DISTRIBUTION OF CARCINOGEN-BINDING PROTEIN IN PRIMARY TUMORS OF THE RAT LIVER INDUCED BY 3'-METHYL-4-DIMETHYLAMINOAZOBENZENE

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Nineteen primary tumors of the liver were investigated by an immunomorphological method using monospecific antibodies against azocarcinogen-binding protein (CBP). The content of this protein was shown to be directly related to the degree of cytotypical differentiation of the tumor cells. CBP was absent in poorly differentiated hepatomas, anaplastic carcinomas, and most adenocarcinomas. This protein was found in highly differentiated hepatomas, in which it was uniformly distributed throughout the cell population.

One stage in the investigation of the role of carcinogen-binding protein (CBP) in malignant growth is the study of its distribution in the cells of normal liver and of tumors developing in that organ.

The writers showed previously that normal liver cells are heterogeneous in their CBP content. Cells rich in CBP are localized in the central parts of the lobules, while cells not containing this protein have a periportal location.

The object of the present investigation was to study the CBP content in primary tumors of the liver by an immunomorphological method.

EXPERIMENTAL METHOD

Male Wistar rats weighing 90-120 g were used in the experiments. For induction of the tumors the animals were kept on the diet suggested by Miller et al. [4], with certain modifications. The composition of the diet (per kg) was as follows: casein 120 g, glucose 290 g, cornstarch 500 g, vegetable oil 80 g, salt 40 g [2], riboflavin 0.001 g, pyridoxine hydrochloride 0.0025 g, thiamine hydrochloride 0.003 g, calcium pantothenate 0.007 g, choline chloride 0.3 g, 3'-methyl-4-dimethylaminoazobenzene 0.6 g.

Multiple tumors of the liver in rats kept on the diet for 4.5 months were investigated.

Pieces of the tumors measuring $3 \times 4 \times 5$ mm from exsanguinated animals were fixed in a mixture of glacial acetic acid and absolute ethanol (1 :100) for 24 h 4°C and embedded in paraffin wax as described previously [2].

Serial sections were then cut to a thickness of 2-3 μ.

The localization of the CBP in the sections was determined by the indirect Coons' method [4]. Monospecific antibodies against CBP were isolated from the serum of rabbits immunized with a preparation of this protein [1]. Ass serum against rabbit γ-globulins, labeled with fluorescein isothiocyanate, prepared at the N. F. Gamaleya Institute of Epidemiology and Microbiology, was used as the fluorescent antibodies. Nonspecific fluorescence was removed by adsorption of the serum with mouse-liver powder.

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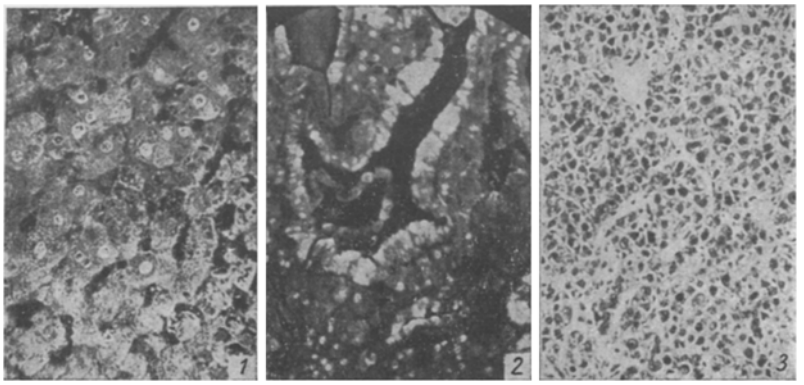

Fig. 1. CBP in sections of highly differentiated hepatoma. Incubation with antibodies against CBP, 60×.

Fig. 2. CBP in section of mixed tumor. Glandular structure lined with high cylindrical epithelium, 30×.

Fig. 3. Section of poorly differentiated hepatoma. CBP absent. Hematoxylin-eosin, 60×.

The dewaxed sections were incubated first with antibodies against CBP and then with labeled serum [2]. The specificity of immunological fluorescence was verified in sections: 1) not treated with antibodies; 2) incubated with labeled serum only; 3) incubated with nonimmune rabbit serum; 4) incubated with antibodies against CBP neutralized with a pure preparation of this protein. The monospecificity of the antibodies was additionally verified by incubating them with sections of the spleen, which were then treated with labeled serum [1].

In each experiment 7-8 serial sections of the tumor were tested, the third or fourth being stained with hematoxylin and eosin.

