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Flow Cytometric Investigations of Human Bladder Carcinoma Compared to Histological Classification

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Summary. The DNA distribution has been investigated in biopsies from human bladder carcinomas by means of flow cytometry (FCM). The FCM analysis was based on a preparation method yielding a suspension of single cell nuclei. Biopsies from 45 patients were analysed. The results demonstrate a correlation between the degree of cellular anaplasia and the occurrence of cell populations with increased DNA content in the tumours.

Cells with diploid DNA content constituted more than 90% of well-differentiated tumours and normal bladder mucosa. Cell populations with aneuploid DNA content occurred in almost all biopsies with cellular anaplasia estimated as grades II and III by microscopic examination. The results indicate that FCM analysis may be a valuable adjunct to histology. The possible prognostic significance of altered DNA distribution in bladder tumours is discussed.

Key words: Flow cytometry - DNA distribution - Human bladder cancer.

In addition to cells with a diploid DNA content, the normal human bladder epithelium contains cells with an increased DNA content. These cells are often described as having tetraploid DNA content. In bladder carcinomas, new heteroploid cell populations often occur resulting in altered distribution of the DNA content (3, 5). The degree of heteroploidy seems to be correlated with the histological differentiation (4) and probably also with clinical malignancy. These studies are based on microspectrophotometric measurements of single cell DNA content, which is a very laborious and time-consuming method and therefore not acceptable as a routine technique.

In recent years, high-speed flow systems have been developed, providing a rapid method for DNA analysis of a large number of cells. The technique has so far been of only limited value as it requires a single cell suspension which has turned out to be difficult to achieve from human solid tumours. Recently, a new preparation method has been elaborated which provides a suspension of single cell nuclei (7). This method has been used in a flow cytometric (FCM) investigation of human prostatic carcinoma (2). In the present study, the same method was applied to a FCM investigation of biopsy specimens from human bladder carcinoma.

MATERIALS AND METHODS

The present study comprises analysis of biopsies from 45 patients with untreated bladder carcinoma admitted to the Radium Center, Aarhus. The patients were clinically staged according to the TNM system (UICC 1974). Biopsies of the tumours were obtained with a conchotome in connection with cystoscopy. The biopsies (approx. 5 mm^2) were divided into 2 parts for FCM analysis and microscopic examination. From 10 patients, biopsies were obtained from apparently normal parts of the bladder mucosa together with the biopsies from the tumours. The specimens were stained with haematoxylin-eosin and cellular differentiation was classified according to Bergkvist et al. (1).

