Allelic Losses of Chromosomes 9, 11, and 17 in Human Bladder Cancer

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ABSTRACT

Twenty-five human bladder tumors were examined for loss of heterozygosity of markers on chromosomes 6p, 9q, 11p, 14q, and 17p. These studies show that all of the markers were reduced to homozygosity in at least some of the tumors. They also confirmed earlier studies by Fearon et al. [Nature (Lond.), 318: 377–380, 1985] that approximately 40% of bladder tumors were reduced to homozygosity for markers on chromosome 11p. However, the greatest frequency of allelic loss was seen for chromosomes 9q (67% of informative cases) and 17p (63% of informative cases) with both chromosomes being lost concordantly in 10 out of 20 informative tumors. Allelic loss of chromosome 9q has not been previously observed with other human cancers; however, deletions of 17p have been reported in breast, lung, and colorectal carcinomas. The data raise the interesting possibility that allelic losses of specific chromosomes might be a feature of cancer in a particular differentiated cell type whereas loss of other chromosomes harboring more generally acting tumor suppressor genes might be a common feature of human cancers.

INTRODUCTION

The potential role of tumor suppressor genes in human cancer has recently received considerable attention (1, 2). DNA markers have revealed that many tumors have undergone a reduction to homo- or hemizygosity for genes located on specific chromosomes. For example, renal cell tumors often show reduction to homozygosity of genes located on chromosome 3p (3), small cell carcinomas of the lung show loss of heterozygosity for genes on chromosomes 3p, 13q, and 17p (4), bladder cancer show loss of heterozygosity for markers on chromosome 11p (5), breast cancer show reductions to homozygosity for markers on chromosomes 11q, 13q, and 17p (6–8), and the detailed work from Vogelstein’s group has shown reduction to homozygosity of chromosomes 5q, 17p, and 18q in human colorectal tumors (9). While the extent of chromosome changes can be extensive as shown in the case of colorectal carcinoma (10) it is clear that losses of the specific chromosomes are nonrandom and may be associated with the development of neoplasia (9).

These studies using recombinant DNA markers have extended earlier classical cytogenetic studies demonstrating marked chromosomal changes in human tumors. Bladder cancer is an example of a tumor type which has been found to show nonrandom chromosomal changes by cytogenetics (11, 12). Taken together, the results of Gibas et al. (13) and Vanni and Scarpa (14) showed monosomy for chromosome 9 in five out of 10 transitional cell carcinomas of the bladder and this was the only karyotypic change which was observed in two tumors. Smeets et al. (15) also found monosomy of chromosome 9 in four out of eight cases of near diploid bladder cancer. However, in a second study using more advanced poorly differentiated bladder tumors Gibas et al. (16) did not observe monosomy of chromosome 9 in seven patients and speculated that the monosomy might be more prevalent in low stage low grade tumors. They also suggested that abnormalities of chromosome 11p appeared to be more frequent in tumors of an advanced nature than those of a superficial noninvasive type. Babu et al. (17) based on cytotypic studies on 15 bladder tumors, further suggested a possible predictive value of 3p duplication, trisomy 7, and 11p deletion with progression of disease. The potential involvement of chromosome 11p in human bladder cancer has also been suggested by Fearon et al. (5) who found five out of 12 patients with transitional cell carcinoma of the bladder had the somatic loss of genes of chromosome 11p resulting in homozygosity or hemizygosity of the nondeleted alleles in the tumor cells. Reductions to homozygosity for multiple markers within human tumors may therefore be responsible for, or at least be strongly associated with, the development of transformation. We have therefore undertaken a restriction fragment length polymorphism analysis of DNA extracted from bladder tumors to extend these studies.

Nine loci on five chromosomal arms (11p, 9q, 14q, 17p, and 6p) were surveyed and a high percentage of the tumors were observed to be reduced to homozygosity for markers located on chromosomes 9, 11, and 17 with many tumors showing multiple deletions of the alleles. These findings are particularly interesting in view of the recent findings by Vogelstein’s group that the p53 gene located on chromosome 17p often contains point mutations in colorectal tumors reduced to homozygosity for 17p (18). The loss of heterozygosity for this chromosome might therefore be particularly significant in human cancer by exposing mutations on the retained allele.

