DNA CONTENT IN SPONTANEOUS HEPATOMA CELLS OF CBA MICE

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Some strains of mice are characterized by spontaneous development of hepatocellular tumors [6, 9, 12, 15]. These tumors characteristically have a high frequency of appearance, similarity of morphological structure to human hepatomas, and ability to metastasize and to induce synthesis of the embryospecific protein α-fetoprotein (α-FP). Unlike tumors of the liver induced by chemical carcinogens, spontaneous hepatomas have received little study. Information in the literature is mainly concerned with immunohistochemical characteristics, and there is virtually no information on the cytogenetic properties of spontaneous hepatomas. Determination of the level of polyploidization of spontaneous hepatoma cells is interesting because polyploidization of hepatocytes is characteristic of normal differentiation of the mouse liver [2].

In the investigation described below the composition of cell populations of spontaneous hepatomas with respect to DNA content in single cells was studied and compared with the size of the tumor and α-FP production.

EXPERIMENTAL METHOD

Experiments were carried out 20 male CBA mice aged from 18 to 22 months. In mice of this strain spontaneous hepatomas develop in 100% of cases after 19 months [6]. Animals in which the diagnosis of hepatoma was made during preliminary clinical examination were sacrificed and autopsied. On examination of the liver, whitish yellow nodules from 1 to 15 mm in diameter were found in all its lobes. The number of nodules varied in individual animals from 3 to 20 or more (the whole liver consisted of multiple confluent nodules of different sizes).

To analyze the DNA content, 15 small nodules 1–3 mm in diameter, 11 nodules 4–6 mm in diameter, and 11 large nodules 7–10 mm in diameter, free from necrosis and hemorrhage, were selected. The whole small nodules, and medium-sized and large nodules cut into halves, together with the adjacent parenchyma, were fixed in 10% neutral formalin. After fixation film preparations of isolated cells were made by the alkaline dissociation method [1] separately from the tumors and surrounding liver. The preparations were stained by Feulgen's method, by hydrolysis in 5 N HCl for 10 min at 37°C, and treatment with Schiff's reagent for 1 h at room temperature. The content of DNA-fuchsine in the tumor and liver cells was measured on a Vickers M-86 scanning integrating microdensitometer (100–150 cells in each case). Mitotic activity in the liver and tumors was determined in films of isolated cells, by examination of 1000–3000 hepatocytes. In parallel experiments the other halves of the nodules were fixed in a mixture of formalin, acetone, and phosphate buffer, pH 6.1 [3], and embedded in Histoplast. Sections 3-4 μ thick were stained with hematoxylin and eosin and investigated histologically. The serum α-FP level of the animals were determined by double immunodiffusion in gel with a standard test system [4]. Furthermore, in four nodules measuring from 3 to 7 mm in three animals the distribution of α-FP in the sections was studied by the direct immunoperoxidase method [13], using monospecific rabbit antibodies and fragments of donkey antibodies against rabbit γ-globulin, labeled with horseradish peroxidase.

EXPERIMENTAL RESULTS

In their histological structure the tumors studied could be classed as highly differentiated hepatomas. Usually the tumor nodule was surrounded by a zone of compressed liver parenchyma. Tumor cells resembled

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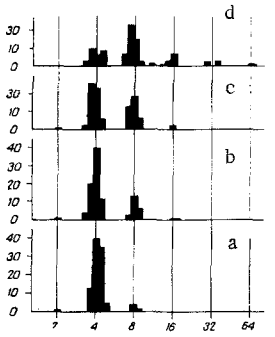

Fig. 1

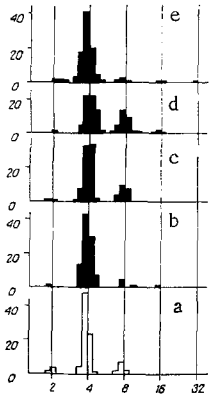

Fig. 2

Fig. 1. Examples of distribution of DNA content in hepatocyte nuclei in spontaneous, highly differentiated mouse hepatomas. a-d) Types of tumors. Here and in Fig. 2: abscissa, DNA content (in ploidy units); ordinate, number of cells (in percent).

