Novel Recurrent Structural Chromosomal Aberrations in Primary Bladder Cancer

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Abstract. *Background: Bladder cancer is a heterogeneous genetic disease and, to date, no specific cytogenetic abnormality has been established. The detection of recurrent genetic changes with common breakpoints is of special interest, facilitating the identification of genes implicated in carcinogenesis. The aim of this study was to investigate recurrent structural chromosomal aberrations with common breakpoints and to correlate them with the histological stage of tumors. Materials and Methods: Fifteen patients with transitional cell carcinoma of the bladder were cytogenetically studied by direct culture of primary tumor cells and G-banding technique. Results: Most of the cases studied exhibited very complex karyotypes. Recurrent structural aberrations were observed involving, according to frequency, chromosomal regions 11p15, 3p12, 14q32, 19q13 and 6q23. Isochromosomes i(8q), i(17q) and i(6p) were also observed. Conclusion: Conventional cytogenetics continues to be valuable in cancer study, detecting common chromosomal breakpoints. Of interest was the detection of novel recurrent structural chromosomal aberrations including involvement of 11p15, 14q32 and 19q13, while a correlation of recurrent abnormalities observed with tumor stage was also evaluated.*

More than 90% of bladder cancers are transitional cell carcinomas (TCC). Other histological types, such as squamous cell carcinoma or adenocarcinoma, are very rare. Histopathologically, TCC is divided into three distinct categories : superficial papillary tumors, invasive tumors and transitional carcinoma *in situ*. The pathogenesis of bladder cancer still remains unclear, while several epidemiological studies have shown that occupational exposure to certain chemicals is linked with bladder cancer (1,2).

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The development of cancer is a multistep process where the disruption of certain specific genes can result in expression of a malignant phenotype. However, the series of mutations leading to malignancy has been elucidated for only a small number of human cancers. Bladder cancer is a heterogeneous genetic disease and, to date, no specific aberration responsible for bladder cancer has been established. The tumors display complex karyotypes with various numerical and structural chromosomal aberrations. However, several non-random chromosomal changes have been reported in bladder cancer, including chromosomes 1,3,5,7,9,11 and Y. Attempts to link certain specific chromosomal aberrations with the clinical course of this disease remain anecdotal and they were not tested in large prospective studies (1,3-7).

We studied cytogenetically, 15 primary bladder tumor specimens by a direct culture and a G-banding technique, investigating the presence of recurrent structural abnormalities and correlating them with the pathological stage.

Materials and Methods

Fifteen patients with TCC were included in this study. None of the patients had ever received chemotherapy or radiothery prior to surgery. Tissue specimens were collected from fresh surgically resected tumors and a routine histopathological examination followed. The tumors were classified histopathologically according to the criteria of the World Health Organization (WHO) classification of urothelial tumors (8). A small portion of each resected tumor was directly processed for cyrogenetic analysis by G-banding technique as described elsewhere (9). As many cells as possible were analyzed in each case, and not fewer than 15. An abnormal clone was defined as two or more cells with either the same trisomy or the same structural anomaly, or as three or more cells with the same missing chromosome. Chromosomal aberrations were designated according to the International System of Human Cytogenetic Nomenclature (ISCN 1995) recommendations (10).

Results

Table I shows the recurrent structural chromosomal abnormalities detected, the modal chromosome number and the histopathological stage. All but three cases presented very

Table I. *Recurrent structural aberrations in bladder cancer involving chromosomes 3,6,8,11,14,17 and 19.*

