Frequent Loss of Heterozygosity in Region of the KIP1 Locus in Non-Small Cell Lung Cancer: Evidence for a New Tumor Suppressor Gene on the Short Arm of Chromosome 12

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Abstract

To refine the chromosomal localization of a putative tumor suppressor gene, we analyzed the loss of heterozygosity (LOH) of chromosome 12 in 36 primary non-small cell lung cancer (NSCLC) samples with matched normal DNA using 22 highly informative polymorphic markers. Twelve cases showed LOH at one or more loci on chromosome 12. LOH of chromosome arm 12p was more frequent in large cell carcinoma than squamous cell carcinoma, indicating molecular genetic heterozygosity within the major NSCLC subtypes. We identified the smallest commonly deleted region on chromosome 12p13. This region is flanked by D12S269 and D12S308, including the KIP1 gene. Mutational analysis of KIP1 using PCR-single strand conformation polymorphism and Southern Blot analysis showed no homozygous deletions, rearrangements, or point mutations, suggesting that the altered gene in this region is not the KIP1 gene. These data suggest that a new tumor suppressor gene which is involved in tumorigenesis of NSCLC is in the region of KIP1.

Introduction

Lung cancer is one of the most common lethal malignancies in the United States. Several alterations of known tumor suppressor genes in lung cancer have been described, including TP53, RB1, p15, and p16 genes (1–4). However, inactivation of multiple tumor suppressor genes is thought to be critical during tumorigenesis. Cytogenetic studies have shown the involvement of multiple lesions in lung cancer (5, 6). Therefore, additional tumor suppressor genes are probably associated with the development of lung cancer.

The paradigm of alterations of many tumor suppressor genes is a mutation of one allele and loss of the second allele. This reduction to homozygosity can be detected as a LOH of informative markers in the region of the tumor suppressor gene. Thus, LOH analysis is an indirect method to search for a tumor suppressor gene.

Deletions of the short arm of chromosome 12 are observed in 10–20% of patients with NSCLC (7, 8). These findings suggest the presence of a putative tumor suppressor gene on chromosome arm 12p. Molecular analysis of 12p deletions has been very limited in scope.

To define the chromosomal localization of the putative tumor suppressor gene, we have performed fine deletion mapping of chromosome 12 in 36 samples of primary NSCLC with matched normal DNA using 22 highly polymorphic markers. We identified the smallest commonly deleted region on 12p.

Materials and Methods

Samples. Thirty-six DNAs from primary NSCLC were obtained at the time of surgery after informed consent from patients. These cancers included 16 adenocarcinomas, 12 squamous cell carcinomas, 4 large cell carcinomas, 3 epidermoid carcinomas, and 1 unclassified carcinoma. The corresponding normal DNAs were obtained from the normal lung tissues surrounding the tumors.

Analysis of LOH Using Microsatellite Markers. The LOH analysis was performed by PCR-amplification of microsatellite sequences. The genetic map of chromosome 12 was compiled mainly from the Genethon microsatellite map and GDB (9–12). Some markers have been assigned to the same location in a 0 cM cluster. Primers were obtained from Research Genetics (Huntsville, AL) or synthesized by the Cedars-Sinai Medical Center Molecular Biology Core and listed in Fig. 1. Each PCR reaction contained 10–100 ng of DNA, 10 pmol of each of the primers, 2 nmol each of the four deoxyribonucleotide triphosphates (Pharmacia, Stockholm, Sweden), 0.5 units of Taq DNA polymerase (Boehringer-Mannheim, Indianapolis, IN), and 2 μCi of [α-32P]dCTP (ICN, Irvine, CA) in 20 μl of the specified buffer with 1.5 mM MgCl2. Samples were amplified by 30–35 cycles of denaturing for 40 s at 94°C, annealing for 30 s at 55°C, and extending for 1 min at 72°C in a programmable thermal controller (MJ Research, Inc., Waltham, MA). After amplification, PCR samples were diluted 5-fold in loading buffer containing 20 mM EDTA, 96% formamide, and 0.05% of both bromophenol blue and xylene cyanol. The products were heated to 95°C for 5 min, and 1.5 μl of each sample was electrophoresed through a 5–6% polyacrylamide gel containing 8.3 M urea for 3–4 h at 85 W. Subsequently, the gels were dried and subjected to autoradiography using Kodak XAR film at −80°C. LOH was inferred when a visible reduction in the ratio of allele radiographic signal intensities occurred in the tumor sample relative to that in the corresponding normal sample.

