Human Colorectal Cancer: High Frequency of Deletions at Chromosome 1p35[1]

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ABSTRACT

Cytogenetic analyses of human colon cancer cells have revealed a high frequency of chromosome 1p deletions among other chromosomal abnormalities. In order to find out whether these chromosomal alterations are manifestations of loss of genetic material, we surveyed DNA of 62 primary tumors, 7 metastases, and matching peripheral blood cells with a panel of polymorphic DNA probes that detect different loci on chromosome 1p. A portion of the probes was derived from a microclone bank generated by microdissection and microcloning of 1p35→pter DNA. In 42% of the colon carcinomas allelic loss was observed with at least one probe. The deletions were of different sizes but always included a region involving band 1p35, except for two tumors in which allelic loss was detected more proximally. The frequency of 1p deletion in the metastases was higher than in the primary tumors. These data indicate that genetic information related to tumorigenesis is located within or nearby region 1p35 and that deletion of this region occurs later in tumor development. Our results add to the number of genetic changes presumably involved in colon cancerogenesis.

INTRODUCTION

Human cancers frequently show nonrandom chromosomal deletions which are thought to affect genetic information whose presence and function prevents the development of the tumor phenotype. This genetic information is referred to as the "tumor suppressor" gene (1). The tumor suppressor gene represents the critical target of the allelic loss event (2, 3). Chromosomal deletions were detected in various cancers, e.g., retinoblastoma (4), Wilms' tumor (5–7), neuroblastoma (8–10), lung cancer (11), and colon cancer (12). In colon carcinomas inactivation of genetic material has been observed in about 75% of the tumors for the gene p53 on chromosome 17 (13), for the gene DCC on chromosome 18q (12), and for DNA on chromosomes 5q and 22q (14).

There are two general strategies to detect gross genetic alterations in tumor cells. First, "allelotyping" can be done, in which one to several probes of each chromosome are used to detect loss of genetic material. This technique requires the availability of a large number of suitable probes. Furthermore, loss of heterozygosity may not be noticed if the probes are located distant from a commonly deleted region. Second, chromosomal aberrations can be identified by cytogenetic analysis. This method reveals nonrandom chromosomal aberrations in different tumor tissues, and subsequently more detailed analyses can be done using a panel of region-specific molecular probes. For this study we have chosen to examine a series of colon tumors by cytogenetic and molecular approaches. Both cytogenetic and molecular analyses revealed alterations of chromosome 1p.

MATERIALS AND METHODS

Tumor Specimens. Colon tumor and normal tissue samples from the same patient were obtained from the operating room. All tumors were spontaneous, *i.e.*, without hereditary background. The primary tumors originated from different portions of the colorectum, and the metastases were localized either in the liver or the lung. All patients were Caucasians aged between 39 and 85 years. One portion of each tumor was immediately put into cell culture; another was used for DNA preparation.

Cell Culture and Cytogenetic Analyses. Tumors were cut into pieces and washed several times with trypsin (15). Single cells were cultivated in Iscove's medium, supplemented with 15% fetal calf serum. Cells were taken immediately and after various times of cell culture, treated with colcemid (0.5 µg/ml final concentration), suspended in hypotonic KCl (0.075 M) for 10 min, and fixed twice with methanol:acetic acid (9:1 and 3:1). Chromosomal spreads were stained with Giemsa (10% in phosphate buffer, pH 6.8).

DNA Analysis. High molecular weight DNA was extracted from tumor samples, corresponding cell lines, and blood samples or normal mucosa using standard procedures. Each DNA (15 µg) was digested with the appropriate restriction endonuclease, fractionated on 0.8% agarose gels, and transferred to GeneScreen Plus membranes (NEN). DNA probes were labeled with ^{32}P by random priming and hybridized to the DNA fixed on the membranes. After hybridization the blots were washed stringently (40 mM sodium phosphate and 1% sodium dodecyl sulfate at 68°C) and exposed to XAR-5 film (Kodak) with an intensifying screen at −80°C for 15–48 h.