Case No	Histological stage	Modal chromosome number	Chromosome						
			3	6	8	11	14	17	19
1	TP$_{1A}$	46						der (17)	
2	TP$_{2A}$	65-69			i(8q)				
3	TP$_2$	55-65		i(6p)		add(11)(p15)			add(19)(q13)
4	TP$_2$	64-69			i(8q)				
5	TP$_2$	60-65	del(3)(p12)	i(6p)	i(8q)	add(11)(p15)			
6	TP$_{1B}$	65-68	del(3)(p12)			add(11)(p15)			
7	TP$_{3A}$	75-78	del(3)(p12)		i(8q)	add(11)(p15)		i(17q)	add(19)(q13)
8	TP$_2$	47-48			i(8q)				add(19)(q13)
9	TP$_{1B}$	65-68	del(3)(p12)	i(6p)		add(11)(p15)		i(17q)	add(19)(q13)
10	TP$_2$	66-69	del(3)(p12)	del(6)(q23)		add(11)(p15)	add(14)(q32)	i(17q)	
11	TP$_2$	58-62		del(6)(q23)			add(14)(q32)		
12	TP$_{1B}$	75-78					add(14)(q32)		
13	TP$_2$	75-80				add(11)(p15)		i(17q)	
14	In situ	70-75	del(3)(p12)				add(14)(q32)		
15	TP$_2$	47-48		del(6)(q23)		add(11)(p15)	add(14)(q32)		

complex karyotypes, with hyperploidization and various unidentified marker chromosomes being the prominent finding. The complexity of the structural aberrations and the hyperploidization did not allow the preparation of a complete karyotype for each of the cases studied. However, we focused on the structural aberrations observed in order to identify non-random aberrations presenting common chromosomal breakpoints. Recurrent structural aberrations were observed involving, according to frequency, chromosomal regions 11p15, 3p12,14q32,19q13 and 6q23. Isochromosomes i(8q), i(17q) and i(6p) were also observed (Figures 1-7). Chromosome 11 was involved as add(11)(p15) in eight cases, del(3)(p12) was detected in six cases, add(14)(q32) in five cases, add(19)(q13) in four cases and del(6)(q23) in three cases. Isochromosomes i(8q), i(17q) and i(6p) were observed in five, four and three cases, respectively. Interestingly, in a case with 46 chromosomes and a pseudodiploid karyotype, monosomy 17 was observed, while an additional unidentified marker chromosome was present. The FISH technique, using an α-satellite probe specific for the pericentromeric area of chromosome 17, showed two copies of chromosome 17, providing evidence that the unidentified marker chromosome was a der(17).

Discussion

The conventional cytogenetic banding technique has proved useful, especially in the area of hematological disorders, elucidating the exact nature of many chromosomal aberrations. However, in solid tumors the detection of recurring genetic changes can be extremely difficult. The tumors display complex karyotypes with many numerical as well as structural chromosomal rearrangements. It is often very difficult, if not impossible, to establish the origin of complex structural chromosomal aberrations. Nevertheless, cytogenetic studies of solid tumors continue to offer valuable information detecting the presence of non-random chromosomal breakpoints. Moreover, suspected abnormalities on the basis of cytogenetic studies could be elucidated by further molecular studies.

No specific cytogenetic aberration has been identified for bladder cancer. However, various non-random deletions, gains or losses of chromosomes and formation of isochromosomes have been observed (1,3-5,11-15).

We studied, cytogenetically, 15 cases of primary bladder cancer by direct culture of tumor cells and the G-banding technique. Most of the cases revealed complex karyotypes with polyploidization and numerous structural aberrations. Recurrent structural abnormalities were detected, according to frequency as follows: add(11)(p15), del(3)(p12), add(14)(q32), i(8q), add(19)(q13), i(17q), del(6)(q23) and i(6p).