Fig. 2. Distribution of DNA content in hepatocyte nuclei in liver (a) and four spontaneous hepatomas (b-e) in the same mouse.

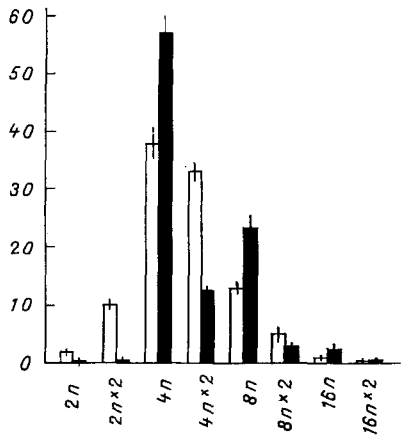

Fig. 3. Comparison of cell composition of liver (unshaded columns) and hepatomas (black columns) by ploidy and number of nuclei. Abscissa, types of cells; ordinate, frequency of occurrence of cells of the given type (in percent).

hepatocytes in their morphology, but they were much larger, highly vacuolated, contained traces of fatty degeneration, and they were virtually identical in size. Often foci of necrosis and hemorrhages were found in the tumors. The mitotic index in the tumors as a rule was an order of magnitude higher than in the surrounding liver, and averaged 0.14 ± 0.6% with variation from 0 to 9.7%. α-FP was found in the blood serum in fairly high concentrations in three mice (titer 1 : 8–1 : 16), and in trace amounts in six mice. α-FP was not detected in the serum of 11 mice. In the sections α-FP was usually contained by single cells of the tumor nodule and, in addition, by single hepatocytes of the surrounding parenchyma.

The distribution of cells by DNA content was studied in 37 hepatomas – 1–4 tumors from 20 mice. In every case the type of distribution of DNA was similar – polymodal, with predominance of the tetraploid class –

and it was independent of the ability of the tumor to produce α-FP. No signs of marked aneuploidy, such as are characteristic of induced hepatocarcinogenesis [5], were present. Despite similarity in principle of the distributions of the cells by DNA content, the tumors nevertheless differed in the ratio between classes of ploidy. According to the degree of development of polyploidy in the tumor cells, all the hepatomas could be subdivided conventionally into three groups (Fig. 1). The first group, including 14 hepatomas, was characterized by minimal values of mean ploidy of the nucleus ($p_n = 4.5 \pm 0.09$) and cell ($p_c = 5.04 \pm 0.11$) (Fig. 1a). The second group consisted of eight hepatomas with $p_n = 5.10 \pm 0.13$ and $p_c = 5.9 \pm 0.15$ (Fig. 1b), and the third group consisted of 14 hepatomas with $p_n = 6.0 \pm 0.2$ and $p_c = 7.24 \pm 0.19$ (Fig. 1c). Mean values of ploidy of the nucleus and cell in the surrounding liver tissue were 4.45 ± 0.13 and 6.34 ± 0.70 respectively. The level of cell polyploidization varied not only in hepatomas from different animals, but also in different hepatomas from the same animal. Sometimes hepatomas which belonged to three different groups by the degree of development of polyploidy could be found in the liver of one mouse (Fig. 2). An extremely high degree of polyploidy ($p_n = 10.1$ and $p_c = 12.0$), much higher than the typical mean values for the liver and for another hepatoma in the same animal ($p_n = 5.0$, $p_c = 5.7$) was observed in one case (Fig. 1d). Comparison of the type of histograms with the size of the tumor showed a certain tendency toward positive correlation between the level of cell polyploidization and the size of the tumor.

Among hepatomas of the first group most were small, whereas among hepatomas of the third group, most were larger, although in each group the sizes of the tumors varied greatly.

