ELSEVIER

Cancer Genetics and Cytogenetics 142 (2003) 25–29

CANCER GENETICS AND CYTOGENETICS

Evaluation of chromosome 8 and 11 aneuploidies in washings and biopsy materials of bladder transitional cell carcinoma

Hasan Acar[a,*], Mehmet Kılınç[b], M. Selman Yıldırım[a], Murat Kaynak[a], Ali Cenker[b]

[a]*Department of Medical Genetics, Selçuk University, Medical Faculty, Konya 42080, Turkey*
[b]*Department of Urology, Selçuk University, Medical Faculty, Konya 42080, Turkey*

Received 18 April 2002; received in revised form 13 September 2002; accepted 25 September 2002

Abstract We compared chromosome 8 and 11 aneuploidies on bladder biopsy tumor tissues and bladder washing samples of transitional cell carcinoma (TCC) and their relationship to tumor malignancy. Interphase fluorescence in situ hybridization (FISH) was applied to nuclei of washing material and biopsy samples of 17 patients with TCC. Incidence of cells having aneuploidy was clearly nonrandom from patient to patient. There was no significant difference in the incidence of aneuploid frequency for chromosomes 8 and 11 between biopsies of bladder tumors and bladder washing samples ($P > 0.05$). For chromosome 8, incidence of disomic cells (having two signals) in grade III tumors was significantly lower than in grade II tumors of both washing samples ($P = 0.004$) and biopsy materials ($P = 0.005$), indicating a high frequency of aneuploidy. The incidence of nuclei with four or more than four signals of chromosome 8 was significantly higher in grade III tumors than in grade II tumors in washing samples ($P = 0.031$ and 0.003, respectively). Similarly, in biopsy material, the incidence of nuclei with more than four signals of chromosome 8 was significantly higher in grade III tumors than in grade II tumors ($P = 0.004$). For chromosome 11, in both washing samples and biopsy materials, the incidence of disomic cells (having two signals) in grade III tumors was significantly lower than that detected in grade II tumors ($P = 0.031$ and 0.014, respectively), indicating a high frequency of aneuploidy. In biopsy materials, the incidence of nuclei with three or four signals was significantly higher than that in grade II tumors ($P = 0.014$ and 0.012, respectively). These findings suggest that FISH analysis of bladder washing samples can be efffectively detected as genetic changes of bladder tumors. It might predict genetic progression of these tumors, which might be related to tumor stage, because higher stages of tumors showed a higher incidence of aneuploidies of chromosomes 8 and 11.

1. Introduction

Bladder cancer, which occurs mostly in developed nations, is a common disease and presently ranks as the 11th most common cancer worldwide. The most common histological type of bladder cancer is transitional cell carcinoma (TCC). Bladder TCC is a heterogeneous group of tumors concerning their biology and clinical behavior. Many cytological tests are important for studying bladder carcinomas although their sensitivities and specificities have a wide range. In addition to cytological tests, many genetic tests such as conventional cytogenetics, flow cytometry, molecular genetics, and molecular cytogenetics (fluorescence in situ hybridization; FISH) have been used for detecting primary and secondary chromosomal abnormalities in bladder cancer. Some genetic events (e.g., monosomy or allelic losses of chromosome 9) seem to occur early and may be associated with tumor initiation [1–6]. Molecular genetic techniques have shown that loss of the short arm of chromosome 8 is associated with invasive growth in bladder cancers [3,7–10]. Conventional cytogenetic studies indicated that numerical and structural alterations also occur in bladder cancers [11–14]. Honglund et al. reported that the aberration patterns were nonrandom but that chromosomal imbalances were highly correlated with tumor stage and grade [15]. In addition, other nonrandom chromosomal aberrations associated with bladder cancer have been identified by different groups [16–18]. These are +7, −9, +10, and structural aberrations of chromosomes 1, 3, 5, 6, 8, 10, 11, and 17. Recent advances in FISH techniques have enabled

* Corresponding author. Tel. +90-332-323-2600, exts. 1662 (office) and 2215 (laboratory); fax: +90-332-323-2641.
E-mail address: yaseminsena@hotmail.com (H. Acar).

0165-4608/03/$ – see front matter
doi:10.1016/S0165-4608(02)00803-8

the investigation of numerical and structural chromosomal aberrations on both metaphase and interphase cells. Interphase FISH analysis of voided urine and bladder washing samples is a promising method that may detect bladder cancer noninvasively [14,19–21]. So far, very few comparative studies using interphase FISH analyses of bladder biopsies and bladder washing samples have been reported [20,22].

