ANTICANCER RESEARCH *24*: 3857-3862 (2004)

Numerical Aberrations of Chromosomes 9 and 11 Detected by FISH in Greek Bladder Cancer Patients

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Abstract. *Background: Bladder cancer is a genetically heterogeneous disease. The chromosomal aberrations observed are non-random and they are often correlated with disease progression. Several environmental risk factors have also been reported to be implicated in the pathogenesis of this disease.The aim of this study was to evaluate, by FISH technique, the numerical aberrations of chromosomes 9 and 11 in Greek bladder cancer patients and to correlate them with grade and histological stage of the tumors. Materials and Methods: FISH with a-satellite DNA probes specific for chromosomes 9 and 11 were applied to 35 primary bladder tumors directly processed for cytogenetic study. Results: Numerical aberrations of chromosome 9 were observed in 23 out of 27 tumors (85.18%). Monosomy 9 was detected in 12 cases (44.45%) and polysomy in 11 cases (40.74%).Statistical analysis showed that polysomy 9 was linked to histological stage (p=0.024) and grade (p=0.01) of the tumors, while monosomy 9 was correlated with tumor stage (p=0.050). Numerical aberrations of chromosome 11 were observed in 25 out of 35 cases (71.43%). Polysomy was detected in 24 cases (68.57 %), while only one case (2.86%) had monosomy 11. Polysomy 11 was found mainly in high-grade and advanced-stage tumors. Conclusion: Numerical aberrations of chromosome 9 could be a potential biomarker for bladder cancer screening. Further studies must be carried out to investigate gene alterations reflected by numerical aberrations of chromosomes 9 and 11 also contributing to the classification of this disease.*

Transitional cell carcinoma (TCC), the most common form of urinary bladder cancer, is presented as superficial papillary tumors (PTa – PT_1) and as invasive tumors (PT_2 – PT_4). Morphologically, PTa and PT_1 tumors are distinguished by invasion of the submucosa. From a clinical point of view, stage PTa and PT_1 are subjected to the same treatment with the exception of PT_1 grade III, which has a high risk of progression to muscle invasive disease. Other types of bladder tumors include carcinoma *in situ* (CIS), squamous cell carcinomas and adenocarcinomas (1,2).

Bladder TCC is a heterogeneous group of tumors in terms of their biology and clinical behavior. The course of disease is often unpredictable and factors affecting tumor progression are not known. It has been suggested that multiple pathways, all including multistep genetic alterations, are involved in bladder tumorigenesis. Conventional cytogenetics and molecular genetics have been used for detecting primary and secondary abnormalities implicated in tumor development and progression. It has been reported that the aberration patterns are non-random and that chromosomal imbalances are highly correlated with tumor stage and grade (2,3).

Alterations of chromosome 9 are the most frequent cytogenetic and molecular finding in TCC of all grades and stages. Of those cytogenetic changes affecting chromosome 9, deletions of the short arm involving loss of $9p_{21}$ are the most common. Other cytogenetic abnormalities detected in TCC are allelic losses of 17p,11p,13q, 8p and 18q and numerical changes of chromosomes 1,7,8,9,11 and Y. Several molecular studies have also highlighted changes to certain genes, some of which have been correlated with tumor type and progression (2-9).

The geographical difference in frequency occurring in some types of cancer may be due to molecular as well as environmental differences. With bladder cancer there are large geographic variations in incidence rates. The disease has been associated with various risk factors of which cigarette smoking, parasitic infections and occupational exposure to chemicals are the most important (1,10-12).

The aim of this study was to evaluate, by the FISH technique, the numerical aberrations of chromosomes 9 and 11 in Greek bladder cancer patients and also to compare incidence of nuclei with aneuploidy in different grades or histological stages of tumors.

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Key Words: Fluorecence *in situ* hybridization, chromosomes 9 and 11, bladder cancer, interphase cytogenetics.

