Sex Chromosome Abnormalities in Bladder Cancer: Y Polysomies are Linked to PT_1-grade III Transitional Cell Carcinoma

ANNA D. PANANI and CHARIS ROUSSOS

Critical Care Department, Medical School of Athens University, Research Unit, "Evangelismos" Hospital, Ipsilandou 45-47, Athens 10676, Greece

Abstract. *Background: Bladder cancer is a heterogeneous group of tumors from both the biological and clinical points of view. Conventional cytogenetics and molecular genetic techniques have shown non-random aberrations in bladder cancer, while certain chromosomal changes have been found to be highly correlated with tumor grade or stage. The aim of this study was to evaluate, by fluorescence in situ hybridization (FISH), the numerical aberrations of chromosomes X and Y in bladder cancer, comparing the incidence of nuclei with aneusomies in different grades or histological stages of the tumors. Materials and Methods: The FISH technique, using DNA probes specific for chromosomes X and Y, was applied to 35 male bladder tumor specimens directly processed for cytogenetic study. Results: Polysomies of chromosome X were observed in 25 out of the 35 cases examined (71.43%), while numerical aberrations of chromosome Y were observed in 22 out of the 35 cases (62.86%). Of those cases with numerical aberrations of chromosome Y, 13 had polysomy (37.14%), while in 9 cases, loss of Y was observed (25.71%). Statistical analysis showed that numerical aberrations on chromosomes X or Y were not linked to histological stage, while a probable correlation was observed between aneusomies X or Y and tumor grade. Comparing the results of PT_1-grade III tumors with those of PT_1-grade II, statistical analysis showed that aneusomies Y and, especially, polysomy Y were correlated with PT_1-grade III tumors, $p=0.023$ and $p=0.010$, respectively. An uncertain correlation between polysomy X and PT_1-grade III tumors was found, $p=0.070$. Conclusion: Our results may suggest that the genetic instability associated with PT_1-grade III tumors may account for the considerable potential for aggression of these tumors. However, to draw definite conclusions, a large number of cases must be studied.*

Correspondence to: Anna D. Panani, Critical Care Department, Medical School of Athens University, Research Unit, "Evangelismos" Hospital, Ipsilandou 45-47, Athens 106 76, Greece. Tel: +30-210-7259307, Fax: +30-210-7259307, e-mail: apanani@med.uoa.gr

Key Words: FISH technique, chromosomes X and Y, bladder cancer, stage PT_1-grade III transitional cell carcinoma.

Bladder cancer is a heterogeneous group of tumors from both the biological and clinical points of view. The factors influencing tumor progression are not known and the outcome of the disease is often unpredictable. It has been suggested that multi-step genetic alterations could be involved in bladder tumorigenesis. Conventional cytogenetics and molecular genetic techniques have shown non-random aberrations in bladder cancer, while certain chromosomal imbalances have been found to be highly correlated with tumor grade or stage. The most common numerical chromosomal abnormalities are loss of chromosome 9, trisomy 7 and loss of Y. Common structural chromosomal changes have also been reported. The fluorescence *in situ* hybridization (FISH) technique, with centromeric-specific DNA probes, allows for the rapid detection of numerical chromosomal aberrations of tumor cells (1-12).

In the present study, the FISH technique, using DNA probes specific for chromosomes X and Y, was applied to 35 bladder tumor specimens in order to compare the incidence of nuclei with aneuploidy in different grades or histological stages of the tumors.

Materials and Methods

Thirty-five male bladder tumors of transitional cell carcinoma (TCC) at the time of diagnosis were included in this study. After surgical resection of the tumors, a routine histopathological examination followed. The tumors were graded histopathologically according to the World Health Organization (WHO) system (13) and were classified according to the International Union Against Cancer (14). Twenty five cases were grade III and 10 were grade II. Fifteen cases were histologically classified as superficial papillary tumors PTa-PT_1, and 20 cases as invasive tumors PT_2-PT_4. Six cases were superficial papillary PT_1-grade III. A small portion of each resected tumor was directly processed for cytogenetic study and FISH, using a dual-labelled cocktail of the DNA probes, DXZ1 specific for chromosome X and DYZ3 specific for chromosome Y (chromosome regions Xp11.1-q11.1 and Yp11.1-q11.1, respectively) (Cytocell Technologies, Cambridge, UK), which was applied to recently made slides from the methanol/acetic acid-fixed cells of all tumors. FISH was carried out according to the manufacturer's instructions. The hybridization of the probe with the cellular DNA site was visualized with a

fluorescence microscope NIKON E600 with a triple filter DAPI/FITC/TEXAS RED. Positive chromosome signals appeared as red (chromosome Y) or green (chromosome X) spots in the nuclei. A minimum of 200 cells were evaluated for each case. The signals were scored using the criteria of Hopman *et al.* (15). A case was counted as aberrant if more than 10% of the cell nuclei showed loss or gain of signals for either chromosomes X or Y. For statistical evaluation, the Chi-square test was used.

