Microsatellite Instability in Bladder Cancer

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Abstract

Somatic instability at microsatellite repeats was detected in 6 of 200 transitional cell carcinomas of the bladder. Instabilities were apparent as changes in (GT)n repeat lengths on human chromosome 9 for four tumors and as alterations in a (CAG)n repeat in the androgen receptor gene on the X chromosome for three tumors. Single locus alterations were detected in three tumors, while three other tumors revealed changes in two or more loci. In one tumor we found microsatellite instability in all five loci analyzed on chromosome 9. The alterations detected were either minor 2-base pair changes or larger (>2 base pairs) alterations in repeat length. All six tumors had low stage (Ta-T1), suggesting that these alterations can occur early in bladder tumorigenesis.

Introduction

Microsatellite markers play an important role in the analysis of LOH in cancer. Microsatellites are tandem iterations of simple di-, tri-, or tetranucleotide repeats, and their usefulness can be attributed to abundance (1), hypervariability (2), fairly even genomic distribution (3), and ease of detection by the PCR.

Microsatellites have been reported to be unstable in some inherited diseases and in some types of cancer. This instability consists of expansion or contraction of DNA within repeat elements (4). Expansion of a trinucleotide repeat is responsible for fragile X syndrome (5), spinobulbar muscular atrophy (6), myotonic dystrophy (7), Huntington's disease (8), and spinocerebellar ataxia type 1 (9). Dinucleotide repeat alterations have recently been linked to predisposition to colorectal cancer (10), in which changes in the microsatellite repeats occurring in a series of 200 TCC of the bladder (13, 14). Recent evidence suggests that LOH of chromosome 9 is an early event in the generation of papillary TCC but not in carcinoma in situ of the bladder. Allelic losses of chromosome 17p and p53 mutations were commonly seen in carcinoma in situ and in invasive tumors (15). The purpose of the current report is to show that microsatellite changes were apparent in some of these tumors, including four tumors with dinucleotide repeat alterations in chromosome 9 and three tumors with changes in a trinucleotide repeat in the androgen receptor gene located on the X chromosome. The cases we report were very interesting since one of them showed genetic alterations at all loci examined in chromosome 9 and all were low stage tumors, suggesting that the genomic instability giving rise to these microsatellite changes might occur as an early event in bladder tumorigenesis.

Materials and Methods

The current results were obtained from the analysis of 200 transitional cell carcinomas of the bladder, 154 of which were previously reported cases (13). Three TCC specimens were obtained from hospitals in Los Angeles County, CA (n = 90), from the Herlev Hospital in Copenhagen, Denmark (n = 64), and from the Johns Hopkins tumor bank, Baltimore (n = 46). Of these, 112 were fresh-frozen and 88 were paraffin-embedded tissues. Tumors were graded according to the criteria of Bergkvist et al. (16) and staged according to the tumor-nodes-metastasis staging system (17). High molecular weight DNA was prepared from fresh-frozen tumor specimens and matching blood samples by proteinase K digestion and phenol/chloroform extraction as described (18). DNA from archival paraffin-embedded specimens was isolated by microdissecting tumor and normal tissues from hematoxylin and cosin-stained frozen sections as described (19).

