Evidence for Two Bladder Cancer Suppressor Loci on Human Chromosome 9

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Abstract

Most carcinomas of the bladder show loss of heterozygosity for markers on human chromosome 9, which suggests that one or more tumor suppressor genes are located on this chromosome. Several observations suggest that such alterations are an important early step in tumorigenesis. We analyzed the pattern of allelic loss in 46 primary carcinomas of the bladder using 19 polymorphic markers from chromosome 9. While most tumors with allelic loss showed loss of heterozygosity for all informative markers that were tested, six tumors demonstrated only partial loss of chromosome 9. Two tumors with partial loss contained deletions that predominately involved the q arm, as shown by previous studies. The other four tumors contained deletions that predominately or exclusively involved the p arm, with a common region of loss between D9S161 (9p21) and the telomere. The results show that there is no single common region of loss on chromosome 9 and identify two distinct regions of loss that may contain bladder tumor suppressor loci.

Introduction

The pathogenesis of neoplasia involves the inactivation of specific tumor suppressor genes (1). For diseases such as familial retinoblastoma and adenomatous polyposis coli, such events appear sufficient to initiate tumorigenesis. The identification of early steps in tumorigenesis may give a better understanding of specific tumor types and could facilitate the development of diagnostic and therapeutic strategies. While tumor suppressor genes can be inactivated by a variety of mechanisms, the loss of large chromosomal segments from tumor cells has proven most useful for gene mapping.

For transitional cell carcinoma of the bladder, previous studies have identified somatic loss of human chromosome 9 material as a candidate initiating event (2–5). Sixty to 70% of either low-grade or high-grade tumors demonstrated LOH (6) of polymorphic markers (6, 7). Localization of a subregion of the chromosome that represents a common region of loss has been hampered by the lack of informative polymorphic markers and by the infrequent occurrence of partial chromosomal deletions (8).

In this study we have used a panel of informative markers to identify and characterize bladder tumors with loss of heterozygosity for portions of chromosome 9. The results suggest that two distinct regions of chromosome 9 are important in the pathogenesis of human bladder cancer and should facilitate the localization of putative tumor suppressor genes on this chromosome.

Materials and Methods

Primary tumors obtained as surgical specimens were processed as described previously (9). DNA from whole blood and from tumor was analyzed for heterozygosity by amplification of dinucleotide repeat-containing sequences using polymerase chain reaction and the conditions described (10). The markers used in this study are shown in Fig. 1. Oligonucleotide primers for D9S12 (1.1, 1.2) were obtained from the American Type Culture Collection (Rockville, MD). D9S145 was previously reported (11). Other primers were obtained from Research Genetics (Huntsville, AL). Primers were labeled with poly-nucleotide kinase (New England Biolabs) and [γ-32P]ATP. Fifty ng of genomic DNA and 50 ng of each primer were subjected to 30 cycles of amplification. Annealing temperatures were 54–58°C. For amplification of D9S165, D9S12 (1.1, 1.2), and D9S180, Taq polymerase (Perkin-Elmer) was added only after preheating samples to 95°C. Products were detected by electrophoresis in denaturing 6% polyacrylamide-urea gels followed by autoradiography. For informative cases, allelic loss was scored if the amount of one allele was significantly reduced in the tumor DNA as compared with normal. Histopathological grade and stage were determined from the pathology report.

Results

46 cases of primary carcinoma of the bladder were examined for loss of heterozygosity of chromosome 9. A number of previous studies have demonstrated the utility of amplification of microsatellite markers for allelic loss analysis (12, 13). Numerous highly informative markers have been identified for human chromosome 9 (14) (Fig. 1). Using these markers, we found that 25 of 46 tumors (54%) had LOH, which agrees with previous studies that determined allelic loss by Southern blotting (5, 8, 15). Nineteen of 46 tumors (41%) had LOH for all informative markers that were tested. On average, six informative markers were tested for each case. For 40 of 46 cases, loss or retention of heterozygosity was concordant for all markers, confirming the reliability of the assay.

For six of 46 cases we found discordancy between markers, indicating a partial LOH. Such partial losses occurred in tumors that were diagnosed as grade II or III, with either papillary or invasive patterns of growth. Because partial LOH may allow better localization of putative tumor suppressor genes, we analyzed these six cases in detail (Fig. 1). Two patterns of loss were apparent. One group of tumors lost sequences predominately from the p arm (cases 1–4), while another group lost sequences predominately from the q arm (cases 5 and 6). Of the 4 tumors with LOH for the p arm, two tumors (cases 3 and 4) demonstrated LOH for all informative p arm markers. However, the 2 remaining tumors (cases 1 and 2) exhibited retention of heterozygosity for the proximal p arm (markers D9S161 and D9S165; Figs. 1 and 2), with LOH confined to the distal p arm. Case 2 was especially informative since heterozygosity was retained at D9S161, while the more distal p arm markers, including the closely linked α-interferon, revealed LOH. The deletions in tumors 1 and 2 are consistent with the presence of a tumor suppressor locus between D9S161 (9p21) and the telomere (Fig. 1, Region A).

Tumors from two patients (Figs. 1 and 2, cases 5 and 6) demonstrated LOH for all informative markers on the q arm and retained heterozygosity for all informative markers on the p arm. The deleted region for tumors 5 and 6 is distinct from region A on the p arm based upon retention of heterozygosity in cases 1, 2, 5, and 6 for the markers D9S165 and D9S161. The results from these four tumors suggest the presence of bladder tumor suppressor functions on chromosome 9 in the two regions designated A and B in Fig. 1.
The deletions in tumors 3 and 4 involved region A and, in addition, overlapped with the deletions in cases 5 and 6 (Fig. 1). Further delineation of the regions lost by these tumors could be obtained by using additional markers in the proximal p and q arms.

Discussion

The possibility that two distinct regions of chromosome 9 are affected by LOH is consistent with previous reports. Cytogenetic studies have detected partial deletions involving either the p arm or the q arm (2–4). Both our study and a previous study have identified tumors with LOH that may be limited to the q arm (Fig. 1, cases 5 and 6; Ref. 8). In addition, Habuchi et al. (15) reported the molecular characterization of one tumor containing a limited deletion involving the region q21.2–34 (15). Taken together, the results suggest the presence of a bladder tumor suppressor in this region of the chromosome. Interestingly, linkage studies have mapped the basal cell nevus syndrome to chromosome 9q22.3–31, and LOH studies implicate a tumor suppressor gene as the etiology of both inherited and sporadic forms of basal cell carcinoma.

Previous analysis of other tumor types suggested that one or more suppressor genes resides on chromosome 9p. Alterations of one or more loci near the interferon gene cluster may contribute to glioma, leukemia, and lung cancer (16). Genetic alterations within 9p21 proximal to the interferon cluster may contribute to both familial and sporadic cases of melanoma (17). Future studies will determine whether the loss of chromosome 9p in these different tumor types is attributable to a single tumor suppressor gene and whether such a gene is altered in bladder cancer.

Allelic loss of chromosome 9 is considered a candidate initiating event for bladder carcinoma. Cytogenetic studies have identified monosomy 9 and trisomy 7 as the only karyotypic deviations in several cases (4). Molecular studies have found chromosome 9 loss as the only frequent alteration in low grade tumors (6, 7, 18). Finally, analysis of multiple primary tumors from single individuals showed that the same chromosome 9 allele was lost in different tumors, whereas other genetic alterations such as loss of chromosome 17 or 18 varied in the different tumors (19). Since the results reported here suggest that two chromosome 9 loci may be involved, it will be important to understand the contribution of each locus to the initiation and progression of carcinoma of the bladder.

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References

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