Urol Res (2000) 28:297–303

ORIGINAL PAPER

Atsushi Uchida · Masaaki Tachibana · Ayako Miyakawa
Kaoru Nakamura · Masaru Murai

Microsatellite analysis in multiple chromosomal regions as a prognostic indicator of primary bladder cancer

Received: 24 April 1999 / Accepted: 28 July 2000

Abstract This study investigated gene abnormalities in bladder cancer patients and the relationship between chromosomal alteration and the clinical outcome using microsatellite analysis. A total of 45 human bladder tumor patients were analyzed. The microsatellite markers for 18q21.1 (D18S46, D18S363, and D18S474), 9p21–22 (D9S171, D9S747, D9S1747, and IFNA), and 17p13.1 (TP53) were used for the loss of heterozygosity (LOH) detection. The clinical outcomes were estimated with univariate and multivariate analyses. The results show that patients with LOHs in 18q21.1 and 9p21–22 exhibited a significantly poor prognosis. LOHs of these chromosomal regions had the most predictable potential compared with the other known prognostic factors, such as tumor grade, TNM stage, and age. It is concluded that microsatellite analysis for 18q21.1 and 9p21–22 is capable of predicting the clinical outcome of bladder cancer patients.

Keywords Loss of heterozygosity · Microsatellite analysis · Bladder cancer · 18q21.1 · 9p21–22

Introduction

Multiple genetic changes may occur during the development of primary bladder cancers [22, 23]. Various mutations in tumor suppressor genes, including p53, have been identified and thought to indicate a progression to a more invasive manner [21, 24]. The relationship between expressing a mutation and/or a deletion of these individual tumor suppressor genes and particular cancer cell types has been relatively well studied. However, the relationships among these possible suppressor genes have yet to be adequately documented.

It has recently been suggested that microsatellite markers are useful for detecting genetic alterations in human cancers [18]. In the present study, we studied the loss of heterozygosity (LOH) of multiple chromosomal regions as possible tumor suppressor genes and their relationship to the clinical outcome in human bladder cancers using microsatellite analysis.

A. Uchida · M. Tachibana (✉)
A. Miyakawa · K. Nakamura · M. Murai
Department of Urology, School of Medicine,
Keio University, Shinanomachi 35,
Shinjuku-ku, Tokyo, Japan
Tel.: +81-3-33531211; Fax: +81-3-32251985

Materials and methods

Tumor specimens and DNA extraction

A total of 45 patients with human bladder tumors that were histologically proven to have transitional cell carcinomas were analyzed between February 1993 and October 1996. Patients were retrospectively selected for this study when a clinical follow-up (mean 37.9 ± 10.5 months) could be made. At clinical presentation, the 33 men and 12 women ranged from 42 to 85 years old (mean age ± SD = 66 ± 11). Overall 3- and 5-year actuarial survival rates were 77% and 72%, respectively. Of all patients, ten patients (2/28 superficial and 8/17 invasive tumor patients) died of bladder cancer.

Sixteen patients underwent radical cystectomy as the primary treatment. Thereafter, resected surgical specimens were studied using routine histological procedures. The extent of tumor invasion was classified according to the TNM [tumor (size), (lymph) node (involvement), metastasis] staging system adopted by the International Union Against Cancer (UICC) [12]. The other patients were treated with transurethral resection as the primary treatment.

Superficial tumors were routinely resected along with the muscle layer, and biopsies were taken from the base of the resected area. According to the WHO classification [20], we determined three of the bladder tumors to be grade 1, 20 to be grade 2, and 22 to be grade 3. Furthermore, we observed 26 superficial (pTa or pT1) tumors as noted and 19 muscle-invasive (beyond pT2) tumors.

Other samples obtained either by transurethral cold cup biopsy or specimens from the resected bladder were immediately frozen and stored at –80 °C. Tumor specimens for DNA preservation were carefully obtained from the superficial layer of the tumors so that the other normal tissue components (muscle, fibrous tissue, and adipose tissue) might be excluded. Genomic DNA was extracted from the paired normal bladder mucosa and tumor specimens by proteinase K digestion (Boehringer Mannheim, Indianapolis, Ind., USA) and phenol/chloroform extraction.

