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EPIGENETIC ALTERATIONS OF ADENOMATOUS POLYPOSIS COLI (APC), RETINOIC ACID RECEPTOR BETA (RARβ) AND SURVIVIN GENES IN TUMOR TISSUES AND VOIDED URINE OF BLADDER CANCER PATIENTS

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Abstract

The CpG promoter methylation has been reported to occur frequently in bladder cancer. Moreover, analysis of gene methylation has been shown to be feasible from voided urine and can be detected with a high degree of sensitivity. The aim of this present study is to determine how methylation patterns of *APC*, *RARβ* and *Survivin* genes change during bladder carcinogenesis and to evaluate whether DNA methylation could be detected in urine sediment. Using the sensitive assay of MSP, we explored the promoter methylation status for the three genes in tumor specimens and urine sediment DNA from 32 bladder cancer patients. Methylation frequencies of the tested genes in tumor specimens were 100%, 75% and 84.4% for *APC, RARβ* and *Survivin*, respectively. Hypermethylation of *APC* was found in all pathological grades and stages of bladder cancer. More frequent promoter hypermethylation of *RARβ* and *Survivin* was observed in high grade tumors and the hypermethylation increased from low to high stages, but there was no significant correlation between stages/grades and hypermethylation of these two gene promoters. In order to investigate clinical usefulness for noninvasive bladder cancer detection, we further analyzed the methylation status in urine samples of bladder cancer patients. Methylation of the tested genes in urine sediment DNA was detected in the majority of cases that were hypermethylated in tumor samples (93.7%) and the frequencies were 79.3% 70.8% and 96.3% for *APC*, *RARβ* and *Survivin*, respectively. Our results indicate that methylation of *APC*, *RARβ* and *Survivin* gene promoters is a common finding in patients with bladder carcinoma. The ability to detect methylation not only in bladder tissue, but also in urine sediments, suggests that methylation markers are promising tools for noninvasive detection of bladder cancer.

Key words: Bladder cancer, MSP, hypermethylation, urine DNA.

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INTRODUCTION

Worldwide, bladder cancer is the seventh most common cancer, approximately accounting 336,000 new cases each year (12, 29). In Morocco, according to the regional cancer registers, bladder cancer is the sixth most common cancer with an incidence of 5.8 and 11.3 per 100,000 persons in Casablanca and Rabat, respectively (4, 52). The average age of bladder cancer occurrence was 62.9 years in women and 63.8 years in men. Urothelial carcinoma (UC) was by far the most frequent histological type (70% in women and 82% in men) while squamous cell carcinoma accounted 10% of cases in women and 4.8% in men (4).

Currently, bladder cancer diagnosis is based on the cystoscopy which relies on morphological, histological and pathological features. This invasive approach provides essential prognostic information, but shows insufficient power to predict precisely the patient outcome. Conventional urine cytology is the standard noninvasive method for cancer detection and disease monitoring. However, this method lacks sensitivity, especially for low grade and stage of bladder cancer (8, 11). Therefore, a more sensitive and noninvasive method is imperative for efficient cancer detection.

Some genetic and epigenetic alterations occur early during tumorigenesis, and could be used as targets for the molecular diagnosis of neoplastic cells in clinical specimens such as biological fluids that are readily accessible (46). Several DNA alterations such as gene promoters methylation (22, 59) have been described in bladder cancer and have shown promising results. DNA methylation occurs on cytosine residues located at the 5' position of guanines in CpG dinucleotides which is not randomly distributed but is especially important in CpG-rich areas, also called CpG islands (55). Hypermethylation has been shown to be an alternative mechanism of tumor suppressor gene inactivation (32, 50). In bladder cancer, a large number of genes have been shown to harbor promoter hypermethylation, including *APC*, *RARβ*, *SFRP* and *RASSF1A* as some of the most consistent and frequent targets (35, 36). The CpG methylation has been also reported to occur frequently in bladder cancer and to be associated with increased tumor stage and grade (7, 36, 42). Moreover, analysis of gene methylation has been shown to be feasible from voided urine (16, 60).

