Allelic Deletion Fingerprinting of Urine Cell Sediments in Bladder Cancer

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Background: Bladder cancer shows frequent nonrandom allelic deletion at various chromosomal regions. Genotypic detection methods could potentially identify patients at risk for recurrent progressive disease. In this study, we examined allelic deletion at specific chromosomal loci in tumor tissue and urine cell sediment samples using a microsatellite-based protocol. Although both allelic deletion and microsatellite instability have been reported in primary bladder cancer, microsatellite instability was not specifically examined in this study. We report a pilot study of 40 patients with bladder cancer in which allelic deletion in tumor tissue and urine cell sediment was compared with conventional urine cytology results.

Methods and Results: Forty tumors were analyzed using a set of microsatellite primers from chromosomes 3, 4, 8, 11, 14, and 17 to construct allelic deletion fingerprints. Cy5.5-labeled PCR products were analyzed using the OpenGene System and GeneObjects software. Eighty-eight percent of tumors showed allelic deletion. In urine cell sediments, the tumor detection rate was 80% compared with 50% for routine urine cytology. The allelic deletion fingerprinting (ADF) procedure identified 69% of incipient tumors, cases initially classified as normal by routine urine cytology.

Conclusion: ADF analysis provides a reliable noninvasive method for the detection and monitoring of recurrent cancer in urine cell sediment samples from patients with bladder cancer.

Key words: bladder cancer, allelic deletion, microsatellite, noninvasive, surveillance.

Transitional cell carcinoma (TCC) of the bladder is the fourth most common human malignancy. The majority of primary bladder cancers are noninvasive superficial tumors that tend to recur as potentially invasive lesions in most patients. The recur rence of potentially malignant invasive bladder cancer in these patients requires repeated expensive surveillance cystoscopy [1,2]. In practice, two phenotypically similar tumors may have different clinical courses or responses to treatment. This situation confounds the ability to predict and individualize a patient's risk for either benign tumor recurrence or progression to recurrent malignant/invasive bladder cancer. For this reason, genotypic detection methods may be able to identify patients likely to develop progressive disease, which could enhance

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tumor surveillance protocols, reduce cost, and potentially improve survival. TCC of the bladder shows frequent nonrandom loss of heterozygosity (LOH) at various chromosomal regions. These allelic deletions have proven useful in the detection of cancer cells. Allelic deletion of highly polymorphic tandem repeat DNA sequences, known as microsatellites, has been used for the detection of LOH in primary tumors. Bladder cancer develops from the expansion of a clone(s) of malignant cells in the urothelium. To date, no unequivocal genetic progression model has been defined for TCC [3], although the accumulation of, rather than order of, genetic alterations likely is critical. LOH at chromosome 17p13, including mutation of the p53 gene, is a frequent event in high-grade bladder cancer [4]. In a study of patients with primary carcinoma *in situ* (Tis) of the bladder, LOH of 9p was found in 61% of lesions, suggesting that this genetic change occurs early in progression [5]. LOH also occurs frequently at 3p, 4q, 8p, 9q, 11p, 14q, and 18q loci, indicating that other important tumor-suppressor genes may be present in these regions [5,6]. Deletions on 3p, 4q, 11p, and 18q have been reported to correlate with more advanced disease [7].

Urine cytology is universally accepted as the noninvasive test for the diagnosis and surveillance of TCC [8]. Although useful in clinical practice, routine voided urinary cytology has an overall sensitivity of less than 50%, which varies with tumor grade, tumor stage, and urine collection and processing methods used. The benefit of urine cytology lies in the high positive predictive value of detecting a tumor. However, the greatest weakness of urine cytology is the lack of sensitivity in the detection of low-stage and low-grade tumors. Furthermore, inflammatory lesions, intravesical therapy, or radiation-associated changes can lead to false-positive urine cytology results [9].