Microsatellite markers

The microsatellite markers D18S46, D18S363, and D18S474 were used for the LOH analysis of 18q21.1 [9, 10, 25]. D9S171, D9S747, D9S1747, and IFNA were utilized for the analysis of 9p21–22. Furthermore, TP53 was employed as the microsatellite marker for chromosome 17p13.1. The designations and sequences used for each primer are shown in Table 1. The sense primer was synthesized and labeled with tetramethylrhodamine isothiocyanate-ATP (XRITC-ATP) or fluorescein isothiocyanate-ATP (FITC-ATP) (Takara, Shiga, Japan). A fluorescence image analyzer (FM BIO-100; Hitachi Software Engineering, Tokyo, Japan, or Fluorimager; Molecular Dynamics, Calif., USA) was used for the detection of PCR products.

Microsatellite analysis

One hundred nanograms of template DNA was used in a 25-μl reaction mixture (AmpliTaq Core Reagent; Perkin Elmer, Norwalk, Conn., USA), which included primer sets (sense and antisense). Both normal and tumor DNA pairs were subjected to 35 cycles of polymerase chain reaction (PCR) under the following conditions: 94 °C for 30 s, 54 °C for 60 s, and 72 °C for 60 s, with a final extension step of 72 °C for 5 min (GeneAmp PCR System 2400, Perkin Elmer). The PCR products were separated by electrophoresis in 7.7 M urea/40% polyacrylamide/formamide gel, and were then visualized with a fluorescence image analyzer.

Each fluorescent intensity of the corresponding band of tumor sample was compared with a normal sample. The intensity of the peak fluorescence area (IOD) was estimated, then the %IOD was calculated using the following formula:

$$\mathrm{IOD} = (\text{IOD of each visible band in tumor sample}) / (\text{IOD from each corresponding band of normal sample}) \times 100$$

An IOD $<$ 50% was considered to be an LOH for the target locus.

Statistical analysis

Correlations between the groups in the presence or absence of LOH for each chromosomal region and other prognostic factors were statistically assessed by Fisher's exact test. The actuarial survival rates depending on the presence or absence of LOH for each chromosomal region were determined by the Kaplan–Meier method. Statistical difference was estimated by the Cox–Mantel test. A P value of less than 0.05 was considered to be significant.

The Cox proportional hazards regression model was utilized to determine which factor might influence patients' survival. These prognostic factors were classified as follows: tumor grade as low-grade (grade 1–2) and high-grade (grade 3), T category as superficial (T1) and invasive (T2), N and M categories as negative metastasis (N0M0) and positive metastasis (N+ and/or M+), and age as young ($\leq$75 years old) and old ($\geq$76 years old).

Results

Representative microsatellite alterations on each marker are shown in Fig. 1 and deletion maps of each chromosomal region are given in Table 2. Eleven of 45 (24%) tumor DNA samples frequently demonstrated an LOH with marker D18S363 from chromosome 18q21.1. The analysis of two additional markers on 18q21.1 (D18S46 and D18S474) confirmed LOH in the tumor DNA samples with nine samples (20%) for D18S46 and six samples (13%) for D18S474. Two samples exhibited LOH for all three microsatellite markers in 18q21.1. The analysis of four microsatellite markers on chromosome 9p21–22 (D9S171, D9S747, D9S1747, and IFNA) confirmed at least one marker loss in 21 (47%) tumor DNA samples. Furthermore, analysis of the TP53 microsatellite marker (17p13.1) also revealed an LOH in 16 (36%) tumor DNA samples. Therefore, 33/45 (73%) bladder tumor DNA samples demonstrated at least one LOH for all of these microsatellite markers. The genetic alteration between exon 8 and 11 of Smad4/dpc4 gene was examined by direct sequencing in 12 tumors showing LOHs on 18q21.1. However, alterations were not found, except for the intron 7 in three tumors (patient 5, 20, and 30) and the intron 10 in one tumor (patient 34) (data not shown). It is not certain whether these alterations reflect the abnormality in Smad4/DPC4 function.

