PLOIDY LEVEL AND ALPHA-FETOPROTEIN PRODUCTION IN SPONTANEOUS MOUSE HEPATOMAS DEPENDING ON THEIR DEGREE OF HISTOLOGICAL DIFFERENTIATION

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Tumors of the mouse liver arise in a cell population which is heterogeneous with respect to its ploidy. The ploidy of a cell is determined by the number of polyploidizing mitoses characteristic of normal tissue differentiation, which it has gone through: the higher the number, the more reproduction cycles accomplished [2]. Preservation of high proliferative powers throughout the life of an animal makes differential polyploid hepatocytes potentially capable of becoming the initial cell of hepatomas, although the likelihood of their transformation falls sharply with an increase in the ploidy level [1]. The diploid part of the population, which has the lowest ploidy and rarely divides, more closely resembles the precursor cells of the hepatocyte. It is interesting to study the ploidy of hepatomas and to correlate it with the stages of hepatocyte differentiation and to identify the possible precursor cells of the tumor.

In the investigation described below methods of quantitative cyto- and immunochemistry were used to determine the DNA content and alpha-fetoprotein (AFP) production in spontaneous hepatomas of hybrid mice and the results were compared with the histopathological type of the tumor. The degree of development of cell polyploidy and the intensity of protein synthesis characteristic of the embryonic liver were regarded as specific markers of hepatic differentiation.

EXPERIMENTAL METHOD

Experiments were carried out on male (CBA × C57Bl/6)(F_1) hybrid mice aged from 15 to 22 months. The DNA content in the isolated tumor cells and the localization of AFP on sections were studied in 26 tumors, 0.4-2 cm in diameter, from 21 mice. Each tumor usually was divided into three parts: one part for histological investigation, one for DNA assay, and one for detection of AFP. The serum AFP level of the animals also was determined [4].

To prepare films of isolated cells by the alkaline dissociation method tissue sections 12-mm thick were taken passing through the center of the fragment. From eight large hepatomas, two samples of tumor tissue, taken from each part of the fragment, were used to prepare film preparations. The DNA-fuchsin content was determined separately in cells of the tumor and surrounding liver tissue, on a Vickers M-86 scanning integrating microdensitometer (100-150 nuclei per specimen) and the number of binuclear cells was counted in films stained by the Feulgen method after counterstaining with azure-eosin, on examination of 500-1000 cells.

EXPERIMENTAL RESULTS

With respect to their morphological criteria all the spontaneous mouse liver tumors investigated were classified as hepatocellular carcinomas, differing in their degree of differentiation. Usually the smaller tumor nodules (from 0.4 to 0.9 cm in diameter) had a homogeneous structure and were highly differentiated; less frequently they were moderately differentiated hepatocarcinomas with a solid or trabecular structure. The large tumors (from 1 to 2 cm in diameter) were distinguished by marked polymorphism of the structure of the individual nodule and they consisted of regions that were heterogeneous as regards their level of differentiation. Analysis of the DNA content in hepatocyte nuclei isolated from tumors homogeneous in their morphology (Table 1) showed that highly differentiated hepatocarcinomas