Indeed, several genes such as *DAPK*, *BCL2* and *TERT* have been shown to be hypermethylated in urine sediment DNA from bladder cancer patients, whereas they were unmethylated in the urine sediment DNA from age-matched cancer-free individuals (16). Also, hypermethylation of

DAPK, *RARβ*, *E-cadherin* and *p16* in urine sediment DNA from bladder cancer patients revealed a good sensitivity and specificity for bladder cancer detection (41).

The *Survivin* gene, mapped at 17q25, has received much attention because of its dual function in apoptosis and cell cycle regulation, and its unique expression pattern (38). This gene is implicated in many types of cancers including bladder cancer (43, 57). The *Survivin* promoter is GC-rich with a canonical CpG island extending into exon 1 (1, 2, 33), which is epigenetically regulated (15, 18, 21).

The Retinoic Acid Receptor beta (*RARβ*) gene, mapped at 3p24, is a member of the thyroid-steroid hormone receptor superfamily of nuclear transcriptional regulators that binds retinoic acid (the biologically active form of vitamin A) and also mediates cellular signaling during embryonic morphogenesis, cell growth and differentiation (48). Retinoic acids exhibit tumor suppressor activity due to their anti-proliferative and apoptosis-inducing effects (5). Several reports have indicated high frequencies of promoter hypermethylation of *RARβ* gene in urinary bladder tumors (varying from 15% to 93%) (7, 8, 17, 22, 36).

The Adenomatous Polyposis Coli (*APC*) tumor suppressor gene is isolated and mapped at chromosomal band 5q21 (27, 31). The binding of APC protein to β-catenin promotes the phosphorylation of highly conserved serine and threonine residues in β-catenin's NH_2 terminus by Glycogen Synthase Kinase 3 (GSK-3) and thereby targeting β-catenin for degradation via the proteosome system (39, 40). Loss of APC function results in nuclear accumulation of β-catenin, which acts as a transcriptional activator by binding to the Tcf / Lef (T cell factor/lymphoid enhancer factor) family of transcription factors, ultimately leading to loss of cellular growth control (37, 49). Many studies have reported a high methylation frequency of *APC* promoter region in bladder cancer (14, 15, 41).

The methylation status of the *APC* and *RARβ* genes was well studied in tissue specimens but only few studies have been reported in urine sediments, whereas, to our knowledge, no study was carried out on promoter methylation of *Survivin* gene in bladder cancer.

The main aim of the present study is to determine the promoter methylation status of the three genes (*Survivin*, *RARβ* and *APC*) in bladder cancer from Moroccan patients and to evaluate their use as epigenetic biomarkers for bladder cancer management. Moreover, this study was performed on both tumor biopsies and their paired urine sediments to evaluate the use of this approach as a non invasive diagnosis method of bladder cancer.

MATERIALS AND METHODS

Patients and Specimens

The study was conducted under the local ethical rules and was carried out on 32 bladder cancer patients (28 males and 4 females; mean age of 64.2 ± 10.7, ranging from 42 to 84 years; median: 66.5) and 4 patients with inflammatory urinary disease (cystitis) of the bladder who underwent surgical treatment at the Urology department of Military Hospital of Instruction Mohamed V (MHIMV) in Rabat, Morocco. Additionally, 12 age-matched healthy volunteers were included as controls.

All the tissue specimens were collected from January 2010 to June 2011 by transurethral resection (TUR) and immediately stored in liquid nitrogen until use. A tumor tissue fragments were fixed by formalin and embedded in paraffin. The corresponding hematoxylin-eosin-stained sections were examined at the Anatomopathology department at the same hospital, staged according to the TNM (Tumor Node Metastasis) classification and assigned the grade according to the WHO (World Health Organization) criteria (14).

To evaluate whether DNA methylation could be detected in urine sediments, paired voided urine samples were obtained before surgery from all patients. The samples from patients and healthy subjects (50 mL fresh urine) were spun down by centrifugation at 800g for 10 minutes at 4°C, and the pelleted urine sediment was washed twice with phosphate buffered saline and stored at -80 °C until use.