Results

The histopathological characteristics of the resected bladder tumors are presented in Table I. The numerical aberrations of chromosomes X and Y, in association with the histopathological characteristics of the resected bladder tumors, are given in Tables II and III. A representative example of FISH analysis is shown in Figure 1. Numerical aberrations of chromosome X, including polysomies, were observed in 25 out of the 35 cases examined (71.43%). Loss of chromosome X was not observed in any of the cases studied. Statistical analysis by the Chi-square test showed that the numerical aberrations of chromosome X were not linked to histological stage ($p=0.567$), while a probable correlation was observed between polysomy X and tumor grade ($p=0.089$). Numerical aberrations of chromosome Y were observed in 22 out of the 35 cases examined (62.86%). Of those cases with numerical aberrations of chromosome Y, 13 had polysomy (37.14%), while in 9 cases loss of Y was observed (25.71%). In all cases with loss of Y, a lack of the Y signal was observed in >60% of the cells examined. With regard to the presence of numerical aberrations of chromosome Y in association with tumor histological stage, statistical analysis using the Chi-square test indicated that there was not a significant correlation ($p=0.920$), while a probable correlation was observed between aneusomies Y and tumor grade ($p=0.090$). In all 6 superficial papillary PT_1-grade III tumors, polysomy X was observed, while in 5 and 1 of these cases, high polysomy or loss of Y were observed, respectively. Comparing the results of PT_1-grade III tumors with those of PT_1-grade II, the results of the Chi-square test showed that there was an uncertain correlation between polysomy X and PT_1-grade III tumors, ($p=0.070$), while aneusomy Y and, especially, polysomy Y were correlated with PT_1-grade III tumors, $p=0.023$ and $p=0.010$, respectively.

Table I. *Histopathological characteristics of bladder cancer patients.*

Histopathological characteristics	Total number of cases (N=35)
Histological grade	
I	–
II	10
III	25
Histological stage	
PTa	1
PT_1	14
PT_2	15
PT_3	3
PT_4	2

Discussion

Bladder cancer is a genetically heterogeneous disease involving multiple genetic alterations in its development. However, no specific aberration responsible for bladder cancer has been established to date. Conventional cytogenetics and molecular cytogenetic techniques have shown several recurrent abnormalities and have highlighted the genetic changes involved in bladder cancer. FISH analysis is a powerful tool for detecting chromosome aberrations using chromosome-specific DNA probes on the interphase nuclei of various tumors (1-6, 16-20). The clinical course of urinary bladder TCC is highly variable and a number of genetic alterations appear to be implicated in the development and progression of tumors. Notably, an association of certain genetic changes with tumor of high grade or advanced stage has been reported, suggesting that genomic alterations leading to tumor progression are most likely to accumulate in advanced tumors. The precise analysis of genetic changes may give insights into tumor biology and may provide better prognostic parameters in clinical practice (7, 10-12, 21).

In the present study, sex chromosome abnormalities were evaluated in relation to the histopathological characteristics in a total of 35 TCC male bladder tumors. Regarding chromosome X, polysomy was found in 25 out of the 35 cases studied (71.43%), while loss of X was not observed in any of the cases studied. Polysomy X was not found to be correlated with tumor stage, though a probable correlation with tumor grade was observed. There are no detailed reported data concerning polysomy X in bladder cancer. Polysomy X has been reported in TCC cell lines, but it has not been found to be related to tumor grade or stage (9). Polysomy Y was found in 13 cases and loss of Y in 9 cases. An uncertain correlation of aneusomies Y with tumor grade, but not with tumor stage, was found. With regard to polysomy Y in relation to the clinical and histopathological data of bladder cancer patients, to our knowledge, there are no detailed reports. A missing Y was reported as a frequent event in bladder TCC, which can occur either early in tumor progression or at advanced stages of the disease. An association between Y losses and the unfavorable course of the disease has also been reported. However, other studies have not shown an increased risk of tumor progression or

Table II. *Chromosomes X and Y copy numbers and histopathological characteristics in bladder cancer patients.*

Histopathological characteristics	Chromosome X (N=35)		Chromosome Y (N=35)		Loss
	One copy	Polysomy	One copy	Polysomy	
Histological grade					
II	5	5	6	1	3
III	5	20	7	12	6
Histological stage					
PT_a-PT_1	4	11	5	6	4
PT_2-PT_4	6	14	8	7	5