The aim of the present study was to compare incidence of nuclei with aneuploidy in biopsies from bladder tumors with bladder washing samples and also to compare incidence of nuclei with aneuploidy in different grades of tumors.

2. Materials and methods

This study was carried out in Selçuk University Medical Research and Application Center (Kombassan Arastirma Merkezi).

The study consisted of 17 patients (15 males and 2 females) ranging from 43 to 74 years in age, with bladder cancer detected between October 2000 and June 2001. Two of four patients with grade III tumors died within 6 months after diagnosis; their grades were T3aN1M0 and T3bN0M0. Another patient died in the first month after diagnosis; his tumor grade was T3aN1M0. All patients having grade II bladder tumors were alive in the first year and their grades were either T1N0M1 or T2N0M0.

The bladder washing samples and bladder biopsy tumor tissues for each case were obtained from the same patients. Before transurethral resection (TUR), the washing samples were obtained by application of cytoscopy were obtained by instilation of 50 mL of saline solution. The specimens were processed for interphase FISH analysis. Bladder washing samples were centrifuged and the pellets were washed twice in saline solution. Mononuclear cells were collected in a centrifugation tube. The pellets were then treated with 0.075 M KCl for 20–30 minutes. The samples were fixed in methanol/acetic acid (3:1). After three applications, the samples were dropped onto clean slides. The slides were aged at room temperature overnight. Bladder tumor samples were obtained from the same patients by application of TUR. One part of the tumor tissue was used for histopathologic examination and the other part was processed for interphase FISH analysis. The slides were prepared by using the touch technique, in which bladder tumor tissue was touched to a clean slide. The slides were fixed in cold methanol, treated with methanol/acetic acid (3:1) for 5 minutes (3 times each), and then air-dried.

2.1. FISH analysis

Standard chromosome preparations of peripheral blood from all patients were used for FISH to check the probe localization on the chromosomes. Five metaphase spreads were analyzed.

For interphase FISH study on bladder bioptic tumors and bladder washing samples, the probes used consisted of chromosome 8 and 11 centromere–specific α-satellite DNA probes labelled with biotin-dUTP (Cambio, Cambridge, UK). The probes were denatured at 70°C for 10 minutes. Interphase nuclei DNA on the slides was denatured in 70% formamide/2× SSC at 70°C for 3–4 minutes. The slides were then dehydrated through a 70, 85, and 100% alcohol series and then air-dried. The denatured probe was applied onto denatured slides and coverslipped. Slides were sealed with rubber cement. Hybridization occurred in a moisture chamber at 39°–41°C overnight. After hybridization, washes for both probes were done in 2×SSC, 50% formamide/2× SSC twice, and 2× SSC at 42°C for 5 minutes each. Detection of the probe mixture was carried out by application of avidin-FITC for 25 minutes at room temperature. Three washes were followed in 4× SSC Tween-20 for 5 minutes each at 42°C. The slides were stained with a counterstain medium containing DAPI or propidium iodide. The slides were examined with epifluorescence microscopy (Opthiphot; Nikon, Japan). For each probe and sample, 200 interphase nuclei were analyzed. Data were analyzed with the Mann-Whitney *U* (corrected *Z*) test.

3. Results

Application of FISH analysis by using chromosome 8 and 11 centromere–specific DNA probes revealed normal localization of their signals on metaphase spreads of peripheral blood lymphocytes from the patients with TCC. To determine the incidence of aneuploidies for chromosomes 8 and 11, FISH analysis was performed on bladder biopsy tumors and washing samples. Based on our experience and published data [23,24] and unpublished observations, hybridization signals for chromosome 8 and 11–specific α-satellite DNA probes for peripheral blood interphase cells were 4 and 4% for monosomies 8 and 11, and 5 and 3% for trisomies 8 and 11, respectively.