Table 1 The PCR primer sequences of each microsatellite marker

Locus symbols	Chromosomes	Susceptible genes	Sequences	Size(bp)
D18S46	18q21.1	DPC4, DCC	Sense 5′-GAATAGVAGGAVVTATCAAAGAGA-3′ Antisense 5′-CAGATTAAGTGAAAACAGCATATGTG-3′	129–153
D18S363	18q21.1	DPC4, DCC	Sense 5′-TTGGGAACTGCTCTACATTC-3′ Antisense 5′-GCTTCATTCTCTCACTGGAT-3′	177–247
D18S474	18q21.1	DPC4, DCC	Sense 5′-TGGGGTGTTTACCAGCATC-3′ Antisense 5′-TGGCTTTCAATGTCAGAAGG-3′	119–139
D9S171	9p21	P16	Sense 5′-AGCTAAGTGAACCTCATCTCTGTCT-3′ Antisense 5′-ACCCTAGCACTGATGGTATAGTCT-3′	159–177
D9S747	9p21	P16	Sense 5′-GCCATTATTGACTCTGGAAAAGAC-3′ Antisense 5′-CAGGCTCTCAAAATATGAACAAAAT-3′	182–202
D9S1747	9p21	P16	Sense 5′-GGCTTTCTCTCTCTTTTTGTCTC-3′ Antisense 5′-GGAATAAATCAGGCTACCAGG-3′	120–140
IFNA	9p22	P16	Sense 5′-TGCGCGTTAAGTTAATTGGTT-3′ Antisense 5′-GTAAGGTGGAAACCCCCACT-3′	138–150
TP53	17p13.1	P53	Sense 5′-AGGGATACTATTCAGCCCGAGGTG-3′ Antisense 5′-ACTGCCACTCCTTGCCCCATTC-3′	103–135

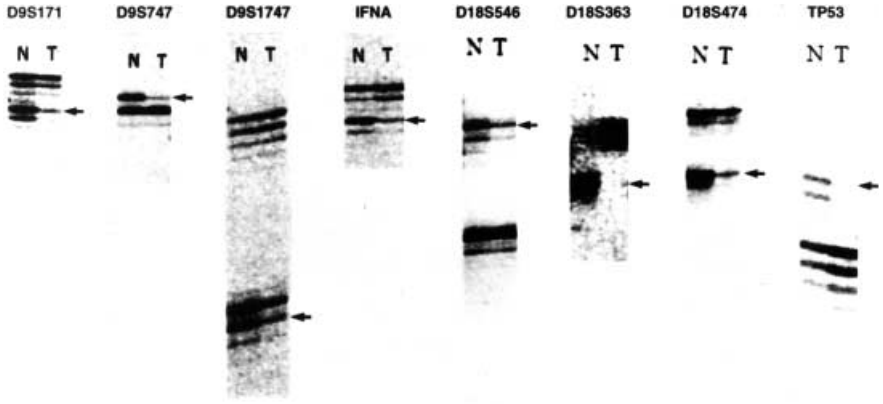

Fig. 1 Representative microsatellite alterations on each marker. *Arrows* indicate abnormal bands. *N* normal DNA sample, *T* tumor DNA samples

Table 2 The frequency of LOH on each chromosome arm determined by microsatellite analysis