Table III. *Chromosomes X and Y copy numbers in stage PT_1-grade III and PT_1-grade II bladder cancer patients.*

Histopathological characteristics	Chromosome X		Chromosome Y		Loss
	One copy	Polysomy	One copy	Polysomy	
Stage PT_1-grade III (N=6)	–	6	–	5	1
Stage PT_1-grade II (N=8)	4	4	4	1	3

recurrences in bladder cancer patients with loss of Y (3, 8, 22). In contrast, Neuhaus *et al.* (23) reported an unexpectedly less frequent progression into muscle-invasive carcinomas in PT_1 tumors with a Y loss than in PT_1 tumors without Y loss. Thus, the role of chromosome Y in bladder cancer still remains obscure.

From a clinical point of view, it is important that highly sensitive methods for the diagnosis, prognosis and follow-up of bladder cancer patients be developed. The identification of genetic alterations in bladder tumors, or the detection of genetically-changed cancer cells in urine are of major importance for the characterization of the biological behavior and diagnosis of bladder cancer (24-26). Our results may suggest that aneusomies X or Y are not related to tumor progression to invasive stages, but they might be correlated with high-grade disease. Notably, 26 out of the 35 cases in the present study had been included in a previously reported study in which abnormalities of chromosomes 9 and 11 were evaluated by FISH (7). In that study, it was shown that aneusomy 9 was linked to tumor stage and grade, while polysomy 11 was mainly found in high-grade and advanced disease. Several other studies have shown that aneusomies of different chromosomes were associated with aggressive tumor behavior (27-28). Therefore, it seems that, to derive sufficient conclusions for the clinical significance of chromosomal abnormalities in bladder cancer and to correlate genetic changes with disease aggressiveness, it is necessary that many more cases be studied and that, in each case, as many chromosomes or chromosomal regions as possible be evaluated by FISH.

Despite a favorable prognosis of non-invasive superficial tumors, a large percentage of patients have tumor recurrence and 10-15% of them develop disease progression. The histological grade constitutes the classic prognostic factor related to tumors. However, in the majority of cases, histopathological characteristics cannot predict which superficial papillary bladder tumors will recur and become invasive. It seems that recurrence and progression of these tumors is associated with the acquisition of certain genetic changes that increase the malignant potential of cancer cells. Thus, several studies have shown, in patients with superficial papillary bladder tumors, aneusomies of certain chromosomes to have a predictive value for tumor progression (21, 23, 26-30). From the clinical point of view, superficial papillary PT_1-grade III tumors constitute a distinct group of bladder cancer characterized by a high risk of progression to muscle

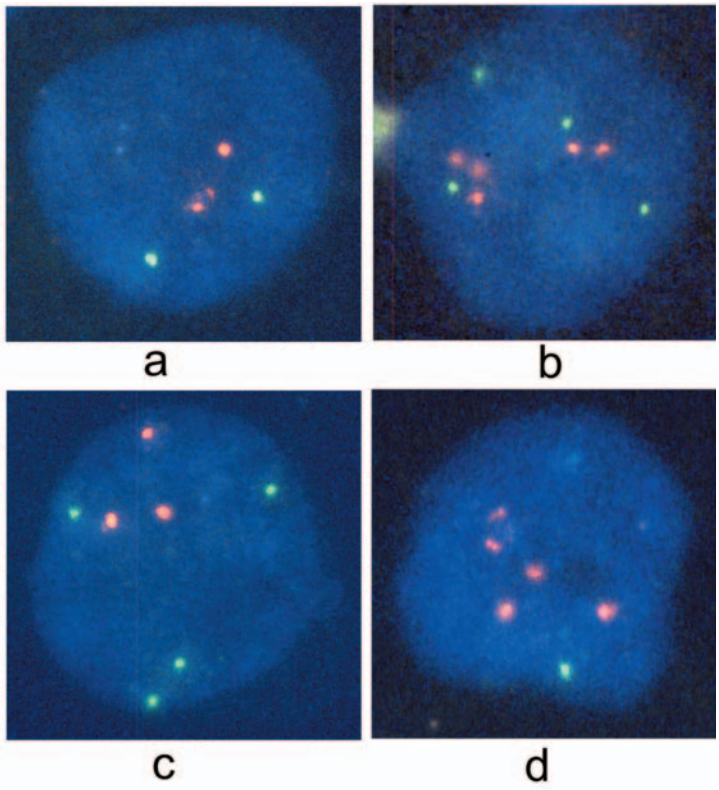

Figure 1. *(a,b,c,d) Copy number of chromosomes X (green spots) and Y (red spots) detected by FISH in bladder cancer cells from four different cases.*