Microsatellite sequence alterations are readily detectable in urine samples using PCR. Mao et al. [10] showed a 95% tumor detection rate with a set of 13 microsatellites. This molecular analysis reliably detected tumors of all grades and stages, although the population studied included many patients with high-grade and advanced-stage disease. The potential for using these molecular markers with exfoliated urothelial cell DNA could establish allelic deletion fingerprinting (ADF) as a method of surveillance for both tumor recurrence and progression and as an adjunct to or even substitute for cystoscopy and cytology. The objective of the present study is to investigate the feasibility of using DNA from exfoliated urothelial cells in urine for both tumor detection and surveillance using a microsatellite-based ADF protocol.

Methods

Sample Collection

Tumor and urine specimens were collected at the Toronto Hospital (Toronto, Canada) from 40 consecutive patients undergoing transurethral resection of primary or recurrent TCC of the bladder. The 40 patients (32 men, 8 women) had a mean age of 73 years (range, 31 to 88 years). This study received approval from the Hospital Research Ethics Board. The study was reviewed at start-up and was subject to annual review and renewal. Included in this annual review was the reporting of all adverse events, patient accrual numbers, expected study outcome, expected study end date, changes in study protocol, and changes in consent protocol. Informed consent was obtained from each patient included on this study before tumor resection. At the time of tumor resection, blood samples and 50-mL voided urine samples were collected from all patients. Tumor samples were snap frozen in liquid nitrogen before DNA extraction. A representative section of tumor was fixed in formalin, stained with hematoxylin and eosin, and graded and staged according to World Health Organization criteria [11] and tumor, node, metastasis (TNM) classification [12]. Urine was collected in disposable 50-mL Falcon tubes and centrifuged at 3,000 rpm for 10 minutes. After decanting the supernatant, sediments were resuspended in physiological saline and recentrifuged. The resulting pellet was stored at −80°C until DNA extraction. Venous blood from each patient was collected in EDTA tubes and peripheral-blood leukocytes were isolated. Peripheral-blood leukocyte DNA served as the normal tissue control.

DNA Extraction

DNA from blood and urine-cell pellets was isolated using the PureGene DNA isolation kit

(Gentra Systems Inc, Minneapolis, MN) according to the manufacturer's instructions. Frozen tumor tissue was cut into sections and digested with proteinase K at 55°C for 24 hours, followed by DNA isolation using the PureGene DNA isolation kit.

Microsatellite Allelic Deletion Analysis

The panel of microsatellite primers used in this study was selected from the Genome Database (http://www.gdb.org) and the current literature [6] based on their preferential allelic deletion in advanced recurrent bladder cancer versus primary superficial/papillary bladder cancer. The microsatellite loci chosen for assessment were d3s1067, d4s43, d8s201, d9s126, pTEN, d11s907, d11s935, d14s267, d14s288, d17s578, and TP53. In all microsatellite sequence primer pairs, only the 3' or reverse primer was end-labeled with CY5.5 fluorescent dye (Visible Genetics Inc, Toronto, ON, Canada). Genomic DNA (20 to 100 ng) was subjected to 25 cycles of PCR in a final volume of 20 µL containing 1 × PCR buffer (10 mM Tris-HCl [pH 8.3], 50 mM KCl, 1.5 mM MgCl2), 3 mM dNTP, 5% dimethyl sulfoxide, 1.0 U Taq DNA polymerase (Life Technologies Inc, Gaithersburg, MD), and 2.5 pmole each unlabeled and CY5.5-labeled primers. The following protocol was used: 94°C for 5 minutes, 25 cycles of 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1 minute, followed by 72°C for 5 minutes. Two microliters PCR product was mixed with 2 µL loading buffer and denatured at 90°C for 1 minute, and 2 µL was loaded on a Microcel in the OpenGene System (Visible Genetics Inc, Toronto, ON, Canada). Run parameters for the OpenGene System were 1,300 V for 30 minutes at a plate temperature of 54°C and a laser setting of 5 mW. Data were processed and analyzed using the GeneObjects Fragment Analysis Tool (Visible Genetics Inc).