Genomic DNA extraction

Genomic DNA from fresh specimens tissue and urine cell sediments were obtained by standard sodium dodecyl sulfate/proteinase K digestion, followed by phenol/chloroform extraction and ethanol precipitation (44). DNA from tissue specimens and urine sediments were precipitated with 2/5 volumes of 7.4M ammonium acetate and 2 volumes of 100% ethanol, followed by an overnight incubation at -20°C and centrifugation at top speed (13,000 relative centrifugal force). DNA was then resuspended in ultrapure Dnase/Rnase-free distilled water and stored at -20°C until use.

Sodium Bisulfite modification

Genomic DNA extracted from tumors and urine sediments were subjected to bisulfite treatment which converts unmethylated cytosine residues to uracil, but methylated cytosines remain unaltered in this process. Briefly, 500ng of genomic DNA from each sample was modified. Sodium bisulfite modification and DNA purification were performed using the EZ DNA Methylation Kit (Zymo Research, Orange, CA, USA) following the manufacturer's protocol.

Methylation-Specific PCR (MSP)

The methylation pattern of promoter regions for *Survivin*, *RARβ* and *APC* genes was evaluated by MSP approach. This highly specific and sensitive method can identify up to 1 methylated allele in 1000 unmethylated alleles (19). For each gene, previously described primers specific to methylated and unmethylated sequences were used (54, 56, 58). Primer sequences, annealing temperatures and the expected product size were listed in Table 1. The mix was prepared in a total volume of 25 µL containing 1X PCR buffer, 2 mM $MgCl_2$, 0.25 mM of each dNTP, 1 µM of each primer and 1 U of AmpliTaq Gold DNA polymerase (Applied Biosystems, CA, USA). 2 µL of modified DNA were taken as template and were amplified at 95°C for 10 min; 40 cycles of 95°C for 30 sec, the specific annealing temperature of each primer set (Table 1) for 45 sec and 72°C for 45 sec; followed by a final extension of 72°C for 10 min. Water was used as negative control. 10 µL of PCR products were loaded onto 2% agarose gel. The gels were then stained with ethidium bromide and visualized under UV illumination.

Statistical analysis

The hypermethylation status of promoters in tumor specimens and urine samples was statistically evaluated

Table1. Primers and annealing temperature for Methylation-specific PCR

Gene	Sense (5' > 3')	Antisense (3' > 5')	Bp	Ta	Ref.
Survivin U	GGTGTGGTGTTGTTGGGTGT	CCAACAAATCCCACAATTCA	200	52	(58)
M	TTCGGTATATTTCGCGTCGT	AACGTCGAAACACCCATACC	180	52	
RARβ U	TTAGTAGTTTGGGTAGGGTTTATT	CCAAATCCTACCCCAACA	232	57	(56)
M	GGTTAGTAGTTCGGGTAGGGTTTATC	CCGAATCCTACCCCGACG	234	57	
APC U	GTGTTTTATTGTGGAGTGTGGGTT	CCAATCAACAAACTCCCAACAA	108	60	(54)
M	TATTGCGGAGTGCGGGTC	TCGACGAACTCCCGACGA	98	57	

PCR indicates Polymerase Chain Reaction; bp: base pairs; Ta: annealing temperature; U: unmethylation-specific primers; M: methylation-specific primers.

using percentages with Confidence Interval (CI) of 95% calculated by EpiInfo software version 6. The correlation between hypermethylation and clinico-histopathological parameters was evaluated by calculating p-values using MedCalc software version 9.

RESULTS

The clinicopathological characteristics of tumor specimens are summarized in Table 2. Regarding histological type, 31 patients had urothelial carcinomas (UC) and one had an adenocarcinoma poorly differentiated. For the 31 UC, histopathological examination revealed that 5 tumors were staged as pTa, 19 as pT1 and 6 as pT2-T4; and 10 tumors were graded as low grade and 20 as high grade carcinomas. Clinicopathological data lacks in only one patient. As presented in Table 2, 100% of the analyzed tumors exhibited aberrant promoter methylation in at least one of the three studied genes. The promoter hypermethylation frequency for individual genes was: 100% (29 of 29) for *APC*, 75% (24 of 32) for *RARβ* and 84.4% (27 of 32) for *Survivin*. Figure 1 shows examples of PCR products obtained after amplification with specific primers of each gene.

