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Flow-Cytometric Demonstration of Tumour-Cell Subpopulations with Different DNA Content in Human Colo-Rectal Carcinoma

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Abstract—*Six consecutively operated colo-rectal carcinomas were subjected to detailed flow-cytometric analysis by taking 6–10 fine-needle aspiration samples from different locations in each tumour. Single-nuclei suspensions were prepared by a detergent technique, and the DNA stained with ethidium bromide.*

In all 6 carcinomas, tumour cells with "near-normoploid" DNA content were found. In addition, a predominant hyperploid cell population was found in 5 of the tumours, often mixed with the normoploid cells. Thus, colo-rectal carcinomas may be composed of more than one tumour-cell stem line. As these subpopulations probably have a different chromosome content, they may behave differently in several important ways, e.g., in malignant potential.

The heterogeneous composition of the tumours may also complicate the interpretation of cell-kinetic and other biological measurements.

INTRODUCTION

By means of flow cytometry (FCM) it is possible to obtain distributions of single-cell DNA content, or DNA histograms, in dispersed cell populations.

Using fine-needle aspiration biopsy and preparation of the material by a new detergent technique, it has recently been demonstrated that FCM can be successfully applied to DNA analysis of human solid tumours [1, 2].

Preliminary FCM investigations of individual human colo-rectal carcinomas have demonstrated wide differences in the DNA histograms when the biopsy specimens were taken from different locations in the same tumour. A series of colo-rectal carcinomas was therefore subjected to more detailed analysis as regards this problem.

MATERIALS AND METHODS

Six to ten fine-needle biopsy specimens were taken from each of 6 consecutively operated colo-rectal carcinomas. Samples from lymph nodes and/or colon mucosa were taken as reference material.

Sampling was performed by aspiration through a 21-gauge needle from different locations in the tumours at a maximal depth of 8–10 mm. A small amount of the aspirate was smeared and stained for cytological examination, and the remainder was suspended in Tris–HCL-EDTA buffer (pH 7.4) by washing the needle and syringe with the buffer.

After centrifugation, the cells were prepared as a single nuclei suspension and stained in one step by incubation at 4°C in a solution of ethidium bromide, RNase and a non-ionic detergent. (For details see [1]).

In a flow cytofluorometer (Cytofluorograph 4800A, Bio-physics Inc.) the relative fluorescence of the nuclei, corresponding to the DNA content was measured, and the results were sorted in a DIDAC 800 (Intertechnique) multi-channel analyser and presented as a histogram with the relative DNA-content on the abscissa and the number of nuclei in each channel on the ordinate. The number of measured nuclei in each sample ranged from 10,000 to 70,000.

The smears were air-dried and subjected to May-Grünwald-Giemsa staining. Each smear

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was examined for the relative content of normal cells, primarily white blood cells.

RESULTS

The reference DNA histograms from lymph nodes or colon epithelium were always very uniform and showed one well-defined peak (Fig. 1), which in each case is defined as the normoploid (or diploid) DNA value (2N) corresponding to the normal diploid chromosome content. An arbitrarily chosen area of ±25% around this value is defined as "near-normoploid" content (or "normoploid area"), and higher values are then taken as hyperploid DNA content. This way of description is in accordance with other investigators [3].

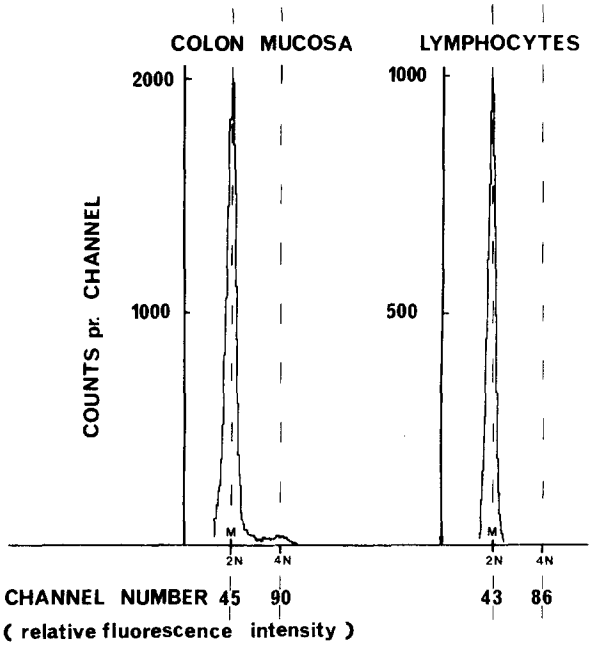

Fig. 1. DNA histogram from normal colon mucosa and from a lymph node. M marks the channel of the modal DNA content in the cell population, which for normal cells corresponds to the diploid chromosome content indicated by 2N.

