Role of Chromosome 9 in Human Bladder Cancer

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ABSTRACT

The tumors of 20 patients with multifocal primary transitional cell carcinoma of the bladder or lymph node metastases were examined for molecular genetic defects which we have previously found to be present in >50% of invasive tumors. These included loss of heterozygosity (LOH) of chromosome 9, which occurs in superficial as well as invasive bladder tumors, and LOH of chromosome 17p and p53 mutations, which are commonly found only in invasive tumors. Analysis of multiple or recurrent primary tumors in 7 patients for these markers was generally consistent with recently published data that the tumors are monoclonal in origin and that p53 mutations occur as a late event in the generation of invasive bladder cancers. Comparison of the primary tumors and metastases to regional lymph nodes in 14 patients demonstrated a complete concordance between the molecular genetic defects present, showing that LOH of chromosomes 9 and 17p and p53 mutations occurred in the primary tumors before metastasis. Because of the importance of chromosome 9 in bladder cancer, we mapped the location of a putative tumor suppressor gene by restriction fragment length polymorphism analysis of 123 cases obtained in this and earlier studies. Most of the tumors showed LOH for more than one marker on chromosome 9. Results of mapping of 4 tumors with partial deletion of chromosome 9 suggests that the tumor suppressor gene is located between 9p12 and 9q34.1.

INTRODUCTION

TCCs of the bladder are clinically managed according to their invasive behaviors. Superficial papillary cancers which are localized to the mucosa or lamina propria often recur and can be controlled by endoscopic surgery and intravesical chemotherapy or Bacille Calmette-Guérin. Invasive cancers which infiltrate into the muscle layer or deeper often metastasize to pelvic lymph nodes or distant organs and are more difficult to treat. We (1, 2) and others (3, 4) have reported that LOH of chromosome 9 is a common genetic defect in superficial papillary and invasive bladder cancers, suggesting that inactivation of a putative tumor suppressor gene(s) on this chromosome plays an important role as an early genetic event during multistep bladder carcinogenesis. On the other hand, allelic loss of chromosome 17p frequently occurs in tumors which have invaded the lamina propria (T1) or muscle (T2-T4) but not in low-grade, low-stage (T0) tumors (2, 5, 6). Invasive tumors often contain mutations in the p53 tumor suppressor gene (6-9), suggesting that inactivation of the p53 tumor suppressor gene(s) on chromosome 9. Results of mapping of 4 tumors with partial deletion of chromosome 9 suggests that the tumor suppressor gene is located between 9p12 and 9q34.1.

MATERIALS AND METHODS

Tumors and DNA Examinations. This study included 62 new TCC cases (cases 69-134) and the further detailed analysis of 61 previously reported cases (1, 2) (cases 1-68). Tumors other than TCC were excluded, and the anatomical sites included 114 bladder tumors (109 of primary sites and 5 cases of lymph node metastases alone), 7 cases of renal pelvic TCC, and 2 cases of ureteral cancer. Twenty cases had multiple tumor sites either synchronously (n = 16, including metastatic sites), asynchronously (n = 3), or both (n = 1). Results from the primary tumor of patient 21 have been reported previously (1). For patient 37, the results of RFLP studies for one of the tumors (BT1) have been previously reported (2). The recurrent bladder tumor (BT2) of patient 37 was obtained subsequently, and we examined both primary and recurrent tumors in this study. p53 mutations had been previously reported for bladder tumor cases 16 (6) and 91 (9). All tumors were graded according to the methods of Ask (11) and Bergkvist et al. (12) and staged according to the Tumor, Nodes, and Metastases system as described by the American Joint Committee on Cancer (13). Tumors were histologically graded and staged without knowledge of the allelic deletion results. High molecular weight DNAs were extracted from tumor and blood by proteinase K digestion and phenol/chloroform extraction as described previously (1). LOH of Chromosome 9 and 17p. Allelic loss of chromosome 9 and 17p was determined in 61 previously reported cases and 62 newly examined cases by RFLP analysis as previously described (1). The following probes with loci and restriction enzymes were used: chromosome 9: HHIH220 (D9S18), Tacl; p72-0.9 (D9S126), Tacl; EKZ130.3 (D9S99), Mspl + Hpall; CMT112 (D9S15), Mspl; LAMP92 (D9S29), Tacl; MCOA12 (D9S16), Mspl + Hpall this study; p53 mutations had been previously reported for bladder tumor cases 16 (6) and 91 (9). 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RESULTS