Comparison of the cell composition of the tumors and liver by ploidy and relative numbers of mononuclear and binuclear cells showed no significant qualitative differences between them (Figs. 2 and 3). The cell series of most highly differentiated hepatomas began with the diploid hepatocyte, although their number was an order of magnitude less than in the surrounding parenchyma. In eight nodules from 5 to 10 mm in diameter, i.e., medium-sized and large, not a single diploid cell was found despite examination of at least 3000 tumor cells in each case. Complete exhaustion of diploid cells in certain tumors may be the result of intensive cell proliferation, in the course of which they underwent polyploidization. Mitotic polyploidization of hepatocytes is a characteristic property of liver cells [2]. The tetraploid level of DNA content, to which most tumor cells are converted, is evidently optimal for tumor growth. On the whole the population of tumor cells differed from that of cells of the parenchyma in the higher level of ploidy of DNA content, accompanied by a reduction on average of two-thirds in the number of binuclear cells ($15.6 \pm 1.1\%$ compared with $45.5 \pm 2.8\%$ in the surrounding liver).

Highly differentiated spontaneous hepatomas studied in this investigation are the commonest liver tumors found. Among spontaneous hepatocellular neoplasms in CBA mice, moreover, poorly differentiated hepatomas consisting of small cells with intensive α-FP production are found [6]. Cases of virtually diploid hepatomas, as well as tumors with high ploidy, the mean level of polyploidization of which exceeds that typical of normal liver [10], have been described in man. Most studies involving karyotype and photometric analysis have been devoted to induced hepatomas [5, 7, 8, 11, 14]. In these cases also, both predominantly diploid tumors and also tumors heterogeneous with respect to their DNA content appear, with a modal class in the region of near-triploid and near-tetraploid values or higher. A characteristic feature of induced hepatocarcinogenesis is chromosomal instability and the development of aneuploidy.

Spontaneous and induced hepatomas of experimental animals and human hepatomas are thus distinguished by considerable variability of their DNA content.

This investigation showed that highly differentiated hepatomas are heterogeneous cell populations with a high level of development of polyploidy. Polyploidization and a decrease in the number of diploid cells are observed in the postnatal period of growth of the liver. It can be tentatively suggested that a high degree of cellular polyploidization in hepatomas is a feature of normal hepatocellular differentiation.

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RATIO BETWEEN PROLIFERATING AND QUIESCENT SPLEEN CELL POPULATIONS DURING DEVELOPMENT OF RAUSCHER LEUKEMIA AND AFTER LOADING OF MONONUCLEAR PHAGOCYTES WITH COLLOIDAL GOLD

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The ratio between the pools of proliferating and quiescent cells is one of the most important critical factors determining the sensitivity of normal and tumor tissues to most cytostatic agents [2]. Correlation between these parameters and the sensitivity of target cells to killer cells, which is evidently connected with modulation of antigens and cell membrane receptors [10, 11], likewise is becoming increasingly evident.

The writers demonstrated previously that a temporary change in functional activity of the mononuclear phagocyte system (MPS) caused by parenteral injection of particles of inert colloids leads to modification of the toxic and antitumor effects of cytostatics: methotrexate, ftorafur,† sarcolysin [3, 9]. In particular, injection of colloidal gold or carbon particles potentiated the antitumor effect of methotrexate on developing Rauscher leukemia. The mechanism of this effect is not clear. It may perhaps be explained by changes in proliferation of the tumor cells connected with disturbance of MPS function. Macrophages are known to be secretory cells, and among the biologically active compounds which they secrete, the so-called monokines, there are factors which affect proliferation of other cells, including hematopoietic cells [7].

To test this hypothesis experiments were carried out, the results of which are described below. In most experiments a technique of nucleoprotein-celite chromatography (NPC chromatography) was used to analyze the ratio between pools of proliferating and quiescent cells; this method enables the proliferative status of the cell population to be assessed [5, 6]. DNA in proliferating cells is very firmly bound with proteins and a high concentration of LiCl and urea (4 M and 8 M respectively) and heating to 96°C are necessary for its elution. When the cells change to the resting state, 1.5 M LiCl and 3M urea at 4°C are sufficient to dissociate the DNA-protein complex [8].

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