Microsatellite Allelic Deletion Criteria

Allele ratios in both normal and tumor samples were calculated and compared using data collected using the GeneObjects Fragment Analysis Tool. The area under each peak, representing each allele in the microsatellite pair, was obtained using the GeneObjects Fragment Analysis Tool, and an allele deletion ratio was calculated by dividing the area of the first/smaller peak/allele 1 by the area of the second/larger peak/allele 2 in each of the normal, tumor, and urine DNA samples. Theoretically, the allelic deletion ratio should be near 1 for cases in which no allelic deletion is present (normal control DNA) and range from 0.5 to 2 for cases in which either allele 1 or allele 2 is reduced by 50% (tumor DNA). Each allelic deletion analysis was repeated in triplicate for each sample for each microsatellite. Ratio values for each microsatellite allelic deletion analysis were compared for reproducibility, and additional confirmatory analyses were performed as necessary. Allelic deletion was scored as present in informative (heterozygous; two alleles of different sizes) cases when the allele ratio value of normal control samples (~1.0) differed by at least 40% from the allele ratio value of tumor samples (<0.6 or >1.4). Allelic deletion was not scored in uninformative (homozygous; two alleles of the same size) cases.

Results

The 40 tumors analyzed in this study were of various, but representative, histopathologic grades and stages (Table 1). Table 2 lists the 11 microsatellites examined in this study and indicates the percentage of informative cases and percentage of allelic deletion for each microsatellite in the 40 tumors. For the ADF study, only microsatellites that were at least 80% informative were used. Because of their low degree of allelic heterozygosity, mic-

Table 1. Distribution of Tumor Grade and Stage in 40 Patients With Papillary Bladder Cancer

	Grade[1]				
Stage*	G1	G2	G3	Cis	Total
Ta	5	11	1	0	17
T1	0	3	9	0	12
T2	0	2	3	0	5
Tis	0	0	0	6	6
Total	5	16	13	6	40

Tis; *in situ* carcinoma; Cis, carcinoma *in situ*.
*Tumor stage and grade according to World Health Organization criteria and tumor; node; metastasis classification.

Table 2. Percentage of Informative and Allelic Deletion Cases at the 11 Microsatellite Loci Examined in 40 Patients With Papillary Bladder Cancer

Locus*	Position†	Informative (%)‡	LOH (%)§
D3S1067	3p14.3-21.1	96	33
D4S43	4p16.3	94	37
D8S201	8pter-23.1	91	43
D9S126	9p21	73	66
PTEN	10q22-q23	51	59
D11S907	11p13	88	52
D11S935	11p12-13	82	51
D14S267	14q32.1-32.2	98	27
D14S288	14q13-21	99	31
D17S578	17pter	84	63
TP53	17p13.1	98	55

LOH, loss of heterozygosity or reduction to homozygosity.

*Chromosomal locus name and microsatellite marker designation from the Genome Database.

†Chromosomal location for each microsatellite locus.

‡Percentage of cases displaying allelic heterozygosity (two alleles of different sizes) for each microsatellite.

§Percentage of cases showing allelic loss (loss of one of the two alleles) for each microsatellite.

rosatellites d9s126 and PTEN were not used for further analysis, leaving nine microsatellites as the reported set of ADF microsatellites. With this set of microsatellites, 88% of the tumors (35 of 40 tumors) showed LOH at one or more loci, and 40% of the tumors (16 of 40 tumors) showed LOH at four or more loci (Fig. 1). To show the tumor allelic deletion fingerprint, we introduce the ADF barcode, listed in Table 3.

The concordance of ADF pattern in urine and matched tumor samples is listed in Table 4. In 32 of these urine samples, one or more allelic deletion was present, providing an overall tumor detection rate of 80% (32 of 40 samples). Urine cytology showed malignant cells in 20 patients, whereas the remaining 20 patients were classified as normal or inconclusive, providing a tumor detection rate with cytology alone of 50% (20 of 40 samples). Of 13 patients classified as normal by routine urine cytology, ADF analysis detected the presence of tumor cells in 69% of these patients (9 of 13 patients).

