Frequent Loss of Heterozygosity for Loci on Chromosome 8p in Hepatocellular Carcinoma, Colorectal Cancer, and Lung Cancer

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ABSTRACT

Frequent loss of heterozygosity at chromosomal loci in a specific tumor type may indicate the presence of a tumor suppressor gene. We have examined loss of heterozygosity on chromosome 8p in paired tumor and constitutional DNA from 346 patients representing seven different types of human cancer. Frequent allelic losses were observed in hepatocellular carcinoma (22 of 46 cases, 47.8%), in colorectal cancer (12 of 26, 46.2%), and in non-small cell lung cancer (14 of 35, 40.0%), in contrast to low frequencies detected in breast cancer (5 of 56, 8.9%) and renal cell carcinoma (2 of 27, 7.4%). Ovarian cancer and gastric cancer showed intermediate frequencies of 33.3% and 22.2%. Subsequent analysis of 120 hepatocellular carcinomas and 94 colorectal cancers with five polymorphic markers along the short arm of chromosome 8 defined commonly deleted regions within the same chromosomal interval, 8p23. 1–8p21.3, suggesting that one or more tumor suppressor genes for both cancers may be present in that region.

INTRODUCTION

Human cancers are now considered to occur through the accumulation of genetic alterations in protooncogenes and tumor suppressor genes (1, 2). Tumor suppressor genes negatively regulate cell proliferation; their inactivation may allow a cell to escape from the normal control of growth. An inactivating mutation in a tumor suppressor gene is considered to be recessive at the somatic cell level; mutations on the mutant allele are unmasked when some chromosomal mechanism eliminates a normal allele (3). This process can be identified as a LOH at specific chromosomal loci in tumor DNA, detected by RFLP markers. When a particular type of cancer exhibits frequent LOH in a specific chromosomal region, one can infer that a tumor suppressor gene important in the genesis of that tumor may be present in the deleted region. This theory received experimental support when a childhood tumor, retinoblastoma, was shown to occur by loss or inactivation of both copies of a single tumor suppressor gene, Rb (4). In common adult cancers, which may involve multiple tumor suppressor genes, the same paradigm appears to apply. For example, after LOH studies had revealed chromosomes 5q, 17p, and 18q as targets of frequent allelic loss in colorectal cancers (5), further investigations led to identification of the APC gene (6, 7), the MCC gene (8), the p53 gene (9), and the DCC gene (10) as the mutated genes at the somatic cell level; mutations on the mutant allele are unmasked when some chromosomal mechanism eliminates a normal allele (3). This process can be identified as a LOH at specific chromosomal loci in tumor DNA, detected by RFLP markers. When a particular type of cancer exhibits frequent LOH in a specific chromosomal region, one can infer that a tumor suppressor gene important in the genesis of that tumor may be present in the deleted region. This theory received experimental support when a childhood tumor, retinoblastoma, was shown to occur by loss or inactivation of both copies of a single tumor suppressor gene, Rb (4). In common adult cancers, which may involve multiple tumor suppressor genes, the same paradigm appears to apply. For example, after LOH studies had revealed chromosomes 5q, 17p, and 18q as targets of frequent allelic loss in colorectal cancers (5), further investigations led to identification of the APC gene (6, 7), the MCC gene (8), the p53 gene (9), and the DCC gene (10) as the mutated genes in the indicated regions.

Several types of human cancer have been under extensive LOH studies in our laboratory, including breast cancer, ovarian cancer, renal cell carcinoma, hepatocellular carcinoma, lung cancer, and colorectal cancer; a number of deleted chromosomal regions important in those cancers have been identified (11–16). The short arm of chromosome 8 was not included in these surveys, because RFLP markers with sufficient informativeness for LOH study were not available in that region. However, a recent observation of LOH on 8p in some colorectal cancers (17) prompted us to examine LOH on this arm in various types of human cancer, in a search for putative tumor suppressor gene(s) on chromosome 8.

Toward this goal, we have recently isolated and mapped a number of new RFLP markers on human chromosome 8 (18). In the present study, we performed RFLP analysis on 346 pairs of tumor and constitutional DNAs from seven types of human cancer using these markers, in order to examine whether alleles on chromosome 8p were specifically lost in any particular type of cancer and, if so, to determine whether a single region was commonly deleted.

