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DNA Profile and Tumour Progression in Patients with Superfical Bladder Tumours

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Summary. 229 patients with Grade 1–2 tumours (WHO), all category Ta or T1 (UICC) and surgically treated, were followed clinically and by flowcytofluorometric DNA-analysis (FCM). The tumours were characterised by their DNA profile. 175 cases were found to be diploid and fiftyfour cases showed aneuploidy. The mean follow-up time with continous FCM analysis was 2.6 years. During this period 19 patients showed tumour progression and 11 of these patients died. No progressive cases were found among 175 patients with repeatedly diploid DNA patterns. Thus tumour progression was exclusively linked to an aneuploid DNA pattern. In these case the degree of ploidy determined the frequency of progression: while 50% of the cases with triploid – hypotetraploid DNA pattern showed progression, only 10% of tumours with a tetraploid amount of DNA were found to be progressive. The degree of ploidy in 33 cases with recurrent aneuploid tumours was in general found to be constant. A fairly high degree of consistency was also found in the number of cells in S-phase, expressing proliferative properties. This indicates that superficial bladder tumours can be well characterised by their DNA profiles, that is the degree of ploidy and the proliferation pattern.

Key words: DNA profile, Tumour progression, Superficial bladder tumours.

Introduction

In recent studies [10] we found that bladder tumours can be subdivided according to their DNA profiles: tumours with DNA values corresponding to a diploid chromosome number, aneuploid tumours with a tetraploid amount of DNA and aneuploid tumours with DNA values deviating from tetraploidy. The proliferation pattern, as measured by the number of cells in S-phase, was low in diploid tumours, moderately elevated in tetraploid tumours and generally high in non-tetraploid aneuploid tumours. In relation to the malignancy grade, it was seen that the diploid tumours encompassed nearly all G1 and about 2/3 of G2 tumours. The tetraploid tumours were almost exclusively found within the G2 group. The non-tetraploid aneuploid group was constituted by G3 tumours and the remaining G2 tumours.

In this study the cellular DNA content was investigated in all patients routinely followed in our department after local resection of G1–2 superficial papillary bladder tumours. The aim was to evaluate whether the original DNA profile is constant in recurrent bladder tumours and correlated to tumour progression.

Material and Methods

The material consisted of 229 patients. The initial treatment was TUR (82%) or open operation (18%). Fifty two patients have been followed by flow cytofluorometry (FCM) since the initial diagnosis and first treatment. The remaining patients had, before the start of regular FCM analyses, already been treated (Fig. 1).

The mean observation time was 7.8 years while the mean follow-up time with continuous FCM analyses was 2.6 years. On the average every patient had been analysed four times by FCM.

The mean age of the patients was 60 years, the same for both sexes, and the male/female ratio 2.5/1.

85% of the patients presented with symptoms of macroscopic haematuria, while 5% of the tumours were diagnosed while investigating microscopic haematuria. Cystoscopy in the examination of patients with recurrent infections, prostatic enlargement, etc. unveiled another 10%.

The tumours were graded according to the WHO grading system [11] and the slides were re-evaluated by one of us (P.L.E.). Fifty one cases were thus classed G1 and 178 cases G2. Clinical staging was performed according to the UICC [11]. All cases belonged to category Ta or T1. At regular intervals all patients were examined by cystoscopy, bimanual palpation, cytological examination of bladder washing and FCM analysis. After a primary or recurrent tumour had been treated, the patient was reviewed after 3 months. In case of tumour absence, the control intervals were gradually increased to 6 and maximally 12 months. When tumour was present, the examination was performed under spinal or general anaesthesia to enable biopsy and/or transurethral resection of the tumour. Resected recurrent tumours were regularly examined histologically. Cytological examinations and FCM analyses were routinely done on bladder