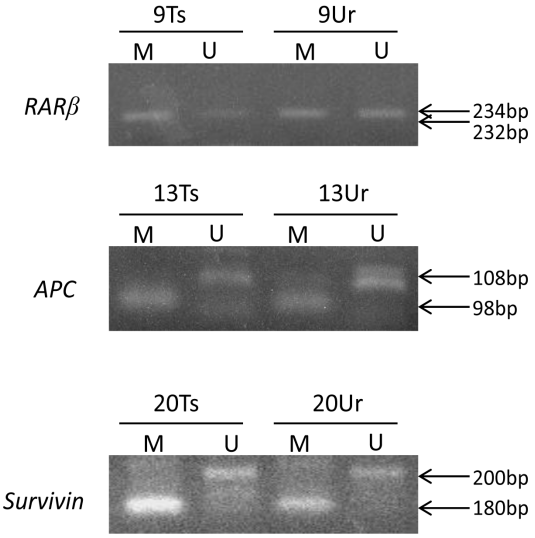

Figure 1. Analysis of *RARβ*, *APC* and *Survivin* methylation in tissue samples and their matched voided urine by MSP using specific primers. The presence of a visible PCR product in lane U indicates the presence of unmethylated genes; the presence of a PCR product in lane M indicates the presence of methylated genes. Ts: tissue DNA; Ur: Urine DNA; bp: base pair.

Regarding to clinicopathological data, our results show that among the 24 tumor tissues with promoter hypermethylation of *RARβ*, 17 were pTa/pT1 and five were pT2-T4. As for tumor grading, 16 were high grade tumors and only six were low grade tumors (Table 3). One case was adenocarcinoma and one was without clinicopathological data. Our data further show that the majority of the 8 unmethylated tumor samples for *RARβ* gene were pTa/pT1 (2 were pTa, 5 were pT1 and only one was pT2) and four tumors were low grade.

Promoter methylation of *Survivin* gene was detected in 27 samples: 19 were classified as pTa/pT1 and 6 as pT2-pT4. Of these, 19 were high grade tumors and only 7 were low grade tumors (Table 3). One case was adenocarcinoma and one has unidentified stage/grade. Among the five unmethylated samples, two tumor specimens were pTa and three were pT1.

The study of each gene promoter hypermethylation was extended to the matched urine sediments and the promoter hypermethylation in one or more of the studied genes was detected in 93.75% (30 of 32) of analyzed samples. Promoter hypermethylation of *APC*, *RARβ and Survivin* genes was detected in 79.3% *(*23 of 29), 53.6% (17 of 32) and 81.3% (26 of 32), respectively.

The methylation status of the three genes in the paired urine DNAs from the 32 bladder cancer patients was compared with the methylation status in the corresponding tumor DNAs. *APC*, *RARβ* and *Survivin* hypermethylation was detected respectively in 79.3% (23 of 29), 70.8% (17 of 24) and 96.3% (26 of 27) of matched urine DNAs within the hypermethylated cases in tumor samples (Table 4). These results, showing that promoter hypermethylation of the three studied genes was detected in tumor DNA of at least two genes and in the matched urine sediment DNA of almost all cases, clearly reflect the high sensitivity of the detection of promoter methylation in urine DNAs.

In paired samples, methylation in urine sediment DNA was always accompanied by methylation of tumor DNA. For the three genes, promoter hypermethylation was absent in urine sediment DNA when the corresponding tumors do not exhibit promoter hypermethylation. Thus, the specificity of methylation detection in urine sediments reached the 100%.