These abnormalities may simply represent unspecified secondary changes associated with tumor progression, but it is possible that they involve genes essential for the malignant evolution of the multipotent ancestral urothelial cell. Some of the abnormalities observed have been previously described, but other recurrent cytogenetic findings detected were novel. The latter included add(11)(p15), add(14)(q32) and add(19)(q13).
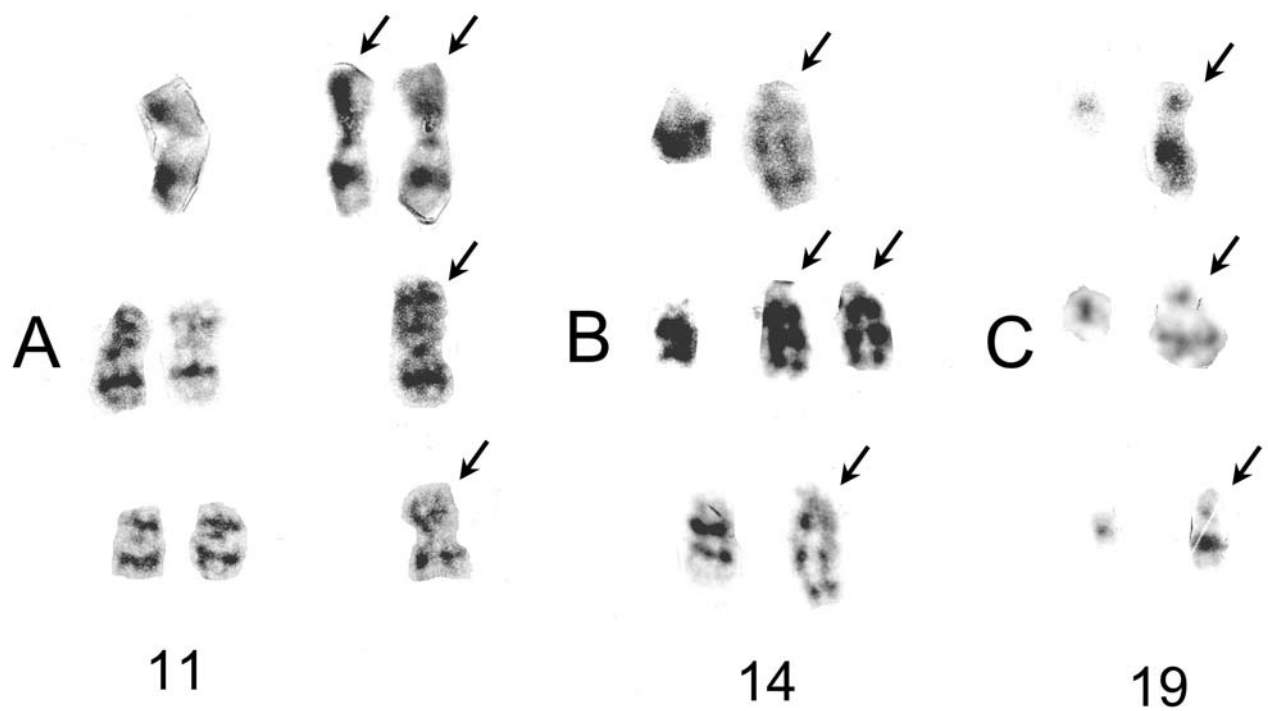