A summary of the LOH and mutational analyses of TCCs at multiple sites is shown in Fig. 1. Seven cases were available with multiple bladder tumors (Fig. 1, left), and a lymph node metastasis was available for one of these (case 124). The same genetic alterations in each of the tumors in the given case were observed in 5 patients, consistent with a common origin for the tumors in each individual. Although not shown in Fig. 1, the same allele was missing in each case scored in the figure. One of the 4 tumors in case 80 (BT4) had a p53 mutation, detected by SSCP, which was not present in the other 3 tumors. The presence of a p53 mutation in BT4 in patient 80 is consistent with our previous observations (2, 6) that these mutations occur as a late stage event in the genesis of this kind of cancer. Case 37 was interesting since BT1 showed LOH of chromosome 9, whereas BT2, which was a recurrent tumor excised 10 months later, had retained both chromosome 9 alleles. These 2 tumors likely shared a common precursor, since both were found to contain an unusual new allele of the androgen receptor gene (result not shown).

Results obtained for LOH for chromosome 17p using three probes located on this chromosome arm are listed in Fig. 1. Tumors were scored as having undergone LOH if they showed deletion of one allele.

Fig. 1. Loss of heterozygosity of chromosomes 9q and 17p and the presence of p53 mutations in multifocal or recurrent transitional cell carcinomas of the bladder (BT) and/or metastatic deposits in the lymph nodes (LN). DNA extracted from multiple or recurrent tumors or lymph nodes was compared to DNA extracted from the WBCs of the patient by standard RFLP analysis for LOH using markers on chromosomes 9q and 17p. Mutations within exons 5–8 of the p53 gene were detected by SSCP analysis as previously described (9). Left, cases with synchronous bladder tumors (cases 134, 133, 131, and 124 and BT2–4 of case 80) or recurrent tumors (cases 104, 80, and 37). All cases with lymph node metastasis are synchronous with the primary tumors, except for case 16.

Table 1. Allelic deletion on chromosome 9 in TCC: summary of Southern blot analysis in combination with our previous studies (1, 2)

<table>
<thead>
<tr>
<th>Tumor grade</th>
<th>Tumor stage</th>
<th>No. of examined case</th>
<th>No. of informative case</th>
<th>No. of LOH case</th>
</tr>
</thead>
<tbody>
<tr>
<td>II T1 or P1</td>
<td>BT1 P1 II</td>
<td>14</td>
<td>13</td>
<td>7 (54)*</td>
</tr>
<tr>
<td>II T1, P1, or P2</td>
<td>BT1 P1 II</td>
<td>5</td>
<td>5</td>
<td>3 (60)</td>
</tr>
<tr>
<td>III T3 or P4</td>
<td>BT1 P1 III</td>
<td>4</td>
<td>4</td>
<td>3 (75)</td>
</tr>
<tr>
<td>III T1 or P1</td>
<td>BT1 P1 III</td>
<td>16</td>
<td>15</td>
<td>9 (60)</td>
</tr>
<tr>
<td>IV T1 or T2, or N+ or M+</td>
<td>BT1 P1 III</td>
<td>41</td>
<td>37</td>
<td>22 (59)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>123</td>
<td>116</td>
<td>72 (62)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses indicate percentages.
informative marker; 8 cases showed LOH for 2 or more markers. Again, case 37 was interesting since BT2 showed LOH for the two markers on chromosome 17p13, where the p53 gene is located, yet retention of the marker on 17p11.2-cen (result not shown), whereas BT1 showed LOH for all 3 markers. These results coupled with the data for LOH of chromosome 9 (Fig. 1) suggested that BT1 may have been derived from BT2 or from a common precursor, even though it had presented earlier in the patient. Altogether, 7 cases (cases 21, 37, 72, 76, 97, 124, and 129) showed LOH of 17p and yet wild-type p53 on SSCP gels. It is possible that these cases had p53 mutations in exons other than 5–8, or alternatively, the mutations were not detectable by the SSCP technique. However, a tumor suppressor gene other than p53 may be involved in these cases.

Also shown in Fig. 1 are our findings from 14 cases, in which single or multiple metastases to lymph nodes were available. In all cases, there was a complete concordance between the defects present in the primaries and metastatic sites. We assume that the same SSCP shifts represent identical mutations based on our earlier experience with the technique on 80 bladder tumors (9). These results clearly show that the 3 defects studied, i.e., LOH of chromosomes 9 and 17p and mutations in the p53 gene, had occurred before metastasis.

The results of LOH analysis of chromosome 9 from 123 cases of TCC examined in this and earlier work (1, 2), arranged according to histological grade and stage, are given in Table 1. As reported earlier, chromosome 9 deletion is common in all TCCs with all tumors showing >50% LOH independently of grade or stage (1–4).

