Frequent Loss of Heterozygosity in Human Primary Squamous Cell and Colon Carcinomas at 7q31.1: Evidence for a Broad Range Tumor Suppressor Gene

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ABSTRACT

Consistent deletions and loss of heterozygosity (LOH) in polymorphic markers in a determinate chromosomal fragment are known to be indicative of a closely mapping tumor suppressor gene. Deletion of the long arm of chromosome 7 is a frequent trait in many kinds of human primary tumors. We studied LOH of 14 markers on chromosome 7q in order to determine the location of a putative tumor suppressor gene in human primary squamous cell carcinoma of the head and neck and in human primary colon carcinomas. Samples were obtained from 18 primary squamous cell carcinomas of the head and neck and 18 primary colon carcinomas surgically removed from patients at the Fox Chase Cancer Center. Loss of heterozygosity was studied performing PCR amplifications of a set of 14 CA microsatellite repeats encompassing 7q21-ter. Of 18 squamous cell carcinomas of the head and neck cases studied, 12 had LOH at one or more loci on 7q. Fifty-three percent of 15 informative cases had LOH of the CA microsatellite dinucleotide repeat marker D7S522 at 7q31.1–7q31.2. Eleven of 18 colon carcinoma cases had LOH of one or more markers assessed, and the maximum LOH (80% of 10 informative cases) was at D7SS52. Percentages of percentage of LOH in both tumor types were normally distributed around microsatellite D7S522. The high incidence of LOH in both tumor types studied suggests that a tumor suppressor gene relevant to the development of epithelial cancers is present on the 7q31.1–31.2, confirming our previous functional evidence for a tumor suppressor gene on chromosome 7.

INTRODUCTION

Alterations in oncopogenes and TSGs are considered to be critical in the multistep process leading to the development of tumors (1, 2) and the succession of these events is very consistent in some types of cancer such as colon carcinomas (3). Even since the idea of recessive-acting TSGs was formulated (4), cytogenetic techniques have been used to reveal their locations. Cytogenetic reports have indicated that human chromosomes are frequently altered in SCCs of the head and neck (1p, 2, 3p, 5, 7, 8p, 10p, 11q, 13, and 18q; Refs. 5 and 6) and in colon carcinomas (1p, 4, 5q, 7, 8p, 12, 13, 15, 17, 18q, and 21; Refs. 7 and 8). Consistent deletions or inversions of part of chromosome 7 are indicative of inactivation of a nearby TSG during neoplastic progression (9). In this regard, deletions of chromosome 7 are common throughout many different types of tumors, including ovarian cancer, gastric carcinomas, and malignant myeloid tumors (10–15). Recently, we reported that LOH of 7q31.1–31.2 is a very common event in human primary breast cancers (16) and primary prostate carcinomas (17), and allelotyping of SCC of the head and neck indicates that 30% LOH is present in the CA microsatellite repeat D7S527 (18). Also, our results using microcell fusion transfer of human chromosome 7 to a murine SCC cell line indicated that chromosome 7 can delay the onset of tumors by 2–3-fold and in some cases even completely repress the tumorigenic potential of the SCC cell line used. In situ hybridization revealed that the clones that reverted to the malignant phenotype had expelled the inserted chromosome (19). Moreover, a recent report demonstrated that insertion of an intact human chromosome 7 into immortalized human fibroblast cell lines with LOH of 7q31–32 restored the senescence properties of the cells (20).

Although cytogenetic techniques are useful, they do not detect the entire spectrum of inactivating events; e.g., microdeletions and homologous recombination with a defective chromatin (21, 22) are beyond the range of detection by karyotyping procedures. More sensitive molecular methods should be used to screen for genetic alterations in any tumors and to determine the smallest chromosome region involved in those alterations. LOH analysis of DNA extracted from solid tumors is the method of choice for determining the smallest regions that harbor TSG (23).

To determine the extent and type of alterations on chromosome 7 in head and neck SCCs and colon cancers, we used an extensive set of highly polymorphic markers on q21-ter. By comparing the results obtained with tumoral and normal DNA, we determined a 1.2 cM SCDR.