Pt no.	Grade	TNM	D18S474	D18S46	D18S3634	D9S171	D9S747	D9S1747	IFNA	TP53
1	3	pT3bN0M1	0	0	0 (RER)	x	1	x	0	1
2	3	pT3aN0M0	0	0	0	x	0	x	0	0
3	3	pTisN0M0	0	0	0	0	x	0	x	1
4	2	pT1bN0M0	0	0	0	x	x	0	0	0
5	3	pT3bN0M0	1	0	1	x	x	0	1	0
6	3	pT4N0M1	x	1	0	x	1	0	1	1
7	2	pT1bN0M0	0	0	0 (RER)	x	x	0	x	1
8	2	pT1aN0M0	0	0	0	x	x	0	x	0
9	2	pTabN0M0	0	0	0	x	x	0	x	0
10	3	pT4N1M0	0	x	0	1	1	0	x	1
11	3	pT3bN1M0	0	x	0	x	x	0	x	1
12	3	pT4N1M1	1	x	1	1	1	1	1	1
13	3	pT4N1M1	0	1	1	x	x	0	0	0 (RER)
14	2	pT1AN0M0	0	0	1	x	x	0	1	1
15	2	pT1bN0M0	0	0	1	0	0	1	1	1
16	2	pT1aN0M0	x	x	0	0	0	0	0	0
17	3	pT1bN1M0	0	0	x	0	x	x	0	x
18	3	pT1bN0M0	1	1	x	0	x	x	0	x
19	2	pT2N0M0	0	0	0	0	1	x	x	0
20	3	pT3aN1M1	1	1	0	0	1	0	1	1
21	2	pT1aN0M0	0	0	0	0	1	1	0	0
22	2	pT1aN0M0	0	0	0	0	x	0	0	0
23	3	pT2N0M0	x	1	0	x	1	0	0	0
24	2	pTaN0M0	0 (RER)	0	0	1	1	0	0	0
25	2	pTaN0M0	0	0	0	x	x	0	1	1
26	2	pT1aN0M0	0	0	0	x	0	x	1	1
27	3	pT1bN0M0	x	0	1	x	0	0	x	0
28	3	pT2N0M0	x	0	1	x	x	0	x	0
29	1	pT1aN0M0	0	0	0	0	0	0	x	0
30	3	pT4N1M1	1	1	1	0	1	0	x	x
31	2	pTaN0M0	0	0	0	x	x	0	x	0
32	1	pTaN0M0	0	0	0	x	x	0	1	1
33	3	pT4N1M1	0	1	0	1	x	x	x	0
34	3	pT3N1M0	0	x	1	1	0	0	0	x
35	3	pT3N1M0	0	x	1	0	0	0	0	0
36	3	pT1bN0M0	0	0	1	x	1	0	1	0
37	3	pT3N0M0	0	1	0 (RER)	1	1	0	1	1
38	3	pT1bN0M0	1	1	0	0	0	0	0	0
39	3	pT1aN0M0	0	x	0	0	0	0	0	x
40	2	pT1N0M0	x	0	x	0	0	0	0	1
41	2	pTaN0M0	0	0	0	0	0	0	0	0
42	2	pT1aN0M0	0	0	x	0	0	0	x	1
43	2	pTaN0M0	0	0	0	0	x	x	0	0
44	1	pT1aN0M0	x	0	0	0	0	0	0	0
45	2	pT1aN0M0	0	0	x	0	0	0	0	0

1, LOH; 0, heterozygosity; x, not informative; RER, replication error

Table 3 Distribution of 45 bladder tumor patients according to LOH of 18q21.1, 9p21–22,and 17p13.1 categorized by the other established prognostic factors. Tumor grade and T category had a significant relationship to the LOH of 18q21.1. Although T category, age, and the LOH of 9p21–22 were significantly correlated, there was no significant relation between the LOH of 17p13.1 and any other prognostic factors

Prognostic factors	Variables	LOH18q21.1			*P* value	LOH 9p21–22			*P* value	LOH 17p13.1			*P* value	Two LOH (18q and 9p)			*P* value
		−	+			−	+			−	+			−	+		
Tumor grade	G1–2	20	2	22		14	8	22		15	7	22		20	2	22	
	G3	8	15	23	< 0.01	10	13	23	0.24	14	9	23	0.76	12	11	23	< 0.01
T category	Superficial (T < 2)	22	6	28		19	9	28		19	9	28		24	4	28	
	Invasive (T2)	6	11	17	< 0.01	5	12	17	0.02	10	7	17	0.75	8	9	17	< 0.01
N and M category	N0M0	23	11	34		20	14	34		24	10	34		26	8	34	
	N1 or M1	5	6	11	0.28	4	7	11	0.3	5	6	11	0.16	6	5	11	0.25
Age	−75	24	12	36		22	14	36		24	12	36		28	8	36	
	75<	4	5	9	0.27	2	7	9	0.06	5	4	9	0.7	4	5	9	0.09
LOH9p21–22		20	4	24						18	6	24					
	+	8	13	21	< 0.01					11	10	21	0.13				
LOH17p13.1	−	18	11	29										22	7	29	
	+	10	6	16	> 0.98									10	6	16	0.49

The LOHs of the microsatellite markers on each respective chromosome were then compared with the other established prognostic factors (tumor grade, TNM classification, and age) (Table 3). When the tumors were classified as either low-grade (grade 1 or 2) or high-grade (grade 3), 15/23 (65%) high-grade tumor samples demonstrated at least one microsatellite marker loss on chromosome 18q21.1. On the other hand, only 2/22 (9.1%) low-grade tumor samples exhibited an LOH with the difference being statistically significant ($P < 0.01$). Although the LOHs of 17p13.1 or 9p21–22 were more frequently observed in high-grade tumors than in low-grade ones, the difference was not statistically significant.