Recombinant DNA Probes. Region-specific probes spanning chromosomal bands 1p22 to 1p36 were used to survey DNA of 69 colon tumors, 24 cell lines, and matching peripheral blood cells or normal mucosa. Some of these probes were obtained from other laboratories: CRI-L336 (D1S47) is a 20 kilobase pair λ clone mapping to the distal part of the 1p arm (16). MS1 (D1S7) is a minisatellite probe (17), mapped to 1p35 by *in situ* hybridization (10). pLMYC10 represents a 1.8 kilobase pair *EcoRI-SmaI* genomic DNA fragment of the *MYCL* gene (18). p1–08 (D1S10) is a 1.7 kilobase pair *EcoRI-EcoRI* fragment, mapping to chromosome 1p22 (19). Other probes were generated in our laboratory by microdissecting 1p35→pter from metaphase preparations of normal chromosomes and microcloning the DNA into a bacteriophage vector (9). This method provided a panel of region-specific DNA probes comprising bands 1p35→pter (10). p1–24 (D1S94), p1–31 (D1S112), and p1–45 (D1S96) are plasmid-subcloned DNA probes from a λ microclone library (9). These probes were mapped to the distal part of chromosome 1p (10). p1–45G1.2B (D1S96) represents a 1.2 kilobase pair *BamHI-BamHI* fragment of the cosmid clone C1–45G. C1–45G was isolated in our laboratory from a human cosmid library with the single copy microclone p1–45 (D1S96).

RESULTS

Cytogenetic Alterations. Our preliminary cytogenetic studies had indicated that cells of colorectal cancers often show deletions of chromosome 1p, in addition to other chromosomal changes[2] (14). In an attempt to identify chromosome 1p alterations in human colorectal cancer cells in more detail, we have chosen to survey a larger series of tumors for chromosome 1p
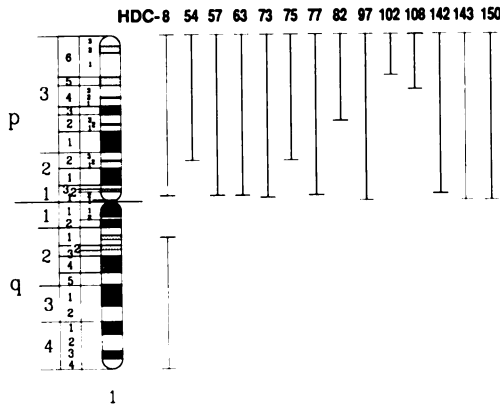
Fig. 1. Schematic illustration of cytogenetic alterations of chromosome 1p in colon carcinomas. *Bars*, aberrations of different sizes. The portion of chromosome 1 distal to 1p35 was common to all deletions.

abnormalities. Cells of primary tumors and of metastases were taken into short-time or long-time culture, and chromosomal spreads were G banded and analyzed by routine cytogenetic procedures. We found that, in a survey of 24 tumors, 14 showed deletions in chromosome 1p (Fig. 1). The deletions had resulted in the loss of various portions of chromosome 1p with breakpoints in 9 cases at 1p11, in 2 cases at 1p22, and in 3 tumors at 1p32, 1p34, and 1p35. Even though the breakpoints varied, the portion of chromosome 1 distal to 1p35 was consistently absent from the tumor karyotype. All deletions appeared to be terminal, and interstitial deletions were not detected. These findings suggested nonrandom deletion of genetic information from 1p35 to pter in a significant number of colon cancers.

Molecular Analyses. With this initial information, we set out to define the architecture of the 1p deletions in a large number of tumors in more detail. Polymorphic probes detecting loci between 1p22→pter were used to analyze whether 69 colon tumors were partially monosomic for chromosome 1p. Homozygous loci, showing only one allele in normal tissue, were designated "noninformative." Allelic loss at the informative loci was scored if one of the two alleles present in normal DNA was absent from the tumor DNA (Fig. 2).

Frequency of Allelic Loss. Of the 69 tumor DNAs, 29 (42%) showed loss of alleles at one or more 1p loci. Thirteen tumors exhibited allelic loss at every informative 1p locus (*e.g.*, tumors 8, 57, 63, 82, and 142 in Fig. 3), confirming our data obtained from cytogenetic analysis that had indicated deletion of large portions of 1p in these tumors. On the molecular level tumor 57 showed the largest deletion that could be detected by our probes. For this tumor the probe p1–08 (D1S10) was informative, and 1p loss was revealed at all loci between 1p22 and pter (data not shown). In tumor 53 the deletion was shorter, extending from 1p32 to pter. Tumors 102 and 108 which cytogenetically had revealed the most distally located breakpoints at 1p34 and 1p35 showed loss of heterozygosity from 1pter to 1p35. More proximally located probes were not informative.