MATERIALS AND METHODS

Samples. Twenty-two primary bladder tumors, one transitional cell carcinoma of the ureter, and five lymph node metastases were obtained from 25 patients at surgery performed in Kenneth Norris Jr. Comprehensive Cancer Center of the University of Southern California. Of the 22 bladder tumors, 18 were transitional cell carcinoma, two were transitional cell carcinomas with extensive neuroendocrine differentiation (19), one was small cell undifferentiated carcinoma without a transitional cell component, and one was a squamous cell carcinoma. All of the transitional cell carcinomas were of histological grade three or higher, classified according to Ash (20) and Bergkvist et al. (21). The tumors were staged according to the TNM pathological staging system (22–24). Seven of the 25 patients had received chemotherapy or radiation therapy prior to radical cystectomy. A whole blood specimen (16 ml) was collected from each patient either before or at least 10 days after surgery for white blood cell DNA preparation to be used as a matching control.

Tissue and DNA Preparation. Tumor or lymph node tissue samples were frozen in OCT embedding compound (Miles Laboratories, Elkhart, IN), and a cryostat section stained with hematoxylin & eosin was prepared for microscopic examination. The best tumor area for extraction was then marked in order to avoid areas of necrosis or normal connective tissue. The percentage of tumor in the marked stained section was estimated and specimens with less than 40% tumor were rejected.

Genomic DNA was extracted from tumors according to the method of Marmur (25) with minor modifications. Briefly, tumor tissues were minced with scalpels and homogenized in a chilled Dounce homogenizer in 0.15 M sodium chloride (NaCl), 15 mM sodium citrate pH 7 (1...
Volumes of absolute ethanol in the presence of 2.5 M ammonium acetate. DNA was solubilized in 10 mM Tris-HCl, 0.1 mM EDTA, pH 8, to be used for restriction endonuclease digestion. DNA was isolated from nuclei of white blood cells according to the method of Bell et al. (26) modified as previously described (27).

It should be noted that we purposely chose to use the Dounce tissue homogenizer instead of a Polytron homogenizer to selectively rupture the epithelial membranes of the transitional cell carcinomas. The stromal tissue present in tumor specimen may resist such gentle homogenization procedures and contribute less normal DNA to the overall yield.

Allelic Deletion Analysis. DNA (2–6 μg) from tumors and white blood cell of each patient was digested with an appropriate restriction endonuclease (Boehringer Mannheim Biochemicals, Indianapolis, IN) and subjected to electrophoresis on 1% agarose gels as described previously (27). The DNA was then transferred to zeta-probe membrane by alkaline transfer according to the manufacturer’s specification (Bio-Rad, Richmond, CA).

The following probes with loci indicated and restriction enzymes were used for analysis:

Chromosome 6-probe CH6 (D6S10) (Taql) (28); chromosome 9-probe MHZ10 (D9S11) (PstI) (29), probe EFD126.3 (D9S7) (PstI) (30), probe A541/1/9 (ASS) (HindIII) (31); chromosome 11-probe HINS 214 (INS) (Rsal) (26), probe T24-C3 (HRAS1) (MspI and HpaII) (32); chromosome 14-probe CMM101 (D14S13) (MspI) (33); chromosome 17-probe HFI2-2 (D17S1) (MspI) (34), probe YNZ22 (D17S30) (MspI or Rsal) (35).

Probe labeling was done according to the procedure of Feinberg and Vogelstein (36). Hybridization and filter washing was done according to the procedure of Church and Gilbert (37). Blots were exposed to Kodak XAR-5 film with either one or two Dupont Lightning-Plus Intensifying screens for 1 to 14 days at —80°C. Patients were considered “informative” for the particular chromosome marker if two alleles appeared in the resultant autoradiograph of the white blood cell DNA. Loss of one of the alleles in the tumor or metastatic node DNA indicated an allelic deletion. An LKB Enhanced Laser Densitometer (Bromma, Sweden) was routinely used to estimate the extent of the allelic loss. Each analysis was repeated at least twice for borderline cases. A value of 60% or less in the normalized densitometric tracing result of an allele was considered a deletion. Normalization of the densitometric tracing was done against the remaining nondeleted allele. Because of the method of calculation, we could not quantitate results in the event of amplification of the nondeleted allele.

RESULTS

The presence of normal stromal cells within a tumor complicates the interpretation of results obtained using an RFLP analysis. We therefore only used tumors which had been confirmed by pathological examination to contain at least 40% of tumor and to be free of large areas of necrotic material. Fig. 1 shows a representative Southern hybridization of the pMHZ10 probe specific for chromosome 9q to matching sets of DNA extracted from white blood cells and tumors from five patients with transitional cell carcinoma. All five patients with the exception of patient number 2 were heterozygous with respect to this probe as shown by the presence of two bands in each of the normal DNA lanes. The tumors obtained from patients 1 and 5 showed marked reduction in the intensity of one allele in the tumor DNA showing that this chromosome had been reduced to homozygosity or hemizygosity in the tumor cells. On the other hand patients 7 and 8 showed no loss of either allele in their tumor cells and patient 2 was not informative with respect to this gene probe. However, patient 2 was informative with respect to another chromosome 9q probe EFD126.3 and showed allelic deletion as scored in Table 1.

