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Cellular DNA content and metastasis pattern in colorectal carcinomas

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Summary. Tissue samples from primary tumours and metastases obtained from surgical specimens and autopsies were investigated by flow cytometry for their DNA content. Of the 149 cases investigated, 124 autopsy cases of colorectal carcinoma were suitable for study. The pattern of metastatic spread in each indiviual case was analysed with reference to the autopsy records. A subgroup of euploid primary tumours was observed which should be considered separately with regard to its biological behavior, tumour location and extent of metastases. In these tumours, the liver was the first and final organ of haematogenic metastatic spread at an above-random frequency and a grossly metastatic liver with less than 15% of residual normal parenchyma was present at the same time. Furthermore, we observed stem-lines deviating in ploidy from the primary tumour in the metastases of nine cases. This indicates the chromosomal heterogeneity of colorectal carcinomas.

Key words: Flow cytometry – Ploidy – Colorectal carcinoma – Metastasis pattern – Liver metastasis

Introduction

The pathological anatomical classification of colorectal carcinomas has remained largely unchanged in recent decades. The evaluation is based on staging of the tumour according to Dukes or the TNM classification, as well as on histological evaluation of the degree of differentiation (Dukes 1932; Dukes and Bussey 1958; Hermanek et al. 1987; Sugarbaker et al. 1985). These criteria permit us to evaluate the current state of spread of local tumour. However, they do not permit an adequate appraisal of biological aggressivity and thus of the individual prognosis. It is necessary to look for further variables which allow a prediction of the potential for metastatic spread of the tumour in adequately treated primary lesions.

In recent years, flow cytometry has attained increasing importance as an informative and rapid measurement for determining the DNA content of tumour cells. In numerous studies, the ploidy of tumour cells from different organs has been determined. Attempts at more far-reaching evaluation of malignancy in connection with the clinical course, histological grading and serum studies (e.g. CEA levels) have been made with the goal of better appraisal of prognosis (Atkin et al. 1979; Barlogie et al. 1983; Feichter et al. 1984; Frankfurt et al. 1984, 1984a).

In the present paper, a systematic workup of metastases obtained at autopsy was performed in addition to an investigation of primary colorectal tumours. Differences were sought in the behavior and the distribution of metastases occuring in euploid and aneuploid primary tumours. In addition, the DNA content was investigated in a large proportion of the metastases and compared with the respective primary tumour.

Method

The study comprised 149 cases of colorectal carcinomas from operative specimens and autopsy material of the Institute of Pathology, University of Munich from 1971 to 1984. In these cases, both the evaluation of the primary tumour (which had mostly been removed surgically) and the autopsy had been performed in our Institute. Cases with secondary malignancies were excluded from the investigation. A precise evaluation both of the distribution of metastases and of the growth behavior of the individual metastases was possible for each individual case from the autopsy protocol. We considered in particular the behavior of the hepatic metastases, which were subdivided

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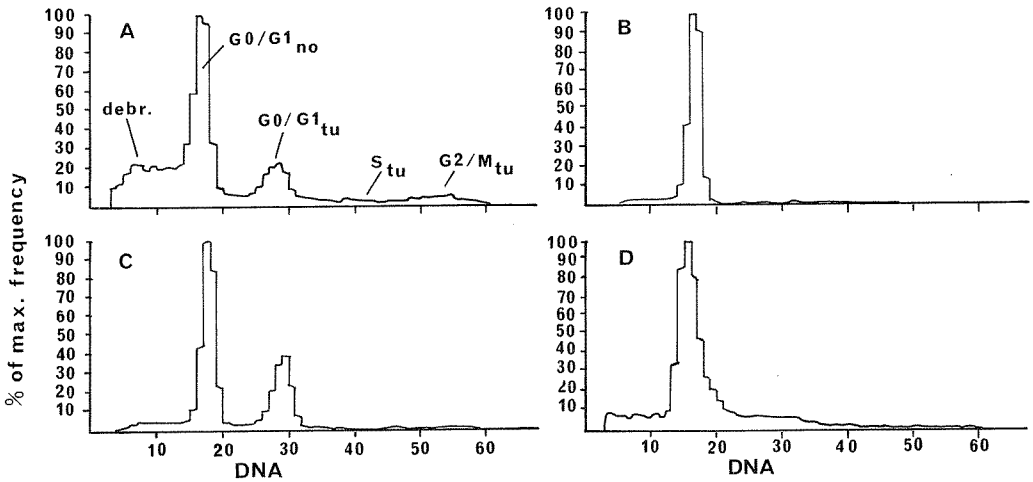