Using chromosome 8 and 11–specific DNA probes, interphase FISH results from biopsies of bladder tumors and bladder washing samples of 17 patients with TCC are given in Tables 1 and 2. Distribution of cells with hybridization signals for chromosome 8 showed a wide range, indicating heterogeneity of cell populations from patient to patient. The incidence of aneuploidy for chromosomes 8 and 11 observed in biopsies from bladder tumors was similar to the incidence of aneuploidy observed in bladder washings ($P > 0.05$). In washing samples, the incidence of disomic cells (having two signals) in grade III tumors was significantly lower than that of grade II tumors ($P = 0.004$), indicating a high frequency of chromosome 8 aneuploidy. Incidence of nuclei with four or more signals of chromosome 8 was significantly higher in grade III tumors than in grade II tumors in washing samples ($P = 0.031$ and 0.003, respectively). Similarly, in biopsy material, the incidence of disomic cells (having two signals) in grade III tumors was significantly lower than in grade II tumors ($P = 0.005$), indicating a high frequency of aneuploidy. Incidence of nuclei with more than four signals of chromosome 8 was significantly higher in grade III tumors than in grade II tumors ($P = 0.004$; Fig. 1).

Table 1
FISH results of chromosome 8 on washing and biopsy materials of 17 TCC patients

Sample type	FISH signals	Grade III (n = 4)	Grade II (n = 13)	Z	P
Washing samples	0	1.00 ± 0.82	1.00 ± 1.00	0.11	NS
	1	2.50 ± 1.91	8.54 ± 13.19	0.68	NS
	2	30.75 ± 8.69	66.62 ± 15.36	2.89	0.004
	3	15.25 ± 10.50	12.15 ± 7.44	0.39	NS
	4	15.50 ± 5.51	7.69 ± 5.60	2.16	0.031
	> 4	35.00 ± 14.21	4.00 ± 4.53	2.98	0.003
Biopsy materials	0	1.00 ± 0.82	0.85 ± 0.90	0.36	NS
	1	3.75 ± 0.96	8.54 ± 13.35	0.45	NS
	2	37.75 ± 9.95	63.38 ± 13.42	2.77	0.005
	3	10.75 ± 5.62	12.85 ± 6.07	1.95	NS
	4	13.00 ± 5.29	7.38 ± 6.04	1.65	NS
	> 4	33.75 ± 8.42	7.00 ± 8.54	2.85	0.004

Abbreviations: FISH, fluorescence in situ hybridization; NS, not significant.

As for chromosome 11, the incidence of disomic cells (having two signals) in grade III tumors was significantly lower than that in grade II tumors in both washing samples ($P = 0.031$) and biopsy materials ($P = 0.014$), indicating a higher incidence of aneuploidy. In biopsy materials, the incidence of nuclei with three and four signals of chromosome 11 was significantly higher than that in grade II tumors ($P = 0.014$ and 0.012, respectively).

4. Discussion

FISH analysis is a powerful tool for detecting numerical and structural chromosome aberrations using chromosome-specific DNA probes on interphase nuclei of various tumors. Different structural and numerical anomalies have been reported by using conventional cytogenetics. Some demonstrated a correlation between genetic abnormalities and development and progression of bladder tumors. Aberrations of chromosomes 9 and 17 have been implicated in the development and progression of TCC of the bladder [25,26]. On the other hand, Neuhaus et al. found polysomy of chromosomes 1 and 17 to be significantly associated with bladder cancer recurrence [27]. In addition, Wang et al. reported that partial or complete loss of chromosome 10 was related to invasive bladder tumor as a nonrandom chromosomal abnormality [18]. Similar findings were reported by Gibas et al. and Smeets et al. [28,29].

Chromosome 8 and 11 alterations have been reported in bladder cancer using different techniques. Numerous conventional cytogenetic analyses revealed frequent aneuploidies of chromosomes 8 and 11 as well as structural aberrations in the bladder tumor tissue [11]. Conventional chromosome analysis of cancer cells by karyotyping is often only possible after tissue culture. This technique, however, may result in a selective growth of cells with the highest mitotic index. The small number of recognizable metaphases, the lack of spreading, poor banding quality, and fuzzy nature of the chromosome also may hamper this analysis. By using an interphase FISH technique, specific genetic aberrations have been reported in bladder cancer using both bladder biopsies and washing samples [14,22,30–33]. Chromosomal alterations also have been detected in voided urine samples of patients with bladder cancer [14,20,34,35]. In this study, we focused on chromosomes 8 and 11 because a number of oncogene and tumor supressor genes such as C-MYC and H-RAS are located on these chromosomes. Seventeen patients with bladder TCC were investigated for the presence of numerical aberrations of chromosome 8. In 11 of 17 patients with bladder TCC, an investigation was conducted to search for chromosome 11 in biopsies of bladder tumors and bladder washing samples.

