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Advances in Brief

Allelic Loss of Chromosome 17p Distinguishes High Grade from Low Grade Transitional Cell Carcinomas of the Bladder[1]

Aria F. Olumi, Yvonne C. Tsai,[2] Peter W. Nichols, Donald G. Skinner, Delver R. Cain, Leon I. Bender, and Peter A. Jones

Urologic Cancer Research Laboratories, Kenneth Norris Jr. Cancer Center, University of Southern California School of Medicine, Los Angeles, 90033 [A. F. O., Y. C. T., P. W. N., D. G. S., P. A. J.], and Departments of Urology and Pathology, Cedars Sinai Medical Center, Los Angeles, 90048 [D. R. C., L. I. B.], California

Abstract

Forty-three transitional cell carcinomas of the bladder of differing grades and stages were examined for reduction to homozygosity for chromosomes 9q, 11p, and 17p. Allelic loss of chromosome 9q was seen in 24 of 38 informative grades II, III, and IV tumors providing further evidence for a bladder tumor suppressor gene on this chromosome. In contrast to the grade-independent involvement of chromosome 9q, allelic losses of chromosomes 11p and 17p were seen only in grade III and IV tumors. The results with chromosome 17p were particularly striking and showed that 0 of 10 grade II *versus* 20 of 31 grade III and IV tumors had allelic losses for this chromosome harboring the p53 tumor suppressor gene often mutated in other human cancers. The data suggest that cumulative genetic damage is sustained in transitional cell carcinomas and that one of the underlying molecular mechanisms distinguishing low grade from high grade tumors involves chromosome 17p.

Introduction

The prognosis for patients with TCC[3] of the bladder is determined largely by the grade and stage of the tumor at diagnosis (1). Low grade tumors (grades I–II) may or may not invade the lamina propria and usually do not invade the musculature. These tumors rarely become life threatening whereas higher grade tumors tend to be more aggressive at presentation, frequently show evidence of muscle invasion, and have a worse prognosis. The pathological grade of the tumor is therefore an essential factor in bladder cancer management since it is linked to the biology of the disease.

The underlying molecular defects in TCC remain unknown, but recent advances in the analysis of other cancers, particularly colorectal tumors, have led to the realization that cumulative genetic damage occurs during tumor progression (2). This damage involves the activation of oncogenes and the inactivation of several putative tumor suppressor genes. The occurrence of multiple genetic lesions therefore seems to be necessary for the formation of a malignant tumor. We have begun an analysis of TCC of the bladder of different histological grades and stages to determine whether cumulative allelic losses, which might implicate inactivation of specific tumor suppressor genes, might also be a hallmark of bladder cancers.

Earlier molecular studies showed that 40% of bladder tumors were reduced to homozygosity for markers on chromosome 11p (3). However, we recently found that the greatest frequency of allelic loss in high grade bladder cancer occurred on chromosomes 9q and 17p, with 67 and 63% reduction to homozygosity in informative tumors, respectively (4). Although colorectal tumors show some loss of chromosome 9q (5), such a high frequency of 9q deletion has not been implicated in other human epithelial tumors. Chromosome 17p, however, which harbors the p53 tumor suppressor gene, is commonly reduced to homozygosity in colorectal (6), breast (7), and lung (8) cancers. It is intriguing that chromosome 17p loss is often associated with late stage cancers, suggesting that the common loss of heterozygosity for this region is associated with tumor progression. We have extended our earlier analysis of high grade bladder tumors to include 10 grade II, 23 grade III, and 10 grade IV tumors to determine whether cumulative genetic deletions are also a characteristic of bladder cancer. The results show that chromosome 9q loss is associated with all bladder cancers regardless of grade but that reduction to homozygosity for chromosome 17p is seen most frequently in tumors classified as grade III or IV.