Fig. 1. The DNA distributions of pepsin isolated nuclei of a colon tumour **A**, the normal adjactent healthy mucosa **B**, a lymph node metastasis **C** and a liver metastasis **D** of the same patient. Primary tumour and lymph node metastasis are aneuploid (DNA index 1.67) while the liver metastasis is euploid. The DNA are standardized to the maximum channel content (100%). The maxima correspond to 1370, 3318, 4146 and 1337 nuclei and a total of 10013, 10156, 22109 and 8606 nuclei and cell debris (debr.) were measured. The coefficients of variation of the G0/G1 peaks of the DNA distributions were **A** 6,7%, **B** 5,4%, **C** 5,5% and **D** 8,9%. They were obtained by fitting a linear Gaussian normal distribution to the G0/G1 peak of the DNA distribution

into three groups in accordance with their frequency in the liver: solitary metastasis, multiple metastases and grossly metastatic liver (residual liver parenchyma not more than 10% to 15%).

Histological sections were used to determine whether the tumour cell population of the material was higher than 30%. If this was the case, preparation of a cell nuclei suspension of the material from the primary tumours, the normal mucosa or of the metastasis embedded in paraffin was carried out in a modification of the method described by Hedley et al. (1983). Two hundred and fifty µl of the suspension obtained was stained with 5 µl propidium iodide (2 mg/ml in HBS buffer, Sigma Chemie, Deisenhofen) for flow cytometric measurement. A Fluvo-Metricell flow cytometer (Kachel et al. 1977) was used for simultaneous determination of the red propidium iodide fluorescence and cell nuclear volume. Monodisperse latex particles of 5 µm diameter (Paesel, Frankfurt) to which fluorescein thiocyanate had been bound covalently, served as internal volume and fluorescence standard in the measurement. The coefficient of variation (CV – standard deviation/mean particle volume × 100(%)) for particle fluorescence and volume was between 1.8 and 2.2%.

A HBO-100 mercury highpressure lamp was used to excite fluorescence between 400 and 500 nm. The cellular fluorescence was measured following epiillumination between 550 nm and 770 nm with a photo-multiplier tube. The maximun height of the logarithmically amplified volume and linearly amplified fluorescent signal of each cell nucleus was digitised with a resolution of 128 channels and transferred to magnetic tape. The evaluation of the measurement was carried out with computer programs which had been developed previously. They allow a representation of the measurements as single-variable or two-variable histograms (Valet et al. 1976, 1979, 1987). Besides the samples from the primary tumour or metastatic tissue, a sample for normal mucosa from the tumour-free resection margin was investigated as reference in each case (Fig. 1).

The statistical analysis was carried out using the exact Fischer test.

Results

Of the 149 cases investigated, 25 could not be evaluated because the number of nuclei was too small or the proportion of necrosis or autolysis was too high. Of the remaining 124 cases, 66% displayed a euploid and 34% an aneuploid chromosome set. In 47% ($n=59$) of the cases, there were non-metastatic primary tumours; of these, 48% showed an euploid and 44% an aneuploid chromosome set (Table 1 A).

Metastatic carcinomas were found in 53% ($n=65$) of the overall investigation material. Here, 52% of the euploid and 56% of the aneuploid tumours had metastasized (Table 1 A).

Our main attention was directed to the cases with metastatic spread verified at autopsy. Here, there was exclusively lymphogenic metastatic spread in 25%; 75% ($n=49$) of the cases showed haematogenous metastatic spread. This type of spread was present in 74% of the group of euploid tumours and in 78% of the cases with aneuploid tumours (Table 1 B). The average age of the patients with euploid primary tumours was 66.0 years and that of the group with aneuploid tumours 66.3 years. The sex ratio female to male was 1.2 to 1.0. The age distribution in relation to the number of haematogenous metastatic sites and the ploidy status did not reveal any significant differences for euploid and aneuploid primary tumours with a single metastasis, or with several metastatic sites. Metastasis distribution and metastatic behav-

Table 1. Distribution of the groups "no metastases/metastases" (**A**), "exclusively lymphogenous metastases/haematogenous metastases" (**B**), and "unilocular metastases/multilocular metastases" (**C**) in % of euploid and aneuploid primary tumours