When the tumors were classified as superficial (pTa/pT1) and muscle-invasive (T2), 11 (65%), 12 (71%), and 7 (41%) of the 17 muscle-invasive tumors exhibited at least one marker LOH on chromosome 18q21.1, 9p21–22, and 17p13.1, respectively. The presence of LOH on chromosome 18q21.1 or 9p21–22 and muscle invasion were found to be statistically significant ($P < 0.05$), but not on chromosome 17p13.1. It should be emphasized that LOH of 18q21.1 was highly related to that of 9p21–22, although neither of them related to that of 17p13.1.

In the subsequent step, the survival rates categorized with the prognostic factors were estimated by the Kaplan–Meier method. The log–rank test results for univariate analyses are shown in Table 4. A statistical significance was found between positive and negative groups of LOH of 18q21.1 (3-year survival; 53% for the positive group and 92% for the negative group, $P < 0.01$). As done for 18q21.1, the group with an LOH of 9p21–22 demonstrated a poor prognosis (3-year survival; 61% for the positive group and 91% for the negative group, $P = 0.02$). No statistical difference was found in the groups with and without an LOH

Table 4 Actuarial survival rates according to various prognostic factors estimated by Kaplan–Meier method and Cox–Mantel test

Prognostic variables	3-year survival			
		n	Rate	*P* value
T category	Ta-1	28	0.96	< 0.01
	T2–4	17	0.49	
N and M category	N0M0	34	0.90	< 0.01
	N+/M+	11	0.42	
Grade	G1–2	22	0.95	0.01
	G3	23	0.61	
Age	< 75	36	0.80	0.05
	> 75	9	0.62	
LOH18q2l.l	−	28	0.92	< 0.01
	+	17	0.53	
LOH9p21–22	−	24	0.91	0.02
	+	21	0.61	
LOH17p13.1	−	29	0.82	0.16
	+	16	0.69	
2 LOH (18q and 9p)	−	32	0.93	< 0.01
	+	13	0.34	

of 17p13.1 (3-year survival; 69% for the positive group and 82% for the negative group, $P = 0.16$). The other known prognostic factors, such as tumor grade, tumor stage (TNM classification), and age were also estimated by the Kaplan–Meier method to ascertain how these factors influenced the prognosis of these patients. There was also significance in the survival rate (Table 4). Patients having multiple LOHs of both 18q21.1 and 9p21–22 exhibited extremely poor prognosis compared with single LOH or no LOH patients ($P < 0.01$, Fig. 2).

Multivariate analysis using Cox proportional hazards model was performed to identify statistically independent factors in patients' survival. When variables considered above were chosen in stepwise reduction, "2 LOHs of 18q21.1 and 9p21–22" (relative death rate = 9.5, $P < 0.01$), and "N and M categories" (relative death rate = 4.5, $P = 0.03$) were the independent prognostic factors.

Discussion

It has been suggested that sequential genetic events are associated with both tumor invasiveness and progression in bladder cancer [6, 22, 23, 26, 29], as has already been described for colon carcinomas [5]. Therefore, this concept may also be applicable to the differentiation between papillary superficial and nodular invasive bladder cancer. Studies in recent years have identified a number of genes as regulators of cancer cell growth. One of the mechanisms for controlling tumor growth might involve direct regulation of the cell cycle. Genes that control the decision to initiate DNA replication, therefore, appear to be attractive candidates as either oncogenes or tumor suppressor genes, depending on whether they play a stimulatory or inhibitory role in the process. One such gene, p53, has emerged as one of the important tumor