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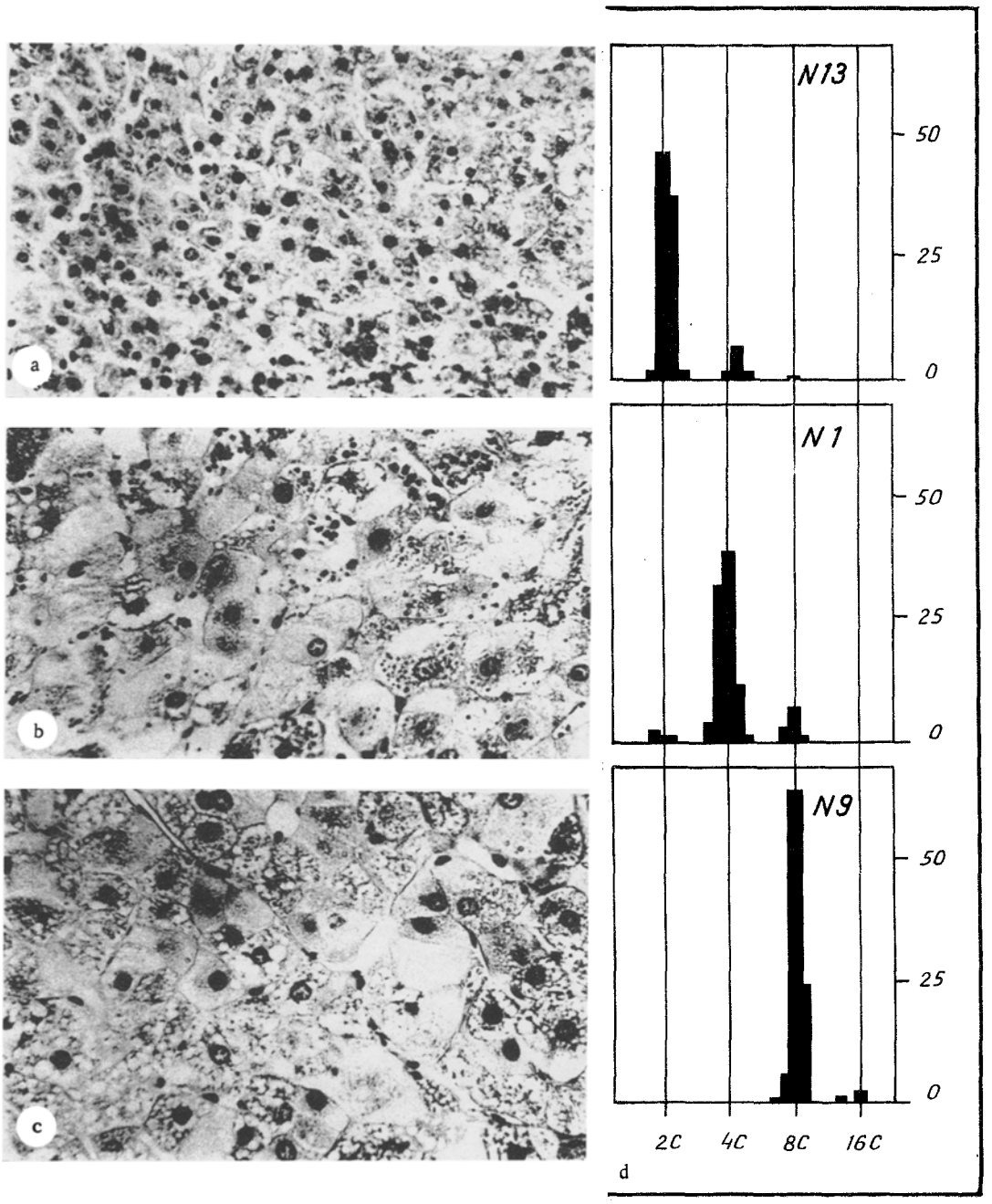

Fig. 1. Histograms of DNA content (d) and microscopic structure of three spontaneous liver tumors in F_1 hybrid mice (a-c). Abscissa, DNA content (in ploidy units); ordinate, number of nuclei (in %). No. 13) Moderately differentiated hepatocarcinoma with predominance of 2C nuclei; No. 1) highly differentiated hepatocarcinoma with predominance of 4C nuclei; No. 9) highly differentiated hepatocarcinoma with predominance of 8C nuclei; Stained with hematoxylin and eosin. 400 ×.

are characterized by a polymodal distribution of DNA content, typical for the liver, with marked predominance of tetraploid (six cases) or octaploid (three cases) nuclei. In the group of moderately differentiated or undifferentiated carcinomas there was a marked increase in the number of diploid hepatocytes) in tumors Nos. 13-15, for example, they were the overwhelming majority. Examples of histograms of DNA content in tumors differing in morphology are given in Fig. 1. In larger, morphologically heterogeneous, spontaneous carcinomas polymodal distributions of DNA content were found more frequently, with the principal peak in the region of tetraploid values of DNA, and with some increase in the proportion of diploid nuclei (Table 1). The frequency of occurrence of binuclear cells was sharply reduced in all the tumors — 6.5 ± 1.2% compared with 33.1 ± 2.6% in the surrounding parenchyma.

TABLE 1. Composition of Nuclei by Ploidy and AFP Content in Spontaneous Hepatocarcinomas of F_1 Mice with Different Degrees of Differentiation

Morphology of tumor	No. of tumor	Nuclei with determined ploidy, %				AFP content in tumors
		2C	4C	8C	16C	
Highly differentiated carcinomas	1	3,7	85,2	11,1	—	Single cells
	2	4,5	86,5	9,0	—	Not determined
	3	2,5	73,3	20,7	3,5	Not determined
	4	1,8	60,7	36,6	0,9	Not determined
	5	2,4	89,0	7,9	0,7	No cells found
	6	—	84,9	14,1	1,0	Single cells
	7	1,3	43,4	45,9	9,4	Not determined
	8	3,5	11,3	84,3	0,9	Single cells
	9	—	—	95,5	4,5	Single cells
Moderately differentiated carcinomas	10	—	94,9	5,1	—	No cells found
	11	0,4	78,0	21,4	0,2	No cells found
	12	41,6	45,3	12,4	0,7	Single cells
	13	85,5	14,1	0,4	—	Single cells
Undifferentiated carcinomas	14	82,6	17,4	—	—	No cells found
	15	88,0	11,1	0,9	—	Many cells
Mixed carcinomas with highly and moderately differentiated and undifferentiated regions	16	21,0	77,5	1,5	—	Single cells
	17	15,1	79,4	5,5	—	Single cells
	18	49,4	40,7	9,9	—	Single cells
	19	18,6	80,1	1,3	—	Many cells
	20	1,4	94,4	4,2	—	Single cells
	21	0,9	95,5	3,6	—	Single cells
	22	0,4	95,1	4,5	—	Single cells
	23	1,5	84,0	13,5	1,0	Many cells
	24	3,8	91,5	4,8	—	Single cells
	25	1,7	90,0	6,7	1,6	Single cells
	26	—	92,6	6,6	0,8	Single cells

