Comparative Genomic Hybridization Analysis Reveals New Different Subgroups in Early-stage Bladder Tumors

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OBJECTIVES To classify bladder tumors according to their genomic imbalances and evaluate their association with patient's outcome.

METHODS Sixty-three superficially and minimally invasive bladder tumors were analyzed by conventional comparative genomic hybridization. Subtelomeric screening in 15 of these tumors was performed by multiplex ligation-dependent probe amplification.

RESULTS Losses of 9q and 9p (32% and 25% of all cases, respectively) as well as gains of chromosomes Xq and Xp (28% and 25%, respectively) were the most frequent chromosome imbalances. Losses of 8p and gains in 1q and 8q were detected in >20% of cases. Tumors were classified into 3 groups according to their individualized pattern of gains and losses. The largest group was characterized by few chromosome imbalances, presenting 77% and 49% of the Ta and T1 tumors, respectively. Another group characterized by chromosomal gains, was composed of equal number of Ta and T1 tumors, with +1q and +17q gains being the most common imbalances. A minority group was characterized by chromosomal losses on 11q, 5q, and 6q. The multiplex ligation-dependent probe amplification study showed good correlation with comparative genomic hybridization results. With regard to the biological significance of this classification, a remarkable fact is that this minority group composed mainly of T1 tumors, showed a significant decrease in patient overall survival.

CONCLUSIONS Our data suggest that superficial carcinomas of the bladder can be subdivided into a larger number of subclasses than had previously been expected. Our results also demonstrate a decreased survival among patients whose tumors show more genomic losses than gains. UROLOGY 75: 347–356, 2010. Crown Copyright © 2010 Published by Elsevier Inc.

Bladder cancer is the fifth most frequent neoplasm in developed countries. The most common form corresponds to urothelial cell carcinomas (BUC, bladder urothelial cell carcinomas), accounting for approximately 90% of all cases. BUC can be classified into 2 categories based on their histopathology and clinical behavior. Approximately 70%-80% of BUC are superficial, noninvasive papillary carcinomas (stage Ta), or microinvasive tumors (stage T1) at initial diagnosis and they are associated with a high risk of recurrence (70%) after treatment. The remaining 20%-30% of bladder cancers showing muscle invasion at the time of diagnosis (≥ T2) have no association with superficial papillary tumors and are thought to arise from carcinomas *in situ*. Approximately 50% of patients with muscle-infiltrating tumors already harbor or will develop metastasis.[1]

Several models of bladder cancer progression have been suggested. According to Spruck et al,[2] 2 distinct molecular pathways seem to exist for the formation of noninvasive bladder cancers: *TP53* inactivation appears to occur early in carcinomas *in situ* and dysplasia tumorigenesis. Conversely, losses involving chromosome 9 may be sufficient for superficial papillary growth in tumors. Van Rhijin et al[3] proposed a modified molecular pathway in which *FGFR3* mutations are involved in low-grade or low-stage tumors. By using computer simulations, Höglund et al[4] indicated the presence of at least 2 cytoge-

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netic pathways in bladder cancer. The first is characterized by the loss of chromosome 9, and the second by the gain of chromosome 7. More recently, Mhawech-Fauceglia et al[5] proposed 3 different progression pathways integrating well-characterized oncogenes and tumor suppressor genes involved in bladder carcinogenesis.

A comprehensive picture of chromosome imbalances, including a number of previously unknown genomic alterations such as high-level amplifications, has been reported in urothelial carcinomas using metaphase comparative genomic hybridization (CGH).[6-10] Although some discrepancies exist, these studies show that losses of 9p and 9q are frequent in tumors of all grades and stages. Gains in 1q, 3p, 6p, 8q, 10p, 17q, and 20q and losses in 2q, 5q, 8p, 10q, 1q, 13q, 14q, and 17p are more frequently observed in T1 and T2 tumors. Several alterations such as loss of 6q and gains in 7p and Xq are more frequently seen in T2-T4 tumors.

Chromosome regions previously reported using conventional CGH and loss of heterozygosity[11] have also been confirmed using array-based CGH.[12-15] In addition, high-resolution mapping of copy number changes in different stages of bladder carcinogenesis allowed for the identification of alterations in many small genomic regions, some of them with high-level amplifications or homozygous deletions.[13] Candidate target genes have been identified in many of these amplified and deleted regions.[5,16,17]

The molecular discrimination between early-stage and invasive bladder cancer has already been described in previous analyses of pools of bladder tumors of different stages and grades using expression profiling analysis.[18,19]

Although both Ta and T1 tumors are considered superficial, they also represent a heterogeneous group of tumors that may differ in their molecular pathogenesis and pathways of progression. By array expression analysis,[20] these tumors were separated into 2 groups. One cluster contained only Ta tumors (mainly low grade), whereas the other contained all the T1 tumors (all high grade) and a subset of Ta tumors (both high and low grade).

