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Advances in Brief

Preliminary Mapping of the Deleted Region of Chromosome 9 in Bladder Cancer

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Abstract

Inactivation of a suppressor gene by deletion of chromosome 9 is a candidate initiating event in bladder carcinogenesis. We have used 13 polymorphic markers spanning the length of chromosome 9 in order to map the region of deletion in human bladder carcinomas. In the majority of tumors loss of heterozygosity was found at all informative sites along the chromosome, indicating deletion of the entire chromosome. Nine tumors had selective deletions of chromosome 9. Mapping of the deleted region in these tumors suggests that the target gene is located between *D9S22* at 9q22 and *D9S18* at 9p12–13.

Introduction

Linkage analysis and LOH[2] studies in familial tumors such as retinoblastoma and Wilms' tumor have led to the isolation of tumor suppressor genes, inactivation of which is involved in initiation of the cancer (1). Loss of specific chromosomes or chromosome regions can also be detected frequently in sporadic cancers using either cytogenetic or molecular techniques. The nonrandom frequency of deletions in sporadic cancers implies the existence of a number of tumor suppressor genes which may be involved in initiation and progression of the disease.

Numerous chromosome losses have been reported in bladder carcinomas, the majority of which are believed to be involved in progression rather than initiation of the disease (2–5). The absence of any defined or common familial form of bladder carcinoma precludes the possibility of utilizing linkage data or analysis of constitutional chromosome abnormalities to identify the chromosome location of the initiating event(s). It might be expected that a primary genetic alteration would be present at a high frequency in all the stages of a neoplasia and would be the prevalent alteration in the earlier stages of the disease.

Cytogenetic studies of bladder tumors have suggested that deletion of chromosome 9 is frequent and may be a primary event (6, 7). There is no clear cytogenetic evidence to suggest the location of the critical gene. Monosomy of chromosome 9 (6–9) has been frequently reported, as has deletion of the q arm (7, 8, 10) and deletion of the p arm (8) or isochromosome 9q (11). Molecular confirmation of the frequent deletion of chromosome 9 has been obtained (2, 11, 12). We have recently reported that loss of chromosome 9 is the only frequent genetic alteration observed in grade I, Ta lesions which are the most clinically benign form of bladder tumor (12). Inactivation of a putative tumor suppressor locus on chromosome 9 is therefore a candidate initiating event in bladder carcinogenesis. We report here the preliminary mapping of the region of chromosome 9 where the critical gene is located.

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[2] The abbreviations used are: LOH, loss of heterozygosity; SSC, standard saline-citrate; SDS, sodium dodecyl sulfate.

Materials and Methods

Tumor specimens were obtained by transurethral resection and immediately frozen. Peripheral blood from each patient was collected in EDTA. Macroscopically pure tumor was dissected from the frozen biopsies and leukocytes were pelleted before extraction of DNA and purification with phenol/chloroform (13). Tumor and leukocyte DNA were separately digested with the appropriate restriction enzyme following the manufacturer's instructions (Boehringer Mannheim UK). DNA was separated in agarose gels of 0.8–1.2% and run as necessary to resolve particular polymorphisms. Blotting was by conventional methods to Hybond-N membrane (Amersham Aylesbury, UK) which was subsequently UV-cross-linked. Probe fragments were labeled by random-priming (14). Prehybridization and hybridization were carried out in 5 × SSC-10% dextran sulfate-5 × Denhardt's solution-0.5% SDS with 0.1 mg/ml carrier DNA. Membranes were washed initially at 65°C in 2 × SSC-0.1% SDS and then washed down to 0.1 × SSC where necessary. Membranes were stripped for reuse by washing in 0.4 M NaOH at 45°C and neutralizing in 0.2 M Tris-HCl-0.1 × SSC-0.1% SDS.

Results and Discussion

In order to identify the most frequently deleted region of chromosome 9, 252 bladder tumors were screened for LOH in 4 regions of chromosome 9. In an attempt to obtain an informative result for every case a number of probes were used in each region. Markers were chosen for their high polymorphic information content although the paucity of markers located at proximal 9q or 9p allowed little choice. All 252 bladder tumors were screened for LOH at distal 9q34 with probes MHZ10, EFD126.3, and MHZ13. All tumors were also screened at proximal 9q34 with MCT136 and 33.1 and some were screened with MCT96.1. At proximal 9q every case was screened with CRI-110 and ASSP3 and on 9p with HHH220, DR6, and IFNβ. Some tumors were also screened for LOH on 9p with OVCO.96 and 72-09. The map location of and polymorphic enzyme used with the DNA markers on chromosome 9 are described in Table 1 (15, 16). Informative results were available with one or more probes for all cases at distal 9q34, for 185 cases (73%) at proximal 9q34, for 181 cases (72%) at proximal 9q and for 228 cases (90%) on 9p.