Size of Deleted Regions. Comparing the deleted portions in all tumors that revealed allelic loss, we found that 26 of 28 informative cases (93%) had deleted 1p35 (Fig. 3). Tumors 15, 38, 74, 89, 120, 129, and 138 showed interstitial deletions of different sizes. Although the probe MS1 (D1S7) revealed loss of genetic material in these tumors, no allelic loss could be detected using the more distally located probes L336 (D1S47), p1–45 (D1S96), p1–24 (D1S94), or p1–31 (D1S112). Tumor 15 showed loss of genetic information only in 1p35 with the probe MS1, while both alleles were present at all other loci
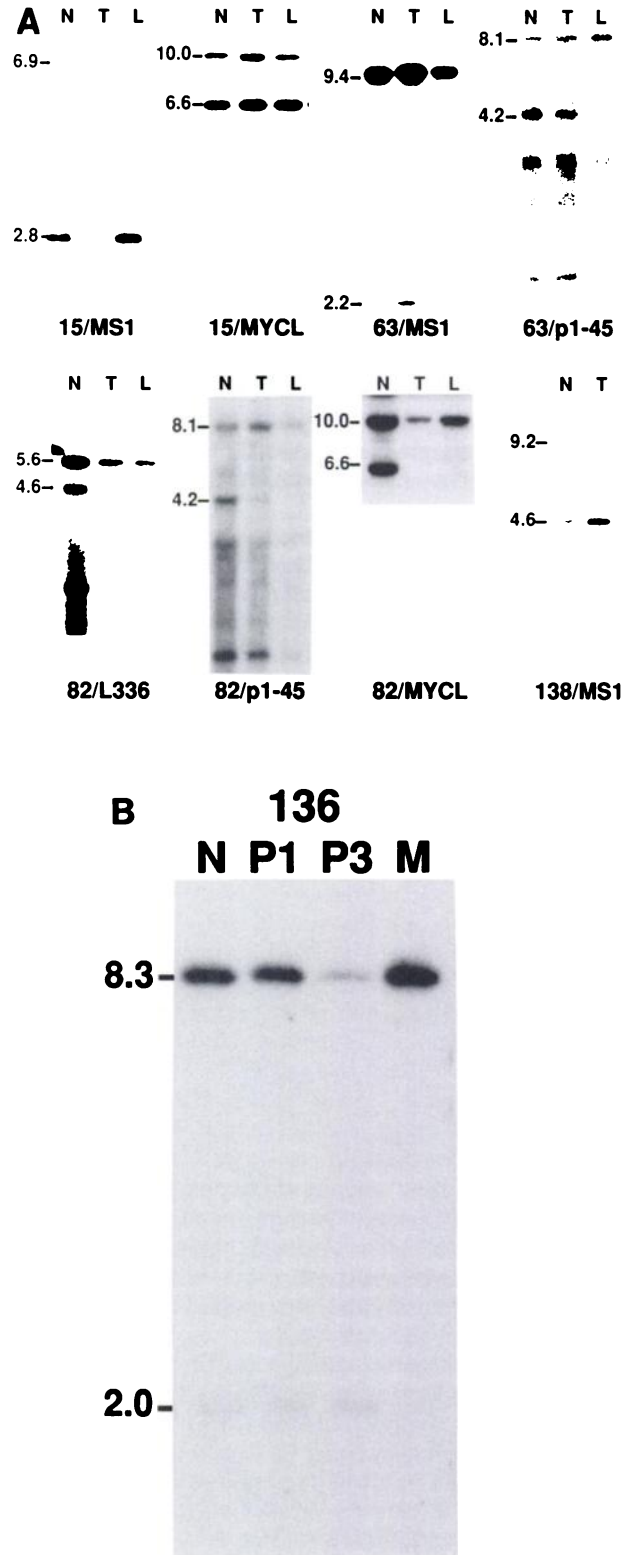