Table 1 shows the results of such an analysis for nonpapillary transitional cell carcinomas of various stages and grades. Almost all of the tumors examined showed loss of heterozygosity for one or more of the markers examined and only three tumors demonstrated no loss of heterozygosity at these loci. The most frequent loss of heterozygosity was seen for markers on chromosome 9q and 17p and many of the tumors showed the concerted loss of heterozygosity for more than one marker. In three cases we were able to compare primary tumors with lymph node metastases (patients 2, 14, and 16) and no further changes occurred at these loci in the metastasis relative to the primary tumor.

Table 2 shows a similar analysis of tumors which had been diagnosed as squamous cell carcinoma, transitional cell carcinomas with neuroendocrine differentiation, or papillary transitional cell carcinomas. Once again it was apparent that there was considerable loss of heterozygosity for markers within this set particularly on chromosomes 9q and 17p.

The results presented in Tables 1 and 2 are summarized in Table 3 which shows that the allelic deletions observed in the bladder tumors varied considerably among the different chromosomes. The most striking finding was the high prevalence of chromosomes 9q (67% of tumors analyzed) and 17p (63% of tumors analyzed) deletions. There also appeared to be the nonrandom loss of heterozygosity for chromosome 11p with chromosomes 6p and 14q being less frequently involved.

Table 1. Loss of heterozygosity in nonpapillary transitional cell carcinomas

<table>
<thead>
<tr>
<th>Patient</th>
<th>Stage</th>
<th>Grade</th>
<th>Specimen</th>
<th>6p</th>
<th>9q</th>
<th>11p</th>
<th>14q</th>
<th>17p</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>P3B4g</td>
<td>Tumor</td>
<td>NI</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>P4Ag4</td>
<td>Tumor</td>
<td>NI</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>P3B4g</td>
<td>Tumor</td>
<td>NI</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>P4Ag3</td>
<td>Tumor</td>
<td>NI</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>P3Ag4</td>
<td>Tumor</td>
<td>NI</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>P3Beg</td>
<td>Tumor</td>
<td>+</td>
<td>NI</td>
<td>NI</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>14</td>
<td>P4Ag4</td>
<td>Tumor</td>
<td>NI</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>16</td>
<td>P3B4g</td>
<td>Tumor</td>
<td>Metastasis</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>18</td>
<td>P3B4g</td>
<td>Tumor</td>
<td>-</td>
<td>NI</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>20</td>
<td>P3B4g</td>
<td>Tumor</td>
<td>NI</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>21</td>
<td>P3Ag3</td>
<td>Tumor</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>22</td>
<td>P3Ag3</td>
<td>Tumor</td>
<td>Metastasis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>24</td>
<td>P3Beg</td>
<td>Tumor</td>
<td>NI</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 2 Loss of heterozygosity in bladder tumors

DNA extracted from tumors or metastases of bladder tumors of the indicated pathological type was analyzed and scored as indicated in Table 1.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Diagnosis</th>
<th>Stage and grade</th>
<th>Specimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
<td>Squamous cell carcinoma</td>
<td>P3A Tumor</td>
<td>6p + 9p + 11p + 14q + 17p -</td>
</tr>
<tr>
<td>4</td>
<td>Transitional cell carcinoma with neuroendocrine differentiation</td>
<td>P3Bq Tumor</td>
<td>6p + 9q + 11p + 14q + 17p -</td>
</tr>
<tr>
<td>13</td>
<td>Transitional cell carcinoma with neuroendocrine differentiation</td>
<td>P4Ag Tumor</td>
<td>6p + 9q + 11p + 14q + 17p -</td>
</tr>
<tr>
<td>17</td>
<td>Small cell undifferentiated carcinoma</td>
<td>P3B Tumor</td>
<td>6p + 9q + 11p + 14q + 17p -</td>
</tr>
<tr>
<td>1</td>
<td>Papillary transitional cell carcinoma</td>
<td>P3Bq Tumor</td>
<td>6p + 9q + 11p + 14q + 17p -</td>
</tr>
<tr>
<td>5</td>
<td>---</td>
<td>P3Bq Tumor</td>
<td>6p + 9q + 11p + 14q + 17p -</td>
</tr>
<tr>
<td>11</td>
<td>---</td>
<td>P3Bq Tumor</td>
<td>6p + 9q + 11p + 14q + 17p -</td>
</tr>
<tr>
<td>13</td>
<td>---</td>
<td>P3Bq Tumor</td>
<td>6p + 9q + 11p + 14q + 17p -</td>
</tr>
<tr>
<td>19</td>
<td>---</td>
<td>P3Bq Tumor</td>
<td>6p + 9q + 11p + 14q + 17p -</td>
</tr>
<tr>
<td>25</td>
<td>---</td>
<td>P3Bq Tumor</td>
<td>6p + 9q + 11p + 14q + 17p -</td>
</tr>
<tr>
<td>10</td>
<td>Transitional cell carcinoma</td>
<td>P3Bq Tumor</td>
<td>6p + 9q + 11p + 14q + 17p -</td>
</tr>
</tbody>
</table>