No hypermethylation of *APC*, *RARβ* and *Survivin* genes was observed in urothelial specimens from two patients with cystitis and in their matched urine sediment DNA. In contrast, promoter hypermethylation of *RARβ* and *Survivin* genes was observed in patients with cystitis (Case 33 and 34) (Table 5), in both TUR specimens of bladder

Table 2. Clinicopathological and hypermethylation detection data from 32 patients with bladder cancer.

Patient ID	Sex	Age	Stage	Grade	Survivin	RARβ	APC
1	F	N/A	pT2	High	M/M	M/U	M/M
2	M	78	pT1	High	M/M	M/M	M/M
3	M	N/A	pT4	High	M/M	M/M	M/M
4	M	74	pT1	Low	M/M	U/U	M/M
5	M	67	pT1	High	M/M	U/U	M/M
6	F	42	pT1	High	M/M	M/M	M/U
7	M	67	pT1	Low	M/M	M/M	M/U
8	M	66	pT1	High	M/M	U/U	M/M
9	M	73	pT1	High	M/M	M/M	M/M
10	M	N/A	pT2	High	M/M	U/U	M/M
11	M	64	pTa	High	M/M	U/U	M/M
12	M	51	pT1	High	M/M	M/M	M/M
13	M	N/A	pT1	High	M/U	M/U	M/M
14	M	84	pT1	Low	M/M	M/U	M/U
15	M	70	pT1	Low	M/M	U/U	M/M
16	M	N/A	N/A	N/A	M/M	M/M	M/M
17	F	64	pT1	Low	M/M	U/U	M/U
18	M	62	pT2	High	M/M	M/M	N/A
19	M	48	pTa	Low	U/U	M/U	M/U
20	M	55	pT1	High	M/M	M/M	N/A
21	M	59	pTa	Low	M/M	M/M	N/A
22	F	75	pTa	Low	U/U	U/U	M/M
23	M	53	pT1	Low	U/U	M/U	M/U
24	M	72	pT2	High	M/M	M/M	M/M
25	M	45	pTa	Low	M/M	M/M	M/M
26	M	71	pT1	High	M/M	M/U	M/M
27	M	67	pT1	High	U/U	M/M	M/M
28	M	70	pT2	High	M/M	M/M	M/M
29	M	63	pT1	High	M/M	M/M	M/M
30	M	N/A	Adenocarcinoma		M/M	M/M	M/M
31	M	N/A	pT1	High	M/M	M/U	M/M
32	M	N/A	pT1	High	U/U	M/M	M/M

M: male; F: female; N/A: not available; U: unmethylated; M: methylated; M/M: tumor DNA methylated/urine DNA methylated; U/U: tumor DNA unmethylated/urine DNA unmethylated; M/U: tumor DNA methylated/urine DNA unmethylated.
Clinicopathological data was not available for No. 16 and No. 30 was an adenocarcinoma

Table 3. Distribution of *Survivin* and *RARβ* hypermethylation according to clinicopathological data.

Parameter	Total	*Survivin* (%)	*RARβ*(%)
Stage			
pTa	5	3 (60)	3 (60)
pT1	19	16 (84.2)	14 (73.7)
pT2-T4	6	6 (100)	5 (83.3)
p value		≥0.35	≥0.85
Grade			
Low	10	7 (70)	6 (60)
High	20	18 (90)	16 (80)
p value		=0.38	=0.46

mucosa and their paired voided urine.

MSP analysis in samples from control cases revealed that urine sediment DNA were predominantly not methylated (10/12) for the three tested gene promoters and thereby 83.33% of control cases were unmethylated, whereas a weak promoter methyation was detected in only 2 cases (2/12).