Overall, 160 of 252 (63%) tumors showed LOH on chromosome 9 indicated by the absence or significant reduction of signal from one allele. This is an accurate figure for the overall frequency of loss of chromosome 9 in bladder carcinoma since the tumors in this series were representative of the grade and stage of bladder tumors seen at presentation (17). Since the detection of LOH by Southern analysis is a relatively crude method of identifying mutations it is possible that inactivation of the putative suppressor gene occurs in more than 63% of bladder tumors. Indeed only 70% of retinoblastomas show LOH at the *RB* locus, while in the other 30% *RB* is inactivated by mutations too subtle to be detected by Southern analysis (18). All bladder tumors may therefore have inactivation of a tumor suppressor gene on chromosome 9.

Of the 160 cases of LOH, 151 appeared to have lost the entire chromosome as suggested by LOH at all informative sites along the chromosome. Monosomy appears to be the predominant deletion event of chromosome 9 in bladder carcinoma. Some deletion mapping

Table 1 *Chromosome 9 DNA markers*

Map location	Locus	Clone	Enzyme
9p24–p23	*D9S47*	OVCO.96	*Rsa*I
9p22–p21	*IFNβ*	SY2051	*Msp*I, *Ban*II
9p22–p21	*D9S126*	72–09	*Taq*I
9p22–p21	*D9S3*	DR6	*Hind*III
9p21	*D9S18*	HHH220	*Taq*I
9q11–q22	*ASSP3*	AS1	*Hind*III
9q22.3	*D9S22*	CRI-110	*Eco*RI
9q34	*D9S49*	33.1	*Hinf*I
9q34	*D9S10*	MCT136	*Pst*I
9q34	*D9S14*	MCT96.1	*Rsa*I
9q34	*D9S7*	EFD126.3	*Hinf*I
9q34	*D9S13*	MHZ13	*Pst*I
9q34	*D9S11*	MHZ10	*Hinf*I

studies have found monosomy to be a common event, for example of chromosome 10 in glial tumors (19, 20) and chromosome 22 in meningiomas (21). Because of the high incidence of monosomy, the high level of loss previously reported at 9q34 (2, 3) does not necessarily imply proximity to the target locus. The frequency of monosomy also meant that it was necessary to screen a large number of tumors in order to identify cases of selective deletion.

Nine tumors showed LOH with some markers and retention of heterozygosity with others. These cases represent selective deletion of chromosome 9 (Fig. 1). These comprised 4 tumors with deletions at loci on 9p but retention of heterozygosity at loci on 9q, 4 tumors with loss of the q arm and retention of the p arm, and 1 tumor with loss of the p arm and proximal q and retention of distal q. These data identify a region of chromosome 9 between HHH220 (*D9S18*) at 9p12–13 and CRI-110 (*D9S22*) at 9q22 as the common region of deletion. The minimum region of overlap between the selective deletions is likely to include the tumor suppressor gene locus assuming that a single gene locus is the target of all the deletions. The suggested common region of deletion spans a large and sparsely mapped region of proximal 9q and centromeric 9p. Deletion mapping with more closely spaced markers within this region of selective deletion may reduce the limits of the deleted region by more closely identifying the deletion breakpoints in the nine tumors. In addition cases of interstitial deletion may be revealed among the 92 cases with retention of heterozygosity.

Two putative suppressor loci have been identified on chromosome 9 in other tumor types. The chromosome region containing the interferon gene complex at 9p21 has been shown to be frequently deleted in leukemias (22), gliomas (23), and lung carcinomas (24). A region of 9q21–22 excluding the interferon gene complex has been reported to be homozygously deleted in melanoma (25). Linkage analysis and LOH studies have implicated the 9q31 region as likely to contain a suppressor gene important in both familial and sporadic basal cell carcinoma (26). Chromosome 9 markers are also lost in colorectal tumors (27) and breast tumors (28).