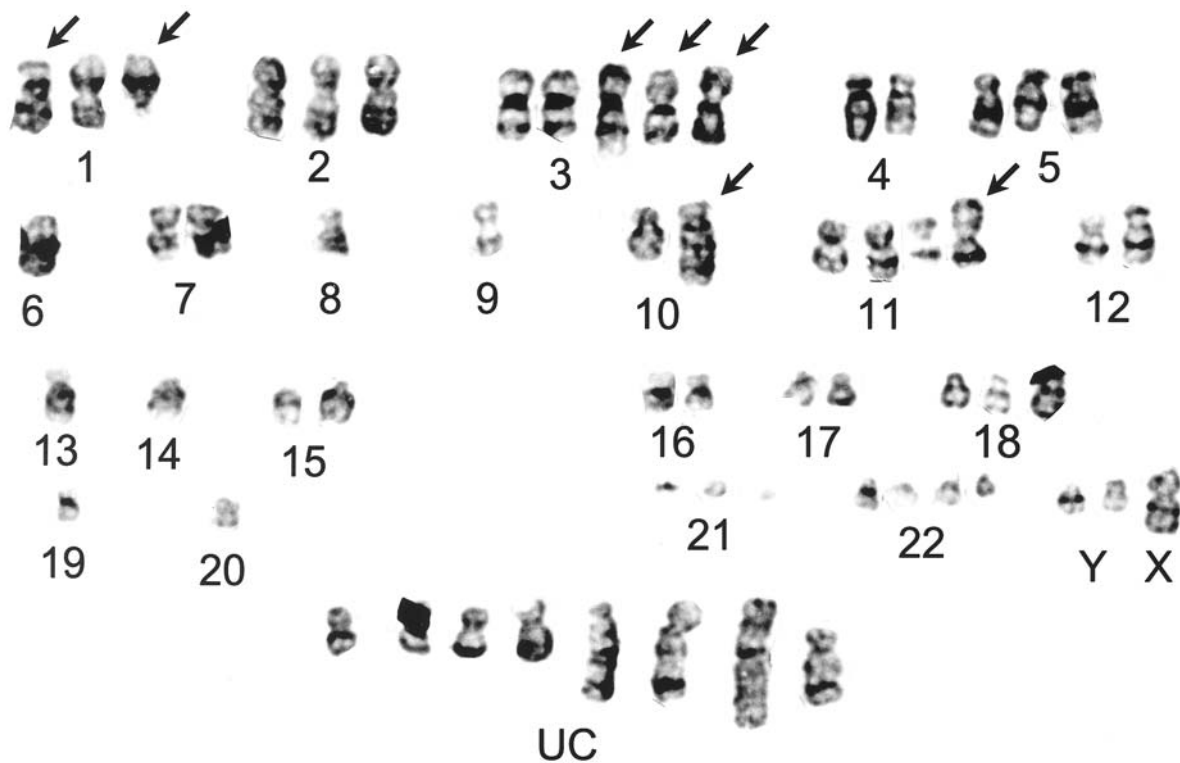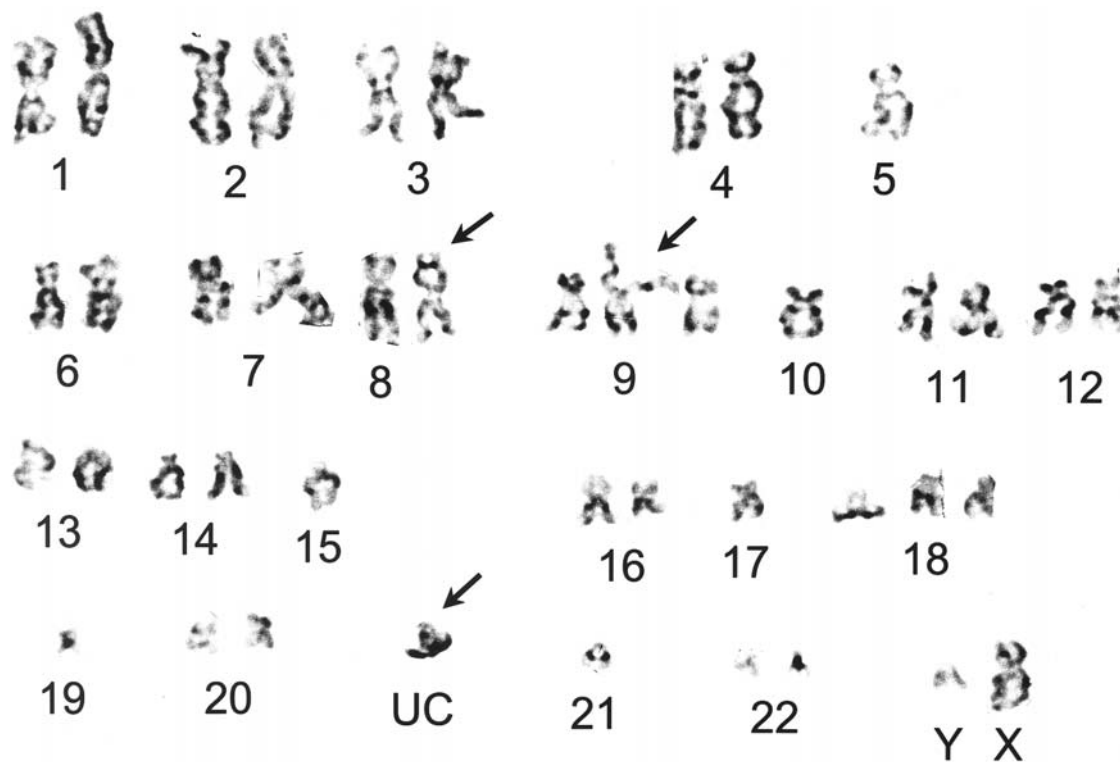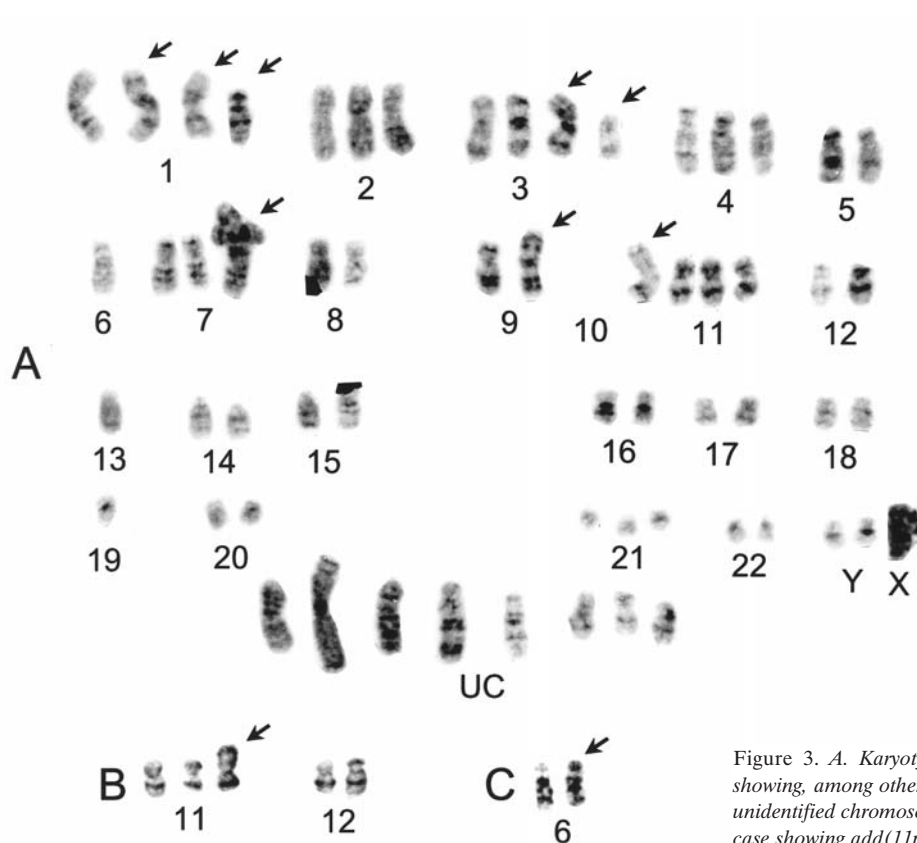Figure 1. *A,B,C. Partial karyotypes showing add(11p), add(14q) and add(19q) respectively (different cases).*