Discussion

Allelic deletion/LOH is the most common genetic alteration detected in primary bladder cancer. Knowles et al. [6] found that 95% of TCCs of the bladder had LOH in at least one locus, suggesting that LOH analysis might be suitable for surveillance of the disease. LOH frequencies observed in the present study agree with those reported for TCCs in the literature [3–6,13,14]. There are pertinent data to support that a target site for primary lesions in

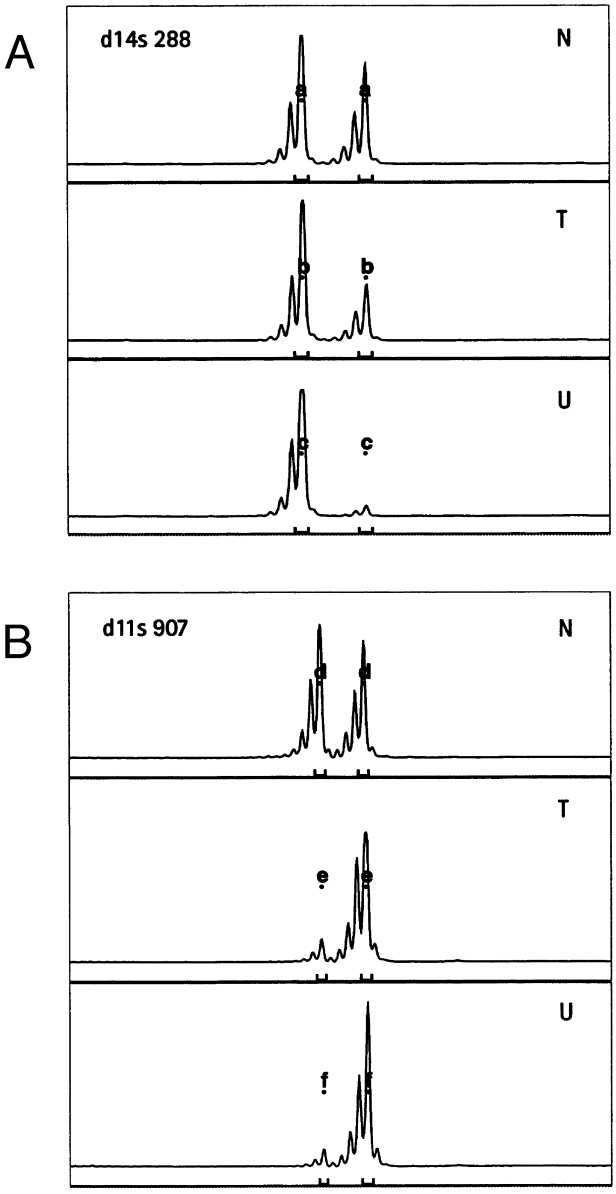

Fig. 1. Allelic loss in one set of matched normal (N), tumor (T), and urine (U) DNA samples from a patient with transitional cell carcinoma analyzed using the GeneObjects Fragment Analysis software. Allele ratios in normal, tumor, and urine samples were calculated and compared. Allelic loss was scored in informative cases when the allele ratio differed by at least 40% between the normal, tumor, and urine samples. (A) Locus primer D14S288 with allelic loss in T and U. (B) Locus primer D11S907 with allelic loss in T and U.

low-grade superficial TCC may reside on chromosome 9 [7,13], and markers for this locus may be considered obligatory when dealing with early detection protocols for primary bladder cancer. Primary reasons for not including microsatellite markers for chromosomes 9p (D9S126) and 10q (PTEN) in the ADF protocol were their low degrees of heterozygosity. The utility of the ADF protocol relies on the ability to easily detect and assess allelic deletion/LOH in normal, tumor, and urine cell sediment DNA samples from the same patient. This ease of detection is predicated on the choice of a microsatellite marker that is routinely informative and routinely shows two alleles of different sizes for every sample tested. Microsatellite markers that are routinely noninformative and consequently have low degrees of heterozygosity routinely show two alleles of the same size, which complicates ADF analysis and confounds allelic deletion/LOH assessment. In addition, assignment of allelic deletion/LOH from homozygous noninformative samples can be further complicated by contamination of samples with normal tissue or normal cells. The final set of ADF microsatellite markers was chosen for their preferential allelic deletion/LOH in recurrent invasive bladder cancer. Although D9S126 and PTEN show allelic deletion in primary bladder cancer and may be useful in generalized screening protocols that have detection of primary bladder cancer as their objective, their use in an ADF protocol for patients already diagnosed with primary bladder cancer as differentiators between benign/recurrent bladder cancer and invasive/recurrent bladder cancer is less clear. Although D9S126 has the greatest degree of allelic deletion in this study, it is also one of the least informative markers in this study. We included this microsatellite marker as an allelic deletion standard to determine whether our primary bladder cancer samples were unusual in their genotype or allelic deletion profile for this chromosomal region. As expected, loci on chromosome 9p, including D9S126, showed a significant degree of allelic deletion in agreement with values published for other primary bladder cancer studies.