The sections were examined in the ML-2 luminescence microscope in blue-violet light and with phase contrast; they were photographed on RF-3 film. Sections stained with hematoxylin-eosin were examined in the light microscope. Altogether 19 tumors were investigated.

EXPERIMENTAL RESULTS AND DISCUSSION

By their morphological structure the tumors investigated were distributed as follows.

1. Highly differentiated trabecular hepatomas (4).
2. Mixed tumors (13). In these tumors the structures of an adenocarcinoma were identified along with foci of a poorly differentiated hepatoma and an anaplastic carcinoma. Areas of highly differentiated hepatoma were found in individual tumors.
3. Anaplastic carcinomas (2).

Investigation of the sections of the tumors by the immunofluorescence method showed that practically all the cells of the highly differentiated hepatomas exhibited fluorescence in the nucleus and cytoplasm. This fluorescence was due to the presence of CBP in the cells, for fluorescence was absent in all the controls used (Fig. 1).

In the sections of the highly differentiated hepatomas the intensity of fluorescence was independent of the distance from the blood vessels, as is characteristic of the normal liver [2].

The content of CBP in the cells of the mixed tumors was nonhomogeneous. Intensive focal fluorescence was observed in the sections of two tumors with the structure of an adenocarcinoma and hepatoma. The glandular structures of these tumors were lined both with cubical and with high cylindrical epithelium with swollen oxyphilic cytoplasm. The cells of the solid and trabecular areas preserved some similarity with hepatocytes (polygonal shape, round nucleus, and oxyphilic cytoplasm). Intensive fluorescence was found in a large proportion of the glandular structures (Fig. 2a) and in individual areas with a solid and

trabecular structure. Under these circumstances many nonfluorescent nuclei were observed in the glandular epithelium and the hepatocyte-like cells against the background of a fluorescent cytoplasm. The dark nonfluorescent zones in these sections were sometimes indistinguishable morphologically from the fluorescent (Fig. 26).*

In the sections of five mixed tumors fluorescent areas were found only as individual foci. These were small groups of polygonal cells of polygonal shape with oxyphilic cytoplasm or epithelium of single gland-like structures. Over the greater part of the sections structures characteristic of dedifferentiated tumors were predominant (Fig. 3). Fluorescence was absent in these structures. Fluorescence also was completely absent in the sections of six mixed tumors and of the two anaplastic carcinomas.

Characteristic processes of structural reorganization, cirrhosis, and cholangiofibrosis were thus observed in the liver tissue surrounding the tumor nodules. The liver cells of the cirrhotic areas always exhibited intense fluorescence which, as a rule, was independent on the distance of the cells from the vessels. No fluorescence was found in the areas of cholangiofibrosis, either in the glandular epithelium of the bile ducts or in the connective tissue. Fluorescence was always absent in the small rudimentary tumors consisting of atypical basophilic cells.

The immunomorphological study of the distribution of CBP in primary tumors of the liver induced by 3'-Me-DAB thus showed that its content is directly connected with the degree of cytotypical differentiation of the tumor cells. This protein was found in tumors which contained cells preserving their similarity with hepatocytes and, therefore, regarded as highly differentiated. A decrease in size of the cells, basophilia of the cytoplasm, a change in shape, and an increase in atypism and morphological dedifferentiation, characteristic of poorly differentiated hepatomas, anaplastic carcinomas, and most adenocarcinomas, were coupled with absence of CBP in the tumor cells.

The relationship thus discovered may signify that the CBP disappears with progression of the tumor. The fact that morphologically identical zones of two mixed tumors differed in the character of their fluorescence could be evidence in support of this view. On the other hand, the possibility cannot be ruled out that the ancestral cells of the highly differentiated and poorly differentiated tumors are hepatocytes located in different parts of the hepatic lobule. Possibly, therefore, the poorly differentiated tumors arise from normal hepatocytes not containing CBP, while the highly differentiated tumors, consisting entirely of fluorescent cells, arise from normal hepatocytes containing this protein.†

LITERATURE CITED

1. G. A. Bannikov and T. A. Chipysheva, Byull. Éksperim. Biol. i Med., No. 6, 77 (1972).
2. G. A. Bannikov and T. A. Chipysheva, Byull. Éksperim. Biol. i Med., No. 2 (1973).
3. J. H. Jones and C. Foster, J. Nutr., 24, 245 (1942).
4. E. C. Miller, J. A. Miller, B. E. Kline, et al., J. Exp. Med., 88, 89 (1948).
5. T. H. Weler and A. H. Coons, Proc. Soc. Exp. Biol. (New York), 86, 789 (1954).

*As in Russian original. There is no "a" or "b" in Fig. 2 – Consultants Bureau.

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