Significant differences in the fraction of genome altered (FGA) have been identified among tumors of different stages, grades, and patient outcome. Stage Ta tumors had the lowest levels of alteration, and stage T1 showed a much higher overall FGA.[14] A subclassification of Ta tumors has also been described on the basis of their gene expression patterns.[19]

In the present study, 63 early-stage carcinomas of the bladder have been analyzed by conventional CGH. Subtelomeric screening in 15 of these tumors was performed by multiplex ligation-dependent probe amplification (MLPA). Analysis of chromosome imbalances in superficially and minimally invasive bladder tumors could add more insight to ascertain events associated with evolutive patterns in these tumors.

MATERIAL AND METHODS

Patients and Methods

A total of 39 tumor samples from 37 men and 2 women were obtained by transurethral resection and were embedded in paraffin blocks. The CGH findings of additional 24 cases (U-1 to U-24) have previously been published.[10] All patient samples were obtained consecutively. Clinical and pathologic data of the patients are presented in Table 1. A total of 26 cases were classified as Ta (11 G1 and 15 G2) and 37 as T1 (16 G2 and 21 G3). All tumors showed a papillary growth pattern. Specimens were graded according to the World Health Organization classification and staged according to the tumor-node-metastasis classification system.[21,22] The median follow-up time of the patients was 60.82 months.

Comparative Genomic Hybridization

For each tumor sample, DNA was extracted from four to five 10-μm paraffin sections using a Qiagen Kit (QIAamp DNA Mini Kit, Basel, Switzerland). Before extraction, the proportion of tumor cells was found to be $>$ 80% as determined by a pathologist. The first and last sections were stained with hematoxylin/eosin to ensure the presence of the tumor in the sections series. CGH analysis was performed according to the method described by Prat et al.[10]

Multiplex Ligation-Dependent Probe Amplification

The MLPA kit P070 (MRC-Holland Amsterdam, The Netherlands) was used according to the manufacturer's protocol. A minimum of 4 normal control samples were included for each MLPA assay. The samples were analyzed using a capillary electrophoresis system (ABI 3100 genetic Analyzer, Applied Biosystems) with Rox 500 size standard. GeneMapper Software v4.0 (Applied Biosystems) was used to quantify the generated peaks. Data from GeneMapper were exported to an Excel file and normalization was achieved by dividing the signal value of each probe by the sum of the signal values of all probes for each sample. A normal copy number should generate a normalized signal value of approximately 1. A reduction of $>$30% in the relative signal strength was considered a deletion of the locus and gains of $>$30% in the signal strength were considered a duplication of the locus. Reduction or gains of 20%-30% in the signal strength were only considered if confirmed by CGH results. Variations of $<$20% were considered normal. The probes for acrocentric chromosomes are indeed located only in the long arms close to the ends, centromere, and telomere.

Statistical Analysis

Overall survival was estimated with the Kaplan-Meier method. The survival curves were statistically compared by a log-rank test. All statistical analyses were performed with SPSS version 15.0, with $P < .05$ considered as statistically significant.

RESULTS

A total of the 63 tumors were analyzed by CGH. The number of imbalances per tumor was 0-17. On average, 5.7 alterations were found per tumor: 3.0 gains (range 0-11) and 2.6 losses (range 0-13).

Losses of 9q and 9p (32% and 25% of all cases, respectively) as well as gains of chromosomes Xq and Xp (28% and 25%, respectively) were the most frequent chromo-