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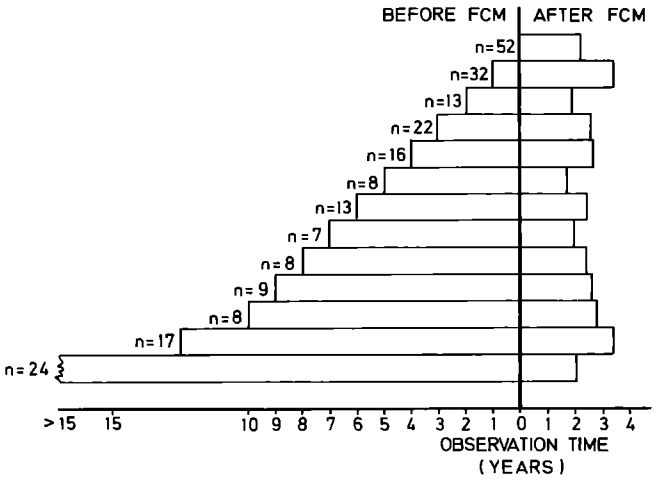

Fig. 1. Mean observation time before and after first cytofluorometric DNA analysis (FCM) in 229 patients. The cases are grouped according to various observation time before start of FCM analyses at year 0

washing material. In addition FCM analyses were performed from tumour biopsies in 48 cases [7, 8].

The DNA content of single cell nuclei was analysed according to a previously described method [6] by a rapid-flow cytofluorometer ICP 11 (Phywe, W.-Germany), with a flow rate up to 1,000 cells/s. The cell material was fixed in alcohol and washed in a buffer solution together with RNase. Single cell nuclei were obtained by pepsine treatment and stained with ethidiumbromide. An average of 30,000–40,000 cells were analysed. The excitation and emission wave lengths were 455–490 nm and 590–630 nm, respectively. A 256 multichannel analyser sorted the output, which was presented as a DNA histogram. After correction for the background the proportion of cells in different phases of the cell cycle was determined from the integrated cell number under the corresponding parts of the DNA histogram. The DNA values of the analysed cells were calculated in relation to the DNA content of normal human lymphocytes (2N).

Evaluations of the number of cells in S-phase have only been made when histograms of high quality, without high backgrounds, were available.

In Fig. 2, three different types of DNA pattern in Grade 2 tumours are shown: (*A*) shows a tumour with diploid DNA pattern, (*B*) a tetraploid type of tumour, (*C*) and (*D*) non-tetraploid tumour types with hypo- and hypertetraploid DNA patterns. A tumour was considered tetraploid when the mean value, calculated from normal bladder urothelium, for the proportion of G_2 + M cells (in bladder washings 4.1 ± 2.0% (n = 45), in biopsies 3.9 ± 1.3% (n = 25)) was exceeded by three standard deviations. In these cases there also existed a G_2 + M maximum in the octoploid region.

Results

The following parameters were studied and correlated to each other:

1) DNA profile – enabling discrimination between diploid and aneuploid cases. The latter were further characterised according to their degree of ploidy and their proliferation pattern.

2) Clinical and morphological findings – with assessment of histological grade and T category.

A case was considered aneuploid if an aneuploid DNA pattern was shown at least once out of multiple investigations.

According to the absence or presence of visible tumour at cystoscopy when FCM investigation was performed the material was divided into two groups. The first group encompassed 88 patients with normal cystoscopic finding. The second group encompassed 141 patients with tumour present at FCM investigation.