invasion (31). In a study by Ribal *et al.* (28), high polysomies of certain chromosomes were found in this group of tumors. Simon *et al.* (21), using a comparative genomic hybridization technique, found a remarkably higher number of amplifications in PT_1-grade III tumors compared with PT_1-grade II carcinomas. Interestingly, in all 6 cases of the present study with superficial papillary PT_1-grade III tumors, polysomy X was observed, while in 5 and 1 of these cases, high polysomy or loss of Y were observed, respectively. Although the number of cases studied was small, comparing results of PT_1-grade III tumors with those of PT_1-grade II, statistical analysis showed a significant association of polysomy Y with PT_1-grade III tumors, while an uncertain correlation between polysomy X and this group of tumors was also observed. Our results may suggest that genetic instability associated with PT_1-grade III tumors may account for the considerable potential for aggression of these tumors. However, a large number of cases must be studied in relation to clinical characteristics to confirm the possible role of polysomies Y, or possibly of other genetic changes, in the biological behavior of PT_1-grade III tumors. Moreover, based on the cytogenetic findings in relation to histopathological characteristics, a new classification of the disease could be proven and new therapy strategies could also be developed.

Acknowledgements

Dr. Athanasios Gialouris is gratefully acknowledged for the statistical analysis. The technical assistance of Mrs. Athanasia Babanaraki is also gratefully acknowledged.

References

1 Van Tilborg AA and Van Rhijn BW: Bladder: Urothelial carcinomas. Altas Genet Cytogenet Oncol Hematol, October 2003. Available at: http://www.infobiogen.fr/services/chromcancer/Tumors/blad 5001.html.

2 Sandberg AA: Cytogenetics and molecular genetics of bladder cancer: a personal view. Am J Med Genet (Semin Med Genet) *115*: 173-182, 2002.

3 Gibas Z and Gibas L: Cytogenetics of bladder cancer. Cancer Genet Cytogenet *95*: 108-115, 1997.

4 Fadl-Elmula I, Kytola S, Pan Y, Lui WD, Derienzo G, Forsberg L, Mandahl N, Gorunova L, Bergerheim USR, Heim S and Larsson C: Characterization of chromosomal abnormalities in uroepithelial carcinomas by G-banding, spectral karyotyping and FISH analysis. Int J Cancer *92*: 824-831, 2001.

5 Poddighe PJ, Pringuier P-P, Vallinga M, Schalken JA, Ramaekers FCS and Hopman AHN: Loss of chromosome 9 in tissue sections of transitional cell carcinomas as detected by interphase cytogenetics. A comparison with RFLP analysis. J Pathol *179*: 169-176, 1996.