Table 3 Allelic deletions in bladder tumors
Summary of results shown in Tables 1 and 2. The percentage of tumors showing allelic losses was calculated based on the total number of informative cases given in parenthesis.

<table>
<thead>
<tr>
<th>Chromosomal arm</th>
<th>% of tumors with deletion (no. informative)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6p</td>
<td>31% (13)</td>
</tr>
<tr>
<td>9q</td>
<td>67% (21)</td>
</tr>
<tr>
<td>11p</td>
<td>39% (23)</td>
</tr>
<tr>
<td>14q</td>
<td>19% (21)</td>
</tr>
<tr>
<td>17p</td>
<td>63% (24)</td>
</tr>
</tbody>
</table>

DISCUSSION

Our data confirm the observation of Vogelstein et al. (10) that allelic losses are highly common in human tumors. Indeed we found that alleles from each of the five chromosomal arms tested were lost in at least some of the tumors. The data also represent a refinement on flow cytometric studies which have demonstrated considerable aneuploidy in bladder tumors (38–40). Since all of the 25 tumors which we examined were obtained from patients undergoing cystectomies for advanced disease the chromosomal changes in these tumors may have been quite extensive.

The 25 tumors investigated also confirmed earlier cytogenetic information suggesting that alterations in chromosome 9 might have relevance to the genesis of human bladder cancer. Indeed more than 60% of the tumors were found to be homozygous for chromosome 9q and the commonality of this loss is strongly suggestive that a gene important for the genesis of bladder cancer resides in this chromosomal location. Currently low grade low stage bladder tumors are being collected in order to elucidate whether loss of this chromosome is important in tumor progression. To our knowledge chromosome 9q has not been implicated in any other form of human cancer suggesting that the loss of this specific chromosome might be important in this particular cell type.

Our results also confirm the earlier report of Fearon et al. (5) that chromosome 11p is often reduced to homozygosity in bladder cancer. Indeed approximately 40% of the informative tumors had undergone reduction to homozygosity for this marker which is a region thought to harbor several tumor suppressor genes (41–43). However, one of the more striking observations which we made was the fact that chromosome 17p was reduced to homozygosity in more than 60% of the cases studied. In their earlier work Fearon et al. (5) found no changes in chromosome 17q in seven informative cases of bladder cancer. Thus, the changes in chromosome 17 appear to be restricted to the short arm, an observation consistent with findings in colorectal tumors where allelic deletions are commonly subchromosomal events which might be mediated by interstitial deletion, mitotic recombination, or gene conversion rather than a loss of a whole chromosome (10). The results obtained with chromosome 17p are particularly intriguing in light of the recent studied by Baker et al. (18). These authors demonstrated that allelic deletions of the short arm of chromosome 17 occurred in over 75% of colorectal carcinomas and provided direct sequencing evidence from two tumors reduced to homozygosity for 17p that the retained p53 gene contained specific point mutations. They interpreted their results to suggest that the p53 gene mutations might be involved in tumorigenesis possibly through inactivation of the suppressor function of the wild type p53 gene. If similar results can be obtained with the bladder tumors we have examined, the results would be important in suggesting that p53 gene inactivation is a common feature of more than one kind of human cancer.

Our data on specific chromosomal deletions in bladder cancer strongly suggest that chromosome 9q loss, which was observed in more than 60% of all informative bladder cancers, might be a specific chromosomal defect associated with this tumor type. On the other hand, 39% of the tumors also showed allelic deletions of chromosome 11 with 63% showing deletions for chromosome 17p. The coincident loss of 11p and 17p are similar to recent findings in breast cancer (8) and in squamous cell carcinoma of the lung (44). Our results raise the intriguing possibility that epithelial tumors might be characterized by the loss of specific chromosomal segments particular for that tumor type but that the genesis of an aggressive tumor might also require the inactivation of a series of other tumor suppressor genes associated with a wider range of human cancers.

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REFERENCES


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