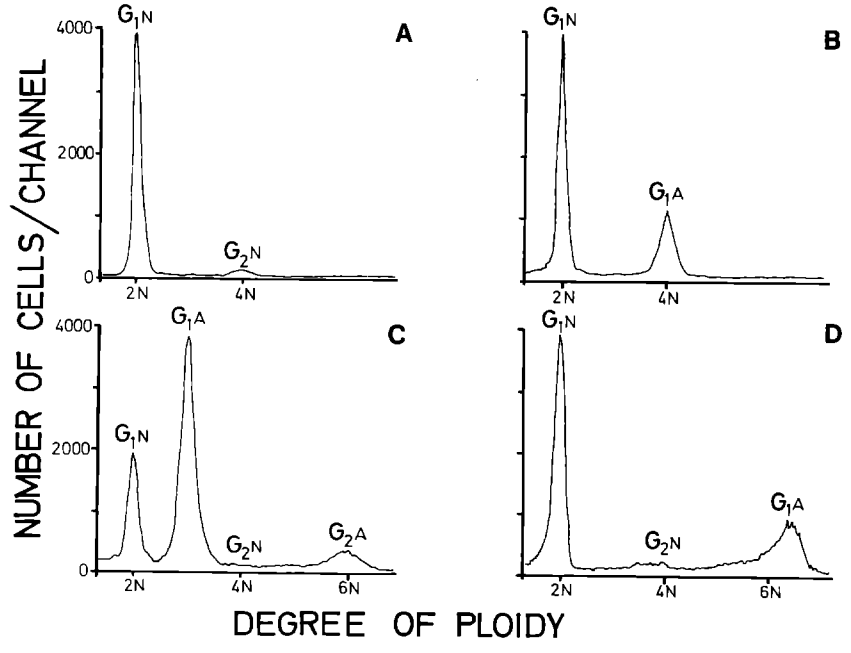

Fig. 2A–D. DNA histograms, (biopsy material), from four cases with Grade 2 tumours. Ordinate: Number of cells/channel. Abscissa: Relative DNA value. The left peaks are made up by diploid G1 cells (2N), corresponding to 46 chromosomes in the cell nuclei. Between the G1 and G2 peaks are the cells in S-phase. The peaks are marked N = diploid 2 N cells and A = aneuploid cell populations. A mean of 45,000 cells/histogram were analyzed. Cases with diploid (A), tetraploid (B) and non-tetraploid aneuploid DNA patterns (C and D) are presented

Table 1. DNA pattern related to initial histological grade in 88 patients without tumour recurrence at FCM investigation

	G1	G2
Diploid	31	54
Aneuploid	1	2

Table 2. DNA pattern related to initial histological grade in 141 patients with tumour at FCM investigation

	G1	G2
Diploid	17	73
Aneuploid	2	49

DNA Pattern and Tumour Grade

The DNA pattern related to the initial histological grade in 88 patients without tumour recurrences at FCM is shown in Table 1. Diploid DNA pattern was found in 85 of these patients. One out of the three remaining patients with aneuploid DNA pattern died from metastatic bladder carcinoma, one proved to have a carcinoma in situ and one is still clinically free of tumour.

Table 2 shows corresponding data for the 141 patients with tumour present. The frequency of aneuploid cases in this group amounting 36% is considerably higher than in the former group. 87% of the tumours were Grade 2 with 40% aneuploid cases.

Degree of Ploidy and Proliferation Pattern in Recurrent Tumours

By comparing the DNA profiles of the primary tumours with those of the recurrences it was possible to study in what way the degree of ploidy and the proliferation pattern could characterise the tumours.

Aneuploidy was found once or several times in 54 cases. In 33 repeatedly treated cases recurrent aneuploid tumours were found. The DNA pattern of every recurrence is shown in Fig. 3a. In the vast majority of these cases a high degree of consistency was found in the degree of ploidy. This means