We detected allelic deletions in 88% of tumor biopsy samples and 80% of urine samples in our study, in concordance with results reported in an earlier study [10]. This similarity may support the feasibility of the microsatellite approach for urine analysis. A follow-up study from the same investigators using 21 patients detected recurrent lesions in ten of 11 patients, and the test was negative in ten of ten patients with no evidence of recurrent tumor [14]. The correlation between paired urine and tumor samples in our study indicates that analysis of urine cell sediments could provide a reliable evaluation of the primary tumor genotype as a complement to the tumor phenotype provided by conventional cytology. The sensitivity of ADF analysis depends on the proportion of tumor cells versus normal cells in the urine cell sediment sample. Results of the pathology report must always be considered when interpreting negative results. In the present report, seven cases (18%) showed an inconsistency between allelic deletion/LOH in paired urine and tumor samples, caused by the presence of normal cells in the tumor biopsy specimen that mask the tumor cell allelic loss [15]. Confounding factors, not easily avoidable or quantifiable, in the ADF analysis are numbers or percentages of normal tissue in tumor biopsy specimens and the ratio of normal and tumor cells in urine cell sediment samples. Although normal urothelial cells are likely to be shed at a lower rate than abnormal tumor cells from Tis or Ta lesions, the presence of this normal cellular component in urine cell sediment complicates ADF analysis. In addition, normal white blood cells from internal bleeding of Tis or Ta lesions also contribute to the normal cellular component in the urine cell sediment. The concordance data (Table 4) presented in this study support this contention.

To facilitate longitudinal risk assessment in our recurrent bladder cancer surveillance protocol, we defined the ADF barcode. This genotypic approach should aid in individualizing a patient's risk for bladder cancer progression in recurrent tumors. Because changes to the ADF pattern are more relevant to the individual tumor than to the group of tumors, allelic deletions in this set of ADF markers, specific to each recurrent tumor, should be indicative of progressive disease. In addition, the ADF barcode provides a means of genotypically subclassifying tumors that appear similar by histological and morphological examination. Microsatellite-based methods previously have been shown useful for the detection of malignant cells from primary tumors in urine samples [16]. As such, ADF analysis provides a reliable noninvasive method for the surveillance of bladder cancer recurrence and progression using urine cell sediments.

Table 3. Allelic Deletion Fingerprinting Barcodes for Matched Primary Bladder Cancer and Urine Cell Sediment Samples