Group		euploid	aneuploid
		($n=82$)	($n=42$)
A	no metastases %	48	44
	metastases %	52	56
	sum %	100	100
		($n=42$)	($n=23$)
B	exclusively lymphogenous metastases %	26	22
	haematogenous metastases %	74	78
	sum %	100	100
		($n=31$)	($n=18$)
C	unilocular metastases %	55	56
	multilocular metastases %	45	44
	sum %	100	100

ior of the haematogenously metastasized carcinomas showed the following picture: in about 55% of the cases, a single metastasis was detected. In about 45%, several sites of metastasis were present. Here, haematogenous spread into more than one organ had occured in 45% of the euploid and 44% of the aneuploid tumours (Table 1 C).

Lymphogenous metastases were investigated by flow cytometry in 35 cases. Of the 49 haematogenously metastasized tumours, a total of 58 metastases from 35 cases (27 liver, 12 lung, 19 other) were investigated by flow cytometry. In 22 euploid primary tumours, 19 showed euploid and three aneuploid metastases, of these one was a lymph node metastasis. In 13 aneuploid primary tumours, aneuploid haematogenous metastases were detected in seven cases and euploid haematogenous metastases in six cases. Altogether, a stem line deviating from the primary tumour was found in the metastases in nine cases (Table 2). In the aneuploid primary tumours, euploid metastases were shown in the liver in three cases, in the liver and lungs in one case, in the lungs in one case and in the spleen in one case (see also Fig. 1). In three cases of euploid primary tumours, aneuploid metastases were found: an aneuploid lymph node metastasis was present with euploid metastatic spread into the liver and lungs in one case. Aneuploid metastases in the liver and lungs with euploid regional lymph node metastases were found in one case and aneuploid metastatic spread into the liver was found in one case.

Table 2. Nine cases with different DNA content in the primary tumor and the metastases: cases 1–6 aneuploid primary tumours, cases 7–9 euploid primary tumours (histogram of case 3 see Fig. 1)

Case
1 euploid liver and lung metastases/aneuploid peritoneal met.
2 euploid lung metastasis/aneuploid liver metastasis
3 euploid liver metastasis/aneuploid lymph node metastasis
4 euploid liver metastasis
5 euploid liver metastasis
6 euploid splen metastasis
7 aneuploid lymph node met./euploid liver and lung met.
8 aneuploid liver and lung metastases/euploid lymph node met.
9 aneuploid liver metastasis

Table 3. Euploidy and aneuploidy of primary tumours in relation to the degree of liver metastases and to the involvement of other organs

	Number of cases	
	euploid	aneuploid
Solitary liver metastasis metastases in:		
1a liver only	o	o
1b liver and other organs	ooooo	– – –
Multiple liver metastases metastases in:		
2a liver only	ooooo	ooooooo
2b liver and other organs	ooooooo	oooooo
*Grossly metastatic liver** metastases in:		
3a liver only	ooooooo	– – –
3b liver and other organs	o	oo

* Less than 15% liver parenchyma

As expected, the liver was the most frequently affected organ. In 83.9% of the euploid and 88.9% of the aneuploid primary tumours with haematogenous metastases, the liver was involved. The frequency of liver involvement remained the same with increasing number of organs affected by metastases. Very much more distinct differences were shown in the subdivision of the hepatic metastases into the groups "solitary metastasis", "multiple metastases" and "grossly metastatic liver" (Table 3). The latter group occurred in eight cases with euploid tumours and in only two cases with aneuploid tumours. In seven out of the eight euploid

cases, there were grossly metastatic livers without an involvement of other organs: The liver was thus the first and sole site of manifestation of haematogenous metastatic spread. A grossly metastatic liver occurred together with metastases in the lungs and ovary in only one euploid case. Despite the small number of cases, this difference is statistically significant ($p=0.015$). However, a grossly metastatic liver did not occur as sole organ of manifestation in the aneuploid tumors: It was only found twice, together with pulmonary and vertebral body metastases.

