ELECTRON-HISTOCHEMICAL INVESTIGATION OF LYSOSOMES IN EXPERIMENTAL HEPATOMAS WITH DIFFERENT DEGREES OF DIFFERENTIATION

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A comparative electron-histochemical investigation of lysosomes in undifferentiated and highly differentiated cancer cells of hepatomas 46 and 22a in C3HA mice showed that the changes were more marked in the lysosomes of the undifferentiated cells of hepatoma 22a. The state of the lysosomes and degree of differentiation of the tumor cells could point to an important role of the lysosomes in the progressive growth of neoplasms.

Various neoplasms may undergo marked changes in their ultrastructure in connection with changes in the degree of differentiation of the tumor cells.

There is now reason to suppose that one mechanism of cell differentiation is linked with the function of lysosomes [4, 6, 8-10, 15, 16].

The study of the lysosomes of tumor cells is accordingly of great interest. Lysosomes were investigated in the cells of malignant tumors differing in their degree of differentiation and in their rate of growth.

EXPERIMENTAL METHOD

The material for this investigation consisted of transplantable hepatomas 22a and 46 of C3HA mice [2] and the liver of healthy C3HA mice. Hepatoma 22a is a rapidly growing strain. It is an undifferentiated solid hepatocellular carcinoma with marked polymorphism of its cells.

The material removed was prefixed for 2 h in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4 at 4°C, and then washed with the same buffer for 12 h at 4°C. Next, sections were cut in a cryostat to a thickness of 40-50 μ and transferred for 40 min to Gomori's incubation medium [11] at 37°C. After incubation, the sections were quickly washed in cacodylate buffer and fixed in 1% osmium tetroxide solution for 30 min at 4°C. The material was then taken through 2.5% aqueous uranyl acetate, alcohols of increasing concentration for dehydration, and acetone and embedded in Epon 812.

Sections taken through the incubation medium without substrate acted as the control. In parallel experiments material was taken for ordinary electron microscopy. Ultrathin sections, 300-500 Å in thickness, were studied in the ÉM-7 electron microscope.

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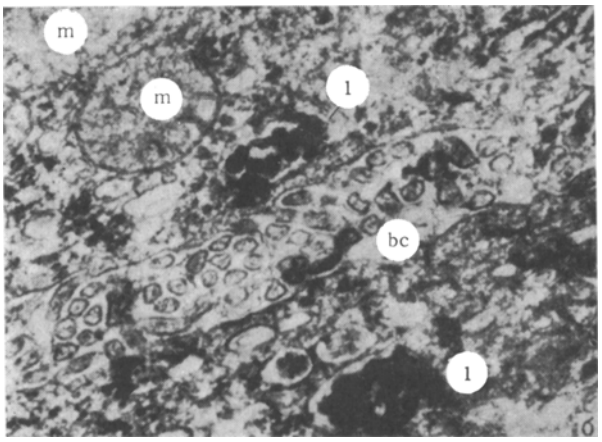

Fig. 1. Liver of C3HA mouse. Reaction for acid phosphatase. Lysosomes with strongly positive reaction for acid phosphatase are clearly visible in the region of a bile capillary. 45,000 ×. Here and in Figs. 2 and 3: n) cell nucleus; ni) nuclear inclusion; m) mitochondrion; l) lysosome; c) cytolysome; bc) bile capillary; mv) microvilli; er) endoplasmic reticulum.

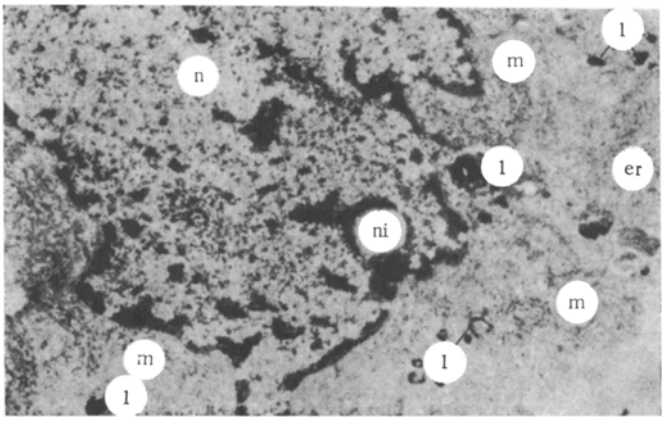

Fig. 2. Hepatoma 46. Reaction for acid phosphatase. Product of the histochemical reaction is clearly visible in lysosomes distributed throughout the cytoplasm. 15,800×.