The cytological examination of the smears corresponding to each sample was in this series done in four of the tumours and showed a uniform picture of tumour cells with an admixture of white blood cells not more than 15% and less than 10% in most smears.

All 8 histograms from tumour No. VI (see Table 1) are shown in Fig. 2, which illustrates that 5 samples were predominated by cells with a modal DNA content in the near normoploid area. The remaining three were bimodal and thus were composed of hyperploid cells with an addition of 20–30% near-normoploid cells. In Fig. 3 are shown the topographical locations of the eight samples, and it is noticed that the hyperploid cell population seems located in one end of the tumour.

The results from all 5 tumours are listed in Table 1. Tumour No. I showed only one near normoploid cell population in all samples. The remainder five were composed of both near-normoploid and hyperploid tumour cells, either alone or mixed, the latter making the histogram bimodal. The modal DNA value of the hyperploid cells were located in the tetraploid area in tumours No. II and III while in the remaining three it was between normoploidy and tetraploidy. No definite topographical pattern, as demonstrated in tumour No. VI appeared in the other four containing different cell types. The size of the tumours ranged from 2.5 × 3 cm to 7 × 7 cm and three different histologic types were represented.

Table 1. Tumour samples grouped according to their content of near-normoploid and hyperploid cell populations

Case No.	No. of samples	No. of samples grouped according to predominant cell populations			
		Near-normoploid	Hyperploid	Mixed near-normo- and hyperploid	Equivocal
I	7	7			
II	7	4		3	
III	8	7		1	
IV	10	1	8	1	
V	6		3	1	2
VI	8	5	1	2	

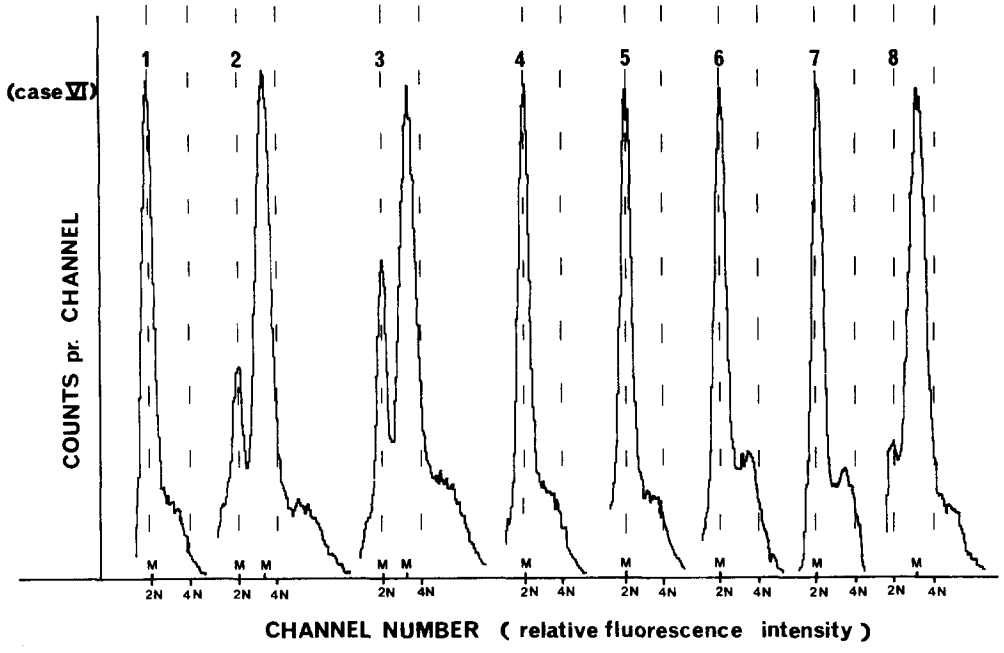

Fig. 2. Eight histograms from a rectal carcinoma (case VI). Nos. 2, 3 and 8 show a hyperploid cell population, and represent one topographic extreme of the tumour (see Fig. 3).