Table 1. Patient data and tumor characteristics

Case	Age/Sex	Stage/Grade	No. Gains	No. Losses	Group	Single/ Multiple	Primary	Recurrence	Survival
U-1	70/*	TaG1	4	0	1	S	+	NO	?
U-2	87/†	TaG2	1	1	3	S	+	NO	?
U-3	61/*	TaG2	0	0	3	S	+	NO	>84
U-4	88/*	TaG2	0	1	3	S	+	NO	>120
U-5	66/*	TaG2	2	1	3	M	+	NO	>60
U-6	93/†	TaG2	2	2	3	S	—	NO	>24
U-7	70/*	TaG2	0	0	3	M	+	(4) CIS	>72
U-8‡	76/†	TaG2	0	0	3	S	+	(8)	>42
U-9	81/*	TaG2	3	4	2	M	+	(28) T1G2; (8) T2G3; (17) T2G3	?
U-10	72/†	T1G2	1	0	3	M	+	NO	>60
U-11	61/*	T1G2	1	2	3	S	+	NO	>72
U-12	59/*	T1G2	5	5	1	S	+	NO	>72
U-13	76/†	T1G2	0	2	3	M	+	(7); (30) TaG1; (2) TaG1; (12) TaG1; (10) TaG1; (12) TaG1	>108
U-14	74/*	T1G2	7	5	1	S	+	(55) TaG2	>84
U-15	71/*	T1G2	8	5	1	S	+	(6)	>108
U-16	80/†	T1G2	1	0	3	S	+	(8); (28); (22); (30); (23)	>120
U-17	68/*	T1G2	2	3	2	M	+	(8); (15); (34) TaG1; (43) TaG1; (3) TaG1; (17) TaG1	>120
U-18	70/*	T1G2	3	3	1	?	—	(19); (20)	>108
U-19‡	60/*	T1G2	3	4	2	?	—	NO	22§
U-20	33/†	T1G2	2	1	3	?	—	(20) TaG2	>96
U-21	77/*	T1G3	4	3	1	M	+	NO	24§
U-22	74/†	T1G3	8	8	2	M	?	NO	16§
U-23	62/*	T1G3	6	6	2	S	+	(17); (13)	>60
U-24	77/*	T1G3	5	2	1	?	+	(12); (1) CIS; (15) TaG3	>60
U-25	46/*	TaG1	1	0	3	S	+	NO	>84
U-26	48/*	TaG1	0	0	3	S	+	NO	>72
U-27	57/*	TaG1	8	4	1	M	+	(19) TaG2; (15)	>72
U-28	68/*	TaG1	1	1	3	S	+	(15) TaG2; (10) T1G1; (13); (8) TaG2; (14)	>84
U-29	63/†	TaG1	5	1	1	S	+	(19) TaG2	>72
U-30	68/*	TaG1	0	0	3	S	+	(28)	>72
U-31	66/*	TaG1	1	1	3	S	+	NO	>60
U-32	45/*	TaG1	11	3	1	S	+	NO	>72
U-33	67/*	TaG1	4	6	2	S	+	(9) TaG3; (11) TaG2	>72
U-34	58/*	TaG1	2	2	3	S	+	(67) T? G2	>60
U-35	60/*	TaG2	1	1	3	S	+	(8); (10); (52)	>84
U-36	66/*	TaG2	12	1	1	S	+	(4) T? G2; (60); (8)	>84
U-37	52/†	TaG2	4	1	1	M	+	(37) TaG1	>84
U-38	67/*	TaG2	0	1	3	S	+	(12) TxG3; (10) T?G3; (30)	>72
U-39	45/*	TaG2	1	2	3	S	—	(8); (13) T1G3	>36
U-40	55/*	TaG2	1	3	3	S	+	NO	?
U-41	73/*	TaG2	4	0	1	S	+	(7) CIS	>60
U-42	73/*	T1G2	0	2	3	S	+	NO	>41
U-43	68/*	T1G2	6	2	1	S	+	NO	>72
U-44	60/*	T1G2	3	0	3	S	+	(10) TxG2; (17); (9) TxG2; (6); (7)	54§

Continued

Table 1. *Continued*

Case	Age/Sex	Stage/Grade	No. Gains	No. Losses	Group	Single/ Multiple	Primary	Recurrence	Survival
U-45	55/*	T1G2	5	3	1	S	+	(10) T?G2; (3) TaG1	>72
U-46	60/*	T1G2	3	12	2	M	+	NO	>60
U-47	49/*	T1G3	3	3	1	M	+	NO	>60
U-48	54/*	T1G3	0	0	3	S	+	NO	>72
U-49	71/*	T1G3	10	1	1	S	+	NO	56§
U-50	40/*	T1G3	4	0	1	M	+	NO	?
U-51	62/*	T1G3	7	4	1	M	+	NO	>30
U-52	62/*	T1G3	1	0	3	M	+	NO	>72
U-53†	77/*	T1G3	6	1	1	S	+	(3)	>39
U-54	63/*	T1G3	0	0	3	M	+	NO	>84
U-55	67/*	T1G3	6	9	2	M	+	(9) TxG3; (4); (10) TxG3	31§
U-56	72/*	T1G3	3	13	2	S	+	NO	>72
U-57†	67/*	T1G3	2	1	3	S	+	(11) T2	?
U-58	63/*	T1G3	2	0	3	M	+	(31) TaG1	>84
U-59	51/*	T1G3	2	0	3	M	+	NO	>72
U-60	44/*	T1G3	1	2	3	S	+	NO	>84
U-61	73/*	T1G3	5	12	2	S	+	NO	3§
U-62	54/*	T1G3	2	13	2	S	+	NO	>42
U-63	71/*	T1G3	5	5	2	M	+	(8) CIS	>60

CIS = carcinomas *in situ*.
Cases are ordered according to stage and grade.
*, Male; †, female.
Recurrence and survival are indicated in month. † indicates cases with cystectomy after the last recurrence.
§ Cancer-related death.
According to the predominance of chromosome gains and losses, tumors were included in groups 1 and 2, respectively.
Group 3 includes tumors with ≤ 4 chromosome imbalances.

some imbalances. Losses of 8p, and gains in 1q and 8q were detected in > 20% of the cases. Gains on 17q and 19p as well as losses on 5q and 11q appeared in > 15% of tumors.

Amplifications were detected at 11q13 (4 tumors), 7q32qter, 12q14q15, 12q24.2q24.3, 19p13.2pter, and 20p13pter (1 tumor in each).