Materials and Methods

Forty-three fresh transitional cell carcinomas and blood samples were obtained from Cedars Sinai Medical Center, Kenneth Norris Jr. Comprehensive Cancer Center, and Los Angeles County/University of Southern California Medical Center. Tumor and blood DNAs were extracted, and allelic deletion analysis was carried out as described previously (4). Tumors were histologically graded and staged without knowledge of the chromosomal arm analysis results.

All of the transitional cell carcinomas were graded according to the methods of Ash (9) and Bergkvist *et al.* (10). The tumors were staged according to the tumor-nodes-metastasis pathological staging system (10). The following probes with indicated loci and restriction enzymes were used for analysis: chromosome 9, probe EFD 126.3 (D9S7) (*Msp*L and *Hpa*II); probe MCT96.1 (D9S14) (*Rsa*I). probe MCT 136 (D9S10) (*Msp*I and *Hpa*II), chromosome 11, probe T24-C3 (HRAS1) (*Msp*I and *Hpa*II), probe HINS (INS) (*Rsa*I); chromosome 17, probe YNZ22 (D17S30) (*Msp*I and *Hpa*II), probe 144D6 (D17S34) (*Msp*I and *Hpa*II).

Results

Table 1 summarizes the results of this study grouped accordingly to histological grade and stage. None of the 10 grade II tumors examined demonstrated invasion of the lamina propria or muscle and were all stage Ta. The 23 grade III tumors were divided into 2 groups; 10 tumors classified as stage Ta, T1, or pT1 were separated from the remaining 13 of stage pT2 or higher, since the latter show invasion into the musculature. The 10 grade IV tumors were all stage pT3 or higher. Tumors were numbered consecutively from 26 to distinguish them from the 25 tumors examined previously (4).

Allelic deletion of chromosome 9q in a tumor is shown in a representative Southern blot in Fig. 1*A*. Table 1 shows that 6

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[2] To whom requests for reprints should be addressed.

[3] The abbreviation used is: TCC, transitional cell carcinoma.

Table 1 *Summary of results from patients with TCC*

Autoradiographs of WBC and tumor DNA were analyzed for loss of heterozygosity (−) or no loss of heterozygosity (+). Markers were noninformative (NI) in some cases. Tumor stages which do not have the prefix p were those in which the entire bladder was not available for pathological diagnosis

Patient	Histological grade	Clinical or pathological stage	Chromosomal region		
			9q	11p	17p
26	II	Ta	NI	NI	+
27	II	Ta	−	NI	+
28	II	Ta	−	+	+
29	II	Ta	+	+	+
30	II	Ta	−	NI	+
31	II	Ta	−	+	+
32	II	Ta	+	+	+
33	II	Ta	−	NI	+
34	II	Ta	+	+	+
35	II	Ta	−	NI	+
36	III	Ta	−	NI	−
37	III	Ta	−	NI	−
38	III	Ta	+	NI	−
39	III	pT1	−	+	−
40	III	T1	+	NI	+
41	III	T1	+	NI	−
42	III	T1	−	NI	+
43	III	pT1	NI	+	−
44	III	pT1	+	+	−
45	III	pT1	−	NI	−
46	III	pT2	+	+	−
47	III	pT2	+	NI	+
48	III	pT3A	NI	−	−
49	III	pT3A	−	+	−
50	III	pT3B	+	+	+
51	III	pT3B	+	−	NI
52	III	pT3B	−	−	−
53	III	pT4A	−	−	+
54	III	pT4A	+	+	+
55	III	pT4A	+	−	−
56	III	pT4A	+	NI	+
57	III	pT4A	−	−	−
58	III	pT4A	−	NI	−
59	IV	pT3B	−	+	−
60	IV		−	NI	+
61	IV	pT3B	−	+	+
62	IV	pT3B	−	−	−
63	IV	pT3B	−	NI	−
64	IV	pT4A	NI	+	+
65	IV	pT4A	−	+	+
66	IV	pT4A	−	+	−
67	IV	pT4A	−	+	NI
68	IV	pT4	NI	+	−

of 9 informative grade II tumors and 5 of 9 informative grade III cancers without muscle invasion were reduced to homozygosity for chromosome 9q. Thirteen of 20 informative cases of grade III and IV tumors showing muscle invasion (stage pT2 and greater) were deleted for chromosome 9q. Deletion of chromosome 11p was not observed in any of the informative grade II or grade III tumors of stages T1 or pT1 or less in this study; however, this was observed previously in a grade III stage pT1 tumor (4). Seven of 18 informative grade III and IV muscle invasive cases were deleted for chromosome 11p.