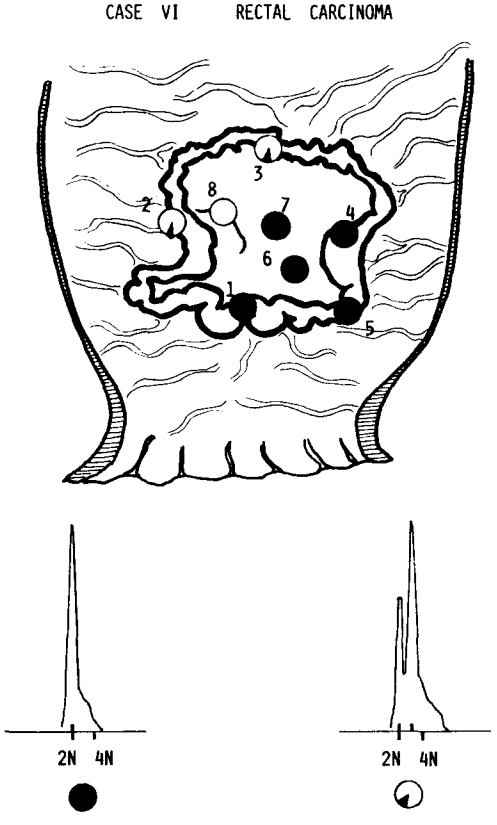

Fig. 3. The sites of the 8 biopsies from the rectal carcinoma in case VI and the two different types of histograms found. The hyperploid cells seem to be predominant in the upper left part of the tumour.

DISCUSSION

The results show that colo-rectal carcinomas may be composed of more than one population of tumour cells as defined by their modal DNA content. These different cell populations may be more or less mixed throughout the tumour, but sometimes confined areas are predominated by cells with nearly the same DNA content. The cytological examinations showed that the contamination of the samples with normal cells was far too low to explain the amount of cells with a near-normal DNA content, which means that both hyperploid and at least most of the near-normoploid cells were tumour cells.

This "mosaic" composition of colo-rectal carcinomas has previously been described only in a few cases. In a survey of the literature of micro-spectrophotometric DNA measurements of all sorts of tumours, Böhm and Sandritter [3] presented their own series of 12 cases of colorectal carcinomas, in which three or four tumours contained an additional small hyperploid population. In one tumour, they found some variation in the cellular composition between the central area and the margin, but in a chapter devoted to the problem of mosaic composition of tumours, they stated that "genuine, coarsely granular mosaics with distinct cell populations (clones) in different sections of the same tumour seem to appear only rarely".

In a paper also concerning mosaic tumour composition, Stich and Steele [4] demonstrated in eight different tumours a common heterogeneity and some examples of simultaneous existence of 2 different stem lines,

especially obvious when the measurement was performed on metaphase or telophase cells. They also observed some regional difference in the composition of one tumour (an osteogenic sarcoma), but in the single case of a colo-rectal carcinoma presented in this selected series, they found only one stem line. Further they stated that "simultaneous existence of several well-established stem lines is rare and probably only a transient stage". In another paper dealing with the DNA content in polyps and adenocarcinomas of the large intestine, Stich *et al.* [5] found a bimodal representation in one of 10 carcinomas and no obvious stem line in another.

The present investigations demonstrate that a mosaic composition of colo-rectal carcinomas may not be rare, and that a single biopsy from a tumour may not be representative of the whole tumour.

It is far from clear whether cells with nearly the same hyperploid DNA content have similar properties in other respects. However, such cells seem to be capable of outgrowing other cells in some parts of a tumour. This may be due to different genetic characters of cells with different DNA content, and raises the interesting question whether similar differences exist in malignant potential, e.g., the ability to grow invasively and to metastasize. In a study of carcinoma of the cervix, Atkin [6] demonstrated a better prognosis in tumours with tetraploid DNA modal values than in those with hyperdiploid DNA modes. In another paper, Atkin reported the opposite finding in cases of breast cancer [7]. Very little is known about that subject in the colo-rectal carcinoma but the present demonstration of a possibly common heterogeneity of the tumours makes the classification by way of ploidy alone rather complex. Also other cell biological measurements, e.g., cell-kinetic analysis may be complicated by this heterogeneity.

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