MATERIALS AND METHODS

Tumor and Lymph Node Samples. Eighty-eight primary head and neck SCC and 18 primary colon carcinomas were surgically removed from patients at the Fox Chase Cancer Center; patients had not undergone previous radiotherapy or chemotherapy. Immediately following surgery, tumor samples were fixed in normal buffered formalin until high-molecular-weight DNA was extracted. Nonmetastatic lymph node samples were also obtained from each patient and stored in a similar way. Eight-μm-thick tissue sections were cut from the paraffin-embedded sample blocks and placed on glass slides. Unstained sections were microdissected to separate normal and non-neoplastic tissue from the tumor. As reference, consecutive serial sections were stained with hematoxilin-eosin. High-molecular-weight DNA was extracted from both lymph node and tumor sections using standard proteinase K digestion followed by chelex extraction (24). DNA from lymph node sample was used as the normal control.

CA Microsatellite Repeat Amplification Analysis. Fourteen CA microsatellite repeats in the 7q21.3-qter region (25) were amplified in a Thermocycler 9600 (Perkin Elmer/Cetus, Norwalk, CT). The 25-μl reaction mixtures contained 2.5 μl 10X standard PCR buffer, 100 ng DNA, 1 unit Taq polymerase, 400 pmol/liter each primer, and 200 mmol/liter each dNTP. In the colon cases, hot start was performed using a Taq polymerase-specific antibody that inactivates the enzyme and is released during the first denaturation cycle (Taq Start; Clontech Laboratory, Inc., Palo Alto, CA). The head and neck SCCs were amplified using a standard PCR protocol. The DNA was amplified with 27 cycles of 20 s of denaturation at 94°C, 30 s of annealing at 50°C, and 15 s of extension at 72°C. The number of cycles used was in the linear part of the amplification process, i.e., before product saturation, permitting us to assume that equal absorbance of both alleles was to be expected if no LOH had occurred.

The PCR products were separated in a 3.5% MetaPhor agarose (FMC Bioproducts, Rockland, ME) gel at 5.5 V/cm for 3 h in TBE buffer (89 mmol/liter Tris-borate, 89 mmol/liter boric acid, and 2 mmol/liter EDTA, pH 7.5) with 0.5 mg/ml ethidium bromide in TBE and a standard loading buffer

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2 To whom requests for reprints should be addressed, at The University of Texas, M. D. Anderson Cancer Center, Department of Cariogenesis, Science Park-Research Division, P.O. Box 389, Smithville, TX 78957.
3 The abbreviations used are: TSGs, tumor suppressor genes; SCCs, squamous cell carcinomas; LOH, loss of heterozygosity; SCDR, smallest common deleted region.
LOH IN SQUAMOUS CELL AND COLON CARCINOMAS

Table 1 Chromosome 7 LOH in 18 primary human SCCs of the head and neck and in 18 primary human colon carcinomas