The high level of monosomy and the presence of a tumor suppressor locus on 9p commonly deleted in other types of tumor raises the possibility that there is more than one target gene on chromosome 9 in bladder carcinoma. This is known to be the case in other carcinomas; for example, critical suppressor genes are known to be located on both chromosome 17p and 17q in breast tumors (29, 30) and on chromosome 11p13 and 11p15 in Wilms' tumor (31). The presence of two target genes could obviously complicate deletion mapping. However, we have not observed any cases of two distinct regions of deletion separated by a retained chromosomal domain.

We have described the preliminary mapping of a tumor suppressor locus deleted in bladder carcinoma to the region between 9p12-13 and 9q22. This is the first step toward positional cloning of this locus. The selective deletions identified here will form a valuable panel for finer mapping studies involving microsatellite repeats and other new polymorphic markers, isolated by techniques such as Alu polymerase chain reaction of radiation hybrids, which should further delineate the minimum region of deletion.

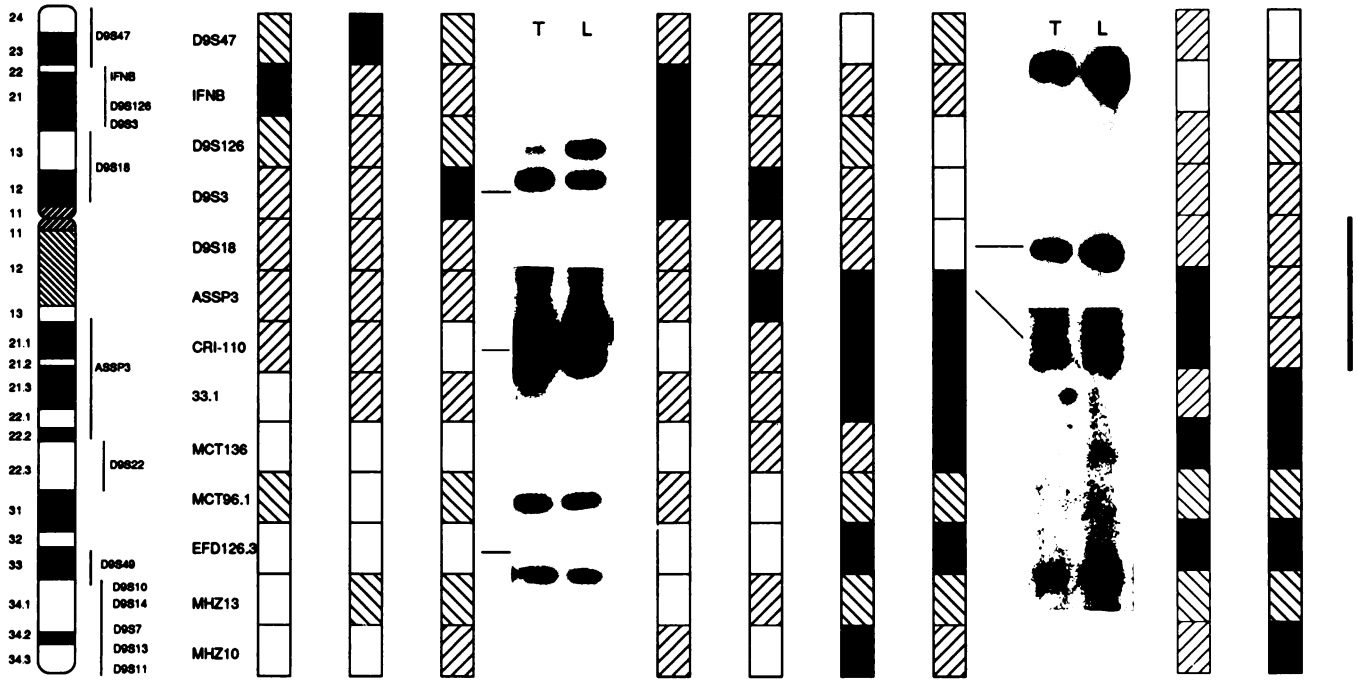

Fig. 1. Deletion map of chromosome 9. Diagramatic representation of the pattern of deletion of chromosome 9 in nine bladder tumors with subchromosomal deletions. *T*, tumor DNA; *L*, leukocyte DNA. *Bar* on *far right*, limits of the deleted region. □, retention of heterozygosity; ■, loss of heterozygosity; ▨, uninformative; ▧, not done.

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