Tumor DNA was examined for genetic alterations at seven separate microsatellites, five localized in chromosome 9 (D9S59, D9S63, D9S64, D9S146, D9S156), one in chromosome 17p (D17S513), and one in the X chromosome (androgen receptor gene locus). Loci D9S59, D9S63, and D9S64 were analyzed for 156 tumors; loci D9S146 and D9S156 were analyzed for 49 tumors; locus D17S513 was analyzed for 90 tumors; the androgen receptor gene locus was analyzed for 25 tumors. The dinucleotide repeat polymorphism, (GT)n, at loci on chromosomes 9 and 17p was analyzed by PCR amplification followed by electrophoresis on denaturing 8% polyacrylamide gels as described (20). The sequences of primers used are: locus D9S59, 5'-TAA CAC CTT ACC AAC ACT GC-3' and 5'-AAG GGA ATT CAT CCC CTG CT-3'; locus D9S63, 5'-TAA TAC CCG TCA ACC TT-3' and 5'-CGG GTA GAT ACT CTA GTC TAC-3'; locus D9S64, 5'-GAA GGA GTC TTT TTT ATT AAC TTA TTA-3' and 5'-AAG CTC GGC GAC ACA GCA A-3'; locus D9S146, 5'-TGC AAT CAA ATT CCC AGC-3' and 5'-GAG GAT GCA TCT CCT CTG CT-3'; locus D9S156, 5'-ATC ACT TTT AAC TCA GGA GG-3' and 5'-AGA TGG TGG TGA ATA GAG GG-3'; locus D17S513, 5'-TTC ACT GTG GGG CTG CTG CT-3' and 5'-GAA AGG CTC CCA ACA GCA A-3'; locus D9S156, 5'-ATC ACT TTT AAC TCA GGA GG-3' and 5'-AGA TGG TGG TGA ATA GAG GG-3'; locus D17S513, 5'-TTC ACT GTG GGG CTG CTG CT-3' and 5'-GAA AGG CTC CCA ACA GCA A-3'; locus D9S156, 5'-ATC ACT TTT AAC TCA GGA GG-3' and 5'-AGA TGG TGG TGA ATA GAG GG-3'; locus D17S513, 5'-TTC ACT GTG GGG CTG CTG CT-3' and 5'-GAA AGG CTC CCA ACA GCA A-3'; locus D9S156, 5'-ATC ACT TTT AAC TCA GGA GG-3' and 5'-AGA TGG TGG TGA ATA GAG GG-3'; locus D17S513, 5'-TTC ACT GTG GGG CTG CTG CT-3' and 5'-GAA AGG CTC CCA ACA GCA A-3'; locus D9S156, 5'-ATC ACT TTT AAC TCA GGA GG-3' and 5'-AGA TGG TGG TGA ATA GAG GG-3'. The trinucleotide repeat polymorphism, (CAG)n, in the androgen receptor gene (22) was analyzed by PCR, performed in a final volume of 25 μl, containing 50 ng of genomic DNA, 1 μM concentrations of each oligonucleotide primer (5'-GTG CCC GAA GTG ATC CAG AA-3' and 5'-TCT GGG AGG CAA CCT CTC CT-3'), 200 μM of the nonradioactive deoxynucleotides, 2 μCi of [α-32P]dCTP, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, 0.01% gelatin, and 1 unit of Taq DNA polymerase (Boehringer Mannhein Biochemicals, Indianapolis, IN). Twenty-four cycles of 94°C for 1 min, 60°C for 1 min, and 72°C for 1.5 min were performed with the initial denaturation step and final elongation step length-
Results

We have recently utilized microsatellite repeat polymorphisms to investigate allelic deletions of chromosomes 9 in 200 TCCs and 17p in 90 TCCs. The microsatellite banding patterns observed for 194 of these cases showed either no changes between normal and tumor DNA or loss of an allele in the tumor DNA. Fig. 1a shows examples of the same banding pattern being present in DNA microdissected from a paraffin-embedded superficial papillary grade II tumor and adjacent normal tissue for two (GT)$_n$ repeat polymorphisms at loci D9S59 and D9S64 (Fig. 1a, Lanes 1, 2, 5, and 6); an example of LOH, detected for the same tumor at locus D9S156 is shown in Fig. 1a, Lane 10. However, differences between normal and tumor DNA banding patterns were observed in the same specimen at loci D9S63 and D9S146 (Fig. 1a, Lanes 3, 4, and 7, 8). These differences consisted of shifts in the electrophoretic mobilities of (GT)$_n$ dinucleotide repeat fragments reflecting a minor 2-base pair expansion of the repeat at locus D9S63 and a >2-base pair expansion at locus D9S146. These alterations were not due to polymerase errors during PCR amplification since results were reproducible in replicate assays, and in mixing reactions in which tumor DNA was added to normal DNA from other patient.