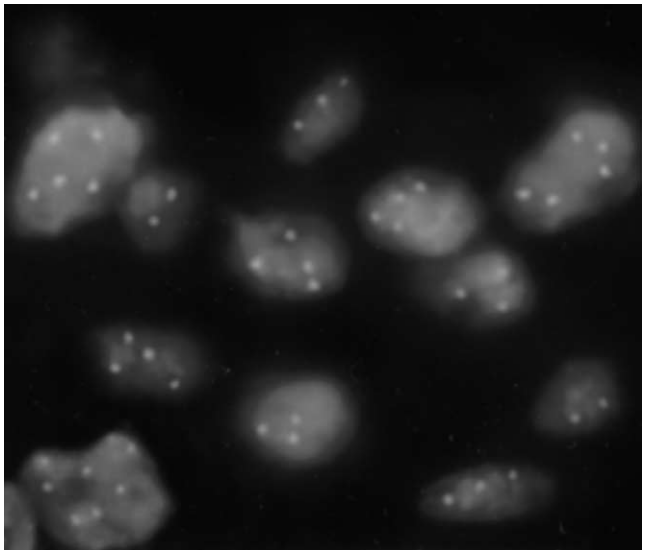

Fig. 1. FISH of interphase cells of patient 1 (grade III) showing two, three, four, and more than four signals, indicating disomic, trisomic, tetrasomic, and polysomic cells for chromosome 8, respectively (DAPI staining).

Table 2
FISH results of chromosome 11 on washing and biopsy materials of 11 TCC patients

Sample type	FISH signals	Grade III (n = 4)	Grade II (n = 7)	Z	P
Washing samples	0	4.00 ± 2.00	2.13 ± 2.30	1.34	NS
	1	8.67 ± 7.02	4.75 ± 3.69	0.82	NS
	2	55.33 ± 2.52	81.38 ± 14.55	2.15	0.031
	3	19.67 ± 5.51	7.25 ± 6.82	1.95	NS
	4	10.33 ± 4.51	4.00 ± 5.83	1.65	NS
	> 4	2.00 ± 3.46	0.50 ± 0.93	0.52	NS
Biopsy materials	0	2.33 ± 1.53	2.75 ± 3.28	0.41	NS
	1	4.00 ± 2.00	4.25 ± 2.96	0.00	NS
	2	55.33 ± 11.37	86.38 ± 8.91	2.45	0.014
	3	24.00 ± 5.57	5.00 ± 4.04	2.50	0.014
	4	13.67 ± 6.66	1.50 ± 2.00	2.50	0.012
	> 4	0.67 ± 1.15	0.13 ± 0.35	0.90	NS

Abbreviations: FISH, fluorescence in situ hybridization; NS, not significant.

The use of both bladder biopsy tumors and bladder washing samples by FISH analysis showed alterations in all cases, although there were small differences among the chromosome 8 and 11 aneuploidies. There was a correlation between structural chromosome 8 abnormalities and higher tumor grades and rates of recurrence [7–9]. In the present study, the results showed a wide range of counts for chromosome 8 signals per tumor cell. This cellular heterogeneity concerning chromosome 8 aneuploidy was higher in grade III tumors than in grade II bladder tumors, although our patients with grade III tumors were limited in number. Similarly, in grade III bladder tumors, the percentage of chromosome 11 aneuploidy was higher than that detected in grade II tumors, although the distribution of cells revealed that the aneuploidy did not show the same range for chromosome 8. These results may indicate that a high percentage of cells having aneuploidy could be related to a higher tumor grade.

In conclusion, this study showed that interphase FISH analysis of touch preparations of fresh bladder tumor tissue and bladder washing samples is a useful method for detecting chromosomal aberrations in individual cancer cells and reveals consistent results. Interphase FISH analysis can be used effectively for the detection of genetic alterations in bladder washing samples.

Acknowledgments

We thank Dr. T. Çora for her valuable assistance, Dr. S. Bodur for the statistical analysis of tha data, and Dr. Unal Egeli for preparation of photograph. This research was supported by Selçuk University Research Fund (grant no. TF-2000/0044).

References

[1] Sandberg AA. Chromosomes changes in bladder cancer: clinical and other correlations. Cancer Genet Cytogenet 1986;19:163–75.