Patient No.	Sample	Pathology Report*	D3S1067†	D4S43	D8S201	D11S907	D11S935	D14S267	D14S288	D17S578	TP53	ADF Barcode‡§
4	t	Ta, G2	1	1	1	1	1	1	1	n	1	1111111n1
	u	Normal	1	x	1	x	1	x	x	x	1	1x1x1xxx1
9	t	Tis, Cis	0	1	0	1	1	1	1	0	1	010111101
	u	G3	0	x	0	x	x	x	1	1	x	0x0xxx11x
10	t	Tis, Cis	1	1	1	1	0	1	1	1	1	111101111
	u	Inconclusive	0	1	1	1	0	x	x	1	1	01110xx11
13	t1	T2, G3	1	0	1	0	0	0	0	1	0	101000010
	t2	T2, G3	1	1	1	1	1	x	1	1	1	11111x111
	u	G3	1	1	1	1	1	0	1	1	1	111110111
14	t	T2, G2	1	n	1	0	1	1	1	1	1	1n1011111
	u	G2	1	x	1	0	0	x	1	1	1	1x100x111
15	t	Ta, G2	1	n	x	1	1	1	1	1	1	1nx111111
	u	Normal	1	x	x	0	0	x	1	0	1	1xx00x101
16	t	Ta, G2	1	0	1	1	0	1	1	0	0	101101100
	u	G2	0	0	1	1	0	x	0	x	1	00110x0x1
18	t	Tis, Cis	1	n	x	1	1	0	0	1	1	1nx110011
	u	G3	0	x	x	0	1	x	0	1	0	0xx01x010
19	t	T1, G3	0	1	0	0	0	1	1	1	0	010001110
	u	G3	0	1	1	0	0	x	0	1	0	01100x010
20	t	T2, G2	1	0	1	0	1	x	0	0	0	10101x000
	u	G2	0	1	0	1	0	x	0	0	0	01010x000
21	t	T1, G3	0	1	0	0	1	x	0	1	0	01001x010
	u	G3	x	0	0	0	0	x	0	0	1	x0000x001
23	t	T1, G3	1	0	1	1	0	x	0	0	0	10110x000
	u	G3	0	1	1	1	0	x	1	0	1	01110x101
24	t1	T1, G3	0	1	1	1	n	x	1	0	1	0111nx101
	t2	T1, G3	x	0	1	1	n	x	0	0	1	x011nx001
	u	G3	0	1	1	1	n	x	1	1	1	0111nx111
25	t	Ta, G3	0	1	0	1	n	x	1	1	1	0101nx111
	u	Inconclusive	1	1	1	1	n	x	1	1	1	1111nx111
26	t	Ta, G2	0	x	x	x	x	x	x	x	0	0xxxxxxx0
	u	G2	1	x	x	x	x	x	x	x	1	1xxxxxxx1
29	t	T2, G3	n	0	0	1	0	n	0	n	1	n0010n0n1
	u	G3	n	0	0	0	0	x	0	n	0	n0000x0n0
31	t	Ta, G2	1	1	0	1	n	1	x	n	1	1101n1xn1
	u	Inconclusive	1	1	1	0	n	1	x	n	0	1110n1xn0
33	t1	T1, G2	n	1	1	n	1	1	1	n	1	n11n111n1
	t2	T1, G2	n	1	1	n	1	1	1	n	1	n11n111n1
	u	G2	n	1	1	n	1	1	1	n	1	n11n111n1
34	t	T1, G3	0	0	0	0	n	0	0	0	0	0000n0000
	u	Inconclusive	1	1	0	0	n	0	x	0	0	1100n0x00
35	t	T1, G2	0	0	1	0	1	1	0	0	1	001011001
	u	normal	x	0	x	0	x	x	0	x	1	x0x0xx0x1
36	t	Ta, G1	1	1	1	n	n	1	1	1	1	111nn1111
	u	Normal	x	1	x	n	n	0	x	0	1	x1xnn0x01
38	t	Ta, G2	1	1	1	1	1	1	1	n	0	1111111n0
	u	Normal	0	1	1	1	0	0	x	n	0	011100xn0