Fig. 1b shows the results obtained for patient B, whose lamina propria invasive grade III tumor DNA, obtained from fresh-frozen tissue, showed changes in banding patterns at all five loci analyzed on chromosome 9 (Fig. 1b, Lanes 2, 4, 6, 8, 10). The alterations detected in this tumor, similarly to the changes observed in the tumor from patient A, were of two types: a single 2-base pair shift was observed at loci D9S59 and D9S146 (Fig. 1b, Lanes 2, and 8), and larger alterations (>2 base pairs) were detected at loci D9S63, D9S64, and D9S156 (Fig. 1b, Lanes 4, 6, and 10). To rule out the possibility of specimen contamination or sample switching, we obtained and analyzed paraffin-embedded tumor and normal material from patient B. When results from the paraffin-embedded material were compared with those obtained from the fresh-frozen tumor the same alteration was detected in DNA from both sources of tumor tissue.

Fig. 2 shows the results of the analysis of a trinucleotide repeat polymorphism in the androgen receptor gene obtained for patients C and D. The tumors from these patients revealed changes in the (CAG)$_n$ repeat at the androgen receptor gene locus. Fig. 2a shows that the two tumor specimens from patient C, superficial (T$_s$) grade III and lamina

![Fig. 1. Dinucleotide repeat polymorphisms in normal (N) and tumor (T) tissue from patients with transitional cell carcinomas of the bladder. The microsatellite markers are located at loci D9S59, D9S63, D9S64, D9S146, and D9S156 in chromosome 9. Each normal allele is represented by a major band surrounded by several other lighter bands. (a) (GT)$_n$ repeat polymorphisms showing microsatellite abnormalities at loci D9S59 and D9S146. Loci D9S59 and D9S64 in Lanes 1, 2, and 5, 6, respectively, showed the same banding pattern for normal and tumor DNA. Locus D9S156 revealed LOH in tumor DNA in lane 10. (b) (GT)$_n$ repeat polymorphisms in patient B. The lamina propria invasive grade III tumor DNA showed alterations in all five loci examined in chromosome 9. Lanes 2 and 8 show a 2-base pair shift at loci D9S59 and D9S146, respectively. Lanes 4, 6, and 10 contain tumor DNA presenting larger alterations of the allele sizes at loci D9S63, D9S64, and D9S156.](image1)

![Fig. 2. Trinucleotide repeat polymorphisms in the androgen receptor gene in the X chromosome in TCC patients. (a) (CAG)$_n$ repeat polymorphism in patient C. Allele sizes are indicated in base pairs. Two alleles of 320 and 299 base pairs are present in the normal DNA (N). A new, truncated allele of 275 base pairs appears in the superficial grade III tumor DNA (Ta) and in the lamina propria invasive grade III tumor DNA (T$_s$) and in the lamina propria invasive grade III tumor DNA (T$_s$) and in the lamina propria invasive grade III tumor DNA (T$_s$) with altered size of the allele.](image2)
Table 1  Bladder TCCs showing microsatellite alterations

<table>
<thead>
<tr>
<th>Patient</th>
<th>Tumor stage</th>
<th>Tumor grade</th>
<th>No. of loci altered/no of loci examined</th>
<th>Names of loci altered</th>
<th>Microsatellite alteration</th>
</tr>
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<tbody>
<tr>
<td>A</td>
<td>Ta</td>
<td>II</td>
<td>2/7</td>
<td>D9S63, D9S146</td>
<td>Minor and major expansions of (GT)n repeat</td>
</tr>
<tr>
<td>B</td>
<td>T1</td>
<td>III</td>
<td>5/7</td>
<td>D9S59, D9S63, D9S64, D9S146, D9S156</td>
<td>Minor and major expansions and contractions of (GT)n repeat</td>
</tr>
<tr>
<td>C</td>
<td>Ta</td>
<td>III</td>
<td>1/7</td>
<td>Androgen receptor gene in X chromosome</td>
<td>Major truncation of (CAG)n repeat</td>
</tr>
<tr>
<td></td>
<td>T1</td>
<td>III</td>
<td>1/7</td>
<td>Androgen receptor gene in X chromosome</td>
<td>Major truncation of (CAG)n repeat</td>
</tr>
<tr>
<td>D</td>
<td>Ta</td>
<td>II</td>
<td>5/7</td>
<td>D9S63, D9S64, D9S146, D9S156, androgen receptor gene</td>
<td>Minor and major contractions of (GT)n repeat; major expansion of (CAG)n repeat</td>
</tr>
<tr>
<td>E</td>
<td>T1</td>
<td>III</td>
<td>1/7</td>
<td>D9S156</td>
<td>Major expansion of (GT)n repeat</td>
</tr>
</tbody>
</table>