Fig. 2. *A*, allelic loss events in colon carcinomas. *Bottom abscissa*, numbers of tumor samples and polymorphic probes are indicated beneath. *Ordinate*, sizes of DNA fragments (in kilobases). Tumor 15 revealed a deletion only with MS1 (D1S7); both alleles were retained at all other loci, as demonstrated by the *MYCL* probe. In tumor 63 loss of heterozygosity could only be detected when using the cell line (*L*), indicated here for the probes MS1 (D1S7) and p1–45 (D1S47). Allelic loss in tumor (*T*) and cell line (*L*) was detected in tumor 82 with all polymorphic probes, as shown by L336 (D1S47), p1–45 (D1S96), and the *MYCL* probe. Tumor 138 represents a primary tumor without analysis of a corresponding cell line. Allelic loss was detected by MS1 (D1S7). *B*, primary tumors 136P1, 136P3 and metastasis 136M. No allelic loss could be detected with probe MS1 in 136P1; 136P3 had lost the larger and 136M had lost the smaller allelic fragment in the tumor tissue.

Fig. 3. Comparison of deleted regions on chromosome 1p in colorectal cancers. The numbers of cases (*abscissa*) refer to the consecutive numbering of tumors in our laboratory. All tumors are shown that revealed loss of heterozygosity with 1p-specific molecular probes. *Ordinate*, the distal part of chromosome 1 with localizations of polymorphic probes used in our study is demonstrated beneath. *White columns*, loss of genetic material with the corresponding probe; *hatched columns*, noninformative probes; *black columns*, both alleles were retained at the locus. Additional analyses of matching cell lines were done for tumors 8 to 142, shown on the left side. All tumors except for two revealed deletions in region 1p35.
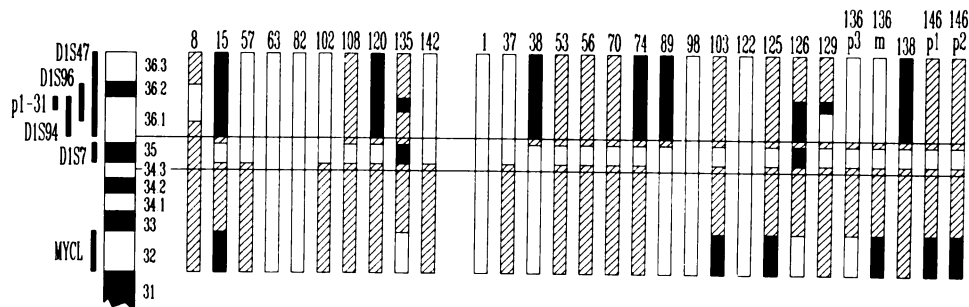
tested. Tumor 38 displayed deleted alleles with probes MS1 and the *MYCL* probe; no loss was found with the more distally located probe L336 and the more proximally located probe p1-08 (data not shown). Tumors 126 and 135 were the only examples that revealed a loss of alleles at the more proximally located *MYCL* locus but not with the MS1 probe. *MYCL* was deleted in 10 of 16 informative cases (63%). The probe L336 (D1S47), mapping to region 1p36, revealed loss of heterozygosity in 12 of 17 informative tumors (71%). Therefore, the smallest region of overlap of deletions in all positive cases is suggested to involve band 1p35. The central part of this region is presumably located in close proximity to D1S7, since in all cases except for two this locus was deleted.

Our cytogenetic analyses had indicated chromosomal alterations involving 1p in >50% of colon tumors. In some cases, however, cytogenetic studies had shown that large portions of the 1p arm were deleted, but loss of genetic material was not detected with molecular probes (tumors 54, 73, 75, 77, 97, 143, and 150 in Fig. 1). For those cases we suggest a translocation event, transferring genetic material from chromosome 1p to another chromosome. In contrast, some of the tumors that cytogenetically appeared to have no chromosome 1p deletion displayed allelic loss when analyzed with molecular probes (tumors 15, 120, and 135 in Fig. 3). These tumors carrying submicroscopic deletions will be valuable tools for identifying a putative tumor suppressor gene.