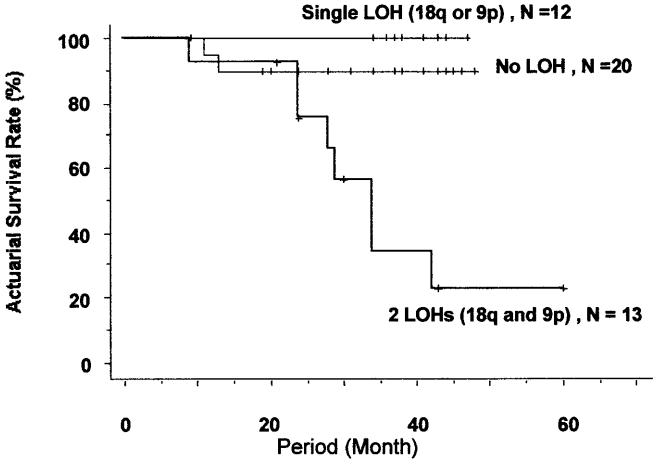

Fig. 2 Actuarial survival rate according to the presence or absence of LOH of 18q21.1 and 9p21–22. The patient group with multiple LOHs on both 18q.21.1 and 9p21–22 tended to have lower survival rates compared with single or no LOH patients (3-year percent survival; 34% vs 93%)

suppressor genes in human cancer [14, 15]. Extensive studies on this gene have been reported, particularly regarding bladder cancer. Accumulated findings taken together point to the conclusion that the frequency of p53 gene mutation is usually low in superficial and low-grade cancer, while it is very high in high-grade and muscle-invasive cancers [7, 24]. According to our univariate analysis, an LOH of 17p13.1 did not clearly demonstrate a statistical significance on patients' survival. It has been reported that patients tended to have a lower survival rate when their p53 genes were mutated according to the PCR-SSCP (single-strand conformation polymorphism) method, whereas no difference in patients' survival was found if the same patients were categorized by microsatellite analysis [27]. Our result is consistent with theirs, suggesting that LOHs are less common in p[4] alterations in bladder cancer. For more sensitive detection, other procedures, such as immunohistochemical detection [4], are recommended.

Unlike 17p13.1, there was a relationship between the clinical outcome and LOH of 18q21 and 9p21 in bladder cancer patients. On 18q21.1, SMAD4/DPC4 is one of the possible tumor suppressors, whose loss or inactivation may contribute to the development of pancreatic cancer [10, 11]. It acts as a mediator of the HGF-β (hepatocyte growth factor β signaling pathway [30]. Since LOH of the locus 18q21 has been demonstrated in bladder cancers [1], the alteration of SMAD4/DPC4 may relate to their carcinogenesis or progression. According to our univariate analysis, LOH of 18q21.1 affected patients' survival significantly. However, we could not find any mutations on the smad4/dpc4 locus except intron sequences in patients who had multiple LOHs in 18q21. It should be noted that other tumor suppressor genes, including bcl-2 and dcc, are encoded on 18q. Further investigations are encouraged to determine which gene on 18q is responsible for poor prognosis of bladder tumor patients.

Previous studies demonstrated the frequency of LOH in 9p as 33–62% [2, 28]. Our result showed LOHs on 9p21–22 as 47% (21/45) of bladder tumors examined and, especially, 71% (12/17) of invasive tumors. Furthermore, LOH of 9p21–22 correlated the pathological invasiveness. Recently, Hartmann et al. [13] studied in situ hybridization on 9p showing LOHs as an early event in the progression of bladder cancer. The discrepancy among the reports may come from the different methods employed. In general, microsatellite analysis tends to underestimate the allelic loss because of amplification of the normal allele by PCR, while technical difficulties of in situ hybridization tend to overestimate it.

Frequent abnormalities in chromosome 9p21 are found in bladder tumors, melanoma, glioma, leukemia, and the other human malignancies that were identified as alterations of p16/CDKN2 locus [2]. The role of P16/CDKN2 appears to be potentially inhibitory of the phosphorylation of Cyclin D by CDK4 and CDK6 [16]. Since P16/CDKN2 consequently inhibits the phosphorylation of pRB [3], it also acts as a tumor suppressor. Inactivation of Rb may have a similar role to that of

P16/CDKN2 in controlling cell cycle in bladder cancer. In addition, not only the deletion, but also other mechanisms, such as 2-hit mutations and hypermethylation of the 5′CpG island upstream of the p16/CDKN2, also inactivate p16/CDKN2 [8]. It is actually difficult to reveal the genetic events in the individual primary bladder cancers. However, despite the low specificity, microsatellite analysis is a useful procedure for demonstrating major genetic alterations [15, 17], because simple DNA repeats can be readily detected in clinical samples by PCR. Tandem repeat DNA sequences, known as microsatellites, represent a very common and highly polymorphic class of genetic elements within the human genome. These microsatellite markers, which contain small repeat sequences, have been used for primary gene mapping and linkage analysis. PCR amplification of these microsatellite markers facilitates the rapid assessment of a loss of heterozygosity and can also greatly simplify the procedures for mapping tumor suppressor genes.

