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C. Deliveliotis · J. Georgoulakis · A. Skolarikos
N. Trakas · J. Varkarakis · S. Albanis · B. Protogerou
A. Bamias

DNA ploidy as a prognostic factor in muscle invasive transitional cell carcinoma of the bladder

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Abstract Radical cystectomy represents the treatment of choice for muscle-infiltrative bladder carcinoma; however, about 50% of patients relapse and die from the disease. In the present study, the prognostic significance of the DNA ploidy in transitional cell carcinoma of the urinary bladder (TCCB) is analyzed. The study was carried out on 66 patients with TCCB who underwent radical cystectomy. DNA ploidy was determined by flow cytometry (FCM) on paraffin-embedded specimens, and the results were analyzed and correlated with the tumor malignancy grade and stage and the clinical course. Forty of the 66 tumors studied (63%) were aneuploid. Aneuploid status was correlated with higher tumor T stage ($P < 0.001$) and grade ($P < 0.001$). Median follow up was 68 months (range: 12–105). Median survival was significantly longer in patients with diploid tumors (> 60 vs 45 months, $P < 0.001$). All patients with diploid tumors were alive and free of bladder cancer during follow-up, in contrast to only 30% of patients with aneuploid tumors. DNA ploidy was an independent prognostic factor, as shown by multivariate analysis ($P = 0.006$). All patients with pT≥3b and diploid tumors were alive at the time of analysis as opposed to none with aneuploid tumors. The results of this study suggest that DNA ploidy can provide prognostic information on patients with muscle invasive carcinoma of the bladder and might represent a means of selection for postoperative management.

C. Deliveliotis · J. Georgoulakis · A. Skolarikos · N. Trakas
J. Varkarakis · S. Albanis · B. Protogerou
Department of Urology, Medical School,
University of Athens, Athens, Greece

A. Bamias
Department of Clinical Therapeutics, Medical School,
University of Athens, Athens, Greece

A. Bamias (✉)
Komninon 31, Haidari, Athens 124 62, Greece,
E-mail: abamias@med.uoa.gr
Tel.: . +30-210-3381546
Fax: +30-210-3381511

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Introduction

Bladder cancer is a common malignancy worldwide. The crude incidence in the European Union is 23 cases/100,000/year and the mortality is ten cases/100,000/year. The natural history of this disease is diverse. Most bladder cancers are initially superficial but 60% recur after local treatment, with 30% of them being more advanced at recurrence and about 10% eventually progressing to muscle infiltrative tumors [1].

Radical cystectomy is the treatment of choice for muscle invasive transitional cell carcinoma of the urinary bladder (TCCB) but 50% of these patients will die from their disease. The current classification systems for TCCB are based on depth of invasion of the bladder wall and metastases to lymph nodes or distant organs (TNM staging), as well as on histopathological features like tumor grade and the presence of an in situ component. Although these features are generally associated with prognosis, their prognostic value is limited when specific subgroups of patients are studied.

DNA flow cytometric analysis has received considerable attention because of its capability of providing important prognostic information in several human malignancies [2, 3, 4]. Bladder cancer represents a potentially successful area for DNA ploidy studies because of the convenience of obtaining tumor cells through urine collection and biopsy specimens through cystoscopy. We have previously shown that there is a good correlation between bladder washings and tumor tissue as well as flow and image cytometry [5]. Other studies have suggested that DNA ploidy could be a useful prognostic indicator in bladder cancer [6, 7, 8, 9, 10, 11, 12]. Most of the studies showing a correlation of DNA ploidy with prognosis included patients with superficial bladder cancer, while its prognostic

significance in muscle invasive bladder cancer remains controversial. Two studies [9, 10] showed a positive correlation but four other studies [13, 14, 15, 16] showed no relationship to prognosis. Nevertheless, the results of these studies are difficult to evaluate since patients had received neoadjuvant chemotherapy or radiotherapy or were entered into bladder preservation protocols. Based on the above data we performed a retrospective study to assess the prognostic value of DNA ploidy in patients undergoing cystectomy for bladder cancer without any additional treatment.

Patients and methods

Patients

A total of 66 consecutive patients who underwent radical cystectomy for TCCB between January 1995 and December 2000 in the Urology Department, Sismanoglion Hospital, were studied. Patients suffered from invasive bladder carcinoma or superficial tumors exhibiting multiple recurrences and/or resistance to intravesical therapy. Pre-treatment staging of the disease was based on bimanual examination, excretory urography, transurethral resection and histological examination of the specimens, and finally on CT scan of the abdomen and pelvis. Patients with inoperable or metastatic disease were excluded. Pelvic lymph node dissection was carried out in all patients. Stage and grade of the tumors were determined according to the American Joint Committee on Cancer criteria [17] and the World Health Organization criteria [18], respectively. Cases before these publications were retrospectively reviewed in order to ensure uniform reporting. Patients did not receive any form of adjuvant or neoadjuvant treatment and were followed-up at regular 6-month intervals.