Table 3. Continued

Patient No.	Sample	Pathology Report*	D3S1067†	D4S43	D8S201	D11S907	D11S935	D14S267	D14S288	D17S578	TP53	ADF Barcode‡§
39	t	T1, G3	n	1	n	1	0	0	1	1	0	n1n100110
	u	Inconclusive	x	0	n	x	x	x	x	x	n	x0nxxxxn
41	t	T2, G3	0	1	0	0	0	1	1	0	0	010001100
	u	G3	0	0	0	0	0	1	0	0	1	000001001
42	t	Ta, G2	1	0	0	0	0	1	1	0	0	100001100
	u	Normal	1	1	1	1	0	1	1	1	1	111101111
43	t	Tis, Cis	0	n	1	0	0	1	1	1	1	0n1001111
	u	Inconclusive	0	n	x	1	0	1	0	x	1	0nx1010x1
46	t	Ta, G2	0	1	x	0	0	1	0	x	1	01x0010x1
	u	Normal	0	0	x	0	0	0	0	x	x	00x0000xx
47	t1	T1, G3	1	n	0	1	n	0	1	0	0	1n01n0100
	t2	T1, G3	1	n	0	1	n	1	1	1	0	1n01n1110
	u	G3	x	n	0	1	n	1	1	x	0	xn01n11x0
48	t	Ta, G2	1	1	0	0	0	1	1	0	0	110001100
	u	G2	1	1	x	0	0	1	0	x	1	11x0010x1
49	t	Ta, G1	0	0	n	0	1	1	1	0	1	00n011101
	u	G1	1	0	n	0	1	1	1	0	1	10n011101
51	t	Ta, G1	1	0	1	n	1	1	1	1	1	101n11111
	u	Normal	1	1	1	n	0	0	0	1	1	111n00011
52	t	T1, G3	0	1	0	1	1	x	1	1	0	01011x110
	u	Inconclusive	0	0	x	0	x	x	x	1	x	00x0xxx1x
53	t	Ta, G2	1	1	0	1	0	x	1	0	0	11010x100
	u	Normal	1	1	x	1	0	x	x	0	1	11x10xx01
54	t1	Ta, G1	x	1	x	x	x	1	1	0	1	x1xxx1101
	t2	Ta, G1	x	0	x	x	x	0	0	0	0	x0xxx0000
	u	Normal	x	1	x	x	1	1	1	1	1	x1xx11111
54	rt	Ta, G2	x	1	x	0	1	1	x	1	0	x1x011x10
	ru	Normal	x	1	x	1	1	1	x	1	1	x1x111x11
55	t	Ta, G1	x	1	x	1	n	1	1	n	0	x1x1n11n0
	u	Normal	x	0	x	0	n	0	0	x	0	x0x0n00x0
57	t	Tis, Cis	x	1	x	0	0	1	n	1	1	x1x001n11
	u	G3	x	0	x	0	1	0	x	0	0	x0x010x00
59	t	T1, G3	x	0	x	0	1	0	1	1	0	x0x010110
	u	G3	x	0	x	0	x	0	0	x	0	x0x0x00x0
61	t	Tis, Cis	x	1	x	1	1	1	1	1	1	x1x111111
	u	G3	x	1	x	x	x	x	x	x	x	x1xxxxxxx
62	t1	T1, G2	x	1	x	0	n	1	x	x	0	x1x0n1xx0
	t2	T1, G2	x	1	x	0	n	1	x	x	1	x1x0n1xx1
	u	Normal	x	1	x	x	x	x	x	x	x	x1xxxxxxx

ADF, allelic deletion fingerprinting; t, tumor; u, urine cell sediment; Tis, tumor *in situ*, Cis, carcinoma *in situ*.

*Tumor stage, tumor grade, and urine cytology according to World Health Organization criteria and tumor, node, metastasis classification.

†The allelic deletion genotype at the specified microsatellite locus; 0, deleted; 1, not deleted; n, not informative; x, no data available (poor or no amplification).

‡Each numeral in the ADF barcode represents the genotype at one microsatellite locus; 0, deleted; 1, not deleted; n, not informative; x, no data available.

§The order of microsatellite loci in the barcode from left to right is D3S1067, D4S43, D8S201, D11S907, D11S935, D14S267, D14S288, D17S578, and TP53.

Table 4. Distribution of Allelic Deletion in Matched Primary Bladder Cancer and Urine Cell Sediment Samples

	Tumor LOH = 0	Tumor LOH >1	Total
Urine LOH = 0	3*	5†	8
Urine LOH >1	2‡	30	32
Total	5	35	40

LOH, loss of heterozygosity/allelic deletion.
*Tumors 4, 33, and 61.
†Tumors 25, 26, 54, 54r, and 62.
‡Tumors 15 and 36.

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