Mao et al. [18] examined various microsatellite sequences in the DNA of normal and tumor pairs from 100 head and neck, bladder, and lung cancer patients, and identified 26 that displayed alterations in at least one locus. They concluded that appropriately selected microsatellite loci are commonly altered in many cancers, and can therefore serve as clonal markers for their detection. Furthermore, they have recently expanded microsatellite DNA markers for the detection of genetic abnormalities of urine sediment from patients suspected of having a bladder tumor [19]. They indicated that microsatellites matching those in the tumor were detected in the urine sediment of 19/20 patients (95%) diagnosed with bladder cancer. Thus, microsatellite DNA markers have been widely used as a tool for the detection of genetic abnormalities.

Non-random chromosome losses demonstrate a stepwise neoplastic transformation in human transitional cells in vitro [29]. Among them, genetic losses in the 18q21.1 are associated with the development of high-grade malignancies of transitional cells. In the present study, we prepared 360 microsatellite analyses for a panel of three different chromosomal regions adjacent to the loci of suppressor genes such as smad4/dpc4, p16/CDKN2, and p53. The clinical data presented herein further support them. Therefore, the results indicate that microsatellite analysis of LOHs on a variety of chromosome regions is clinically useful for determining prognostic factors for patients with bladder cancer.

References

1. Brewster SF, Gingell JC, Browne S, Brown KW (1994) Loss of heterozygosity on chromosome 18q is associated with muscle-invasive transitional cell carcinoma of the bladder. Br J Cancer 70: 697
2. Cairns P, Polascik TJ, Eby Y, Tokino K, Califano J, Merlo A, Mao L, Herath J, Jenkins R, Westra W, et al (1995) Frequency of homozygous deletion at p16/CDKN2 in primary human tumours. Nat Genet 11: 210
3. Dryja TP, Rapaport JM, Joyce JM, Petersen RA (1986) Molecular detection of deletions involving band q14 of chromosome 13 in retinoblastomas. Proc Natl Acad Sci USA 83: 7391
4. Esrig D, Spruck CHD, Nichols PW, Chaiwun B, Steven K, Groshen S, Chen SC, Skinner DG, Jones PA, Cote RJ (1993) p53 nuclear protein accumulation correlates with mutations in the p53 gene, tumor grade, and stage in bladder cancer. Am J Pathol 143: 1389
5. Fearon ER, Cho KR, Nigro JM, Kern SE, Simons JW, Ruppert JM, Hamilton SR, Preisinger AC, Thomas G, Kinzler KW, et al (1990) Identification of a chromosome 18q gene that is altered in colorectal cancers. Science 247: 49
6. Fearon ER, Feinberg AP, Hamilton SH, Vogelstein B (1985) Loss of genes on the short arm of chromosome 11 in bladder cancer. Nature 318: 377
7. Fujimoto K, Yamada Y, Okajima E, Kakizoe T, Sasaki H, Sugimura T, Terada M (1992) Frequent association of p53 gene mutation in invasive bladder cancer. Cancer Res 52: 1393
8. Gonzalez-Zulueta M, Bender CM, Yang AS, Nguyen T, Beart RW, Van Tornout JM, Jones PA (1995) Methylation of the 5′ CpG island of the p16/CDKN2 tumor suppressor gene in normal and transformed human tissues correlates with gene silencing. Cancer Res 55: 4531
9. Gyapay G, Morissette J, Vignal A, Dib C, Fizames C, Millasseau P, Marc S, Bernardi G, Lathrop M, Weissenbach J (1994) The 1993–94 Genethon human genetic linkage map [see comments]. Nat Genet 7: 246
10. Hahn SA, Hoque AT, Moskaluk CA, da Costa LT, Schutte M, Rozenblum E, Seymour AB, Weinstein CL, Yeo CJ, Hruban RH, Kern SE (1996) Homozygous deletion map at 18q21.1 in pancreatic cancer. Cancer Res 56: 490