[2] Spruck III CH, Ohneseit PF, Gonzalez-Zulueta M, Esreg D, Miyao N, Tsai YC, Lerner SP, Schmutte C, Yang AS, Cote R, Dubeau L, Nichols PW, Hermann GG, Steven K, Horn T, Skinner DG, Jones PA. Two molecular pathways to transitional cell carcinoma of the bladder. Cancer Res 1994;54:784–8.

[3] Simon R, Bürger H, Brinkschmidt C, Böcker W, Herthle L, Terpe HJ. Chromosomal aberations associated with invasion in papillary superficial bladder cancer. J Pathol 1998;185:345–51.

[4] Erbersdobler A, Friedrich MG, Schwaibold H, Henke RP, Huland H. Microsatellite alterations at chromosome 9p, 13q and 17p in nonmuscle-invasive transitional cell carcinomas of the urinary bladder. Oncol Res 1998;10:415–20.

[5] Zhao J, Rechter J, Wagner U, Roth B, Schraml P, Zellweger T, Ackermann D, Schmid U, Moch H, Mihatsch MJ, Gasser TC, Sauter G. Chromosomal imblanced in noninvasive papiplar bladder neoplasms (pTa). Cancer Res 1999;58:3555–60.

[6] Erbersdobler A, Kaiser H, Friedrich MG, Henke RP. Numerical aberrations of chromosome 8 and allelic loss at 8p in non-muscle-invasive urothelial carcinomas of the urinar bladder. Eur Urol 2000;38: 590–6.

[7] Knowles MA, Shaw ME, Proctor AJ. Deletion mapping of chromosome 8 in cancers of the urinary bladder using restriction length polymorphism and microsatelite polymorphisms. Oncogene 1993;8: 1357–64.

[8] Knowles MA, Elder PA, Williamson M, Cairns JP, Shaw ME, Law MG. Allelotype of human bladder cancer. Cancer Res 1994;54:531–8.

[9] Takle LA, Knowles MA. Deletion mapping implicates two tumor supressor genes on chromosome 8p in the development of bladder cancer. Oncogene 1996;12:1083–7.

[10] Wagner U, Bubendof L, Gasser TC, Moch H, Gorog J-P, Richter J, Mihatssch MJ, Waldman FM, Sauther G. Chromosome 8p deletions are associated with invasive tumorgrowth in urinary bladder cancer. Am J Pathol 1997;151:753–9.

[11] Sandberg AA, Berger CS. Rewiev of chromosome studies in urological tumours. II. Cytogenetics and molecular genetics of bladder cancer. J Urol 1994;151:545–60.

[12] Atkin NB, Baker MC. Cytogenetic study of ten carcinomas of the bladder: involvement of chromosomes 1 and 11. Cancer Genet Cytogenet 1985;15:253–68.

[13] Fadl-Elmula I, Gorunova L, Lungdren R, Mandahl N, Forsby N, Mitelman F, Heim S. Chromosomal abnormalities in two bladder carcinomas with secondary squamous cell differentiation. Cancer Genet Cytogenet 1998;102:125–30.

[14] Meloni AM, Peier AM, Haddad FS, Powell IJ, Block AW, Huben RP, Todd I, Potter W, Sandberg AA. A new approach in the diagnosis and follow-up of bladder cancer. FISH analysis of urine, bladder washings, and tumors. Cancer Genet Cytogenet 1993;71:105–18.

[15] Hoglund M, Sall T, Heim S, Mitelman F, Mandahl N, Fadl-Elmula I. Identification of cytogenetic subgroups and karyotypic pathways in transitional cell carcinoma. Cancer Res 2001;61:8241–6.

[16] Sandberg AA. The chromosomes in human cancer and leukemia. 2nd edition. New York: Elsevier Science Publishing Co., 1990, pp. 805–13.

[17] Georgina B, Rosa M, Maria RC, Jose S, Jose E. Trisomy 7 may be a primary change in non-invasive transitional cell carcinoma of the bladder. Cancer Genet Cytogenet 1990;50:9–14.

[18] Wang MR, Perissel B, Taillandier J, Kemeny J-L, Fonck Y, Lautier A, Benkhalifa M, Malet P. Nonrandom changes of chromosome 10 in bladder cancer: detection by FISH to interphase nuclei. Cancer Genet Cytogenet 1994;73:8–10.