Figure 2. *Karyotype by G-banding technique from case 5 showing, among other abnormalities, add(11p). UC: unidentified chromosomes.*

Figure 3. *A. Karyotype by G-banding technique from case 5 showing, among other abnormalities, del(3p) and add(11p). UC: unidentified chromosomes. B,C. Partial karyotypes from the same case showing add(11p) and i(6p), respectively.*

Figure 4. *Karyotype by G-banding technique from case 4 showing, among other abnormalities, i(8q) . In this case the modal chromosome number was nearly triploid. UC: unidentified chromosome.*
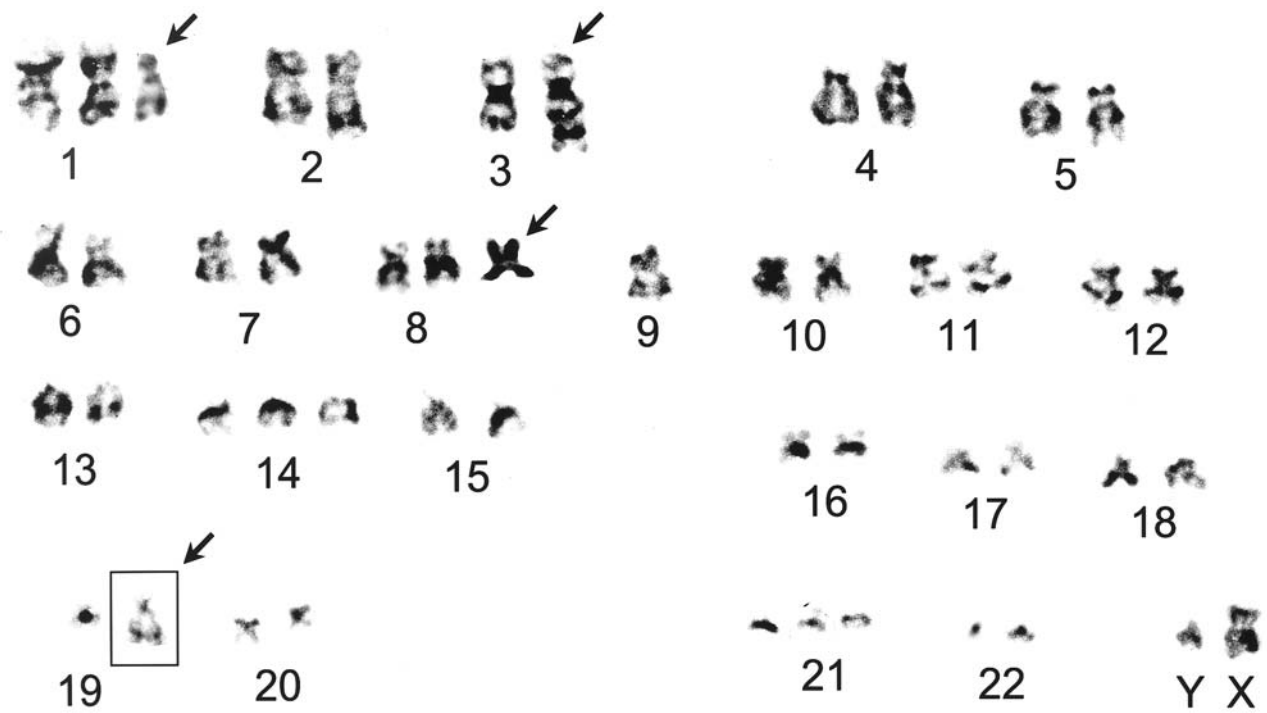
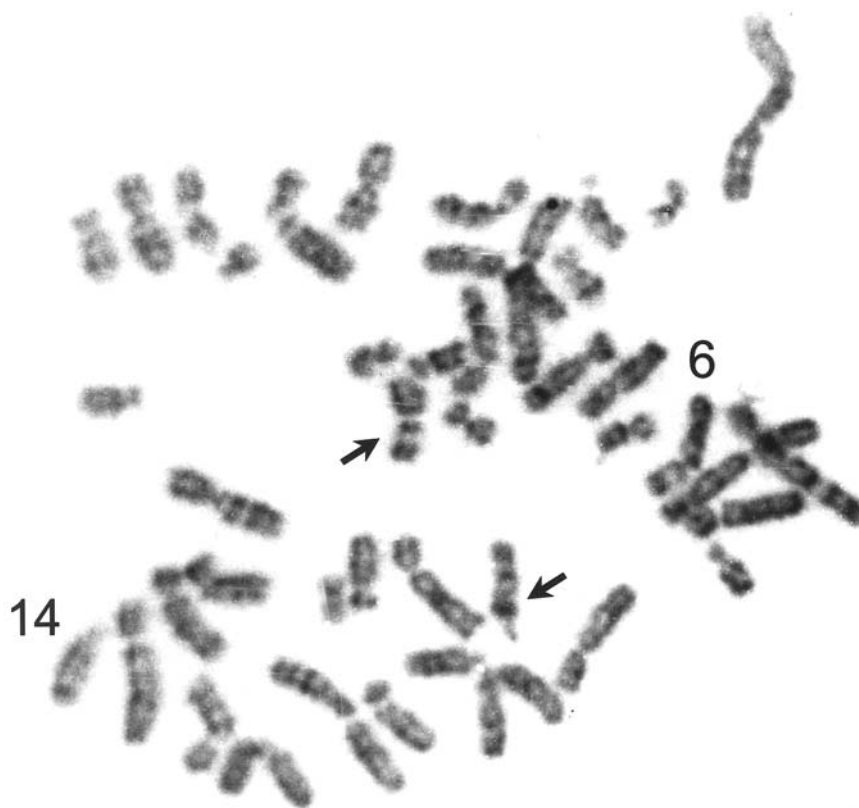Figure 5. *Karyotype by G-banding technique from case 8 showing i(8q) and add (19q).*