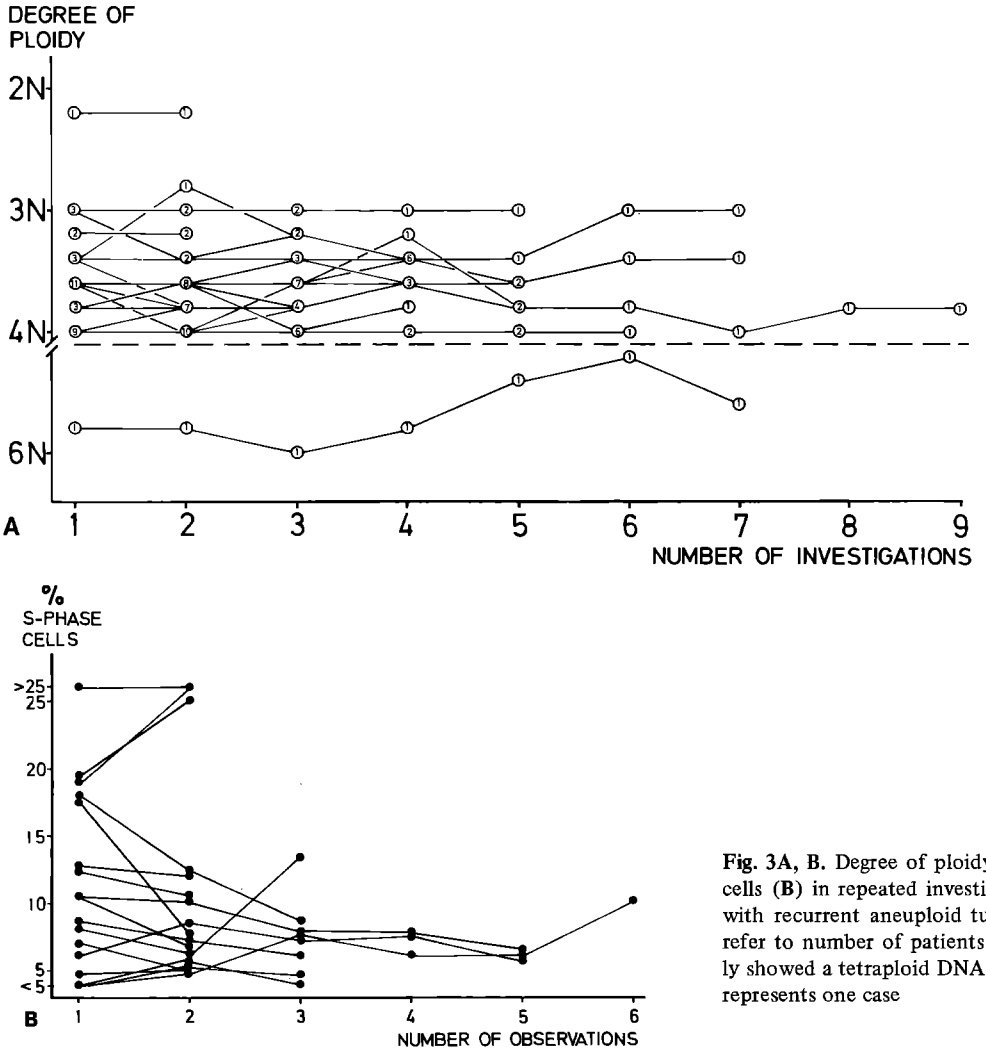

Fig. 3A, B. Degree of ploidy (**A**) and per cent S-phase cells (**B**) in repeated investigations of individual cases with recurrent aneuploid tumours. The figures in (**A**) refer to number of patients, i.e. nine patients originally showed a tetraploid DNA pattern. In (**B**) every mark represents one case

