Chromosome 9 Allelic Losses and Microsatellite Alterations in Human Bladder Tumors

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Abstract

Chromosome 9 allelic losses have been reported as a frequent and early event occurring in bladder cancer. It has been postulated that a candidate tumor suppressor gene may reside on this chromosome, alterations of which may lead to the development of a subset of superficial bladder tumors. More recently, the involvement of two different regions harboring suppressor loci, one on each of both chromosome 9 arms, has been proposed. We undertook the present study with the objectives of better defining the deleted regions of chromosome 9 in bladder tumors, as well as evaluating the frequency of microsatellite alterations affecting certain loci on this chromosome in urothelial neoplasia. Seventy-three primary bladder tumors were analyzed using a set of highly polymorphic markers, and results were correlated with pathological parameters associated with poor clinical outcome. We observed that, overall, 77% of the tumors studied showed either loss of heterozygosity for one or more chromosome 9 markers and/or microsatellite abnormalities at chromosome 9 loci. Detailed analyses showed that two regions, one on 9p at the interferon cluster, and the other on 9q associated with the q34.1–2 bands, had the highest frequencies of allelic losses. Furthermore, Tm lesions appeared to present mainly with 9q abnormalities, while Tt tumors displayed a mixture of aberrant 9p and 9q genotypes. These observations indicate that loss of heterozygosity of 9p may be associated with the development of superficial tumors with a more aggressive biological behavior or, alternatively, they may be related to early disease progression. In addition, microsatellite alterations were documented in over 40% of amplified cases. Taken together, these data suggest that two different tumor suppressor gene loci on chromosome 9 are involved in tumorigenic events in bladder cancer and that chromosome 9 microsatellite alterations are frequent events occurring in urothelial neoplasia.

Introduction

Cytogenetic and molecular genetic analyses of bladder cancer have identified abnormalities in a number of chromosomes which appear to be involved in the development and progression of these tumors (1–4). Loss of heterozygosity of chromosome 9 is a frequent mutation occurring in both superficial and muscle invasive lesions. Allelic losses in 9p (5–7) and 9q (3, 8, 9) have been reported, estimating the area of deletions between 9p22 and 9q34.1. The involvement of two different regions containing suppressor loci in bladder tumors has been recently proposed (10). In addition, somatic instability at microsatellite repeats, detected in low stage bladder tumors, has been reported as an early alteration associated with tumorigenic events in such neoplasias (11). The microsatellite instability observed in the hereditary nonpolyposis colorectal cancers (Lynch syndrome) suggested that these tumors may arise through a mechanism different than the inactivation of a tumor suppressor gene (12–14). The inherited basis of these neoplasia appears to be due to an inactivation of DNA damage recognition/repair pathways. However, even in hereditary nonpolyposis colorectal cancers, multiple hits are necessary for the transformed cells to acquire the malignant phenotype. The objectives of this study were to better define the deleted regions of the chromosome 9 in bladder tumors, to delineate the possible role of the loss of heterozygosity (LOH) in 9p and 9q arms, and to evaluate the existence and frequency of microsatellite alterations in urothelial neoplasias. We examined genetic alterations occurring on chromosome 9 using a well-characterized cohort of patients affected with superficial and muscle invasive bladder tumors. We centered our study on the analyses of RFLP, VNTR, and microsatellite markers assigned to both arms of chromosome 9.

Materials and Methods

Tissues. Seventy-three primary bladder tumors were obtained from radical cystectomy samples (n = 64) and transurethral resection specimens (n = 9). Tissues were embedded in tissue TEK OCT cryopreservative (Miles, Inc., Elkhart, IN) and stored frozen at −70°C. Normal tissue was obtained from the same bladder in an area free of tumor or, in 5 cases, from other normal tissue (i.e., peripheral blood or prostate). Five-μm sections were used for hematoxylin and eosin staining. The sections were examined by a pathologist (V. E. R.) to confirm the presence of tumor, evaluation of tissue morphology, and pathology staging. The 73 cases selected showed at least 50% of tumor cells on the sample and were included in the study. All specimens were graded by WHO classification and staged according to the TNM pathological staging system.