A significant increase in the number of detectable aberrations in relation to stage-grade of the tumors was not observed: TaG1 tumors had, on average, 3.4 gains and 1.6 losses, resulting in a total of 5.0 aberrations per tumor. This average was 3.0 (1.9 gains, 1.1 losses) in TaG2 tumors; 5.9 (2.9 gains and 3.0 losses) in T1G2 and 7.8 (3.8 gains and 3.9 losses) in T1G3 tumors. As previously described by Prat et al,[10] losses on 5q and 6q were significantly more frequent in T1G3 than in T1G2 tumors ($P = .01$ and $P = .004$, respectively).

Although gains predominated over losses in Ta tumors, the relationship between gains and losses was similar in T1 carcinomas (Fig. 1A). However, when tumors were classified according to their individualized pattern of gains and losses (Fig. 1B), it was observed that the distribution of both types of imbalances was not homogeneous showing 2 well-separated groups, one characterized by gains (group 1) and the other by losses (group 2). An intermediate third group with few imbalances (≤4) was also observed (group 3). Chromosomal imbalances detected in each group are shown in Table 2.

Group (1) included 20 tumors: 7 Ta/G1G2 (7/20, 35%), 6 T1G2 (6/20, 30%), and 7 T1G3 (7/20, 35%). This group seems to be characterized by gains on 1q and 17q (55% and 50% of tumors, respectively); both imbalances were detected in 45% of these tumors. Group 2, which was characterized by the predominance of losses, included 12 tumors: 2 Ta/G1G2 (2/12, 16%), 3 T1G2 (3/12, 25%), and 7 T1G3 (7/12, 58%). Losses on 6q where only detected in this group (5/12, 42%), other characteristic losses were on 11q and 5q (66% and 58% of the tumors, respectively). Gains of chromosome X were detected in 58% of the tumors from this group. Cases with equal number of gains and losses were included in the first or second group according to the presence of +1q (U-12, U-18, and U-47) or −5q/−11q, respectively (U-22, U-23, and U-63). Group 3 was characterized by a low number (≤ 4) of genomic imbalances; in this group, 55% (17/31) of the tumors were Ta/G1G2, 22.5% (7/31) T1G2, and 22.5% (7/31) were T1G3. Cases with no imbalances (U-3, U-7, U-8, U-26, U-30, U-48, and U-54) were included in this group. Cases with 4 gains and no losses (U-1, U-41, and U-50) were included in group 1. The average number of alterations per tumor was 8.1, 12, and 1.7 in groups 1, 2, and 3, respectively.

To add more insights, subtelomere screening was carried out in 15 tumors (5 from each group defined by CGH). Most of the results obtained by MLPA confirmed those of CGH (Table 2). Deletions of 9p and/or 9q were detected in 6 of 15 tumors. Gains of 1q and 8q were detected in 2 of 15 and 3 of 15 tumors, respectively. Few or no imbalances were detected in tumors of group 3. Despite the low number of tumors analyzed, the distribu-

