Frequent Allelic Deletion at a Novel Locus on Chromosome 5 in Human Lung Cancer

Ilse Wieland² and Malte Böhm³
Institut für Zellbiologie (Tumorforschung), Universitätssklinikum Essen, Virchowstrasse 173, 45122 Essen, Germany

ABSTRACT

Frequent allelic deletions in tumor cells are indicative of the inactivation of tumor suppressor genes. Recently, we isolated the single-copy sequence del-27 (I. Wieland, M. Böhm, and S. Bogatz, Proc. Natl. Acad. Sci. USA, 89: 9705–9709, 1992). Here we show that del-27 detects a restriction fragment length polymorphism that allows examination for loss of heterozygosity (LOH) in tumor specimens. LOH at the del-27 locus occurred in 57% (4 of 7) of the informative cases independent of the histopathological differentiation grade. LOH for exon 11 of the APC gene occurred in 71% (5 of 7) of the informative cases but was not associated with LOH at the del-27 locus. The del-27 sequence was localized to chromosomal region 5p13–5q14, proximal to the MCC/APC region, using a somatic cell hybrid panel. Together with our previous finding that del-27 is deleted homozgyously in a lung carcinoma cell line, these results suggest that del-27 is linked closely to a novel putative tumor suppressor gene.

INTRODUCTION

Genetic alterations on chromosome 5 have been demonstrated in a variety of human neoplasias. Loss of an entire chromosome 5 or interstitial deletions in the long arm (5q-) are observed frequently in patients with acute myogenous leukemia or myelodysplastic syndrome (1). These are probably mutagen-induced leukemias occurring as a late complication of cytotoxic therapy or as a result of occupational exposure to carcinogens (2). The smallest commonly deleted region in these neoplastic diseases has been narrowed to 5p31. This region contains the IRF-1 (interferon regulatory factor 1) gene and possibly another putative tumor suppressor gene (2, 3). In some solid tumors, allelic deletions of the MCC and APC tumor suppressor genes have been reported at 5q21. The MCC and APC genes originally were discovered as potential tumor suppressor genes of sporadic and hereditary forms of colorectal carcinomas (4, 5). Also, a high incidence of allelic deletions in the MCC/APC region is found in esophageal and small cell lung carcinoma, both correlated with excessive mutagen exposure (6, 7). From cytogenetic studies on lung carcinomas it appears that probably a larger chromosomal region (5q13–5q21) is involved in lung tumorigenesis (8).

To identify DNA sequences that are specifically deleted in tumor cells, we recently isolated a DNA probe (del-27) from human chromosome 5 by using genomic difference cloning (9, 10). This sequence is deleted homozygously in a lung carcinoma cell line, suggesting its linkage to a putative tumor suppressor gene (9, 10). Here we show that the del-27 sequence is located in chromosomal region 5p13–5q14 and detects a RFLP. Using the del-27 sequence as a RFLP probe, we observe allelic deletion in 57% of the squamous cell carcinomas of the lung.

MATERIALS AND METHODS

PCR on Human/Hamster Somatic Cell Hybrids. Human/hamster somatic cell hybrid cell lines GM 10114, GM 11443, GM 11444, GM 11437, GM 11442, and GM 11436 were obtained from the Human Genetic Mutant Cell Repository (Camden, NJ). High molecular weight DNA was isolated (11) and 100–200 ng were used as template DNA in PCRs. Amplifications were carried out in a 50-μl reaction volume containing 20 pmol of each del-27 primer (5’GGATAACAAAAAGGGATGTC3’ and 5’GATTGATCTAGCTG-CACC’C’C) (10), 200 μM concentrations of deoxynucleotide triphosphates, and 1 unit of Taq DNA polymerase (Boehringer Mannheim) at 95°C for 5 min followed by 35 cycles at 94°C for 1 min, 58°C for 2 min, and 72°C for 1.5 min. PCR products were analyzed on 2% agarose gels.