propria invasive (T1) grade III, contained a new shortened 275-base pair allele that was not present in the normal tissue DNA. These results for patient C were confirmed in tumor DNA obtained from paraffin-embedded material. The new fragment represented a deletion within the trinucleotide repeat element. Sequencing of each of the three alleles revealed that the first two, present in the normal DNA, contained 24 and 17 CAG repeats, respectively, while the third, new allele contained only 9 CAG repeats. In contrast, an expansion within the trinucleotide repeat element is shown in Fig. 2b for the tumor obtained from patient D.

Table 1 summarizes the stages and grades as well as the genetic alterations detected in the tumors in which microsatellite changes were found. All six RER+ tumors were low stage (T2a–T1), grades II–III, with three tumors (from patients C and E) showing alterations in only one of the seven loci analyzed, while three other tumors (from patients A, B, and D) revealed alterations in more than one locus.

In contrast to the results obtained for chromosome 9 and the androgen receptor gene in the X chromosome, the analysis of locus D17S513 in chromosome 17p did not reveal any microsatellite alteration in 90 TCCs analyzed.

Discussion

Our data show that genomic instability as measured by changes in microsatellite repeats occurs in TCC of the bladder. Since alterations were detected in low stage TCCs, including two low grade tumors, genomic instability might be an early event in bladder tumorigenesis. The low number of bladder tumors in which we observed microsatellite changes could be due to the fact that only seven markers were analyzed, five of them on the same chromosome. Thus, our results may reflect only a small part of a genome-wide instability in bladder cancer. However, further studies with a larger number of microsatellite markers would be important to verify this interpretation.

The tumor obtained from patient B was particularly interesting in that it contained microsatellite changes in all five loci analyzed on chromosome 9. This specimen was obtained from a patient who had a history of a kidney TCC and a ureteral TCC resected 7 and 3 years, respectively, before diagnosis of the tumor analyzed in our study, suggesting an association between somatic instability in chromosome 9 and susceptibility to multiple primary tumors.

The data obtained for patient C were informative in relation to the timing of the instability in tumorigenesis since the T2a tumor showed LOH for chromosome 9 whereas the T1 tumor, which was excised 10 months later, had retention for this chromosome (13). Since both tumors contained the new truncated trinucleotide repeat fragment in the androgen receptor gene, it is likely that they were derived from the same transformed cell and that the allelic loss for chromosome 9 occurred after the instability, resulting in a faster growing tumor which was detected earlier. The development of a shortened CAG repeat allele is intriguing and may be a manifestation of RERs and genomic instability associated with transformation. RER during tumor development may also result in expansions of trinucleotide repeats, such as those which occurred in the tumor from patient D. In contrast to tumors from patient C, the tumor from patient D contained an expansion of the (CAG)n repeat in the androgen receptor gene, and also showed expansions and deletions at (GT)n repeats on chromosome 9.

Genetic alterations similar to the ones detected in our study have recently been reported for hereditary nonpolyposis colorectal cancer and one associated ovarian carcinoma (10, 11). Microsatellite changes have not only been linked to familial predisposition to colon cancer, in which a tendency to this type of alteration could be inherited, but have also been detected in sporadic colon carcinomas (10, 11). The changes in repeat lengths that we detected in bladder carcinomas were of two types: major alterations (>2 base pairs) and minor alterations (2-base pair change) in the repeat fragment size, similar to the changes observed in colon cancer (11). The tumors in which we detected microsatellite alterations could be grouped according to the number of loci affected: 3 tumors showed DNA alterations at multiple loci; and 3 tumors showed alterations at only 1 locus.

Our findings that microsatellite instabilities are present as an apparently early event in the development of bladder cancer, for which a hereditary predisposition has never been described, suggest that this kind of instability might be common to sporadic human cancers, and that RER-containing tumors might not be unique to hereditary nonpolyposis colorectal cancer families. Thus, it will be interesting to search for microsatellite alterations in other tumor types to investigate the hypothesis that sporadic tumors can acquire this genotype during tumor development and progression.

References


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