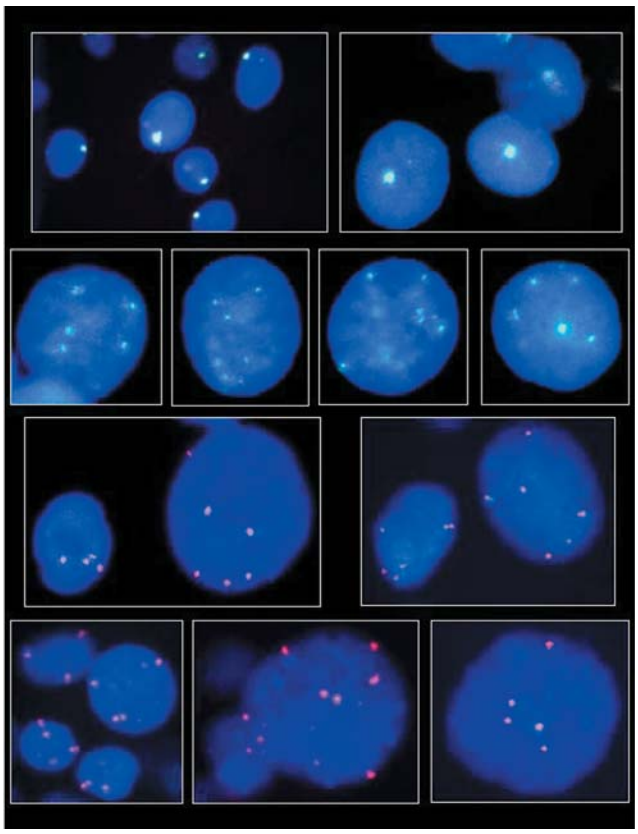

Figure 1. *Copy number of chromosomes 9 (green spots) and 11 (red spots) detected by FISH in bladder cancer cells from different cases.*

Table I. *Histopathological characteristics of bladder cancer patients (n=35).*

Histopathological characteristics	Total number of patients (%)
Histological Grade	
I	1 (2.85%)
II	12 (34.28%)
III	22 (62.85%)
Histological Stage	
Superficial papillary (PTa-PT_1)	12 (34.28%)
Superficial papillary PT_1, grade III	3 (8.57%)
Invasive type (PT_2-PT_4)	19 (54.28%)
In situ	1 (2.85%)

Materials and Methods

Thirty - five patients with TCC of the bladder were included in this study. None of the patients had ever received chemotherapy or radiation prior to surgery. Tissue specimens were collected from surgically resected tumors and 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 classification of the International Union Against Cancer (14). Twenty-two patients were grade III, 12 were grade II and one was grade I. Twelve patients were histologically classified as superficial papillary tumors PTa - PT_1, 19 patients as invasive tumors PT_2 - PT_4 and one patient had *in situ* carcinoma. Three patients were superficial papillary PT_1 - grade III. Because this histological type from the clinical point of view has a high risk to progress to muscle invasion (15), it was considered as an invasive type. A small portion of each resected tumor was directly processed for cytogenetic study and FISH was applied on recently made slides from the methanol/acetic acid-fixed cells of all patients, as described elsewhere (16).The DNA probes D9Z3 specific for chromosome 9 (chromosome region 9q12) and D11Z1 specific for chromosome 11 (chromosome region 11p11.1 – q11.1) were used. The hybridization of the probe with the cellular DNA site was visualized by fluorescence microscopy NIKON E600 with a triple filter DAPI/FITC/TEXAS RED. Positive chromosome signals appeared as red or green spots in nuclei. A minimum of 200 cells from each slide were evaluated for each case and for each chromosome probe. Signals were scored using the criteria of Hopman *et al.* (17). To avoid misinterpretation due to technical error, normal lymphocyte nuclei were used as a control. Approximately 97% of control lymphocyte nuclei showed two signals for probes specific for chromosomes 9 and 11. A case was counted as aberrant if more than 10% of cell nuclei showed loss or gain of signals for chromosomes 9 or 11. For statistical evaluation, the Kruskal Wallis and the Mann-Whitney tests were used.