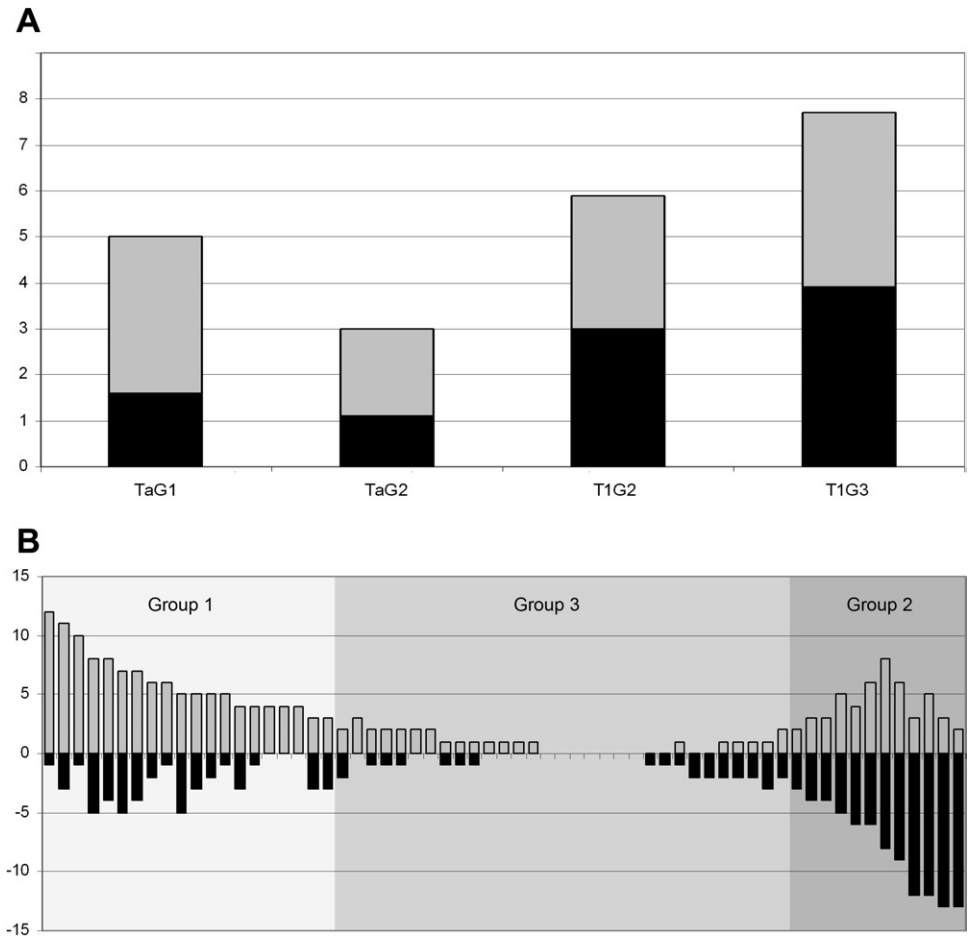

Figure 1. **(A)** Chromosomal imbalances by tumor stage and grade. **(B)** Tumor classification according to their individualized pattern of gains and losses, showing 3 separate groups as explained in the text (Bars in gray indicate gains and those in black indicate losses).

tion of duplications does not seem to be random: duplication of 15q was only observed in tumors of group 2, whereas duplication of 1q and 3p or 3q was only observed in tumors of group 1. In addition, MLPA study also showed new dosage imbalances not detected by CGH. Cancer-related death was found in 7 patients; 4 of them belonging to group 2. Significant differences were observed when comparing overall survival between groups ($P = .005$), confirming that group 2 had the worst outcome (Fig. 2). The life-table analysis showed a survival rate after 48 months of 60% for group 2, 94% for group 1, and 100% for group 3. A decreased overall survival was also observed in patients in whom tumors have imbalances ≥ 7 ($P = .007$).

COMMENT

Bladder cancer consists of a heterogeneous group of tumors that differ in grade and stage according to clinical characteristics. In the present study, the chromosome imbalances shown in 63 early-stage carcinomas of the bladder were analyzed. Conventional CGH was used to dissect the spectrum and frequency of alterations in bladder cancer and to classify them in different subsets of tumors according to their genomic imbalances (Fig. 1B, Table 2).

Most of the chromosome imbalances detected in the present study have already been reported by other authors.[6-10] Deletions of 9p and 9q are the most frequently deleted regions in papillary bladder tumors. The loss of the whole chromosome 9 or specific cytobands is thought to be one of the initial events in bladder carcinogenesis.[4] Recent CGH array data have suggested that clones at 9p21 might be the pinpoint of a candidate gene to act as tumor suppressor gene in bladder cancer.[14,15] Our results agree with the initiation of bladder cancer by the loss of chromosome 9, showing imbalances affecting this chromosome Ta and T1 bladder tumors. Whole chromosome or partial gains affecting chromosome X were found in approximately 25% of the tumors that were analyzed. Chromosome bands Xp11p13 and Xq21q22.2 had already been described as probable locations of oncogenes with relevance for bladder cancer.[8,23] Unfortunately, recent studies of bladder cancer using CGH array discard the sexual chromosomes due to misinterpretations.[12]

According to the present study, 3 groups of noninvasive bladder urothelial cell carcinomas may be described