Deletion of chromosome 17p (see example in Fig. 1*B*) was not observed in any of the 10 informative cases of grade II tumors (Table 1). On the other hand, stage Ta, T1, and pT1 grade III tumors showed reduction to homozygosity for chromosome 17p in 8 of 10 informative cases. The loss of one allele of chromosome 17p therefore appears to be linked to the histological transition between grade II and grade III TCC. Table 1 also shows that 7 of 12 grade III tumors showing muscle invasion and 5 of 9 informative grade IV tumors had chromosome 17p deletions.

Table 2 is a summary of the chromosomal analysis combining the present results with those previously published for high grade TCC (4). Chromosome 9q was reduced to homozygosity in all groups, with the highest rate of loss seen in grade IV tumors (88%). There appeared to be no large differences in the frequencies of chromosome 9q loss in each group and overall 36 of 54 (67%) of informative TCCs were reduced to homozygosity for this chromosomal arm. The number of informative grade II and III tumors for chromosome 11p was low but there appeared to be an increased frequency of loss of 11p in grade III tumors of stage P2 and greater. Table 2 also shows an apparent discontinuity in the frequency of reduction to homozygosity for chromosome 17p between grade II and higher tumors. Thus 0 of 10 grade II tumors compared to 31 of 50 (62%) of the remaining tumors showed reduction to homozygosity for 17p. This difference is statistically significant (2-sided P value = 0.0013).

Discussion

Our molecular analysis confirms cytogenetic studies (12–14) that chromosome 9 abnormalities are implicated in TCC of the bladder. The results extend our earlier investigations with high grade tumors to show that reduction to homozygosity for chromosome 9q occurs in approximately 65% of all TCCs of grade II or higher. The data suggest that this chromosome may harbor a tumor suppressor gene, the inactivation of which is responsible for the initial loss of growth control in urothelial

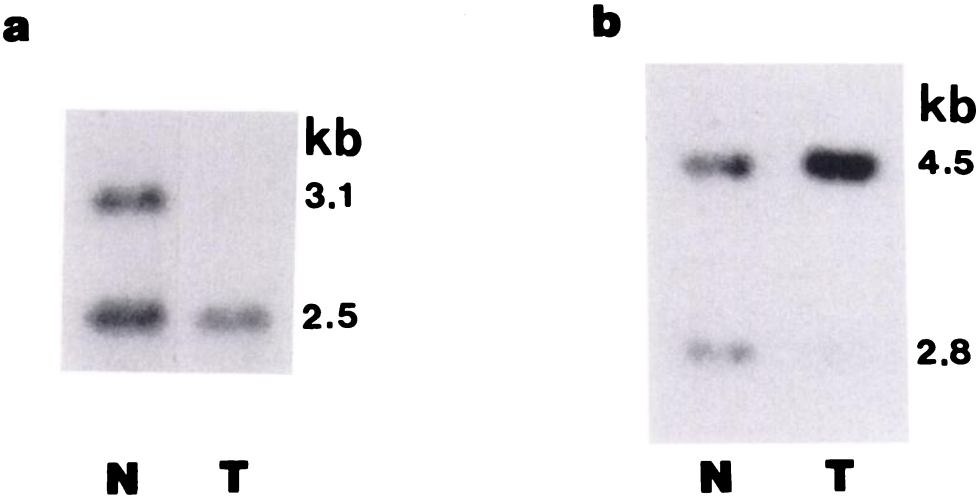

Fig. 1. Representative Southern hybridizations of chromosome 9q probe (*a*) or chromosome 17p probe (*b*) to normal control (*N*) or primary tumor (*T*) DNA. (*a*) Genomic DNA from patient 27 was digested with *Rsa*I and hybridized to probe MCT96.1. (*b*) Genomic DNA of patient was digested with *Msp*I and *Hpa*II and hybridized to probe 144 D6. Molecular weights of the allelic fragments are given in kilobase (*kb*) pairs.