11. Hahn SA, Schutte M, Hoque AT, Moskaluk CA, da Costa LT, Rozenblum E, Weinstein CL, Fischer A, Yeo CJ, Hruban RH, Kern SE (1996) DPC4, a candidate tumor suppressor gene at human chromosome 18q21.1 [see comments]. Science 271: 350
12. Harmer M (1987) TNM classification of malignant tumors. International Union Against Cancer, Geneva
13. Hartmann A, Moser K, Kriegmair M, Hofstetter A, Hofstaedter F, Knuechel R (1999) Frequent genetic alterations in simple urothelial hyperplasias of the bladder in patients with papillary urothelial carcinoma. Am J Pathol 154: 721
14. Hollstein M, Sidransky D, Vogelstein B, Harris CC (1991) p53 mutations in human cancers. Science 253: 49
15. Jones MH, Nakamura Y (1992) Detection of loss of heterozygosity at the human TP53 locus using a dinucleotide repeat polymorphism. Genes Chromosomes Cancer 5: 89
16. Kamb A, Gruis NA, Weaver-Feldhaus J, Liu Q, Harshman K, Tavtigian SV, Stockert E, Day RS III, Johnson BE, Skolnick MH (1994) A cell cycle regulator potentially involved in genesis of many tumor types [see comments]. Science 264: 436
17. Kwiatkowski DJ, Diaz MO (1992) Dinucleotide repeat polymorphism at the IFNA locus (9p22). Hum Mol Genet 1: 658
18. Mao L, Lee DJ, Tockman MS, Erozan YS, Askin F, Sidransky D (1994) Microsatellite alterations as clonal markers for the detection of human cancer. Proc Natl Acad Sci USA 91: 9871
19. Mao L, Schoenberg MP, Scicchitano M, Erozan YS, Merlo A, Schwab D, Sidransky D (1996) Molecular detection of primary bladder cancer by microsatellite analysis. Science 271: 659
20. Mostofi FK (1973) Histological typing of urinary bladder tumours. International histological classification of tumours
21. Nigro JM, Baker SJ, Preisinger AC, Jessup JM, Hostetter R, Cleary K, Bigner SH, Davidson N, Baylin S, Devilee P, et al (1989) Mutations in the p53 gene occur in diverse human tumour types. Nature 342: 705
22. Sandberg AA (1986) Chromosome changes in bladder cancer: clinical and other correlations. Cancer Genet Cytogenet 19: 163
23. Sidransky D, Messing E (1992) Molecular genetics and biochemical mechanisms in bladder cancer. Oncogenes, tumor suppressor genes, and growth factors. Urol Clin North Am 19: 629

24. Sidransky D, Von Eschenbach A, Tsai YC, Jones P, Summerhayes I, Marshall F, Paul M, Green P, Hamilton SR, Frost P, et al (1991) Identification of p53 gene mutations in bladder cancers and urine samples. Science 252: 706
25. Straub RE, Speer MC, Luo Y, Rojas K, Overhauser J, Ott J, Gilliam TC (1993) A microsatellite genetic linkage map of human chromosome 18. Genomics 15: 48
26. Tsai YC, Nichols PW, Hiti AL, Williams Z, Skinner DG, Jones PA (1990) Allelic losses of chromosomes 9, 11, and 17 in human bladder cancer. Cancer Res 50: 44
27. Uchida T, Wada C, Ishida H, Wang C, Egawa S, Yokoyama E, Kameya T, Koshiba K (1995) p53 mutations and prognosis in bladder tumors. J Urol 153: 1097
28. Williamson MP, Elder PA, Shaw ME, Devlin J, Knowles MA (1995) p16 (CDKN2) is a major deletion target at 9p21 in bladder cancer. Hum Mol Genet 4: 1569
29. Wu SQ, Storer BE, Bookland EA, Klingelhutz AJ, Gilchrist KW, Meisner LF, Oyasu R, Reznikoff CA (1991) Nonrandom chromosome losses in stepwise neoplastic transformation in vitro of human uroepithelial cells. Cancer Res 51: 3323
30. Zhang Y, Feng X, We R, Derynck R (1996) Receptor-associated Mad homologues synergize as effectors of the TGF-beta response. Nature 383: 168