Table 2. Summary of results obtained by CGH and MLPA*

Case	Stage/Grade	Group	Gains	Losses
U-36*	TaG2	1	1q21qter, 5q34qter, 12q22qter, **12q24.2q24.3**, 15q22q24, 16pterq13, 16q23qter, 17p12q12, 19p13.3, 20q, 21q22, 22q12 *MLPA: dup 1q, 4q*	2q36q37 *MLPA: del 2q, 19p, Xp(PAR1)*
U-32	TaG1	1	2p25, 2q37, 11q13, 16, 17, 19, 20q, 21, 22, Xq21, Xq26qter	3p12, 5q14q23, 13q32
U-49	T1G3	1	1q43, 2p24pter, 2q36qter, 11q12q13, 16p13.1pter, 17p13, 19, **19p13.2pter**, 22, Y	5p15.2q23
U-15*	T1G2	1	1p32p34, 1q, 8q, **11q13,** 13q13qter, 19, 20, **20p13pter** *MLPA: dup 1q,8q,19q*	5q, 8p, 11, 15q15q25, Y *MLPA: del 8p, 11p*
U-27	TaG1	1	1p34.2pter, 4p16, 4p11q24, 7q11, 17pterq12, 19, Xpterq25, Y	5p15, 6p22, 11q23, 12p12
U-14	T1G2	1	1, 4q27qter, 5, 8q, 17q24qter, X, Yq11.2pter	2q33qter, 8p, 9q22qter, 13q11q14, 18
U-51*	T1G3	1	1q43qter, 4p15.2q21, 5q11.2q12, 7p21pter, 7q11.2, 10p11.2p10, 19 *MLPA: dup 11p, 13q*	7q33q35, 9q21q31, 10q24q25, 16q22q23 *MLPA: del 16p*
U-43	T1G2	1	2q37, 5q27, 9q34, 17p, 19p, 20q13.2q13.3	4q26q27, 12q15
U-53	T1G3	1	1q21q24, 1q31q32, 2p14p15, 7p15, 8q21.3q23, 17q21	9p13p23
U-12*	T1G2	1	1q21qter, 3p22pter, 3q, 8q22qter, 17q11q21 *MLPA: dup 3p, 3q, 8q*	2q32qter, 3p13p14, 9, 17p13, Y *MLPA: del 9p, 9q, 17p, Xp (PAR1), Xq (PAR2)*
U-45	T1G2	1	5p14pter, 10p11p12, 19p, 20, Y	5q34, 9, 11
U-24	T1G3	1	1q, 3, 8p21q13, 17q, Y	8p22pter, 9q31qter
U-29	TaG1	1	1q25q42, 3p26, 14q24qter, 17q, Xp22.1pter	Y
U-21	T1G3	1	1q32qter, 8q, 15q, Y	2q21q24, 8p, 9q
U-37	TaG2	1	3p26, 3q28, 9p21pter, Xq21q22	Y
U-50	T1G3	1	1p32pter, 17q11.2q12, 19, Xp22.2pter	
U-41	TaG2	1	1p36.2pter, 16p13.2pter, X, Y	
U-1	TaG1	1	4p22pter, 4q32qter, 7, 16	
U-47*	T1G3	1	1q31, 4q10q31.3, X *MLPA: dup 3q*	1p36.1pter, 16, Y *MLPA: del 9q*
U-18	T1G2	1	1q21qter, 13q21qter, Y	8p, 9p11p22, 19
U-6	TaG2	3	1q23qter, 8q	4q31qter, 8p
U-44	T1G2	3	3p24p26, 6p25, 11q13	
U-57	T1G3	3	3p25pter, 4q21	5q32qter
U-20	T1G2	3	**7q32qter**, 20	13q22q32
U-5*	TaG2	3	19, Y *MLPA: no dup*	9q21qter *MLPA: del 9q*
U-59	T1G3	3	1p36.2, 9q34	
U-58	T1G3	3	3p26, Xq21	
U-35	TaG2	3	1q42	Xq10q13
U-31	TaG1	3	X	14q31
U-28	TaG1	3	8q24.1qter	Y
U-25	TaG1	3	Xp11.3q25	
U-52	T1G3	3	X	
U-16*	T1G2	3	19p *MLPA: no dup*	*MLPA: no del*
U-10*	T1G2	3	19 *MLPA: no dup*	*MLPA: no del*
U-4	TaG2	3		Y
U-38*	TaG2	3	 *MLPA: no dup*	Y *MLPA: del Xp (PAR1), Xq (PAR2)*
U-2	TaG2	3	19	9
U-42	T1G2	3		9p23p24, Y
U-13	T1G2	3		9q21q31, 14q23qter
U-11	T1G2	3	8q	8p, Y
U-60	T1G3	3	X	2q12q22, Y

Continued

Table 2. *Continued*

Case	Stage/Grade	Group	Gains	Losses
U-39	TaG2	3	15	9, Y
U-40	TaG2	3	6p21	12q15, 18q22, 21q21
U-34	TaG1	3	3p21, Xq	9, 13q22q31
U-17*	T1G2	2	**11q13**, 15q13qter *MLPA: dup 15p, 15q*	9q13q22, 11p, 11q14qter *MLPA: del 9p, 9q, 11p, 11q*
U-9	TaG2	2	8q, 17q, Y	8p, 9, 11p, 17p
U-19	T1G2	2	1q, 8q, 20	9, 11p14pter, 13q31q32, 17p
U-63*	T1G3	2	4q12q13, 8q24.3, 19p, X, Y *MLPA: no dup*	2p23p24, 2q22, 9p, 9q31q32, 11q22q23 *MLPA: del 9p*
U-33*	TaG1	2	4p11q13, 19p, X, Y *MLPA: dup 15q*	2p21p24, 8p23, 11q23, 12p12, 13q31, 14q24q31 *MLPA: del 9q*
U-23	T1G3	2	**11q13**, 12, 14q21qter, 15, 19, 20	5q12q31, 9, 11p, 11q14qter, 18q12qter, Y
U-22	T1G3	2	6p22p23, 8q21q22, 10p, 11q, 12p, 17, 19, 20p11.2qter	4q, 5q13q23, 6q13q22, 7q21, 9q, 11p14pter, 18q21qter, X
U-55*	T1G3	2	7q11q22, 8q13qter, **11q13**, **12q14q15**, 17p12pter, Xpterq23 *MLPA: dup 8q, 9p*	4p15.3p15.1, 4q22qter, 5q33q34, 6p12, 6q21qter, 8p21p22, 9p13, 9q31, 13q21q31 *MLPA: del Xp(PAR1)*
U-46	T1G2	2	14q13, X, Y	4p15.3, 5q31qter, 7q34q35, 8p22p23, 10p13p14, 10q25, 11p15, 11q23q24, 13q31, 14q31, 17p12, 18q12.3qter
U-61	T1G3	2	7q11, 8q10q21.3, 19, X, Y	2p14p24, 2q21, 4p15.1p15.2, 5q12q13, 6q25, 7q31q35, 8p21p22, 9p12q23, 10q25, 11q23, 14q24, 15q24
U-56*	T1G3	2	5p13q11.2, 10p12, Xp21q25 *MLPA: dup 4q, 5p, 10p, 11p, 13q, 21q*	2p24p23, 3p13p14, 3q32q33, 5q34, 6q24.1q24.2, 8p12p22, 9p12p22, 9q31q33, 10q24q26, 11q23q24, 15q23q24, 17p12, 18q21q22 *MLPA: del 16p, 17p, 18q, Xp(PAR1)*
U-62	T1G3	2	4q10q21, X	1q31q41, 2q12q14.3, 5q23qter, 6q25, 7p12p14, 7q32q35, 9p12, 10q25, 11q23, 12p12, 12q24.1q24.2, 15q24q25, Y