RFLP Analysis by Southern Blot Hybridization and PCR. High molecular weight DNA (11) from randomly chosen normal blood donors was cleaved to completion with 14 different restriction enzymes in independent reactions. The digests (5 μg) were electrophoresed on 0.8% agarose gels and transferred onto a nylon membrane (Gene Screen Plus, Du Pont) using a vacuum blotting unit (Vacugene; LKB). The blots were hybridized to a 32P-labeled del-27 probe (9) for 18 h at 64°C and washed three times in 2 × standard saline-citrate-0.2% sodium dodecyl sulfate (11) and once in 0.5 × standard saline-citrate-0.2% sodium dodecyl sulfate at 64°C. Autoradiography was performed as described (9).

RFLP analysis at the APC locus (a RsaI polymorphic site in exon 11 of the APC gene) was performed by PCR on isolated DNA from blood donors as described (12). The generated 133-base pair PCR product is cleaved (alleles b) to an 85- and a 45-base pair fragment by RsaI digestion if the polymorphic restriction site is present, and it remains uncleaved (alleles a) if the site is absent (12). RsaI-digested PCR products were electrophoresed on 3% agarose gels.

Cell Lines and Tumor Specimens. Human lung cancer cell lines SK-LU-1 (HTB 57), SW 900 (HTB 59), and NCI-H69 (HTB 119) were obtained from the American Type Culture Collection; all other cell lines were obtained and cultivated as described (10). Matching normal blood and tumor biopsies from lung cancer patients were collected as described (13). To determine allelic deletions, tumor tissue was purified for Southern blotting by microscopy-guided gross dissection (14) and for PCR analysis by a microdissection method (13). Southern blotting, hybridizations, and PCRs were carried out as described above.

RESULTS

Subchromosomal Localization of the del-27 Sequence. Recently, the del-27 sequence was assigned to human chromosome 5 using a human/hamster somatic cell hybrid panel (10). To localize the del-27 sequence more closely, human/hamster somatic cell hybrids retaining only parts of human chromosome 5 were analyzed by PCR. In all hybrids having deletions of the short arm of human chromosome 5 (GM 11437, GM 11442, and GM 11436), a 356-base pair PCR product of the del-27 sequence was amplified (Fig. 1). In the hybrid GM 11443, containing human chromosome 5 with an interstitial deletion [del(5)(q15q21.3)] which encompasses the MCC/APC region (data not shown), the del-27 PCR product also was detected. However, in hybrid GM 11444, retaining only the distal part of 5q (5q22-ter), no del-27 PCR product was amplifiable (Fig. 1). The same results were obtained by genomic Southern blotting (data not shown). This most likely places del-27 proximal to the MCC/APC region. From these results we conclude that the del-27 sequence maps to human chromosome 5p13–5q14.

RFLP Analysis at the del-27 Locus. A RFLP was detectable in HindIII-cleaved DNA from randomly chosen normal blood donors. Seven of 13 donors (54%) were heterozygous at the del-27 locus with.

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2 Recipient of a Deutsche Forschungsgemeinschaft-Habilitationsstipendium. To whom requests for reprints should be addressed.
3Urologische Klinik, Universitätssklinikum Hufelandstrasse 55, 45122 Essen, Germany.
4 The abbreviations used are: RFLP, restriction fragment length polymorphism; PCR, polymerase chain reaction; LOH, loss of heterozygosity; NSCLC, non-small cell lung carcinoma.

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Fig. 1. Subchromosomal localization of the del-27 sequence on human chromosome 5 by PCR. Diagram depicts human/hamster somatic cell hybrid cell lines retaining different parts of human chromosome 5. +, presence; -, absence of the del-27 PCR product.

The frequencies for the 4.8-kilobase fragment (allele a) of M = 0.27 and for the 3.9-kilobase fragment (allele b) of M = 0.73. These donors showed a similar frequency of heterozygosity for the APC RsaI polymorphism (54%). However, occurrence of the del-27 and APC polymorphisms was distinct. This was most apparent in human lung carcinoma cell lines. Although 7 of 8 (88%) NSCLC cell lines tested were homozygous at the del-27 polymorphic site, 4 of the 8 (50%) NSCLC cell lines were heterozygous at the APC locus (Fig. 2, Lanes b–h and m). In the small cell lung carcinoma cell line SK-LC-17, which has a homozygous deletion of the del-27 polymorphic site (10), allele a of the APC RFLP is still contained (Fig. 2, Lane i). Since no matching normal DNA of these lung cancer cell lines was available, allelic deletion at the del-27 locus was investigated in tumor biopsies.