Results

The histopathological characteristics of the resected bladder tumors are shown in Table I. Table II shows numerical aberrations of chromosomes 9 and 11 in association with the histopathological characteristics of the resected bladder tumors. A representative example of FISH analysis is shown in Figure 1. Numerical aberrations of chromosome 9 were observed in 23 out of 27 cases examined (85.18%). Of those cases with numerical aberrations, 12 presented monosomy (44.45 %) and 11 polysomy (40.74 %). Statistical analysis using the Kruskal - Wallis and the Mann-Whitney tests showed that polysomy 9 was linked to histological stage ($p=0.024$) and grade ($p=0.01$) of the tumors while monosomy 9 was correlated with tumor stage ($p=0.050$). A probable correlation was observed between monosomy 9 and

Table II. *Copy number of chromosomes 9 and 11 and histopathological characteristics in bladder cancer cases.*

Histopathological characteristics	Chromosome 9 (n=27)			Chromosome 11 (n=35)		
	Disomy n (%)	Monosomy n (%)	Polysomy n (%)	Disomy n (%)	Monosomy n (%)	Polysomy n(%)
Histological Grade						
I	1 (100%)			1 (100%)		
II	3(27.27%)	6 (54.55%)	2 (18.18%)	5 (41.66%)		7 (58.33%)
III		6 (40%)	9 (60%)	4 (18.18%)	1 (4,55%)	17 (77.27%)
Histological Stage						
Superficial Papillary ($PTa-PT_1$)	4 (40%)	4 (40%)	2 (20%)	6 (50%)		6 (50%)
Invasive Type * (PT_2-PT_4)		8 (50%)	8 (50%)	4 (18.18%)	1 (4.55%)	17 (77.27%)
In situ			1 (100%)			1 (100%)

* In this group 3 cases of superficial papillary PT1-grade III are also included

tumor grade, $p=0.078$. Numerical aberrations of chromosome 11 were observed in 25 out of 35 cases examined (71.43%). Of those cases with numerical aberrations of chromosome 11, 24 presented polysomy (68.57 %) and only one case had monosomy 11 (2.86%). Regarding the presence of polysomy 11 in association to grade or histological stage of the tumors, statistical analysis using the Kruskal - Wallis and the Mann-Whitney tests showed that there was no significant correlation, $p=0.127$ and $p=0.133$, respectively.

Discussion

Bladder cancer is a genetically heterogeneous disease with heterogeneous growth properties. It has been suggested that multiple pathways with multistep genetic alterations are involved in the development of this disease but, to date, a specific aberration responsible for bladder cancer has not been established. The tumors display complex karyotypes with various numerical and structural abnormalities. However, several non - random chromosomal aberrations patterns have been suggested (2,3). Conventional chromosome analysis of cancer cells by karyotyping is very difficult. The small number of recognizable metaphases, the poor banding quality and the fuzzy nature of the chromosome hamper this analysis. The application of molecular cytogenetic techniques has resolved some of the above difficulties and has highlighted the genetic changes of bladder cancer (4,5,7,9,18-20). Some of these changes have been well correlated with tumor grade and stage. FISH analysis is a powerful tool for detecting chromosome aberrations using chromosome-specific DNA probes on interphase nuclei of various tumors. Regarding the pathogenesis of bladder cancer, several studies have also shown that various environmental factors are associated with this disease.

In the present study, we evaluated the numerical aberrations of chromosomes 9 and 11 in a total of 35 TCCs of Greek bladder cancer patients. We focused on these chromosomes because a number of oncogenes and tumor suppressor genes are located on them. We used the FISH technique, which is considered to be a valuable method for the detection of numerical chromosomal aberrations. Regarding chromosome 9, in most specimens (85.18 %) numerical aberrations were detected. In 40.74% of the cases an increase of the copy number of chromosome 9 was detected, while 44.45% of the cases presented loss of chromosome 9. Changes involving loss of genetic material of chromosome 9 are the most frequent genetic alterations in TCC of the bladder. They have been reported in early as well as in advanced disease and they are frequently seen as the sole cytogenetic abnormality, suggesting that they are primary events in uroepithelial carcinogenesis. Besides loss of material from both arms of chromosome 9, loss of the entire chromosome copy is seen in about 50% of the reported cases. Several loci were defined by RFLP - analysis and microsatellite studies: $9p_{21}$, $9q_{22}$ and $9q_{32-34}$. In $9p_{21}$ the tumor suppressor gene P_{16} was identified,