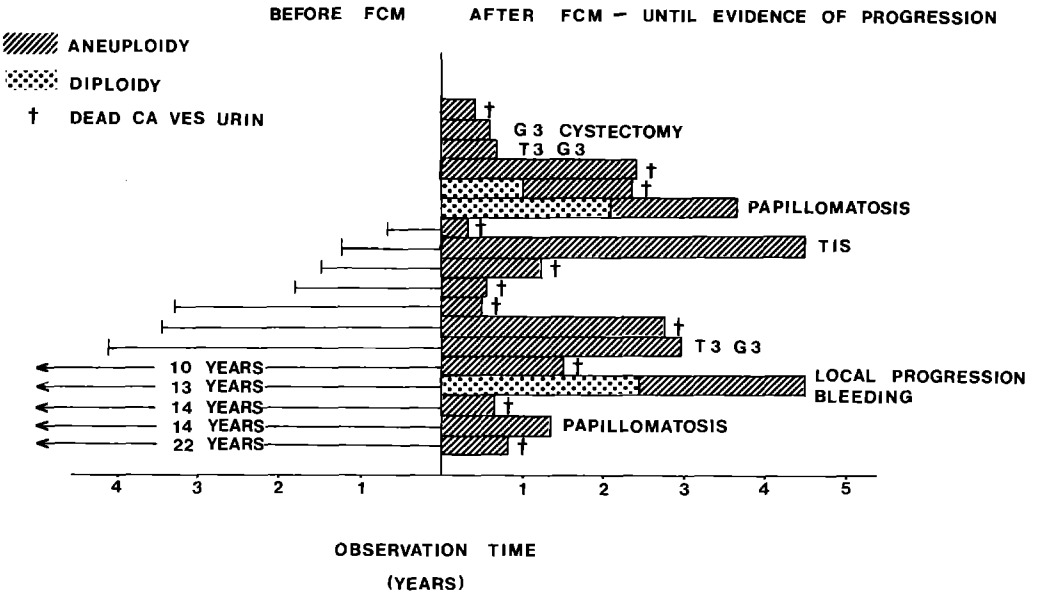

Fig. 4. Clinical course related to FCM findings in 18 progressive cases. One case with clinical evidence of progression before first FCM investigation was excluded

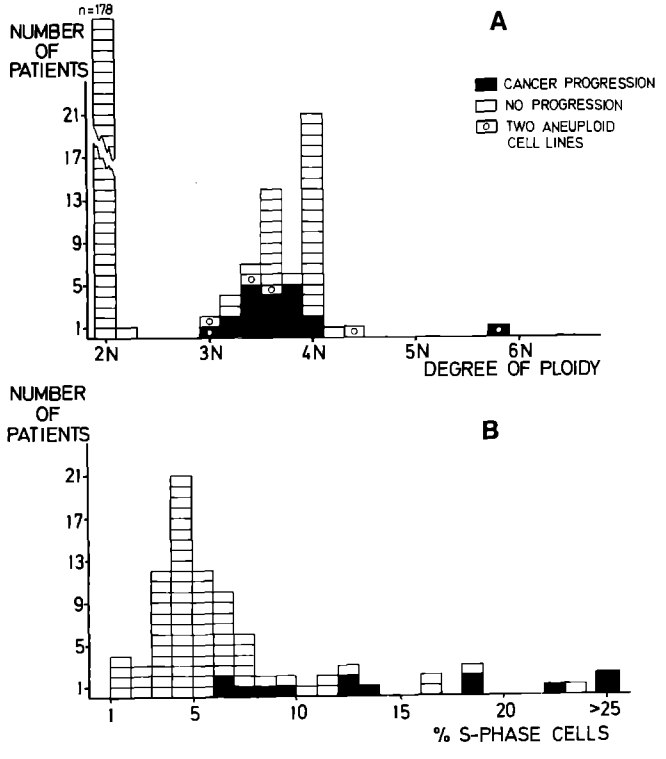

Fig. 5A, B. Degree of ploidy (A) and per cent S-phase cells (B) in the total material. Progressive cases and cases with more than one aneuploid cell line are specially indicated

that a tumour seems to be well characterised by its degree of ploidy. In cases where minor changes can be observed, it is impossible to decide whether these are due to real changes in ploidy pattern or to inaccuracies of the estimations. Nineteen cases with an initially diploid DNA pattern have changed into an aneuploid DNA pattern.

Out of 21 cases with an initially aneuploid DNA pattern, no tumour recurrence was seen in 12 cases and the DNA pattern from bladder washing material was continuously diploid during follow-up. In 8 cases tumour recurrences appeared and the DNA patterns of these recurrent tumours were now found to be diploid. One patient died with progressive disease before further FCM studies were done.

In 16 aneuploid cases where repeated S-phase determinations were possible, there also existed a fairly high degree of consistency (Fig. 3b). The proportion of S-phase cells in diploid cases, which is not shown here, was constantly in the range of 3%–6%.

DNA Pattern and Tumour Progression

The clinical courses in progressive cases related to DNA analysis are summarised in Fig. 4. Nineteen patients showed progression and 11 of these patients died. Progression is defined as: a) increased grading or T-category (n = 14), b) development of flat carcinoma in situ (n = 4), local progession such as papillomatosis necessitating more radical treatment (n = 3).