[19] Awata S, Sakagami H, Tozawa K, Sasaki K, Ueda K, Kohri K. Aberration of chromosomes 8 and 11 in bladder cancer as detected by fluorescence in situ hybridization. Urol Res 2000;28:185–90.

[20] Bubendorf L, Grilli B, Sauter G, Mihatsch MJ, Gasser TC, Dalquen P. Multiprobe FISH for enhanced detection of bladder cancer in voided urine specimens and bladder washings. Am J Clin Pathol 2001;116:79–86.

[21] Ishiwata S, Takahashi S, Homma Y, Tanaka Y, Kameyama S, Hosaka Y, Kitamura T. Noninvasive detection and prediction of bladder cancer by fluorescence in situ hybridization analysis of exfloited urothelial cells in voided urine. Urology 2001;57:811–15.

[22] Eleuteri P, Grollino MG, Pomponi D, Guaglianone S, Gallucci M, De Vita R. Bladder transitional cell carcinomas: a comparative study of washing and tumor bioptic samples by DNA flow cytometry and FISH analysis. Eur Urol 2000;37:275–80.

[23] Acar H, Kaynak M, Yakut T, Uçar F, Egeli U. Determination of allelic delition of multiple endocrine neoplasm type 1 (MEN1) gene in acute myeloid leukemia (AML) by application of FISH-TSA technique. Teratog Carcinog Mutagen 2002;22:369–75.

[24] Acar H, Dundar M, Yuce H, Stewart J, Connor MJ. Sreening for trisomy 12 in bone marrow from patient with CLL by the application of FISH to interphase nuclei. Tr J Med Sci 1996;26:461–5.

[25] Tsai YC, Nichols P, Hiti AL. Allelic loss of chromosome 9, 11 and Y7 in human bladder cancer. Cancer Res 1990;50:44–7.

[26] Ghaleb AF, Pizzola JG, Melamed MR. Aberations of chromosome 9 and 17 in bilharzial bladder cancer as detected by fluorescence in situ hybridization. Am J Clin Pathol 1996;106:234–41.

[27] Neuhaus M, Wagner U, Schmid U, Ackermann D, Zellweger T, Maurer R, Alund G, Knönagel H, Rist M, Moch H, Mihatsch MJ, Gasser

TC, Sauter G. Polysomies but not Y chromosome losses have prognostic significant cancer in pTa/pT1 urinary bladder cancer. Hum Pathol 1999;30:81–6.

[28] Gibas Z, Prout GR, Connoly JG, Pontes JE, Sandberg AA. Non random chromosomal changes in transitional cell carcinoma of the bladder. Cancer Res 1994;44:1257–64.

[29] Smeets W, Pauwels R, Laarakkers L, Debruyne F, Geraedts J. Chromosomal analysis of bladder cancer: nonrandom alteration. Cancer Genet Cytogenet 1987;29:29–41.

[30] Cajulis RS, Frias-Hidvegi D. Detection of numerical chromosomal abnormalities in malignant cells on fine needle aspirates by fluorescence in situ hybridization of interphase cell nuclei with chromosome specific probes. Acta Cytol 1993;37:391–6.

[31] Cajulis RS, Haines III GK, Frias-Hidvegi D, McVary K. Interphase cytogenetics as an adjunct in the cytodiagnosis of urinary bladder carcinoma: a comparative study of cytology flow cytometry and interphase in bladder washes. Anal Quant Cytol Histol 1994;16:1–10.

[32] Whelless LL, Reeder JE, Han R, O'Connell MJ, Frank IN, Cocket AJ, Hopman AH. Bladder irrigation specimens assayed by fluorescence in situ hybridization to interphase nuclei. Cytometry 1994;17:319–26.

[33] Sauther G, Gasser TC, Moch H, Richter J, Jiang F, Alberecht R, Novotny H, Wagner U, Bubendorf L, Mihatsch MJ. DNA aberrations in urinary bladder cancer detected by flow cytometry and FISH. Urol Res 1997;25:37–43.

[34] Betz J, Meloni AM, Sandberg AA. FISH studies on the chromosome in male urinary cells. Cancer Genet Cytogenet 1996;88:155–7.

[35] Inoue T, Nasu Y, Tsushima T, Miyaji Y, Murakami T, Kumon H. Chromosomal numerical aberrations of exfoliated cells in the urine detected by fluorescence in situ hybridization: clinical implication for the detection of bladder cancer. Urol Res 2000;28:57–61.