DISTRIBUTION OF α-FETOPROTEIN AND ALBUMIN IN PARAFFIN SECTIONS OF ZAJDELA'S ASCITES HEPATOMA CELLS

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The localization of α-fetoprotein (αFP) and albumin in the cells of a Zajdela's ascites hepatoma of rats was studied. Examination of paraffin sections of cells fixed with a mixture of acetic acid (1%) and 96° (99%) ethanol showed that 9.3% of hepatoma cells contain αFP and 0.6% contain serum albumin; a few cells contain both proteins simultaneously. Cells containing αFP or albumin are distributed in the islets of tumor cells without any regular pattern.

KEY WORDS: Zajdela's ascites hepatoma; α-fetoprotein; albumin.

Investigations have shown that α-fetoprotein (αFP) is synthesized during embryogenesis by cells of the liver [1, 3] and yolk sac [14, 15]. In the adult αFP is synthesized by cells of the regenerating liver [3], hepatoma, and teratocarcinoma [4, 12, 13, 18]. The problem of whether αFP is produced by all cells of the normal organs or tumor or by only a certain number of cells specialized for the synthesis of this protein has not yet been solved [2]. Attempts have been made to answer this question in the past. An investigation of mouse liver cells in the postnatal period showed that they differ sharply in their αFP content [10]. αFP also was found in only some of the tumor cells in a study of human and mouse hepatomas [12]. However, the problem requires further more careful study.

Zajdela's transplantable ascites hepatoma is a good producer of αFP. In tissue culture the cells of Zajdela's hepatoma synthesize αFP [9]. However, the tissue culture method does not give the answer to the question whether all cells or only some of them synthesize this protein.

EXPERIMENTAL METHOD

Zajdela's ascites hepatoma was transplanted into Wistar rats. The animals were killed on the 5th-7th day after transplantation of the tumor. The ascites fluid obtained was immediately centrifuged for 2 min at 300-500 g, the supernatant was discarded, and the residue (ascites cells) was washed in cold Hank's solution during centrifugation under the same conditions 5 times. Unless the preparations were made from cells washed to remove ascites fluid, they were unsuitable. Numerous serum antigens were found on the surface of all the cells and this complicated the analysis of the intracellular distribution of the antigens.

After vital staining with trypan blue 0.2-0.3% of dead cells were found in the suspension of washed cells. The washed cells were fixed for 1 h at 4°C in a mixture of 96° ethanol (99%) and glacial acetic acid (1%) and for 30 min in a second batch of the same fixative [16]. The subsequent procedure of taking the cells through alcohols and xylols followed the routine laboratory technique [11], but with low-speed centrifugation, as described above, and the cells were kept for 5 min in each portion of alcohol and xylol. The cells quickly settled to the bottom. After 30-60 min the paraffin was removed and replaced by a second portion. The cells were shaken. This change of paraffin was repeated twice further, after which a mixture of paraffin

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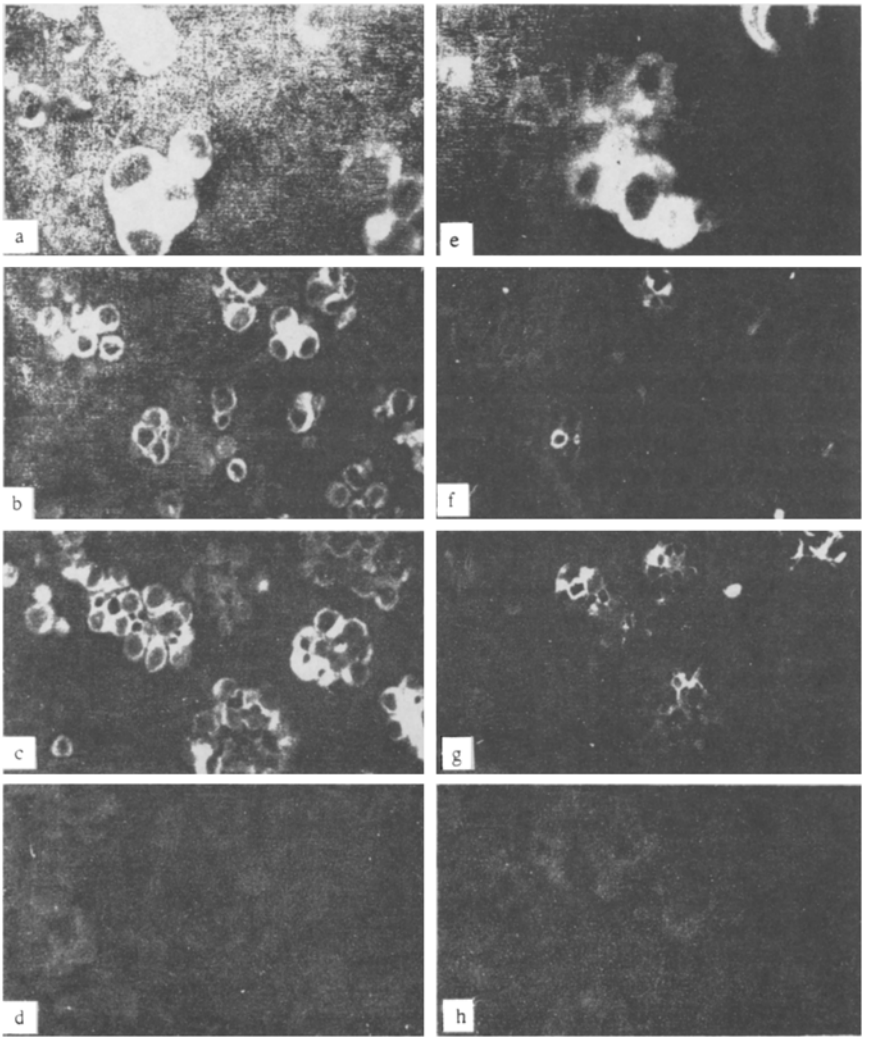