MATERIALS AND METHODS

Samples. Tumors and their corresponding noncancerous tissues were obtained at surgery from 120 patients with primary hepatocellular carcinoma, 94 with colorectal carcinomas, 59 with non-small cell lung cancer, 24 with ovarian cancer, 24 with gastric cancer, 82 with breast cancer, and 40 with renal cell carcinoma. All tissues were dissected in the operating room, frozen immediately, and stored at −80°C until the DNA was isolated.

DNA Extraction and Southern Blotting. Frozen tissue samples were ground to a very fine powder in liquid nitrogen, suspended in lysis buffer, treated with protease K, and extracted by phenol-chloroform-isooamyl alcohol as described elsewhere (11). Five μg of DNA were digested overnight with a 10× excess of restriction enzymes (Boehringer Mannheim) and fractionated by electrophoresis using an 0.8% agarose gel. The DNAs were then transferred to nylon membranes (Biodyne; Pall) in 0.1 N NaOH-0.1 N NaCl and fixed by UV cross-linking (11).

Probes and Hybridization. The five markers used in this study are shown in Table 1. Cosmid markers cCl8-1, cCl8-277, cCl8-319, and cCl8-512 were recently developed at the Cancer Institute and localized on chromosome 8 by fluorescent in situ hybridization (18). cMSR32, a cosmid clone at the MSR locus on human chromosome 8 (19), detected a Mrpl polymorphism with four alleles and was localized to 8p22 by fluorescent in situ hybridization (18). A polymorphic restriction fragment of each cosmid was purified by agarose gel electrophoresis and used as a probe for hybridization to detect LOH. Probes were labeled with [32P]dCTP by random primer extension (20). Prehybridization, hybridization, and autoradiography were carried out as described elsewhere (11). The membranes were stripped in 0.1 N NaOH and repeatedly hybridized.

Definition of Loss of Heterozygosity. The signal intensity of the polymorphic alleles was quantified with a Hoefer GS-300 scanning densitometer; the peak areas corresponding to each hybridizing signal...
were calculated by electric integration using a GS-370 1-D electrophoresis data system (Hoefer Scientific Instruments, San Francisco, CA). After the DNA loading difference was corrected for, the signal intensity of alleles of tumor tissue DNA was compared to that of normal tissue DNA. When reduction in signal intensity was >50%, it was judged to be a loss of heterozygosity. Loss of heterozygosity was distinguished from chromosome duplication by normalizing the signal for the chromosome sis data system (Hoefer Scientific Instruments, San Francisco, CA).

RESULTS

Allelic Loss on Chromosome 8p in Various Types of Cancers. As an initial step to test the involvement of genes on chromosome 8p in human carcinogenesis, we carried out a RFLP analysis with probe cMSR32 (8p22) to examine allelic loss with seven types of human adult cancers, i.e., cancers of the liver, stomach, colorectum, kidney, lung, breast, and ovary. Fig. 1 shows representative autoradiograms demonstrating LOH on 8p in cancers of the liver, colon, lung, ovary, and stomach. Loss or significant reduction of one allele was observed clearly in each case. Table 2 summarizes the frequency of allelic losses in each of the seven type of cancers studied. Breast cancer and renal cell carcinoma showed allelic losses at a frequency no greater than 10%. In contrast, losses were frequently observed in hepatocellular carcinoma (47.8%), colorectal cancer (46.2%), and non-small cell lung cancer (40.0%). Although ovarian cancer and stomach cancer showed moderately frequent LOH, the numbers of samples examined were probably too small to substantiate the significance of these values.

Detailed Analysis of LOH on Chromosome 8p in Hepatocellular Carcinoma. Five markers, cCI8-1 (D8S140), cCI8-512 (D8S238), cMSR-32 (MSR), cCI8-319 (D8S220), and cCI8-277 (D8S194), were chosen to represent different segments of the short arm of chromosome 8 (Table 1). Paired constitutional and tumor DNAs from 120 patients with hepatocellular carcinoma were examined for allelic losses with this panel of markers. Of these, 97 cases were informative with at least one marker; 39 tumors (40.2%) showed LOH with at least one marker on chromosome 8p. Table 3 shows the frequency of LOH at the five marker loci, which are listed according to linear order along the chromosome. The frequency of LOH increased toward the center of the short arm.