Cell Lines. Colorectal cancers often contain a high proportion of normal cells in addition to the tumor cells. To find out whether the normal cells would interfere with our analyses of allelic loss in 1p, we have compared 24 tumors with matching cell lines established from these tumors. Of the 24 tumor cell preparations 8 showed allelic loss at 1p; coincidentally the cell lines established from these tumors also revealed corresponding loss at 1p. Additionally, two cell lines revealed loss of 1p sequences that remained undetectable in the corresponding tumors (*e.g.*, tumor 63 in Fig. 2*A*). When these tumors were examined histologically, a major proportion of the tissue proved to consist of normal cells that presumably had masked detection of a 1p deletion. Our data reveal that 1p deletion is present in the tumor and is not a tissue culture artifact.

Allelic Loss and Tumor Progression. To find out whether 1p deletion is an early or a late event during colon tumorigenesis, we compared the allelotypes of primary tumors and 7 metastases. Five metastases revealed deletions with one or several 1p-specific probes (tumors 15, 63, 82, 102, and 136M in Fig. 3), while 24 of 62 primary tumors showed loss of heterozygosity. Hence, the percentage of allelic loss in the metastases was higher than in the primary tumors (71% compared to 39%). When we analyzed three primary carcinomas and a metastasis

obtained from the same patient (136P1, 136P2, 136P3, and 136M in Fig. 2*B*), in two of the primary tumors loss of genetic material could not be detected. The third primary tumor, however, and the metastasis revealed a loss of heterozygosity. The two deletions were of different sizes. The primary tumor 136P3 lacked sequences at loci corresponding to L336, MS1 and *MYCL*. The metastasis 136M revealed allelic loss with probes L336 and MS1 but had retained the two *MYCL* alleles. Moreover, the comparison with the normal tissue showed that 136P3 and 136M had lost different alleles, although the deletion overlapped the 1p35 region in both cases. These results show that the deletions were not of clonal origin and thus had arisen later in tumor development.

DISCUSSION

The present study was designed to determine whether chromosomal deletion in 1p is a common event in colon tumorigenesis. By using a panel of 1p-specific molecular probes we found allelic loss involving chromosome 1p in 42% of all colon tumors. The percentage is likely to be higher, since in several cases probe MS1 (D1S7) which detected most of all deletions was not informative. Moreover, allelic losses in tumors might have been masked by normal cells in the tumor tissue. Furthermore, alterations disrupting a putative tumor suppressor gene on chromosome 1p might be too small to be detected.

A previous analysis of colon carcinomas by Vogelstein *et al.* (20) revealed loss of heterozygosity with a 1p-specific probe in only 3 of 36 informative tumors. Therefore, aberrations in chromosome 1p appeared to be insignificant compared to other chromosomes in the allelotype analysis. The discrepancy between the previously reported results and our present results may be due to the fact that the locus identified by probe YNZ2 used in the analysis has not been finely mapped and may be localized distant from the smallest region of overlap for deletions which we detected in 1p35. Analysis of a 5q-specific probe (C11p11) on our colon tumors revealed allelic loss in 13% of all informative cases (data not shown).

Comparison of primary tumors and metastases revealed a higher frequency of chromosome 1p deletions in the metastases. Furthermore, 1p deletions in a primary tumor and a metastasis obtained from the same patient were not of clonal origin because they differed in size and both tumors had lost different alleles. These results indicate that allelic loss in chromosome 1p is a late event during colon tumorigenesis. Therefore, 1p deletion might combine with other genetic changes in colon carcinomas (21) which may lead to the development of the later tumor stages.

Alterations of chromosome 1p have been detected in other

human tumors, and they were localized to 1p36 in neuroblastomas (9), 1p36 and 1p22 in melanomas (22), 1p36 in breast carcinomas (23), and 1p21–22 in malignant mesothelioma (24). These data may indicate that deletion of different portions of chromosome 1p are instrumental in different types of human cancer. The full spectrum of cancers with significant 1p deletions is unknown at present. However, at least in renal cell carcinomas that we analyzed in a parallel study, 1p deletion was detected in only 1 of 8 cases (data not shown). Our studies suggest that deletion of the smallest region of overlap at chromosome 1p35 may contribute to the progression of colorectal cancer.

ACKNOWLEDGMENTS

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