6 Hoglund M, Sall T, Heim S, Mitelman F, Mandahl N and Fadl-Elmula I: Identification of cytogenetic subgroups and karyotypic pathways in transitional cell carcinoma. Cancer Res *61*: 8241-8246, 2001.
7 Panani AD, Babanaraki A, Malianga E and Roussos C: Numerical aberrations of chromosomes 9 and 11 detected by FISH in Greek bladder cancer patients. Anticancer Res *24*: 3857-3862, 2004.
8 Sauter G, Moch H., Wagner U, Novotna H, Gasser TC, Mattarelli G, Mihatsch MJ and Waldman FM: Y chromosome loss detected by FISH in bladder cancer. Cancer Genet Cytogenet *82*: 163-169, 1995.
9 Yu DS, Chen HI and Chang SY: Chromosomal aberrations in transitional cell carcinomas: its correlation with tumor behavior. Urol Int *69*: 129-135, 2002.
10 Wagner U, Bubendorf L, Gasser TC *et al*: Chromosome 8p deletions are associated with invasive tumor growth in urinary bladder cancer Am J Pathol *151*: 753-759, 1997.
11 Richter J. Jiang F, Gorog J-P *et al*: Marked genetic differences between stage pTa and stage pT1 papillary bladder cancer detected by comparative genomic hybridization. Cancer Res *57*: 2860-2864, 1997.
12 Placer J, Espinet B, Salido M., Sole F and Gelabert-Mas A: Correlation between histologic findings and cytogenetic abnormalities in bladder carcinoma: a FISH study. Urology *65*: 913-918, 2005.
13 Mostofi FK, Sobin LH and Torloni H: Histological Typing of Urinary Bladder Tumors. International Histological Classification of Tumors, No 10. World Health Organization, Geneva, pp. 16-17, 1973.
14 Harmer MH: TNM classification of Malignant Tumors. Geneva: International Union Against Cancer, pp. 111, 1978.
15 Hopman AHN, Ramaekers FCS, Raap AK, Beck JLM, Deville P, Ploeg M and Vooijæs GP: *In situ* hybridization as a tool to study numerical aberrations in solid bladder tumors. Histochemistry *89*: 307-316, 1988.
16 Fadl-Elmula I, Gorunova L, Mandabl N, Elfving P, Lundgren R, Mitelman F and Heim S: Karyotypic characterization of urinary bladder transitional cell carcinomas. Genes Chromosomes Cancer *29*: 256-265, 2000.
17 Nemoto R, Nakamura I, Uchida K and Harada M: Numerical chromosome aberrations in bladder cancer detected by *in situ* hybridization. Br J Urol *75*: 470-476, 1995.
18 Inoue T, Nasu Y, Tsushima T, Miyaji Y, Murakami T and 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 *28*: 57-61, 2000.
19 Acar H, Kilinc M, Yildirim MS, Kaynak M and Cenker A: Evaluation of chromosome 8 and 11 aneuploidies in washings and biopsy materials of bladder transitional cell carcinoma. Cancer Genet Cytogenet *142*: 25-29, 2003.
20 Awata S, Sakagami H, Tozawa K, Sasaki S, Veda K and Kohri K: Aberration of chromoses 8 and 11 in bladder cancer as detected by fluorescence *in situ* hybridization. Urol Res *28*: 185-190, 2000.
21 Simon R, Burger H, Brinkschmidt C, Bocker W, Hertle L and Terpe H-J: Chromosomal aberrations associated with invasion in papillary superficial bladder cancer. J Pathol *185*: 345-351, 1998.
22 Betz J, Meloni AM and Sandberg AA: FISH studies on the Y chromosome in male urinary cells. Cancer Genet Cytogenet *88*: 155-157, 1996.
23 Neuhaus M., Wagner U, Schmid U, Ackermann D, Zellweger T, Mauer R, Alund G, Knonagel H, Rist M, Moch H, Mihatsch M and Gasser TC: Polysomies but not Y chromosome losses have prognostic significance in pTa/pT1 urinary bladder cancer. Hum Pathol *30*: 81-86, 1999.
24 Ishiwata S, Takahashi S, Homma Y, Tanaka Y, Kameyama S, Hosaka Y and Kitamura T: Noninvasive detection and prediction of bladder cancer by fluorescence *in situ* hybridization analysis of exfoliated urothelial cells in voided urine. Urology *57*: 811-815, 2001.
25 Junker K, Werner W, Mueller C, Ebert W, Schubert J and Claussen U: Interphase cytogenetic diagnosis of bladder cancer on cell from urine and bladder washing. Int J Oncol *14*: 309-313, 1999.
26 Bartlett JM, Watters AD, Ballantyne SA, Going JJ, Grigor KM and Cooke TG: Is chromosome 9 loss a marker of disease recurrence in transitional cell carcinoma of the urinary bladder? Br J Cancer *77*: 2193-2198, 1998.
27 Watters AD, Going JJ, Grigor KM and Bartlett JM: Progression to detrusor-muscle invasion in bladder carcinoma is associated with polysomy of chromosomes 1 and 8 in recurrent pTa/pT1 tumors. Eur J Cancer *38*: 1593-1599, 2002.
28 Ribal M J, Alcaraz A, Mengual L, Carrio A, Lopez-Guillermo A, Mallofre C, Palou J, Gelabert A and Villavicencio H: Chromosomal high-polysomies predict tumor progression in T1 transitional cell carcinoma of the bladder. Eur Urol *45*: 593-599, 2004.
29 Watters AD, Ballantyne SA, Going JJ, Grigor KM, Cooke TG, JM and Bartlett TG: Aneusomy of chromosomes 7 and 17 predicts recurrence of transitional cell carcinoma of the urinary bladder. Br J Urol *84*: 1-8, 2000.
30 Kruger S, Mess F, Bohle A and Feller AC: Numerical aberrations of chromosome 17 and 9p21 locus are independent predictors of tumor recurrence in non-invasive transitional cell carcinoma of the urinary bladder. Int J Oncol *23*: 41-48, 2003.
31 Holmang S, Hedelin H, Anderstrom C, Holmberg E and Johansson SL: The importance of the depth of invasion in stage T1 bladder carcinoma: a prospective cohort study. J Urol *157*: 800-803, 1997.

Received September 7, 2005
Revised November 11, 2005
Accepted November 23, 2005