Flow cytometric analysis

Nuclear suspensions for flow cytometry (FCM) measurements were prepared separately in each case from paraffin sections selected by one pathologist after microscopic examination. After deparaffinization, the specimens were enzymatically disaggregated, stained with propidium iodide according to the manufacturer's protocol, and analyzed on a FACScan flow cytometer (Becton Dickinson, San Jose, Calif.). Chicken erythrocytes were used for instrument calibration. Lymphocytes from healthy donors were also used as a control for the normal diploid peak. An average of 40,000 cells were analysed from each tumor sample at flow rates varying from 30 to 100 cells/s. The DNA ploidy status was expressed as the DNA index, which was the ratio of the G0/G1 peak of the neoplastic cells over that of the lymphocytes. A population was characterized as diploid (DNA index 1) when the neoplastic cells and the lymphocytes provided the same, single G0/G1 peak. Aneuploid populations were characterized by a different peak containing at least 15% of the total number of the neoplastic cells.

Statistical analysis

For statistical analysis, SPSS software was used (SPSS for Windows, version 10, SPSS, Chicago, Ill.). Correlation of DNA ploidy with baseline characteristics was performed using the χ^2-test and Fischer's exact test, where appropriate. Life tables were estimated by Kaplan-Meier statistics and survival curves were compared using the log-rank test. Multivariate analysis was performed according to Cox's proportional-hazard model. Survival was calculated from the day of surgery. All *P*-values were two tailed and 5% was chosen as the level of statistical significance.

Results

Patients

The median age of patients was 62 years (range 40–75). All patients were male. Of the 66 TCCBs, 14 (21%) were stage T_1, 16 (24%) stage T_2, 12 (18%) stage T_{3a}, 18 (28%) stage T_{3b}, and six (9%) stage T_4. The tumor grades were as follows: 12 (18%) were G_1, 18 (28%) G_2, and 36 (54%) G_3. Six out of the 66 patients (9%) had lymph node involvement.

Flow cytometry

Aneuploid tumors were found in 40 patients (61%) and diploid tumors in 26 patients (39%). Aneuploid status was strongly correlated with a higher tumor T stage and grade. No tumor with lymph node metastasis was diploid, as opposed to 43% of tumors without lymph node involvement but this difference did not reach statistical significance (Table 1).

Table 1 Correlation of DNA ploidy with baseline characteristics

	DNA ploidy		*P*
	n (%)		
Stage	Diploid	Aneuploid	
T1 + T2 + T3a	22(52)	20(48)	< 0.001
T3b + T4	4(17)	20(83)	
Grade			
1	12(100)	0(0)	< 0.001
2	8(44)	10(56)	
3	6(17)	30(83)	
LN involvement			
Yes	0(0)	6(100)	0.074
No	26(43)	34(57)	

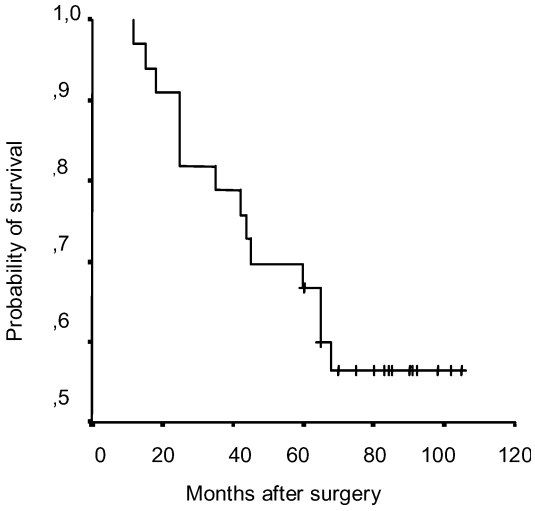

Fig. 1 Survival of all 66 patients treated with cystectomy

Survival analysis

The median follow up was 60 months (12–105). During follow-up, 30 patients died from bladder cancer. The median survival time of the whole population was 77 months (95% CI: 68–85). Median survival of patients with diploid tumors was significantly longer than that of patients with aneuploid tumors (>60 vs 45 months, $P<0.001$) (Fig. 1). During follow-up, all 26 patients with diploid tumors were alive, in contrast to only 12 (30%) among 40 patients with aneuploid tumors.

Univariate analysis showed that lymph node involvement, high tumor grade, advanced tumor stage and aneuploidy were associated with significantly shorter survival ($P<0.0001$ for all factors). Multivariate analysis showed that lymph node involvement and ploidy were independent prognostic factors for survival [$P<0.0001$, hazard ratio: 18 (95% CI: 5.6-57) and $P=0.006$, hazard ratio: 72 (95% CI: 3.3-266)].