Among 39 tumors which lost heterozygosity for at least one locus, 22 had lost a part of chromosome 8p. Fig. 2 presents examples of autoradiograms showing partial deletion of 8p. Tumor 107 showed LOH at MSR while retaining heterozygosity at D8S238 (Fig. 2a). Tumor 25 retained heterozygosity at D8S194 but showed LOH at MSR (Fig. 2b). The LOH data among the 22 tumors are summarized schematically in Fig. 3. Four cases (tumors 63, 79, 93, and 126) showed a pattern of interstitial deletions. Tumor 93 showed the smallest deleted region defined by D8S238 and D8S220. All 17 tumors informative at MSR lost heterozygosity, but eight of them retained heterozygosity at D8S238 (tumors 67, 79, 93, 107, 114, 126, and 128), indicating that the distal limit for the commonly deleted region lies between these two loci. Three tumors defined the proximal limit: tumors 63 and 93 lost heterozygosity at D8S194 while retaining heterozygosity at D8S220; tumor 30 lost heterozygosity at D8S140 and D8S238 but retained heterozygosity at D8S220. Furthermore, 12 other tumors that lost heterozygosity at MSR or D8S220 retained heterozygosity at D8S194. Taken together, the results indicated that the common region of deletion lies within 8p23.1–8p21.3, in a region flanked by D8S238 and D8S220.

Fig. 1. Autoradiograms from Southern blot analyses, demonstrating LOH in hepatocellular carcinoma (a), colorectal cancer (b), lung cancer (c), ovarian cancer (d), and gastric cancer (e), with probe cMSR32. N, DNA from normal tissue; T, DNA from tumor tissue; c, constant band; 1, 2, and 3, alleles 1, 2, and 3, respectively.

Table 1 Chromosome 8 RFLP markers

<table>
<thead>
<tr>
<th>Locus</th>
<th>Marker</th>
<th>Location</th>
<th>Fragment</th>
<th>Enzyme</th>
<th>Allele size (kilobases)</th>
<th>Heterozygosity</th>
</tr>
</thead>
<tbody>
<tr>
<td>D8S140</td>
<td>cCI8-1</td>
<td>8p23.3</td>
<td>Msp 3.0</td>
<td>Msp1</td>
<td>3.0/2.7</td>
<td>0.41</td>
</tr>
<tr>
<td>D8S238</td>
<td>cCI8-512</td>
<td>8p23.1</td>
<td>Msp 1.6</td>
<td>Msp1</td>
<td>2.8/1.6</td>
<td>0.35</td>
</tr>
<tr>
<td>MSR</td>
<td>cMSR-32</td>
<td>8p22</td>
<td>E-H.9</td>
<td>Msp1</td>
<td>6.3/3.1/2.9/2.7</td>
<td>0.61</td>
</tr>
<tr>
<td>D8S220</td>
<td>cCI8-319</td>
<td>8p21.3</td>
<td>Taq 6.8</td>
<td>Msp1</td>
<td>6.8/3.5/3.3</td>
<td>0.43</td>
</tr>
<tr>
<td>D8S194</td>
<td>cCI8-277</td>
<td>8p11.2</td>
<td>Msp 4.6</td>
<td>Msp1</td>
<td>4.6/2.5/2.1</td>
<td>0.49</td>
</tr>
</tbody>
</table>

Table 2 Allele loss on 8p in various cancers

<table>
<thead>
<tr>
<th>Types of cancer</th>
<th>No. of patients tested</th>
<th>Allelic losses/informative cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>79</td>
<td>22/46 (47.8)</td>
</tr>
<tr>
<td>Colon</td>
<td>38</td>
<td>12/26 (46.2)</td>
</tr>
<tr>
<td>Lung</td>
<td>59</td>
<td>14/35 (40.0)</td>
</tr>
<tr>
<td>Ovary</td>
<td>24</td>
<td>4/12 (33.3)</td>
</tr>
<tr>
<td>Stomach</td>
<td>24</td>
<td>4/18 (22.2)</td>
</tr>
<tr>
<td>Breast</td>
<td>82</td>
<td>5/56 (8.9)</td>
</tr>
<tr>
<td>Kidney</td>
<td>40</td>
<td>2/27 (7.4)</td>
</tr>
</tbody>
</table>
Table 3 Allelic loss at loci on 8p in hepatocellular carcinoma