Analysis of LOH Using a Polymorphism in Exon 1b of the KIP1 Gene. The KIP1 gene has been mapped to 12p (13–17). As we reported previously, exon 1b of the KIP1 gene contains a polymorphism; at codon 109, guanine is substituted for thymine (GtC→GGC), resulting in an amino acid substitution of glycine for valine (Val→Gly). By analyzing this polymorphism using PCR-SSCP, we examined LOH of the KIP1 gene. PCR-SSCP was performed as described previously (18).

Statistical Analysis. Fischer’s Exact test was performed for statistical evaluation of data. Probability values were two tailed, with P ≤ 0.05 regarded as statistically significant.

Results

We examined thirty-six NSCLC samples for LOH of chromosome 12 using 22 highly informative polymorphic markers. Fig. 2 displays a representative autoradiograph showing LOH. The patterns of LOH are shown in Fig. 3. Twelve samples showed LOH at least at one locus on chromosome 12. Of these 12 cases, three of them (nos. 75, 121, and 135) showed LOH at all informative loci on chromosome 12. This is consistent with
Discussion

In this study, we analyzed the LOH of chromosome 12 in 36 primary NSCLC samples with matched normal DNA using 22 highly informative polymorphic markers. All of our samples were obtained at the time of initial surgery for the primary lung cancer; therefore, these results reflect the genetic changes that are important in development, rather than in metastatic spread, of NSCLC.

Twelve samples showed LOH at least at one locus on chromosome 12. This frequency is higher than that reported by previous allelotype analysis, which is 10–20% (7, 8).

We found that LOH of 12p was significantly more frequent in large cell carcinomas than in squamous cell carcinomas. LOH on 17q was found more frequently in adenocarcinomas than squamous cell carcinomas (19). However, for most of the other chromosome arms, LOH was more frequently reported in squamous cell carcinoma than in adenocarcinoma (8). These observations indicate molecular genetic heterozygosity within the major NSCLC subtypes.

We identified the smallest commonly deleted region on chromosome 12p13. This region is flanked by D12S269 and D12S308. This smallest commonly deleted region on 12p contains the cyclin-dependent kinase inhibitor gene KIP1. This gene was considered to be a good candidate for a tumor suppressor gene in NSCLC.
candidate for a tumor suppressor gene because it acts as a negative regulator of cellular proliferation (13, 14). We previously examined the mutational status of the KIP1 gene by PCR-SSCP and Southern blot analysis using most of the samples used in this study (18). No detectable point mutations or homozygous deletions were found. These findings suggest that the KIP1 gene is unlikely to be the target tumor suppressor gene of NSCLC. Taken together, another tumor suppressor gene that is important in tumorigenesis of NSCLC is probably close to the KIP1 gene.

We found recently that the same region as identified in the present study was frequently deleted in acute lymphoblastic leukemia of childhood (20). The LOH of the same region both in NSCLC and in acute lymphoblastic leukemia of childhood suggest that either a critical tumor suppressor gene exists in this region with a broad range of activity or more than one tumor suppressor gene is present in this region.

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Note Added in Proof

Recently, a new chromosome 12 map was published [(K. Krauler et al., Nature (Lond.), 377 (Suppl 3): 321, 1995), in which KIP1/p27 was mapped between D12S98 and D12S358. Nevertheless, KIP1/p27 continues to be in the frequently deleted region in most cases with LOH of chromosome 12.}

References

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