When patients were stratified according to stage ($T \leq 3a$ vs $T>3a$), grade (1+2 vs 3) and lymph node involvement, DNA ploidy was associated with a better prognosis in all subgroups of stage and grade as well as in the subgroup of tumors without lymph node involvement (Table 2). The most striking differences in the percentage of surviving patients were observed between diploid and aneuploid tumors within the poor prognosis groups (pT≥3b, grade III).

Discussion

TCCB has a diverse natural history. The main goal of frequent follow-up is to retain an intact bladder in patients whose tumors will remain superficial, while at the same time to proceed early to cystectomy in patients who will progress to muscle invasive disease in order to achieve a high probability of cure. Histopathological stage and grade have long been used to guide surgeons in their decisions but the prognostic information they offer is limited, since cystoscopy staging can be inaccurate [19, 20], while tumors which progress are almost universally of high grade. For this reason, new methodologies and techniques have been applied to supplement the prognostic information provided by stage and grade.

The determination of the nuclear DNA content of TCCB has been frequently investigated, and many of the reports published in recent years have considered the role of DNA ploidy in studies that evaluated a number of prognostic factors in well-characterized groups of patients [6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 21, 22, 23]. Most studies investigated the role of DNA ploidy in superficial bladder cancer. Nevertheless, prognostic information is also necessary in patients undergoing cystectomy for muscle invasive or high grade T1 bladder cancer, since mortality from this disease is high and there is a suggestion that adjuvant or neoadjuvant chemotherapy might be of benefit provided that the selection of appropriate candidates is possible [24, 25]. We attempted to study DNA ploidy estimation with FCM as a potential selection criterion by selecting patients who did not receive any other form of treatment apart from cystectomy. We believe that this represents an advantage of our study, since the inclusion of patients who were treated using radiotherapy or chemotherapy in addition to cystectomy in previous reports [10, 13, 14, 15, 16, 26] may complicate the interpretation of the results.

Table 2 Outcome according to ploidy within specific subgroups of patients

Subgroup		Median survival	Log rank *P*	Patients alive (%)
T1 + T2 + T3a	Diploid	>60	<0.001	100
	Aneuploid	73		70
T3b + T4	Diploid	>60	<0.001	100
	Aneuploid	31		0
Grade I + II	Diploid	>60	<0.001	100
	Aneuploid	63		40
Grade III	Diploid	>60	0.005	100
	Aneuploid	47		27
No lymph node involvement	Diploid	>60	<0.001	100
	Aneuploid	58		35

DNA aneuploidy was found in 63% of our cases, which is in agreement with the results of Lee and Park [9], but different from the DNA aneuploidy rate of 44% reported by Bittard et al. [11] and the rate of 82% reported by Campanella et al. [7]. These differences are most probably due to a number of factors, such as difference in patient selection, number of cases, sampling method and analytical techniques. Our study confirms the prognostic significance of DNA ploidy in this group of patients. All patients with diploid tumors survived the follow-up period with no evidence of disease and the prognostic significance of DNA ploidy was independent of other prognostic factors, i.e. stage, grade and lymph node involvement.

While radical cystectomy remains the standard therapy for muscle-invasive bladder cancer, the high incidence of treatment failure with locally advanced cancers continues to present a therapeutic challenge. Although the role of adjuvant chemotherapy has not yet been defined, it is frequently offered to patients with extravesical extension (pT≥3b) or regional lymph node involvement [27, 28]. The evaluation of selection criteria for adjuvant therapies is of paramount importance in order to achieve the maximum benefit, while patients with a low probability of benefit could be spared the side effects of chemotherapy. Several molecular markers are currently being evaluated in human malignancies, including bladder cancer [1]. Our subgroup analysis showed that all patients with pT≥3b and diploid tumors were alive at the time of analysis as opposed to none with aneuploid tumors. Although the number of patients included in our study is relatively small, this difference is highly significant and the follow-up long enough to support the potential of tumor ploidy evaluation by FCM as a useful marker for selection for adjuvant treatment within this poor prognosis group: adjuvant treatment seems to be unnecessary for patients with diploid tumors, while such treatment might be useful in order to improve the outcome in patients with aneuploid tumors. Nevertheless, it should be stressed that our study was not designed to answer this question, which can only be addressed in properly designed prospective randomized trials. In addition, an important aspect of the application of DNA ploidy as a prognostic marker in bladder cancer is its reproducibility. DNA ploidy estimation cannot substitute for stage and grade, since it could not be considered an equally important prognostic marker based on existing data. Nevertheless, data from other studies as well as ours underline the prognostic significance of tumor behavior and indicate that if these results are confirmed in large, prospective trials, DNA ploidy might be useful in selecting patients for postoperative management within groups of similar stage and grade.

In conclusion, the results of this study show that DNA ploidy is an important prognostic factor for patients with TCCB and a possible selection criterion for adjuvant treatment following cystectomy.

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