<table>
<thead>
<tr>
<th>Locus</th>
<th>Probe</th>
<th>Location</th>
<th>No. of patients tested</th>
<th>Allelic losses/informative case (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D8S140</td>
<td>C18-1</td>
<td>p23.3</td>
<td>101</td>
<td>7/39 (17.9)</td>
</tr>
<tr>
<td>D8S238</td>
<td>C18-512</td>
<td>p23.1</td>
<td>111</td>
<td>7/37 (18.9)</td>
</tr>
<tr>
<td>MSR</td>
<td>MSR-32</td>
<td>p22</td>
<td>108</td>
<td>28/59 (47.5)</td>
</tr>
<tr>
<td>D8S220</td>
<td>C18-319</td>
<td>p21.3</td>
<td>100</td>
<td>16/43 (37.2)</td>
</tr>
<tr>
<td>D8S194</td>
<td>C18-277</td>
<td>p11.2</td>
<td>92</td>
<td>5/45 (11.1)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>120</td>
<td>39/97 (40.2)</td>
</tr>
</tbody>
</table>

Detailed Analysis of LOH on Chromosome 8p in Colorectal Cancer. The five probes on 8p (see Table 1) were used to examine allelic losses in 94 colorectal carcinomas. Table 4 presents the frequency of LOH detected at each locus. LOH was observed in 34 of 88 informative cases (38.6%) with at least one probe on 8p. The frequency of LOH was highest in the middle part of the short arm at MSR (37.5%) and D8S220 (37.1%) and lowest at D8S140 (18.2%) and D8S194 (15.0%). Fifteen tumors showed loss of a subchromosomal region of 8p. For example, tumor 36 showed LOH at MSR while retaining heterozygosity at D8S220 (Fig. 2c); tumor 148 retained heterozygosity at D8S238 but showed LOH at MSR (Fig. 2d). Fig. 4 summarizes the LOH data from the 15 colorectal cancers showing regional deletions on 8p, which centered around the MSR locus as well. Five cases (tumors 12, 18, 28, 133, and 148) showed a pattern of interstitial deletions. The distal limit of the commonly deleted region was located between D8S238 and MSR from the observation of four tumors (tumors 7, 12, 28, and 148) that retained heterozygosity at the D8S238 locus and lost an allele at MSR. The proximal limit was defined by four tumors: tumors 4 and 36 lost heterozygosity at MSR but retained heterozygosity at D8S220, while tumors 39 and 141 lost heterozygosity at D8S238 but retained heterozygosity at D8S220. The data indicate that the commonly deleted region on 8p in colorectal cancer, as in hepatocellular carcinoma, lies at p23.1-p21.3, between D8S238 and D8S220.

DISCUSSION

We report here that alleles at loci on chromosome 8p were frequently lost in three common adult cancers: hepatocellular carcinoma; colorectal cancer; and lung cancer. Commonly deleted regions were defined at 8p23.1-p21.3 in hepatocellular carcinoma and in colon cancer. Two lines of evidence support the significance of these observations. First, 46 of the hepatocellular carcinomas in this study previously had been allelotyped on almost all chromosomal arms by Fujimori et al. (14), who reported that loci on 5q, lOq, lip, 16q, and 17p showed a LOH frequency of more than 40%. The nonrandom nature of allelic losses in colorectal cancer examined in the present study represents a specific genetic change and that a putative tumor suppressor gene might exist on 8p.

Our previous allelotype study of hepatocellular carcinoma detected LOH on chromosomes 5q, 10q, 11p, 16q, and 17p (14). Others have described LOH on chromosomes 4q, 11p,
prostate cancers using four probes on 8p; they defined the commonly deleted region for prostate cancer from 8pter to 8q11.2. Since this region includes the entire short arm of chromosome 8, it obviously overlaps the commonly deleted region described here. In any case, deletion mapping with a large number of probes from the 8p23.1–p21.3 region will be warranted, to identify tumor suppressor genes for these types of cancer on chromosome 8p.

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