Table 2 *Allelic deletions in TCC*

Summary of Southern blot chromosomal analysis in combination with our previous study (4).

Tumor grade	Tumor stage	No. deleted/no. informative on chromosomal arm		
		9q	11p	17p
II	Ta	6/9 (67)[a]	0/5 (0)	0/10 (0)
III	Ta, T1 or pT1	7/11 (64)	1/5 (20)	9/12 (75)
III	pT2 and greater	9/18 (50)	11/18 (61)	12/21 (57)
IV	pT3 and greater	14/16 (88)	3/15 (20)	10/17 (59)

[a] Numbers in parentheses, percentage.

cells. Chromosome 9 deletions or translocations have been observed in association with some leukemias, melanoma, glioma, and some colorectal cancers (5, 15, 16) but not with other human solid malignancies of epithelial cells. We have not yet mapped the chromosome 9 deletions but all of the markers used were on chromosome 9q suggesting that a tumor suppressor unique to bladder cancer may be located in this region.

In contrast to the apparent involvement of chromosome 9 in all grades of tumors examined, chromosome 11p and 17p deletions were restricted to grade III and IV tumors. The number of stage Ta, T1, or pT1 grade II or III tumors informative for markers on 11p was too low to determine whether reduction to homozygosity was associated with increasing grade or stage. However, frequent losses of this chromosome arm were seen in grade III and IV tumors which had invaded the musculature. The most striking result appeared to be the clear difference in the frequency reduction to homozygosity of chromosome 17p between grade II and higher grade tumors ($P = 0.0013$). It seems reasonable to postulate that a gene located on chromosome 17p is responsible for the differing biological behaviors of grade II and the higher grade tumors. However, our data are not yet comprehensive enough to exclude the possibility that this apparent transition is associated with stage rather than grade because we obtained no grade II tumors higher than stage Ta and only three grade III stage Ta tumors. Nevertheless chromosome 17p losses are clearly most frequent in higher grade and stage TCC.

The results with chromosome 17p are particularly intriguing since the p53 gene located on 17p often contains mutations in colorectal and other tumors homozygous for chromosome 17p. Indeed, we have recently found that the p53 gene is frequently mutated in primary invasive bladder cancers.[4] There is therefore evidence that the molecular event demarcating the differing biologies of grades II and III tumors is loss of normal p53 gene function induced by a mutational event. The mechanisms for the induction of p53 mutations is of relevance for the development of several late stage human malignancies. More than 35% of these mutations seem to be caused by the deamination of 5-methylcytosine to form thymine (17). The methylation of cytosine residues in the dinucleotide sequence CpG, within the p53 gene in human DNA, may therefore predispose them to point mutations capable of altering p53 function.

Our results suggest that multiple accumulative genetic defects seen in other human tumors may also be a feature of TCC of the bladder. Early low grade cancers contain chromosome 9 deletions, with higher grade tumors containing additional chromosome 11 and 17 deletions. The results do not preclude the participation of other genes such as *ras* (18) and *Rb* (19) which have been suggested to be relevant to bladder cancer development. Thus further study of bladder cancer is likely to be of value in determining the nature of genetic alterations important to this disease in particular and cancer in general.

[4] D. Sidransky, A. Von Eschenbach, Y. C. Tsai, P. A. Jones, I. Summerhayes, F. Marshall, M. Paul, P. Green, S. R. Hamilton, P. Frost, and B. Vogelstein. The p53 gene is frequently altered in primary inactive bladder carcinoma and can be identified in urine sediment, submitted for publication.

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