DNA Isolation and Southern Blotting. Twenty to 30 consecutive 30-μm-thick sections were cut from each tissue block. The DNA was isolated, digested, and transferred to nylon membrane as described previously (2, 3). The DNA was fixed to the membrane using a UV linker (UV Stratalink 1800; Stratagene, La Jolla, CA).

Probes. The following probes with chromosome map positions, loci, and restriction enzymes were obtained from the ATCC (Rockville, MD) and were used for RFLP analysis in this study: p08782 (9q34–qter; Asgltl; HindIII); pabl K2 (9q34; v-abl; TaqI); PMCOA12 (9q; D9S28; MspI); p04580 (9q34.3; D9S10; TaqI) VNTR; pED126.3 (9q34; D9S7; TaqI) VNTR; DR6 (9p21–ter; D9S5; HindIII) (provided by Jane Fountain, Massachusetts Institute of Technology-Center for Cancer Research, Cambridge, MA); pINIF A3 (9p22; interferon α-2; MspI) (provided by Dr.P.M. Pitha Rove, The John Hopkins University-Oncology Center, Baltimore, MD).

Primers. The following primers were used for the present study: IFNA cluster (5′-TGCGCGTTAATGTTGT-3′ and 5′-AGTTAAGGGGGTCTCACCCTTAC-3′; provided by Dr. Manuel Diaz, Loyola University School of Medicine, Chicago, IL); D9S54 (ATCC); ABL 1 (9q34.1) (ATCC); and ASS (9q34.1) (ATCC).

Hybridization, PCR Amplification, and Autoradiography. The probes were labeled to a high specific activity with [α-32P]dCTP by the random oligolabeling technique using the Primed DNA Labeling kit (Boehringer Mannheim, Indianapolis, IN), and membranes were pretreated and hybridized...
as described previously (15). The dinucleotide repeat polymorphism was analyzed by PCR amplification according to the Gene Amp kit protocol (Perkin-Elmer Cetus, Norwalk, CT) with the following modifications. The final reaction volume was reduced to 20 μl; 2 μCi [α-32P]dCTP were added per reaction; and annealing temperatures ranged from 55 to 60°C, depending on the set of primers used. This was followed by polyacrylamide gel electrophoresis on 8% acrylamide gels. For samples in which the normal tissue showed heterozygosity for a given probe or set of primers, the presence of the nondeleted allele in the tumoral tissue was determined by a second reaction using primers that produced a different fragment. The relative decrease or the absence of the allele was established, as reported previously, comparing the nondeleted alleles in the normal and tumor tissue counterparts (2, 3).

Statistical Methods. Fisher's exact test (16) was used to assess the association between clinicopathological factors and chromosome 9 alterations, including 9p and 9q allelic losses, as well as microsatellite abnormalities at chromosome 9 loci.

Results

In the present study, 73 cases of primary bladder tumors were evaluated for LOH and microsatellite alterations on chromosome 9. The probes and primers were selected on the basis of chromosomal location and rate of polymorphism. Table 1 summarizes the results obtained by RFLP and allelic losses identified by PCR allelotyping. A summary of the LOH and microsatellite alterations is shown in Fig. 1. Fig. 2 illustrates some of the altered patterns observed in this study. LOH was defined by the complete or partial reduction in signal of one allele in the normal and the tumor tissues was assessed by densitometry using an Ultrascan XL Laser Densitometer (Pharmacia LKB Biotechnology, Piscataway, NJ). The relative decrease or the absence of the allele was established, as reported previously, comparing the nondeleted alleles in the normal and tumor tissue counterparts (2, 3).

Table 1 Association between LOH, microsatellite alterations, and pathological parameters.

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* Number of allelic losses/number of informative cases.

* Number of alterations/number of amplified cases.