The commonly deleted region of chromosome 9 was mapped by analyzing the tumors with LOH with two or more markers on this chromosome. Fig. 2 shows that 68 of the 123 tumors had deletions of 1 or more markers, including 54 tumors showing LOH with 2 or more markers. As indicated in the legend to Fig. 2, at least 3 of these tumors represented complete monosomies. Only 4 tumors in our study showed partial deletions of chromosome 9. RFLP results for case 54 showed retention of the distal q arm and LOH of the remaining chromosome segment (Fig. 3). A summary of the mapping results is shown in Fig. 4, which shows that the common region of deletion was localized to the region between 9p12 and 9q34.1. However, the distal 9p arm, i.e., 9p22 to p24, was not examined with the available probes.

![Fig. 2. Pie chart summarizing the deletion data for chromosome 9 (n=14) with regard to numbers of probes used in this and previous work (1, 2). Of the 54 tumors that had deletions of 2 or more markers, 3 tumors had deletions between 9p22 and 9q34, 10 tumors had deletions between 9q13 and 9q34, 20 tumors had deletions between 9q31 and 9q34, 5 tumors had deletions between 9q32 and 9q34, and 16 tumors had deletions between 9q34.1 and 9q34.3.](image_url)

DISCUSSION

We have traced the genealogies of multicentric, recurrent and metastatic bladder tumors in this study by the use of molecular genetic approaches. Our results from 5 patients with synchronous primary tumors are in accordance with recent work by Sidransky et al. (10) who used X chromosome inactivation studies to demonstrate that multifocal TCCs are indeed monoclonal. Chromosome 9 deletions, when they occur, therefore, appear to be early events in the development of papillary TCC. Furthermore, since the same chromosome 9 was lost in each deleted case, this was consistent with a monoclonal origin for the tumors.

The two cases with a discordance between the molecular genetic defects present within the tumors were particularly interesting. Case 80 showed that one of 4 tumors had a p53 mutation, suggesting that the mutation, which is known to be a late event in the generation of TCC, might predispose this particular tumor to further progression and invasion. Another discordant case was patient 37, in whom BT1 showed LOH for chromosome 9 and 17p, whereas BT2, which was excised 10 months later, had retention for chromosome 9 and 17p11.2-cen. Since both tumors contained a new truncated trinucleotide repeat marker within the androgen receptor gene (results not shown), it is likely that they were derived from the same transformed cell. The LOH for chromosome 9, which had occurred in BT1, might have resulted in a faster growing tumor which presented before its predecessor still heterozygous for chromosome 9. If this interpretation is correct, then it suggests the presence of a precursor cell which gave rise to these 2 tumors and that LOH of chromosome 9 may not always precede papillary tumor development. In support of this, recent studies in our laboratory have shown that, when carcinoma in situ and other TCCs occur synchronously, they often do not share the same genetic defects with respect to chromosome 9 loss. Indeed, carcinoma in situ, which by definition is noninvasive, commonly contains p53 mutations, whereas chromosome 9 deletions are less common in these tumors.

Few studies have compared the molecular defects in metastases with those of the primary tumors. Our results show quite clearly from the 14 cases available that the lymph node metastases were derived from the original tumors and that the 3 defects studied existed within the primary tumor before metastasis had occurred. It will be interesting to examine these metastases with other markers to determine whether specific changes associated with metastasis can be identified.

Mapping and cloning of the putative bladder tumor suppressor gene on chromosome 9 will be essential for a more complete understanding...
of bladder cancer. Our data show that the majority of cases which have undergone LOH for chromosome 9 appear to have lost a significant amount of the chromosome. Indeed, earlier cytogenetic studies (15) and fluorescence in situ hybridization studies (3) have shown that chromosome 9 monosomies are common in bladder cancer. In the four cases which showed partial deletions, the putative tumor suppressor gene appears to be located between 9p12 and 9q34.1, which is consistent with recent studies by Cairns et al. (16) who mapped the deletion to between 9p12 and 9q22.2. Several potentially interesting genes have been reported to be localized within the q arm of chromosome 9. Recent linkage analysis revealed that the gene for Gorlins syndrome located on 9q31 or 9q22.3-3q31 is located in this region (17, 18). Gorlins syndrome is an autosomal dominant disorder characterized by basal cell carcinoma of the skin, ovarian fibroma, medulloblastoma, skeletal abnormalities, and developmental malformations. The gene for ABH tissue antigen is also located in 9q34, these antigens are expressed on normal transitional cell surfaces, and loss of antigenicity in bladder cancer appears to correlate to some extent with the risk of progression (19).

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REFERENCES

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