Out of 52 patients followed with DNA analysis from the initial diagnosis, diploidy was found in 30 cases and aneuploidy in the remaining 22 cases. In six of these 22 cases progression in tumour disease was found, most commonly during the first year of observation. Thirteen cases with progression were found in the remaining material of 176 patients, initially treated before the start of FCM analyses.

The most striking finding was that progression was invariably found within the group of aneuploid tumours. In 16 progressive cases aneuploidy was present during the whole

time of FCM follow-up. Three cases initially were diploid, changing into an aneuploid DNA pattern after 1–3 years.

The significance of degree of ploidy and proliferation pattern in progressive cases is shown in Fig. 5. It can be seen from Fig. 5A that the majority of progressive cases were found among tumours with DNA content in the tri-tetraploid region, while in the relatively large group of tetraploid tumours (4N) only two progressive cases were found.

Aneuploid cases exhibited widely varying S-phase values – from 4% to more than 25%. Determination of the number of cells in S-phase was possible in 13 progressive cases. Fig. 5B shows that the relative incidence of tumour progression was higher as the proliferation rate increased. The mean values for progressive and non-progressive cases (16.1 ± 10.9 and 10.4 ± 5.6) respectively differed significantly. In contrast to the wide range of values found in aneuploid cases, all diploid cases – irrespective of the presence or absence of tumours – showed a low proliferation rate, with a mean value of about 4%.

Discussion

229 cases of operatively treated bladder tumours, category Ta or T1, have been followed clinically and by FCM-analyses. The material is representative for the large group of cases with well or moderately well differentiated bladder carcinoma continuously followed in urological departments.

By repeated FCM-analysis in cases of recurring tumours we found that the degree of ploidy as well as the proportion of S-phase cells was fairly constant. This shows that it is possible to characterise a bladder tumour by determining the degree of ploidy and degree of proliferation. This is in conformity with Sandberg et al. [4] who showed by chromosome analysis that the chromosomal pattern in recurrent papillary non-progressive tumours did not differ from that of the original tumour.

The most striking finding was that tumour progression was never seen in cases with diploid DNA-pattern. Among the non-diploid cases, tumours with tetraploid DNA-patterns constituted the largest single group. In this group however, only 2/21 (10%) of the cases showed progression, while progression was seen in no less than 17/33 (51%) of aneuploid, non-tetraploid cases. These results are in agreement with Tavares et al. [5] using single cell cytophotometry in the investigation of bladder tumours. They showed that di- or tetraploid cases had a substantially better prognosis than tri-hexaploid cases. In a similar study Fossa [3] has shown in biopsy material from 123 patients that diploid cases exhibited a better prognosis than the non-diploid ones. A possible explanation for this may be found in differences in proliferation rate – tetraploid tumours on the average having a lower percentage of S-phase cells than tumours in the tri-tetraploid region [9]. It is also shown in our material that progression was closely related to proliferation rate (Fig. 5B).

In the total material tumour progression was recorded in 19 cases. Within Grade 2 tumours one would normally expect at least 30% progressive cases [1 and 2]. The relatively small number seen here is a direct reflection of our selection method, excluding all patients who progressed or died before onset of FCM investigations.

In the group of 52 patients followed with DNA-analysis from the initial diagnosis aneuploidy was found in 22 cases. Although the observation time is short, progression was seen in six of these cases, most commonly during the first year of observation, which indicates highly aggressive properties in these Grade 2 tumours.

In the group of 88 patients with one or more tumour recurrences not studied from the initial diagnosis by FCM, aneuploid DNA-patterns were found in 31 cases. Eleven of these patients showed tumour progression, all but one exhibiting aneuploidy from the onset of FCM-analysis. Apparently the patients with aneuploid, specially non-tetraploid, DNA-patterns represent a high risk group. Since in a considerable number of cases a shift from diploid to aneuploid DNA-pattern was seen, all cases must be continuously followed.

Three cases with aneuploid DNA patterns were found in the group of 88 patients without visible tumour. This indicates that the method can be helpful in early detection of potentially progressive disease.

In conclusion it has been shown that the DNA-profile can characterise a tumour and bears a clear significance in predicting progression in cases of well and moderately well differentiated superficial bladder carcinoma.

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