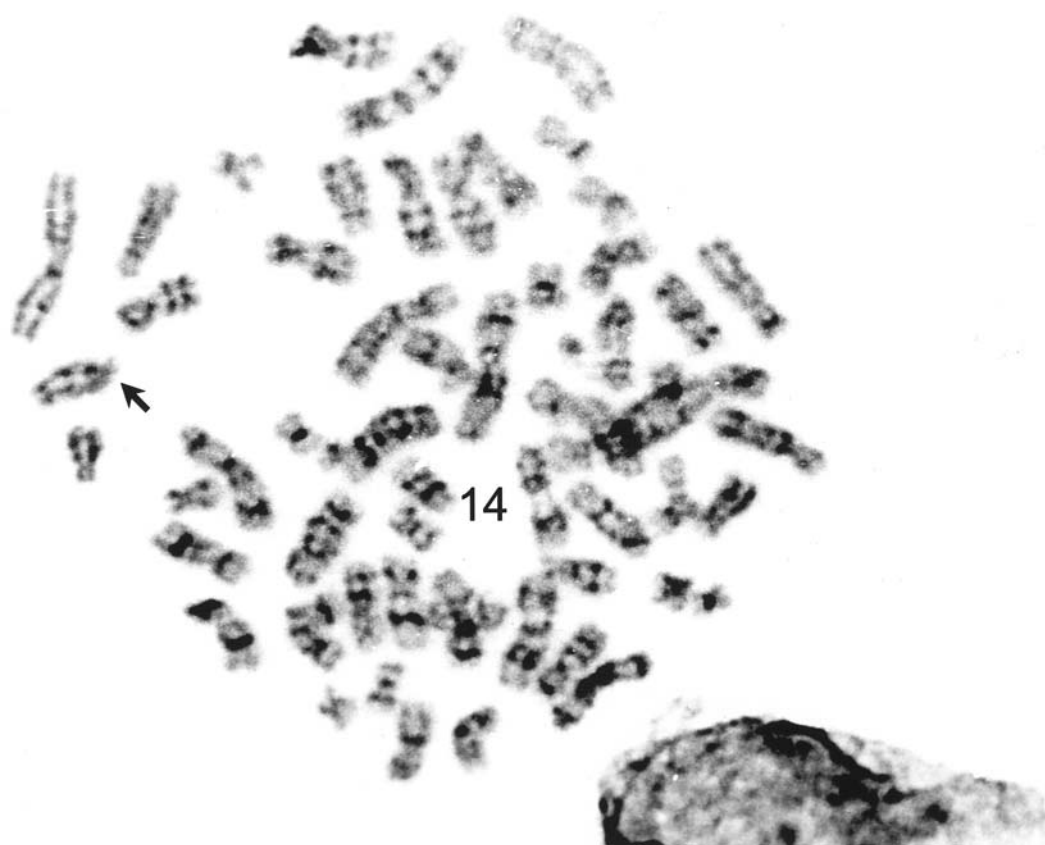Figure 6. *A metaphase from case 15 showing del(6q) and add(14q)*

Figure 7. *A metaphase from case 11 showing add(14q).*

Abnormalities of chromosome 3 have been reported in about 30% of urothelial carcinomas and they are often observed in cases with complex karyotypes. Previous studies have shown that deletions of 3p involve bands 3p11 ~ p21. Molecular studies have demonstrated frequent deletions of 3p in invasive bladder tumors, but not in papillary superficial lesions (1,6,14-17). In this study del(3)(p12) was observed in two papillary superficial carcinoma, in three invasive and in one *in situ* case.