Fig. 1. Diagrammatic representation and summary of the frequency of chromosome 9 alterations in bladder tumors. The approximate physical location of each marker is indicated. The percentage of alteration relates to the number of detected mutations per informative cases studied. n, number of informative cases studied with each marker.
In order to better define the deleted region(s) on chromosome 9 in bladder cancer, we undertook the present study mapping the candidate regions using a set of highly polymorphic markers. The cases selected included both superficial and muscle invasive urothelial neoplasias. We observed that chromosome 9 alterations were indeed common events in bladder tumors. The highest frequency of allelic losses was found in the short arm of chromosome 9 with a 65% LOH in the IFNA gene cluster. This frequency increased to 80% when superficial lesions were considered as a subgroup. Detailed analysis at two other loci on this arm, D9S54-telomeric and D9S3-centromeric to IFNA, indicated that only muscle invasive tumors had allelic losses in these regions. This suggests that a candidate tumor-suppressor gene on 9p for certain superficial bladder tumors is closely linked to the IFNA locus. It has been reported that the locus for familial melanoma susceptibility is in the chromosomal region 9p13-p22 (18). In addition, genetic alterations near the interferon gene cluster have been documented in other human neoplasias, such as gliomas (19), nonsmall cell lung cancer (20, 21), leukemias (22), and lymphomas (23). Allelic losses on the long arm of chromosome 9 were frequently observed, with an overall LOH of 45% for the 9q34.1-3 region. The frequency also increased to 56% when superficial tumors were analyzed. Interestingly, only 13% of informative cases showed allelic losses at the D9S28 locus, centromerically located in reference to the 9q34.1-3 region. Furthermore, it should be noted that all of these tumors were muscle invasive bladder cancers. These data indicate that a candidate tumor suppressor gene on 9q for a subset of superficial bladder tumors is located in the 9q34.1-3 region. The galactosyl transerase that codes for the ABO histoblood group-related antigens maps to this area (24). Altered patterns of expression of these antigenic determinants has been reported as a common phenotype in bladder cancer (25, 26). More recently, it has been reported that the target gene for the Gorlin syndrome maps to chromosome 9q22.3-q31 (27, 28). This is an autosomal dominant disorder predisposing to basal cell carcinomas of the skin and other neoplasms, including bladder tumors.

Allelic losses reported here on 9p and 9q are in concordance with previous findings from other groups (5, 6, 8-10). In addition, we observed that 68% of 9p LOH samples showed losses for 9q. However, T1 lesions appeared to have 9q LOH as the main genetic alteration, whereas T2 tumors presented with a mixture of aberrant chromosome 9 genotypes (i.e., 9p-, 9q-, and 9p/9q). Moreover, all allelic losses observed at D9S54 occurred in muscle invasive tumors. Taken together, these data suggest that 9p LOH may be associated with a more aggressive biological behavior or early disease progression.

We also designed this study to evaluate the existence and frequency of microsatellite alterations occurring on chromosome 9 in urothelial neoplasias. These abnormalities have been associated with the replication error phenotype, a reflection of genetic instability (12-14). The ASS microsatellite displayed normal patterns in all amplified samples, whereas two expansions were identified at the ABL locus. However, we found that 41% of the amplified cases for the D9S54 locus exhibited instability, indicating that this is not a random event in bladder cancer. The alterations characterized on D9S54 did not seem to relate to 9p and/or 9q status. Gonzalez-Zulueta et al. (11) have recently documented somatic instability at microsatellite repeats on chromosome 9 in four bladder tumors (11).

In the present study and considering all types of genetic alterations, we found that 77% of the informative cases had chromosome 9 mutations, representing the largest percentage documented to date. 

4. R. Chaganti et al., personal communication.
This may be due to the combination of assays used as well as the loci and regions analyzed. It is possible that the cohort of patients selected, based on histopathology evaluation and good tissue preservation, may have also contributed to the results reported here. Further studies centering on the role of these abnormalities, mainly as they occur in and regions analyzed. It is possible that the cohort of patients selected, will be needed in order to better understand their involvement as either primary or secondary events in urothelial neoplasias.

Acknowledgments

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References

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