<table>
<thead>
<tr>
<th>Markers</th>
<th>Colon Cases</th>
<th>Informative (% of total)</th>
<th>% LOH</th>
<th>Head and neck SCCs</th>
<th>Informative (% of total)</th>
<th>% LOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>D7S527</td>
<td>1</td>
<td>8 (44.4)</td>
<td>12.5</td>
<td>2</td>
<td>9 (50)</td>
<td>22.20</td>
</tr>
<tr>
<td>D7S528</td>
<td>0</td>
<td>8 (44.4)</td>
<td>0</td>
<td>2</td>
<td>13 (72.2)</td>
<td>15.4</td>
</tr>
<tr>
<td>D7S496</td>
<td>4</td>
<td>13 (72.2)</td>
<td>30.7</td>
<td>1</td>
<td>10 (55.5)</td>
<td>10</td>
</tr>
<tr>
<td>D7S523</td>
<td>4</td>
<td>9 (50)</td>
<td>44.4</td>
<td>5</td>
<td>13 (72.2)</td>
<td>38.3</td>
</tr>
<tr>
<td>D7S486</td>
<td>9</td>
<td>18 (100)</td>
<td>50</td>
<td>3</td>
<td>10 (55.5)</td>
<td>30</td>
</tr>
<tr>
<td>D7S633</td>
<td>4</td>
<td>7 (38.8)</td>
<td>57.1</td>
<td>2</td>
<td>6 (33.3)</td>
<td>33.3</td>
</tr>
<tr>
<td>D7S677</td>
<td>6</td>
<td>9 (50)</td>
<td>66.6</td>
<td>4</td>
<td>10 (55.5)</td>
<td>40</td>
</tr>
<tr>
<td>D7S522</td>
<td>8</td>
<td>10 (55.5)</td>
<td>80</td>
<td>8</td>
<td>15 (83.3)</td>
<td>53.3</td>
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<tr>
<td>D7S655</td>
<td>4</td>
<td>8 (44.4)</td>
<td>50</td>
<td>3</td>
<td>7 (38.8)</td>
<td>43.8</td>
</tr>
<tr>
<td>D7S480</td>
<td>7</td>
<td>16 (88.8)</td>
<td>43.7</td>
<td>4</td>
<td>10 (55.5)</td>
<td>40</td>
</tr>
<tr>
<td>D7S490</td>
<td>4</td>
<td>10 (55.5)</td>
<td>40</td>
<td>4</td>
<td>12 (66.6)</td>
<td>25</td>
</tr>
<tr>
<td>D7S487</td>
<td>2</td>
<td>8 (44.4)</td>
<td>25</td>
<td>0</td>
<td>10 (55.5)</td>
<td>0</td>
</tr>
<tr>
<td>D7S498</td>
<td>2</td>
<td>9 (50)</td>
<td>22.2</td>
<td>0</td>
<td>8 (44.4)</td>
<td>0</td>
</tr>
<tr>
<td>D7S550</td>
<td>0</td>
<td>9 (50)</td>
<td>33.3</td>
<td>0</td>
<td>6 (33.3)</td>
<td>0</td>
</tr>
</tbody>
</table>

(24). The gel was photographed with a Fotodyne 3–4400 UV transilluminator (Fotodyne Inc., New Berlin, WI) and Polaroid Positive-Negative 4×5 Instant Film (Polaroid Corp., Cambridge, MA).

Allelic Loss Determination. Determination was only done on informative patients. Normal DNA samples that were polymorphic at a given locus were considered to be informative, whereas the homozygotes were considered noninformative. The signal intensity of fragments was determined by densitometry or by visual examination by two reviewers.

We considered a sample to have LOH if an entire band was absent or the band intensity was less than 30% of the normal intensity (26). Although PCR amplification cannot be considered quantitative, we optimized the PCR conditions so that equal amounts of template produced equal amounts of amplified product. We used 27 amplification cycles, which we demonstrated to be in the linear part of the amplification process, i.e., before product saturation (data not shown). We also conducted a series of titrations using different proportions of homozygous and heterozygous templates to assess the influence of stromal tissue contamination of our amplification reactions. We determined that we could detect as little as 30% contamination by heterozygous template in the tissue contamination of our amplification reactions. We determined that we

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...normality of the percentage of LOH distributions was tested using the Kolmogorov-Smirnov continuous cumulative distribution test (27). To this end, we used both the raw-percentage LOH data and the adjusted data by using the informative:total cases ratio as the adjustment factor. No significant difference was found between the two analyses. The distributions obtained with the two different tumor types were compared using Tukey’s test.

RESULTS AND DISCUSSION

Fourteen CA microsatellite repeats (25) amplified by PCR were used to screen 18 SCCs of the head and neck and 18 colon carcinomas samples for LOH in 7q21-qter (Table 1). Figs. 1 and 2 are representative photographs of CA microsatellite amplifications in SCCs of the head and neck and in colon carcinomas, respectively. The nonradioactive method used in this study has several advantages over its radioactive counterpart (18). In addition to avoiding the use of radioisotopes, the separation of the PCR products is done in a horizontal high-resolution agarose gel that allows the screening of 96 reactions in a single electrophoretic run, dramatically increasing the speed of the overall process. The use of this system did not decrease the sensitivity of the allele detection, since this agarose type resolves up to 2 base pairs in horizontal runs. This observation seems to be supported by the fact that the percentage of heterozygosity obtained in this study correlates with the heterozygosity reported by Weissenbach et al. (25). The amounts of template used for the PCR reactions was within the range used by other groups performing this type of analysis (18, 28).