Isochromosome i(8q) was observed in five cases. The presence of i(8q) has been reported in some bladder cancer cases. Deletions with a breakpoint at band 8 p12 ~ pter have also been reported and they have been associated with a higher grade and a more advanced stage. It has been hypothesized that loss of tumor suppressor genes located on 8p could lead to more infiltrating forms of this malignancy. A previous M-FISH study of bladder cancer cell lines showed loss of 8p to be correlated with an advanced stage of the disease (11-14,18,19). All our cases with i(8q) were invasive cancers.

Isochromosome i(17q) was observed in four of our cases, while loss of chromosome 17 with an additional der(17) chromosome was found in one pseudodiploid superficial

papillary case. Previous cytogenetic studies have indicated that 17p losses are mainly found in advanced TCC. At the molecular level, there have been many reports of alterations of chromosome 17. Alterations of the p53 gene located on 17p have been found mostly in high grade/stage tumors and *in situ* bladder carcinoma (1,14,16,17,20-22). In this study, i(17q) was found in three invasive cases and in one superficial papillary case.

Deletions of 6q are common in various human tumors and 6q is a probable site of tumor suppressor genes. Previous studies showed that the presence of the 6q deletion was associated with muscle invasion (1,16,23). We found del (6)(q23) in 3 cases, all of which were of invasive type, while i(6p) was observed in three additional cases.

Non-random involvement of chromosome 11 has been found in several cytogenetic studies of bladder cancer. Deletions of 11p is the most common change and it was observed more frequently in invasive high stage tumors. Molecular studies have shown that approximately 40% of bladder tumors show LOH on 11p, seen predominantly in high grade/high stage tumors, while the HRAS1 gene located on 11p15 was found mutated in 15% of bladder cancer cases

(1,12,14,23). In our study we found add(11)(p15) in eight cases. Six of our cases were invasive tumors and two were superficial papillary. To our knowledge add(11)(p15) has not been previously described as a recurrent abnormality in bladder carcinoma (15). This abnormality might lead to gene alterations, but loss of genetic material from 11p could not be excluded.

Abnormalities involving $14q_{32}$ as add(14)(q32) were found in five of our cases. To our knowledge, add(14)(q32) has not been described in bladder cancer. Loss of heterogosity was reported on 14q in about 25% of bladder cancers and two common deletion regions on $14q_{12}$ and $14q_{32}$ were defined (1,24). Add(14)(q32), observed in our cases, could either lead to genes alterations or to loss of genetic material from 14q. Allelic losses on 14q showed significant association with tumor stage, suggesting that this change is a late event in tumor progression (1,24).Three of our cases with add(14)(q32) were of invasive type, one was *in situ* cancer and another was superficial papillary cancer.

Also of interest in our study was the detection of add(19)(q13), found in four cases. Abnormalities of chromosome 19 are not common in bladder cancer and only sporadic cases with add(19)(q13) have as yet been described. Array-based Comparative Genomic Hybridisation (CGH) studies detected high-level amplification of 19q13 (7,14,25). The fragile site FRA 19A is located on 19q13. An association between environmentally-induced chromosomal fragile sites and cancer have been reported. It is believed that fragile sites provide regions of the genome that are more susceptible to damage and that this contributes to the carcinogenic process, because of subsequent changes to gene function or dosage (26). Cases with add(19)(q13) in this study were histologically of invasive type (3 cases) as well as superficial papillary cancer (1 case).

Successful conventional cytogenetic techniques require a high mitotic index and high-quality chromosome preparations but, even when this is achieved, the complete characterization of chromosomal aberrations remains very difficult or impossible. Nevertheless, conventional cytogenetics continues to be valuable in the study of cancer, detecting common chromosomal breakpoints, which could be further investigated by the application of molecular techniques for identification of genes important in carcinogenesis. Moreover, the cytogenetic investigation of cancer continues to offer useful information to clinicians. Bladder cancer is a genetically heterogeneous disease and certain cytogenetic aberrations could be very useful in predicting disease outcome.

Although the number of cases studied was small and the clinical data restricted to histological staging, of interest was the detection of novel recurrent structural chromosomal aberrations, while a correlation of the chromosomal abnormalities observed with tumor stage was also evaluated.

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