Cases are ordered according to Figure 1B. Amplifications are in bold.
Cases U-3, U-7, U-8*, U-26, U-30, U-48, and U-54 (not included in the table) showed no imbalances.
* Cases studied by MLPA. Subtelomere duplications and deletions are indicated in italic. *Dup* = duplication, *del* = deletion, *15p* = *15q* centromere proximal, *PAR* = pseudoautosomal region. Case U-8* also showed no subtelomere imbalances.

in relation to the quantity and quality of the chromosome alterations: 1 group of tumors is mainly represented by chromosomal gains, another group by chromosome losses, and the third group shows minor changes. The last group includes 70% of the total Ta low-grade (G1, G2) tumors that were analyzed in the present study. This result coincides with the predominance of low chromosome imbalances in Ta bladder tumors as it has been previously described in the recent World Health Organization classification.[24] Interestingly, a relationship between *FGFR3* mutations and chromosome stable tumors has also been suggested.[25] The few subtelomere dosage imbalances detected in tumors of this group also confirms their chromosome stability.

The presence of a high number of gains in a group of tumors, predominantly T1 but also Ta (group 1), suggest that the pattern of chromosome gains considered as characteristic of advanced stages could even appear in early stages of bladder carcinogenesis.

In fact, thorough statistical analysis of karyotypic patterns have demonstrated that BUC, as well as most solid tumors, show at least 2 distinct evolutionary routes, 1 characterized by genomic losses, and the other by genomic gains.[4] In both pathways, the driving forces behind the karyotypic profiles are likely to be associated with some type of chromosome instability.[26]

It has been demonstrated that early aberrations, to a large extent, determine the spectrum of subsequent, late aberrations. This indicates that different modes of chromosomal evolution may be initiated at an early stage of tumorigenesis, leading to an accumulation of chromosomal abnormalities along distinct pathways.[4,27,28] Our results suggest that gains of chromosome 1q could define a pathway of gains in which +17q would be frequently

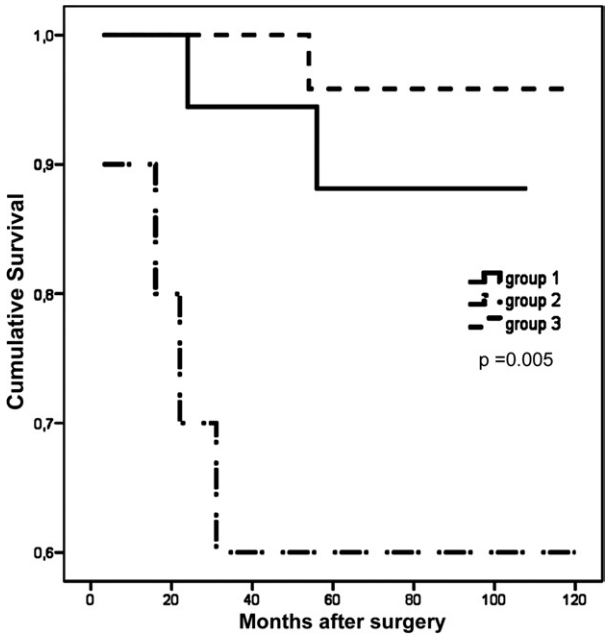

Figure 2. Kaplan-Meier curves of the 3 different groups of patients.

associated. The implication of 1q gain in early stages of urothelial carcinogenesis has been confirmed by CGH array.[15] In contrast, chromosome 11 loss, with -5q and -6q also being associated, could drive another pathway with a predominance of losses. These 2 pathways are not mutually exclusive and they could converge at more advanced phases of tumor development.