which is a negative cell cycle regulator encoding an inhibitor of cyclin - dependent kinases. This gene seems to be a major deletion target in bladder cancer (2,3,21-23). Concerning polysomy 9 in bladder cancer, there are no detailed reported data. Ishiwata *et al.* (24) reported that losses of chromosome 9 are generally found in low-grade and early-stage tumors. In contrast, chromosomal gain is often observed in patients with high-grade and advanced-stage tumors. Besides tumor suppressor genes on chromosome 9, which are suggested to play a role in bladder carcinogenesis, the presence of other oncogenes reflected by polysomy 9 and participating in the neoplastic process could not be excluded. Of interest in this study was the detection of an association of polysomy 9 with both histological grade and stage of the bladder tumors, while monosomy 9 was linked to tumor stage. From a clinical point of view, it is important to develop highly sensitive and non - invasive methods for the follow - up of bladder cancer patients, because 70 - 80% of all bladder cancer recur. Detection of genetically changed tumor cells in urine is one of the new approaches for characterization of biological behavior and diagnosis of carcinomas (24-26). Numerical aberrations of chromosome 9 could be a potential biomarker for bladder cancer screening. Bartlett *et al.* (27), using the FISH technique, proposed that loss of chromosome 9 from primary TCC of the bladder identified patients at high risk of recurrence and possible progression.

Regarding chromosome 11, in most cases (71.43 %) numerical aberrations were found. All of these cases except one presented polysomy 11. However, a correlation between polysomy 11 and histopathological stage or grade of disease was not detected by statistical analysis. Numerical aberrations of chromosome 11 are a frequent finding in bladder cancer and they frequenty include monosomy. Also deletion of 11p is commonly found in bladder cancer cases (2,3,6,8). Mutations of the H-RAs gene located on 11p have been frequently reported in bladder cancer (1). In some studies, a correlation was shown between the numerical aberrations of chromosome 11 and the grade or stage of the tumor (28,29), but other studies were unable to confirm such a correlation (30). Acar *et al.* (29) showed that, in washing samples and biopsy material of bladder cancer, the incidence of nuclei with three or four signals of chromosome 11 was significantly higher in grade III than that in grade II tumors. The authors consider that FISH analysis can be used effectively for the detection of genetic alteration in bladder washing samples.

Numerical abnormalities involving chromosome 11, in which cyclin - D is located, could be a mechanism for the increase of cyclin - D copy number. Array-based comparative genomic hybridization detected high level amplification of 11q13 (CCND1) in bladder cancer. Molecular studies have also revealed a good correlation between DNA copy numbers and cyclin-D1 expression in amplified areas. However, a high cyclin-D1 protein expression has also been observed without simultaneous amplification (1,31-33). Furthermore, the numerical aberrations of chromosome 11 might reflect alterations in other genes implicated in the genesis and progression of bladder cancer.

Although the number of cases we studied was not very large, we showed that polysomy 9 in Greek bladder cancer patients is correlated with tumor histological grade and stage, while monosomy 9 was linked to tumor stage. Regarding numerical aberration of chromosome 11, monosomy was found in only one case while polysomy was the prominent finding. It was mainly found in high-grade and advanced-stage patients (77.27 %), but this was not statistically significant.

Bladder cancer is a genetically heterogeneous disease proceeding *via* different pathways of tumorigenesis and progression and the possible genetic causes of this heterogeneity have to be thoroughly investigated, contributing also to the classification of this disease.

Acknowledgements

Dr Athanasios Gialouris is gratefully acknowledged for the statistical analysis. The assistance of the staff of the Department of Pathology in Evangelismos Hospital, Athens, Greece, is also gratefully acknowledged.

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Received July 27, 2004
Accepted October 27, 2004