, in part, to differences in the type and number of primers (6 to 36 pairs) used.

Random amplified polymorphic DNA (RAPD) is a PCR-based fingerprinting technique that amplifies random DNA fragments with single short primers of arbitrary nucleotide sequence under low annealing stringency (13). This technique has been used extensively for DNA-fingerprinting, as well as for species classification and strain determination (14). It has also been demonstrated that the instability within the genome can be detected with this fingerprinting method (15). The main difference between RAPD and microsatellite instability is that RAPD may serve to detect genetic changes in the entire genome, and the sequences being amplified are anonymous, arbitrary and unique, whereas microsatellite instability only detects base-pair expansion or contraction in specific microsatellite loci. In the present study, the relationship between genomic instability/change and human lung cancer was investigated using RAPD analysis. The usefulness of this DNA fingerprinting method for the detection of genomic instability in lung cancer tissues was also evaluated.

Materials and methods

Tissue samples

The 20 lung cancer tissues used for the study were biopsy samples obtained from Guangzhou Medical College Hospital in Guangzhou, China. Surrounding normal tissue from each biopsy sample was dissected and used as the corresponding normal tissue. Samples 10 and 14 were SCLCs and the remaining 18 samples were NSCLCs.

DNA extraction

DNAs were extracted from cancer and corresponding normal tissues using the standard phenol/chloroform extraction and ethanol precipitation method (16). In brief, normal or cancer tissues were incubated with 2 ml lysis/digestion buffer (1% sodium dodecyl sulfate, 1 mM EDTA, 50 mM Tris at pH 8.5, and 100 µg proteinase K/ml) at 52°C for 16 h. The digested lysate was subjected to two further extractions with an equal volume of chloroform: phenol:isoamyl alcohol (24:25:1). After centrifugation, DNA was precipitated from the aqueous phase by two vol. of cold absolute ethanol and collected with a glass rod (17). The DNA was further purified with RNase digestion, two phenol/chloroform extractions, and precipitated and collected as described above. The concentration of DNAs was determined with both spectrophotometric and fluorometric methods.

RAPD analysis

Seven arbitrary primers were used for RAPD analysis. The sequences of these primers were: (i) 5′ CCG GCT ACG G; (ii) 5′ CAG GCC CTT C; (iii) 5′ AAC GGT CAC G; (iv) 5′ AAG TGC CGG G; (v) 5′ AGG CAT TCC C; (vi) 5′ GGT CTG AAC C; and (vii) 5′ AAG GCT AAC C. Normal and tumor DNAs (0.5 µg) from each patient were amplified with each arbitrary primer (50 pmol), Taq DNA polymerase (1.25 U), dNTPs (100 µM) and MgCl2 (2.5 mM) in 50 µl of reaction buffer for 40 cycles. PCR conditions were: denaturing at 94°C for 0.5 min, annealing at 40°C for 1 min and extension at 72°C for 1 min. PCR products (5 µl each) mixed with loading buffer were loaded in 2% agarose gels and electrophoresed with 100 V for 1 h. The gels were stained with ethidium bromide (0.5 µg/ml) and visualized under UV light.

Results and discussion

Results of genomic instability detected by RAPD analysis in human lung cancer tissues are shown in Table I. With the...
Table I. Genetic instability in lung cancer detected by RAPD analysis

| Primer number | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|---------------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
|               | +  | -  | -  | -  | +  | -  | -  | -  | +  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  |
|               | -  | -  | -  | -  | -  | -  | -  | -  | +  | -  | +  | -  | -  | -  | -  | -  | +  | +  | +  | -  |
|               | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | -  | +  | +  | +  | +  | +  | -  | -  | +  | -  |
|               | -  | -  | -  | -  | -  | -  | -  | -  | +  | -  | +  | -  | -  | -  | -  | -  | +  | +  | +  | -  |
|               | -  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  |
|               | -  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  |

+ , Genetic instability detected; −, no genetic instability detected.

seven arbitrary primers used, all cancer samples (95%) except sample 20 demonstrated genomic instability, which was detected by at least one primer (Table I and Figure 1). Among these cancer tissues tested, sample 9 showed the highest genomic changes since it could be detected by six out of seven primers used. The ability to detect genomic instability by each primer ranged from 15 to 75% and the average detection by these primers was 42%. Figures 2 and 3 show the banding profiles of cancer and corresponding normal DNAs. Banding shifts, missing bands and/or banding intensity changes, which indicate genomic instability, were demonstrated in these figures. Although the number of cancer tissues used in this study is relatively small, these results seem to indicate that there is a close relationship between genomic instability detected by RAPD analysis and NSCLC.

Figure 1. Genomic instability in lung cancer detected by RAPD analysis.

Figure 2. DNA banding patterns of 20 lung cancer tissues (T) and corresponding normal cells (N) with RAPD analysis using primer 1 (banding pattern changes are marked by arrows; M, ØX174/Hae III marker, banding sizes from top: 1353, 1078, 872, 603, 310, 281, 234 bp).

Figure 3. Banding pattern changes in lung cancer tissues with RAPD analysis (A, sample 3, with primer 3; B, sample 16, with primer 6; C, sample 18, with primer 7; 1, band missing; 2, band shift; 3, band-intensity change).

Microsatellite instability is known to be involved in the development of hereditary non-polyposis colorectal cancer and in certain cases of sporadic cancers (4). It has also been shown that defective mismatch repair is associated with microsatellite instability (18–24). Microsatellite instability has been studied extensively in a variety of human cancers using the microsatellite-DNA fingerprinting method. However, this fingerprinting method is considerably time-consuming and labor intensive since many pairs of primers have to be used and one primer from each pair has to be labeled. Furthermore, in some human cancers, such as NSCLC, microsatellite instability was detected only in a small fraction of the cancer tissues (5,6,10–12). The low frequency of microsatellite instability reported for NSCLC, according to Fong and coworkers (11), may not be due to the intrinsic susceptibility of various microsatellite markers to instability. It seems that tandem repeats of DNA in microsatellite regions may not be good markers for studies of the association between genomic instability and some human cancers, such as lung cancer. RAPD, a PCR-based DNA fingerprinting technique, has been used for detecting genetic changes, including DNA rearrangement, DNA addition or deletion and ploidy changes in cells (15). This technique is much easier to perform and may detect DNA changes in the whole genome. However, it is not known what, if any, mutator genes are involved in the genomic instability detected by RAPD. Furthermore, the quality of DNA samples may affect the reproducibility of RAPD banding patterns (17). Nevertheless, this problem can be simply resolved by collecting precipitated DNA with a glass rod (17). Based on the results reported here, it seems that RAPD is a useful tool for the detection and analysis of genomic instability in lung carcinogenesis. Studies with larger numbers of SCLC, NSCLC tissues from Caucasian patients, and the comparisons between microsatellite instability and genomic instability detected by RAPD in lung cancer tissues are in progress.
Genomic instability in lung cancer tissues

References


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