When the DNA content was compared in cells isolated from different regions of the same carcinoma (eight cases) the character of the histograms was uniform in type. The similarity of distributions of DNA content in two specimens from the same tumor means that the histograms can be used to judge the averaged characteristics of the tumor as regards DNA content, and that they do not reflect regional differences in its structure. Although on microscopic investigation some of these tumors had considerable polymorphism of structure of the nodule, as reflected in a combination of morphological variants of hepatocellular carcinomas with different degrees of differentiation, it was not reflected in the type of the histograms, for a sufficiently large volume of tissue was used to prepare preparations of isolated cells. The question of evaluation of the DNA content in the tumor on the basis of single measurements, which may not reflect possible regional variations in the level of ploidy, becomes particularly important when these data are used for diagnostic purposes and for choice of optimal tumor therapy [8].

The coefficient of variation of the DNA content in the modal classes of nuclei averaged 7.5% (max = 10.1) for highly differentiated tumors, 9.6% (max = 13.1) for the group of undifferentiated tumors, and 9.3% (max = 12.1) for tumors heterogeneous with respect to morphology (in the surrounding liver 8.1%). The scatter of the DNA content in the tumor cells compared with normal may, on the one hand, indicate the development of aneuploidy in the course of malignant growth, and on the other hand, it may reflect variations in the intensity of their staining due to heterogeneity in the structural organization of their chromatin [5]. Previously the writers demonstrated the absence of any marked aneuploidy in spontaneous highly differentiated hepatomas of CBA mice [3].

The serum AFP level in nearly all the mice was average and varied from 2 to 20 μg/ml. Many AFP-positive cells were found in only three large tumors, one of which was a small-cell undifferentiated carcinoma with modal DNA content in the 2C region, whereas the other two were extremely heterogeneous hepatocarbinomas as regards their morphology, with predominance of nuclei with a tetraploid DNA content (Table 1). Besides ordinary hepatocytes, in such tumors AFP was present also in small cells which, in their morphology, resembled embryonic hepatocytes. In the remaining tumors AFP either was absent altogether or was present in single cells (Table 1).

This comparative study of spontaneous hepatomas in F_1 mice showed that the level of cell ploidy and the intensity of AFP production may vary sharply in tumors which are similar in

morphology and degree of morphological differentiation. Previously the writers found high degrees of development of polyploidy in spontaneous benign neoplasms of the liver in mice of one of the parental lines (CBA) and absence of correlation of this feature with AFP production [3]. However, the intensity of AFP synthesis in moderately differentiated and undifferentiated spontaneous hepatomas in CBA mice was much higher [4]. Induced hepatomas also showed a broad spectrum of level of AFP production [11]. The mechanisms of irregular reexpression of AFP in certain hepatomas and the role of this phenomenon are not clear, despite intensive study of the molecular, cellular, and tissue principles of its synthesis [6, 10].

If relations between the DNA content and morphology of spontaneous hepatomas in mice of the two lines studied are analyzed, a tendency will be noted for the proportion of diploid nuclei to rise with a decrease in the degree of histological differentiation, whereas in adenomas and highly differentiated carcinomas the ability, characteristic of histogenesis of the liver, to undergo intensive cellular polyploidization is more often preserved. It has also been shown that the mechanisms of regulation of AFP synthesis in highly differentiated hepatomas are similar to those acting in the normal liver [5]. These data indicate the greater phenotypic similarity of morphologically highly differentiated tumors with the normal hepatocyte population with respect to a number of differential features, in close harmony with the view that carcinogenesis is blocked ontogeny [9]. The appearance of polyploid cells with a euploid DNA content in spontaneous mouse hepatomas can most probably be regarded as an indicator of hepatocellular differentiation, and not a sign of malignant growth, although the DNA content and the degree of its variation in induced hepatomas may increase considerably during development of the tumor [7].

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