Discussion

The variables determining the evaluation of the nature and course of metastatic spread of colorectal carcinomas are primarily the extent of lymph node involvement and the depth of penetration of the tumour into the intestinal wall (Dukes and Bussey 1958). Histological grading provides additional information without unequivocal prognostic relevance (Sugarbaker et al. 1985). Establishment of the DNA content of tumour cells has been used in connection with the criteria specified above as a further variable useful in determining the prognosis (Rognum et al. 1982, 1984; Tribukait et al. 1983; Wirsching et al. 1985 and 1986). A very much poorer course of aneuploid carcinomas in Dukes stages A, B and C has been shown by several investigators on colorectal primary tumours (Kokal et al. 1986; Wolley et al. 1982).

In advanced tumours with hepatic metastases, prognostic differences between euploid and aneuploid tumours could not be demonstrated (Finan et al. 1986). The present study deals with autopsy and surgical material which considers tumour spread at the time of death without knowledge of the clinical course thus, evaluation of the prognosis is not possible.

In this material, the ratio of euploid to aneuploid tumours was 2:1. In the correlation of the DNA content of the primary tumours and metastases with the patterns of metastatic spread, it was shown that euploid and aneuploid primary tumours did not differ in the incidence of lymphogenous or haematogenous spread. The two tumour types did not deviate significantly from each other with regard to the number of sites of haematogenous metastases: Single and multiple metastases were equally frequent in the two populations.

As the first "filter station" of the venous drainage of the entire colon and the rectum (with the exception of the distal one third of the rectum) the liver assumes a special place in haematogenous metastatic spread (Walther 1948). The overall percentage involvement of this organ in manifest haematogenous metastatic disease in colorectal carcinoma is between 80% and 84% (Eder 1984; Weiss et al. 1986). It has been shown in earlier investigations that numerous large metastases up to the state of grossly metastatic liver occur in almost half of those cases with exclusively hepatic involvement (Eder 1984).

In our material, the group with the latter type of metastases consists exclusively of euploid tumours (Table 3, group 3a). Four of seven of these patients died in coma due to liver failure. Further sites of haematogenous metastasis were found in only one euploid case with grossly metastatic liver (Table 2, group 3b).

However, only two cases with grossly metastatic liver were found amongst the aneuploid carcinomas; other organs were also affected in both of these cases (Tab. 3, group 3b). The small number of metastatic livers in group 3b is explained by the fact that the death rate in multi-organ involvement must be higher and that complete metastatic infiltration of the liver has no time to develop.

Amongst the euploid tumours, a group thus crystallizes which involves the liver exclusively and destroys this organ without having produced further haematogenous foci (Table 3, group 3a). In a high percentage of cases, the cause of death is coma due to liver failure: The liver is the first and final organ for haematogenous spread.

According to the classical cascade theory of metastatic behavior in colorectal carcinomas, there is secondary metastatic spread from the metastasis in most cases, after a latency period has elapsed (Weiss 1985). This principle is confirmed in our investigation in a large number of the tumours. In the euploid group with grossly metastatic liver, a variant behavior is present: despite almost complete infiltration of the organ by carcinoma, further spread (e.g. into the lungs) does not occur.

The coefficients of variation of the DNA distributions were between 4.9 and 10.5%. This is higher than CV's obtained from DNA measurements on freshly digested speciments but comparable to results obtained by others on autopsy material (Hedley et al. 1983).

The phenomenon of the heterogeneity of colorectal primary tumours has been extensively described in the literature (Hiddemann et al. 1986; Peterson et al. 1978; Quirke et al. 1985; Spremulli 1983). We can only comment it indirectly on the basis of retrospective investigation of autopsy material. However, the occurrence of euploid metas-

tases in aneuploid primary tumours and vice versa in nine cases (see Table 2 and Fig. 1) is an indication of the presence of heterogenous stem lines with possibly different metastatic potential in the primary tumour, or the development of various stem lines from the primary tumour in the metastases. In our material, euploid stem lines were present in 46% of the metastases investigated in aneuploid primary tumours. However, only 14% of cases showed aneuploid metastases from euploid primary tumours. This may be an indicator of a more pronounced heterogeneity in aneuploid tumours.

To summarize, the observations support the suspicion that individual stem lines of the primary tumour may show differences both with regard to the route of metastatic spread as well as in the metastatic behavior depending on their potential to form metastases. This observation might be of clinical significance for example, with regard to the establishment of indications for selective perfusion of cytostatic drug in liver metastases. Further investigations with larger numbers of cases and precise analysis of the primary tumours and their associated metastases are necessary to clarify these questions.

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