Our results indicated that the loss of part or all of 7q was a common event in both types of human cancer. LOH occurred in at least one 7q locus in 12 (66.6%) of 18 SCCs of the head and neck and in 11 (61.7%) of 18 colon carcinomas. This incidence is higher than that at other frequently deleted regions in these tumor types (29, 30). As can be seen in Fig. 3, LOH occurred most frequently (in 53.3% of SCCs and 80% of the colon carcinomas) in D7S22 (7q31.1–31.2). Furthermore, the segment delimited by D7S486 and D7S480 (7q22-q31.2), which includes D7S22, had an average percentage of LOH of 40.1% in head and neck SCCs and of 57.3% in colon carcinomas. These results are consistent with the observation published recently indicating that CA microsatellite D7S527 has a 30% LOH in SCCs of the head and neck (18). No evidence of genetic instability of microsatellites (replication errors, RER+) was found in the present study.

Analysis of the histograms of the data for each tumor type by the Kolmogorov-Smirnov test (27) indicated that they both were normally distributed as would be expected for a stochastic process such as the inactivation of a tumor suppressor gene. Comparison of the two distributions using Tukey’s test (27) revealed significant differences (P < 0.005). Thus, the distributions for the two tumor types were qualitatively identical but quantitatively different. Both head and neck SCCs and colon carcinomas had a high frequency of LOH in...
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Fig. 2. Representative PCR amplification of the CA microsatellite repeats in DNA from colon carcinoma. Case numbers are shown over top of the respective lanes; T and N, matched DNA samples isolated from tumor tissue and lymph nodes, respectively. A, D7S518; B, D7S522; and C, D7S498.

154 bp — 142 bp —
220 bp —
200 bp —

Fig. 3. Representation of 7q21.3-qter and approximate position of the microsatellite repeats (24). Histograms show the percentage of LOH for each of the microsatellites in both tumor types. Solid columns, head and neck SCCs; checkered columns, colon carcinomas.

7q31.1–31.2, confirming the presence of a TSG on chromosome 7 that affects a wide range of tumor types. This segment has also been reported to be deleted in breast cancer (16) and in other types of cancer (10–15).

Fig. 4 displays a schematic representation of the deleted regions of human chromosome 7 in 16 cases studied. In these cases, large deletions can be predicted by the molecular techniques used. The probability of three or more allelic losses in the same fragment caused by independent events is very small, so the occurrence of such series of LOH of contiguous markers is more likely due to deletion of an entire segment than to separate small deletions. In our samples, these deletions were frequently interstitial. Superimposition of those deletions defined a SCDR for each type of neoplasia studied. In the colon carcinoma cases, the SCDR was flanked by D7S633 and D7S655, which are 1.2 cm apart. The SCC SCDR was defined by microsatellites D7S486 and D7S655, which are 2.4 cm apart; this fragment includes the colon carcinomas SCDR, and extends 1.2 cm further into the proximal end of the long arm of chromosome 7. The superimposition of both SCDR allowed us to narrow the location of the tumor suppressor gene to 7q31.1-q31.2, which is about 1.2 cm (25). Given the size of this SCDR, more than one gene could be involved as has recently been shown was the case in the retinoblastoma locus in head and neck SCCs (31).
The high frequency of LOH occurrence in the long arm of chromosome 7 at 7q31.1-31.2 in both types of neoplasias studied, along with our previous observations of repression of tumorigenicity by microcell-mediated transfer of chromosome 7 to a murine SCC cell line (19), indicate that human chromosome 7 harbors a tumor suppressor gene distal to c-met at 7q31.1-31.2. This tumor suppressor gene seems to be relevant to several types of human neoplasias, as can be inferred by our previous report on LOH in human primary breast cancer (16) and primary prostate carcinomas (17) and cytogenetic evidence of chromosome 7 deletions in other neoplasias (10-15).

REFERENCES
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