DISCUSSION

DNA hypermethylation is a common mechanism for inactivating tumor suppressor and other cancer genes in tumor cells (6). The aberrant methylation patterns have been used as targets for detection and diagnosis of tumor cells in clinical specimens such as tissue biopsies or body fluids

Table 4. Hypermethylation Frequencies in promoters of *Survivin, RARβ* and *APC* genes in tumor and urine DNAs from bladder cancer patients.

gene	Number of tumors with methylation/Total number of tumors (%, 95%CI)	Number of patients with methylation in urine/Total number of tumors (%, 95% CI)	Number of patients with methylation in urine/Number of patients with methylation in primary tumor (%Sensitivity, 95%CI)
Survivin	27/32 (84.4, 67.2-94.7)	26/32 (81.3, 63.6-92.8)	26/27 (96.3, 81.0-99.9)
RARβ	24/32(75, 56.6-88.5)	17/32 (53.1, 34.7-70.9)	17/24 (70.8, 48.9-87.4)
APC	29/29 (100, 88.1-100)	23/29(79.3, 60.3-92.0)	23/29 (79.3, 60.3-92.0)

CI = confidence interval.
The sensitivity reflects the fraction of patients in which the urine DNA was methylated among cases with methylation in matched tumor DNA of the same gene promoter.

Table 5. hypermethylation detection in inflammatory cases of bladder.

Patient ID	Sex	Age	Clinicopathological data	*Survivin*	*RARβ*	*APC*
33	M	65	inflammatory urinary disease (cystitis)	M/M	M/M	M/U
34	M	86	inflammatory urinary disease (cystitis)	U/U	M/M	N/A
35	M	71	inflammatory urinary disease (cystitis)	U/U	U/U	U/U
36	M	61	inflammatory urinary disease (cystitis)	U/U	U/U	U/U

N/A: not available; M/M: tumor DNA methylated/urine DNA methylated; U/U: tumor DNA unmethylated/urine DNA unmethylated; M/U: tumor DNA methylated/urine DNA unmethylated; M: tumor DNA methylated.

(e.g. urine) (45).

Using the sensitive assay of MSP, we have explored the promoter methylation status of three important genes (APC, RARβ and Survivin) in both tumor specimens and urine sediments DNA from Moroccan patients with bladder cancer.

In this panel of genes, we obtained 100% diagnostic coverage in all grades and stages tumors and our results indicate that bladder tumors showed promoter hypermethylation frequency of individual genes in: 100% for APC, 75% for RARβ and 84.4% for Survivin.

Our investigation on promoter methylation of APC gene showed that the aberrant methylation is constantly present in tumors regardless to clinicopathological features.

Worldwide, the frequency of gene promoter methylation of APC is controversial. In our investigation, promoter hypermethylation of APC was detected in all analyzed bladder specimens (100%). These findings are comforted by several studies showing a high prevalence of APC gene promoter hypermethylation (3, 13, 22, 27, 59). However, some others found that promoter of APC was methylated at lower rate (34, 36, 59).

The frequency of promoter hypermethylation of RARβ gene was 75%. This finding is in good agreement with many published studies on bladder cancer showing a high frequency of promoter hypermethylation of RARβ gene (93% and 87.8%) (8, 22) and contrast with other studies detecting a lower frequency (15% and 24%) (7, 36).

Despite of the attractive interest of Survivin gene, the epigenetic study of this gene is restricted to some types of cancer but to our knowledge, there is no investigation in bladder cancer. Targeting the same promoter region of CpG island, already explored in endometrial cancer by Nabilsi et al. (38) showing hypermethylation in this region, we detected aberrant methylation of Survivin promoter at 84.4% of analyzed tumor specimens. In contrast, a low frequency of Survivin promoter methylation was detected in glioblastoma multiform (30%) (20) and ovarian cancer (13.9%) (18); and no evidence of Survivin promoter methylation was found in cervical and oral squamous cell carcinomas (9, 51). Furthermore, it has been reported in previous studies that this anti-apoptotic gene was overexpressed in bladder cancer (57). This is in sharp contrast to the majority of genes examined in this regard, which show an inverse correlation between methylation and expression. However, some genes were found to be upregulated while the CpG sites were hypermethylated as it is the case of Survivin in endometrial cancer (38), GPH-α in a variety of tumor cell types and Hs.137007 in breast cancer (10, 30). Many studies described important links between gene promoter hypermethylation and clinical features of bladder cancer proposing that these hypermethylation markers are clinically important (25).