Fig. 1. Distribution of αFP, albumin, and γ-globulin in cells of Zajdela's ascites hepatoma: a, b, c) treatment of sections with antibodies against αFP, d) with antigens against αFP, neutralized by pure αFP; e, f, g) against albumin, h) against γ-globulin; a, e) 270 ×; b, c, d, f, g, h) 120 ×.

and wax was added. The cells were kept at 56°C for 30 min, then at room temperature until the paraffin solidified, after which they were transferred to a refrigerator. Serial paraffin sections 3μ in thickness were cut on a Reichert microtome.

The presence of αFP in the ascites fluid and serum of the rats was determined by the agar precipitation test [5] using a test system for rat αFP [7].

Rabbit antibodies (AB) against rat αFP obtained by the method described in [8] were used; rabbit AB against rat αFP were exhausted with a pure preparation of αFP obtained by a method also described previously [6]; rabbit monospecific serum (AS) against rat albumin was obtained by immunization of rabbits as described in [7] with a pure preparation of albumin isolated by electrophoresis in polyacrylamide gel; the AS was used in immunofluorescence analysis in a dilution of 1:10; finally, rabbit AB against mouse γ-globulin, giving a cross reaction with rat γ-globulin, were used.

Antigens were determined by the indirect immunofluorescence method [17, 20], using monospecific sheep AB against rabbit γ-globulin, labeled with fluoresence isothiocyanate [11]. Inhibition of fluorescence on neutralization of AB against rat αFP and albumin respectively by pure preparations of αFP and albumin and the absence of rat γ-globulin in the cytoplasm of the ascites cells were used as the specificity of fluorescence control. The localization of αFP and albumin was determined in neighboring serial sections. The preparations were examined in the ML-2 microscope and photographed on RF-3 film. After examination, the dewaxed sections were counterstained with hematoxylin-eosin for morphological analysis.

EXPERIMENTAL RESULTS

Zajdela's ascites hepatoma, strain C, consists of single cells and spherical islets of cells, each containing from 3 to several dozen cells (Fig. 1). The cells were of different sizes and were round or oval with a large nuleus. As a rule the nuclei contained one nucleolus. Analysis of the ascites fluid and blood serum from an animal inoculated with Zajdela's hepatoma in the agar precipitation test showed that they contained αFP on the 6th day of growth in identical titers of 1:8-1:16. The specificity of fluorescence of the hepatoma cells containing αFP and albumin was confirmed by the two controls. First, neutralization of rabbit AB against the test proteins by pure preparation of these proteins completely inhibited fluorescence of the cytoplasm of the cells (Fig. 1d). Second, hepatoma cells containing αFP, albumins, or both these proteins were never stained by treatment with antibodies against γ-globulin (Fig. 1h).

Examination of 10,631 cells in 5 experiments showed that αFP is contained by 9.3% of single cells or cells composing islets of ascites hepatoma. The localization of αFP in the cytoplasm of the cells was homogeneous or punctuate (Fig. 1a, b, c). Punctate fluorescence was observed nearer to the nuclear membrane. In some cases αFP was located in one strip of the cell (Fig. 1c), either externally or internally to the other cells of the islet; this was evidently the result of displacement of the nucleus. Such a distribution of αFP was characteristic of cells forming a large islet. The intensity and character of fluorescence of the cells stained with antibodies against αFP were independent of their size, shape, or position in the islet. The most widely different combinations were observed. Some of the islets consisted completely of cells containing αFP, but other islets were found which had only one or two such cells or more. No regular pattern could be observed in the αFP content of the cells depending on the size of the islet. Most islets (67%) contained no such cells.

Only exceptionally rarely (0.6% of cases) was albumin present in the cytoplasm of the ascites cells (Fig. 1e). Fluorescence of albumin was observed mainly in large islets and it was distributed in the space between the cells as distinct and bright lines (Fig. 1f, g). Such fluorescence was not considered to be specific. Since the albumin concentration was higher than the αFP concentration in the ascites fluid, fluorescence of this sort can be explained conjecturally by inadequate washing of the cells. Sometimes fluorescence was distributed as a network in the islet.

In some cases single cells or whole islets containing both αFP and albumin were observed. However, only in 7 of 10,631 cells were both proteins found simultaneously, and they did not contain γ-globulin.

The comparatively high content of αFP in the blood serum of the rats with transplanted Zajdela's hepatoma could be considered to be connected with a disturbance of the normal function of the liver and the switch of some hepatocytes to αFP synthesis. To test this hypothesis paraffin sections of the liver and ascites cells of one rat were prepared. Staining these sections with antibodies against αFP showed that the cytoplasm of the hepatoma cells contained αFP in the ordinary percentage, whereas no αFP was found in the liver cells. Remembering that γ-globulin was never found in ascites cells containing αFP, the presence of αFP in the hepatoma cells could not be explained by the nonspecific uptake of this proein from the ascites fluid. The pattern of αFP distribution observed in the hepatoma cells and the results of the control tests suggest that the ascites hepatoma cells containing αFP in fact synthesized it. The results show that only some of the hepatoma cells are responsible for the αFP production. Further investigations are required to give the final answer to these questions.

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