Hoglünd et al[4] identified a subgroup of urothelial carcinomas initiated by -9 and followed by -11p and +1q. Our results support this pathway and also suggest that tumors with +1q will accumulate gains, whereas tumors with -11p accumulate losses, defining 2 new subgroups in the -9 pathway.

Three tumors with few chromosomal changes (group 3), showed chromosome imbalances commonly found in the group of gains (+1q, U-6 and U-35) or in the group of losses (-5q, U-57), suggesting that these tumors may evolve to accumulate more gains or loses during carcinogenesis.

Results of the present study also indicate the presence of recurrent aberrations in subtelomere regions, some of them not detected by conventional CGH analysis. The non-random distribution of these abnormalities suggests that they could be relevant in tumorigenesis.

A relationship between chromosomal imbalances and survival rates was observed in the present study, confirming that superficial BUC with few chromosome changes have a good prognosis. Tumors with predominant losses over gains could represent a minority group with poor clinical outcome among superficial urothelial tumors.

CONCLUSIONS

In summary, our results suggest that superficial (Ta and T1) BUC can be subdivided into a larger number of subclasses than had previously been expected. Our results also demonstrate a decreased survival among patients whose tumors show more genomic losses than gains.

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EDITORIAL COMMENT

Bladder tumors show widely differing histopathology and clinical behavior. This is reflected in the molecular genetic alterations they contain. Much information has accumulated on somatic genomic alterations in bladder tumors of all grades and stages. Heterogeneity and unpredictable behavior of this type of tumors make necessary the use of combinations of markers that may provide information predictive of prognosis or response to specific forms of therapy.

Deletion of chromosome 9 was one of the first alterations identified cytogenetically in bladder tumors. This suggested that chromosome 9 genes might participate early in the process of tumor development, but subsequently a great deal of information has come from comparative genomic hybridization (CGH) and array-CGH studies of large tumor panels, although till date, functional validation for most of these studies is absent.

The purpose of this study was to evaluate associations between measures of genomic instability and bladder cancer clinical phenotype by array-based CGH in 63 early-stage carcinomas of the bladder (Ta and T1). Subtelomeric screening in 15 of these tumors was performed by multiplex ligation-dependent probe amplification. This expects to find out the evolution patterns of superficially and minimally invasive tumors. Although authors' findings do not differ from previous studies, they have made a classification that gives us a new tool that could be useful for clinical classification.

In this study, a significant increase in the number of detectable aberrations in relation to stage-grade of the tumors was not observed. Although gains predominated over losses in Ta tumors, the relation between gains and losses was similar in T1 carcinomas. The relation between gains and losses allowed the authors to create a tumoral subclassification and identify a subgroup that was characterized by the predominance of losses.

The authors have observed that the subgroup including 2 Ta/G1G2 (16%), 3 T1G2 (25%), and 7 T1G3 (58%) they had the worst outcome. Moreover, losses on 6q were only detected in this group (42%). Other characteristic losses were on 11q and 5q (66% and 58% of the tumors, respectively). Gains of chromosome X were detected in 58% of the tumors from this group. In addition, multiplex ligation-dependent probe amplification study showed new dosage imbalances not detected by CGH.

It could be interesting to conduct a study of multifocal tumors in a single patient or analyze chromosome imbalances in samples from the same patient at different times during their disease course and see whether it is possible to predict a high risk of progression, although this would extend the study to a large number of patients and a longer study period. Analysis of chromosomal alterations of various synchronous lesions in a single patient could shed light on possible monoclonal origin of individual lesions, the timing of chromosomal alterations, and the growth benefits that it could provide. In the same way, albeit with few cases, analysis of chromosomal alterations in benign or preneoplasic lesions in bladder is being carried out, as well as on morphologically normal urothelium from bladder cancer patients who required cystectomy.

The findings of the authors open a door for possible use in every day clinical practice because it could allow us to predict a worse evolution in a group of tumors that, with the current histopathological grading methods that are used routinely, may seem homogeneous but which have significant genetic differences.

Although the number of cases is reduced to make decisions regarding the management of patients with bladder cancer, later the sum of findings about the genetic changes detected by other authors and its correlation with the clinical course of patients could be a useful tool for its implementation in daily practice because it would allow us to adopt different therapeutic and follow-up guidelines, depending on the chromosomal abnormalities found.

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REPLY

We are grateful for the positive editorial comment regarding the clinical potential of classifying bladder tumors according to the predominance of gains or losses. Understanding the genetic events leading to bladder cancer progression is important in decreasing the overall morbidity and mortality associated with the disease.

The existence of 2 pathways, one with predominance of losses and the other with predominance of gains, has previously been proposed in other solid tumors; our results suggest that these pathways also operate in bladder cancer. These 2 pathways are not mutually exclusive and they could converge at more advanced phases of tumor development. Considering that losses on 5q and 6q are frequent in muscle-invasive bladder tumors, we suggest that these chromosome imbalances could be responsible for the poorer evolution of tumors that show a predominance of loss.