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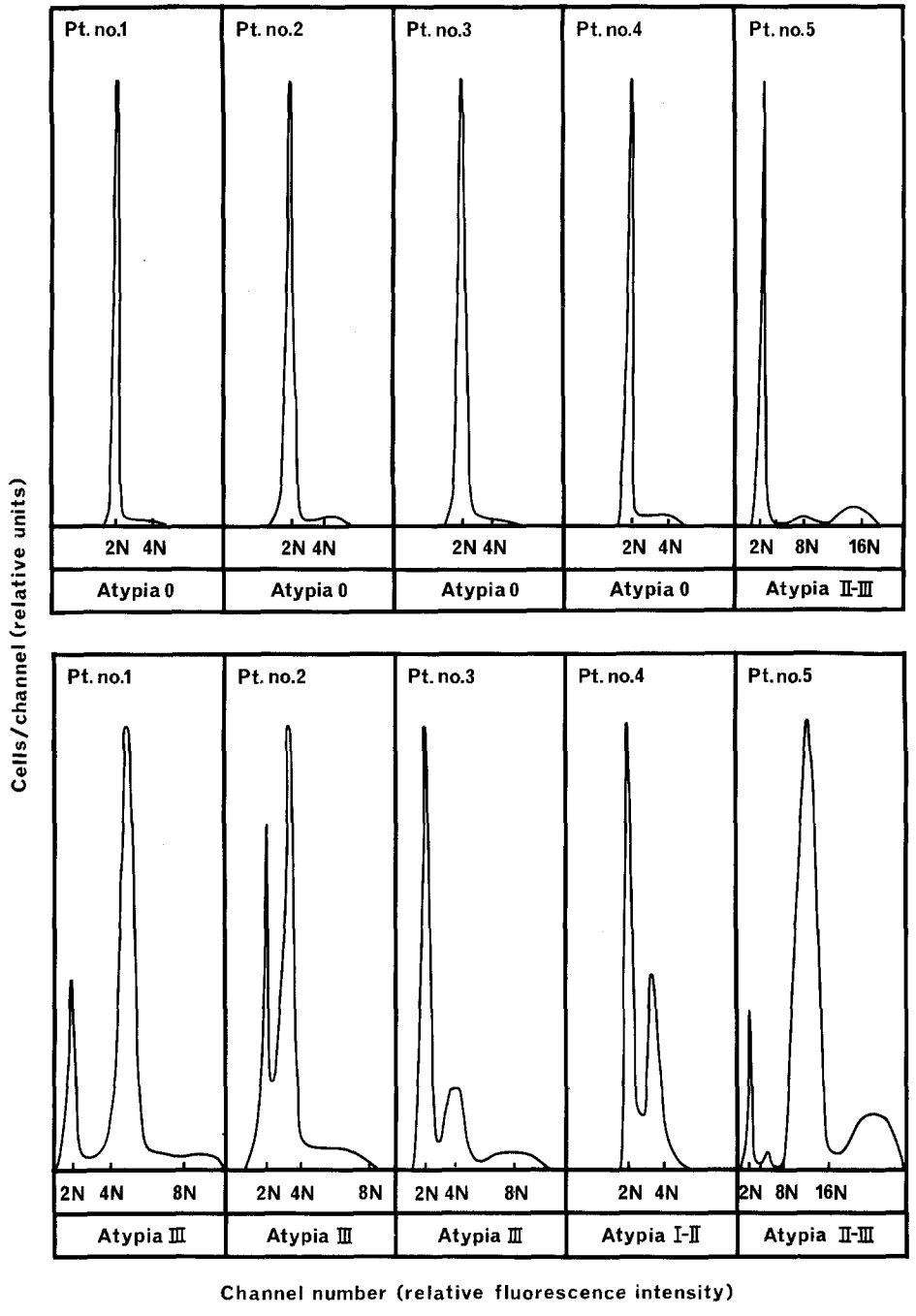

Fig. 1. The distribution of nuclear DNA content in apparently normal biopsies from 5 patients (upper) and in biopsies from the tumours from the same patients (bottom). The degree of cellular anaplasia is indicated below each histogram. Ordinate: Cells/channel (relative units). Abscissa: Channel number (relative fluorescence intensity). Each histogram is based on the analysis of about 30,000 cells

FCM Analysis

The biopsy specimens were collected in 5 ml Tris-EDTA buffer (pH 7.4) at 4°C. After centrifugation (200 g, 5 min), the specimens were comminuted with a scalpel. The staining procedure was carried out according to Vindelöv (7). The cells were suspended at 4°C in a solution containing sodium citrate 1000 mg, ethidium bromide (BDH) 10 mg, sodium chloride 584 mg, Nonidet-P40 (BDH) 300 µl, RNase (BDH) 10 mg, and distilled water to make 1000 ml (pH 7.6). The cells were stained for at least 2 h and passed through a 100-µm filter. Microscopic examination ensured a suspension of single cell nuclei. The flow cytometer was a Cytofluorograph 4802 (Biophysics, Inc.) connected with a DIDAC 800 multichannel analyser (Intertechnique). The signals were presented as a plot of the number of measured cells versus relative fluorescence intensity. The percentage distribution of the various cell populations was calculated by integration performed on the DIDAC 800. To ensure comparable results a suspension of unstimulated human lymphocytes was used as standard.

RESULTS

Figure 1 shows the results of the FCM analysis of specimens from apparently normal parts of the bladder mucosa. The degree of differentiation as estimated by microscopic examination is shown below each histogram. The DNA histograms show that the greater part of the cells have a DNA content corresponding to a diploid (2N) cell population (compared to unstimulated human lymphocytes). Integrations in the histograms show that the cells with increased (non-diploid) DNA content constitute less than 10%. In one exception (the biopsy from patient No. 5), the cells with non-diploid DNA content constituted 30%. It appears from the figure that the microscopic examination showed atypia grades II-III. This biopsy was the only one of 10 apparently normal specimens which showed any cellular dedifferentiation and more than 10% non-diploid cells. The lower part of the figure shows the results of FCM analysis of the specimens from the tumours from the same 5 patients. The degree of anaplasia is shown below the histograms. It appears that these biopsies contain considerable cell populations with increased DNA content. Most of these cell populations have aneuploid DNA content and constitute in all 5 cases more than 10% of the analysed cells.