In our study, more frequent promoter hypermethylation of RARβ and Survivin is observed in high grade tumors (16/20, 80% and 18/20, 90%; respectively) than in low grade (6/10, 60% and 7/10, 70%; respectively) (Table 3). Moreover, for these two genes, hypermethylation frequencies increased from low to high stages. However, no significant correlation between stages/grades and hypermethylation of the two genes promoters was found (Table 3), probably due to the small size of studied group. It was demonstrated that the RARβ can be involved in bladder cancer progression due to its hypermethylation in high stages and grades (25) and can inform on bladder cancer prognosis. The same pattern was observed for Survivin in endometrial tumors where methylation progressively increased from low to high grade (38).

The APC aberrant methylation is observed in all clinicopathological features including low grades and stages, which suggests that hypermethylation of APC gene promoter could be used as a biomarker for early diagnosis

of bladder cancer. This finding is in good agreement with previous study (22), indicating that hypermethylation in promoter of this gene can be relatively an early event in bladder tumorigenesis.

Hypermethylation was also detected in inflammatory lesions (cystitis). The same profile was observed in chronic inflammation in other studies (23, 24), suggesting that this epigenetic alteration may preexist in morphologically normal cells (47) and reflecting a pre-malignant characteristic of the bladder (53). Therefore, molecular tests that target these alterations have conceptual advantages for the successful early detection of bladder neoplasias (6).

The study of each gene promoter was extended to the matched urine sediment DNA and the promoter hypermethylation in one or more of the studied genes was detected in 30 of 32 analyzed samples, thereby the detection coverage of methylation in urine reached 93.7%. Hypermethylation was not detected in two urine DNAs corresponding to patients with Ta and T1 stages. This is in good agreement with the published study by Dulaimi et al. (11) where the methylation anomaly was not detected in some urine sediment DNA and most likely due to a low amount of neoplastic DNA in these urine samples (11).

For the three genes, the use of urine sediment DNA as template to detect hypermethylation provides high sensitivity (Table 4). Gene promoter methylation of APC was detected in 79.3% (23/29) of voided urine samples of patients and not detected in six urine DNAs (one was Ta, and five were pT1) where the APC gene was hypermethylated in tumor DNA. This high sensitivity is supported by a study conducted by Hoque et al. showing that methylation in urine was detected in 73% of hypermethylated cases in tumors (22).

The frequency of promoter hypermethylation of RARβ in all analyzed urine sediment DNA was 53.6%, whereas the hypermethylation prevalence in matched urine, among hypermethylated cases for the promoter of RARβ in tumor tissue, reached 70.8%. This result is in agreement with data reported by Chan et al. (8) and suggests that hypermethylation of RARβ has a good sensitivity and specificity for bladder cancer detection (41).

The Survivin promoter hypermethylation in the voided urine was 81.3% in the 32 urine sediment DNAs and 96.3% in the 27 hypermethylated tumor cases (Table 4).
The methylation analysis of APC, RARβ and Survivin genes in control cases from age-matched subjects has shown that the majority of cases (83.33%) were not methylated. The low level of APC and RARβ methylation observed in our control group was supported by Hoque et al. investigation (22). The Survivin gene promoter was found to be unmethylated in normal samples comparing to endometrial tumors (38) that were hypermethylated. These findings suggest that aberrant methylation of such genes is a rare event in normal DNA and is related to bladder malignancy.
A noninvasive test with good sensitivity and specificity could prescreen, before cystoscopy, patients with clinical symptoms or those who are at high risk, and would also be useful in monitoring patients for recurrence. Furthermore, because early detection may successfully identify potentially lethal lesions (T1 or Tis) before they become muscle invasive; such test could significantly impact the morbidity and mortality of the disease (26, 28). Therefore, DNA methylation in urine sediment may be a promising method for non invasive cancer detection and should reliably detect bladder cancer. It might also reduce the number of cystoscopies in surveillance of bladder cancer. More comprehensive epigenetic studies in lager cohort are necessary to validate these findings.

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