Allelic Deletion at the del-27 Locus in Human Squamous Cell Carcinomas. Of 14 patients with squamous cell lung carcinoma, 7 were heterozygous (i.e., informative) for the del-27 polymorphic site. In 4 of these 7 informative cases (57%) LOH was observed in the carcinoma (Fig. 3A; Table 1). This is a high frequency of allelic loss compared with other loci (15–17) and considering the small sample size. These four carcinomas consisted of one well, two moderately, and one poorly differentiated squamous cell lung carcinoma (18), which does not suggest a correlation between allelic loss at the del-27 locus and differentiation grade. We also looked for allelic loss at the APC locus at 5q21 by PCR. To exclude contaminating normal stromal cells and leukocytes, which were responsible for visible background bands in Southern blot hybridizations (Fig. 3A, P11, P19, P56) and might have obscured PCR results, tumor islets free of contaminating normal tissue were isolated by a microdissection method (13). With this procedure, a high frequency (71%) of loss of heterozygosity was observed at the APC gene (Fig. 3; Table 1).

At the del-27 locus also showed allelic deletion at the the APC locus (Table 1). This demonstrates that a high frequency of allelic loss can occur at either del-27, APC, or at both loci on chromosome 5 in human squamous cell carcinoma of the lung.

DISCUSSION

The main result of this study is the identification of another region on human chromosome 5 (5pl3–5ql4) that shows frequent allelic

Table 1 Allelic deletion at the del-27 and APC loci in human squamous cell lung carcinomas

<table>
<thead>
<tr>
<th>Patient</th>
<th>Histology</th>
<th>del-27</th>
<th>APC exon 11</th>
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<tr>
<td>19</td>
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<td>LOH</td>
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<tr>
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<tr>
<td>47</td>
<td>G3</td>
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* Differentiation grade: well (G1); moderately (G2); or poorly (G3) differentiated according to World Health Organization guidelines.

** LOH, loss of heterozygosity (i.e., allelic loss observed); n.i., not informative; n.d., not determined.
deletion in squamous cell lung carcinomas. Loss of heterozygosity in this region was detected with the del-27 sequence, which also is deleted homozygously in a human lung carcinoma cell line. This supports the close linkage of the del-27 sequence to a novel putative tumor suppressor gene involved in the development of lung cancer.

Previous studies have demonstrated that recurrent allelic deletions in tumors are indicative of the inactivation of a tumor suppressor gene. These tumor-specific allelic deletions can be detected by RFLP probes that show LOH in the tumor cells. Apparently, the closer a RFLP probe is to the relevant tumor suppressor gene, the higher is the incidence of LOH (16). In human lung cancer, LOH frequently has been observed in the region of the p53 gene (17p13), the MCC/APC region (5q21), and at least 3 regions on the short arm of chromosome 3 (7, 19). In addition to allelic deletions, homozygous deletions are considered evidence for the identification of tumor suppressor genes (20–22). Homozygous deletions in the p53 gene and on chromosome 3p were reported in few lung carcinoma cell lines and lung carcinomas, implying the truly recessive way of gene inactivation (13, 23–25).

The del-27 RFLP shows 54% heterozygosity in normal DNA. A similar frequency of heterozygosity was observed in exon 11 of the APC gene in normal DNA (12) (Fig. 2). Heterozygosity rates well deleted homozygously in a human lung carcinoma cell line. This may be a result of different purity grades of the examined tumor tissue. A similar frequency of heterozygosity was observed for the APC gene and 20–30% LOH was detected (with del-27 locus, whereas only one-half of these tumor cell lines were over 50% have been reported for other tumor suppressor loci (12).

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