In Table 1, the degree of cellular anaplasia is compared to the results of the FCM analysis. It is evident that the occurrence of cell populations with aneuploid DNA content is related to the degree of cellular anaplasia. All 6 biopsies, atypia grade I, were diploid, while almost all biopsies, atypia grades II and III, contained aneuploid cell populations.

The results of the FCM analysis and the extension of the tumours are compared in Table 2. It appears that all invasive tumours but one contained aneuploid cell populations. Of 19 cases with tumour confined to the epithelium, 9 biopsies contained cell populations only with diploid DNA content, while cell populations with aneuploid DNA content were found in 10 biopsies.

DISCUSSION

The NP-40 preparation method described by Vindelφv has previously been used for FCM analysis of fine-needle biopsies. The results presented here indicate that the method can also be used for the preparation of larger conventional biopsies. This is an obvious advantage as it is often easier to ensure representative biopsies from solid tumours with a conchotome.

The method seems to give reliable DNA histograms with clear separation between cell populations with different DNA content. The mechanical comminution of the specimens does not bring all

Table 1. Correlation of FCM and cytology. The table includes 45 patients. The biopsies denoted "diploid" contained more than 90% cells with diploid DNA content. The biopsies denoted "aneuploid" contained more than 10% cells with aneuploid DNA content

No. of biopsies	Micr. Atypia	FCM	
		Diploid (>90%)	Aneuploid (>10%)
6	I	6	0
4	I-II	2	2
7	II	1	6
2	II-III	0	2
26	III	1	25

Table 2. Correlation of FCM and T category. The table includes 45 patients. T category is according to the TNM system (UICC 1974). For diploid and aneuploid, see text to Table 1

No. of biopsies	T category	FCM	
		Diploid (>90%)	Aneuploid (>10%)
19	TIS	9	10
10	T1	1	9
2	T2	0	2
11	T3	0	11
3	T4	0	3

cells into single cell suspension. Consequently, it cannot be excluded that the cells examined are subject to some selection. Furthermore, in addition to epithelium, the biopsies contain other types of cells, i.e. leucocytes and fibroblasts. These factors may obviously influence the DNA distribution, but are probably of only minor importance as they do not explain the occurrence of aneuploid cell populations.

Earlier microspectrophotometric measurements have demonstrated that the normal human bladder epithelium is polyploid. Levi et al. (5) presented data indicating that cells with tetraploid or octoploid DNA content constitute about 30%. These results were not confirmed by Fossås (3). Investigating 13 normal biopsies, she found no case with more than 9% tetraploid cells. The reason why a higher percentage of tetraploid cells is reported in some microspectrophotometric investigations might be that bi-nucleated cells are included among those investigated. In the present study, a suspension of single-cell nuclei was obtained. This probably explains why only a few cells in the normal biopsies were found to have an increased DNA content.

The results presented here demonstrate that poorly differentiated tumours (atypia grades II and III) contain cell populations with aneuploid DNA content. These cell populations are not found in the normal bladder mucosa or in well-differentiated tumours (atypia grade I). Similar results were reported by Lederer based on microspectrophotometric measurements (4). However, no clear distinction was obtained between tumours with atypia grades I and II. The aneuploid cell populations probably represent tumour cell stem lines. According to Levi et al. (5), bladder tumours arise as diploid and finally progress to aneuploid populations. The data in Table 2, however, demonstrate that aneuploid cell populations may occur early in tumour development. Almost half of the tumours classified as TIS contained such populations. These tumours might represent the more malignant part.

Compared to microspectrophotometry, FCM seems to be a time-saving technique which can give valuable information about the DNA distribution in solid tumours. FCM analyses of prostatic carcinomas have revealed heteroploid cell populations with only 2N, 4N and 8N DNA content. Most bladder tumours contain populations with aneuploid DNA content. This discrepancy is likely to be due to tissue differences.

The clinical significance of cell populations with increased DNA content is not yet clear. Levi et al. (5) found no correlation between the DNA stem line ploidy and the clinical course. However, Tavares et al. (6) found a better prognosis for carcinomas with a tumour DNA stem line in the diploid or tetraploid region than for tumours with a triploid or hexaploid DNA stem line. Further studies are needed to determine whether the DNA distribution in tumours influences the prognosis. FCM analysis seems to be a valuable tool in such investigations.

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