EXPERIMENTAL RESULTS

The parenchymatous cells of normal liver showed polarity in the distribution of their lysosomes, which were mainly located in the zone of the bile capillaries. The lysosomes were round or slightly oval in shape. The electron-dense homogeneous matrix of the lysosomes was surrounded by a single elementary membrane. The number of lysosomes in the cell varied from one to five.

The lysosomes of normal liver cells of C3HA mice gave a fairly strongly positive reaction for acid phosphatase (Fig. 1).

In the control sections the reaction for acid phosphatase was negative.

In the highly differentiated cancer cells of the hepatoma 46 staining by Gomori's method revealed a varied picture of the lysosome. The main mass of the cancer cells contained more lysosomes than normal hepatocytes, and the arrangement, shape, and size of the lysosomes, differed very considerably (Fig. 2).

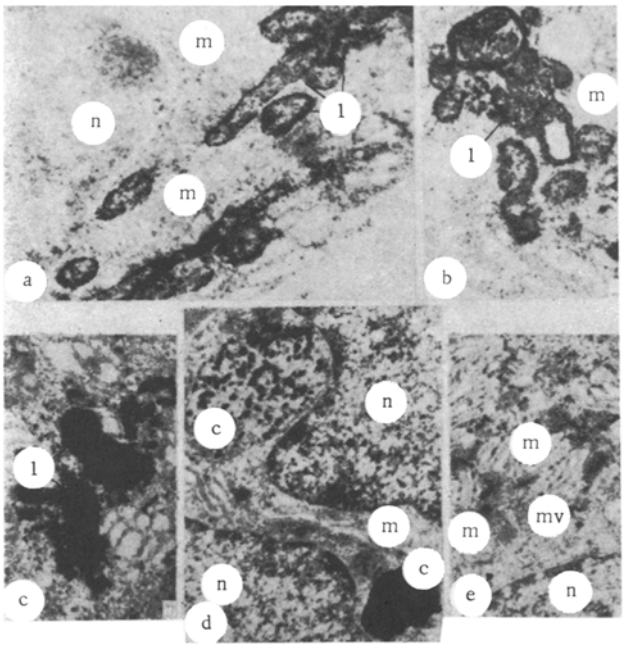

Fig. 3. Hepatoma 22a: a) reaction for acid phosphatase: many large, elongated lysosomes in cytoplasm of cell, product of histochemical reaction revealed particularly intensively beneath lysosome membrane (section without counterstain, 31,500×); b) reaction for acid phosphatase: group of lysosomes join together into a complex (section without counterstain), 30,000×; c) reaction for acid phosphatase: expulsion of product of histochemical reaction from large lysosomes into cytoplasm (30,000×); d) reaction for acid phosphatase, cytolysomes in adjacent tumor cells, varied arrangement of product of histochemical reaction in cytolysomes: continuous in one, in discrete areas in another (11,700×); e) reaction for acid phosphatase: product of histochemical reaction can be observed in microvilli of tumor cells (15,000×).

Large and small, oval and round, horseshoe-and glove-shaped lysosomes were seen. The staining for acid phosphatase in all lysosomes of these cells was comparatively intense. Often the lysosomes contained vacuoles and various cytoplasmic inclusions. Many cells had large lysosomes, arranged in groups at the boundary with the amorphous zone. Individual cells with single lysosomes were seen occasionally.

In sections not reacting for acid phosphatase the lysosomes could not be identified. In control sections incubated without substrate it was also impossible to detect the lysosomes.

In the undifferentiated cancer cells of the hepatoma 22a the histochemical reaction for acid phosphatase revealed numerous lysosomes occupying a large part of the cytoplasm (Fig. 3a); as a rule they were large and oval or, frequently, curiously shaped. Frequently the lysosomes were grouped into complexes (Fig. 3b). Lysosomes and cytolysomes with acid phosphatase liberated outside their membranes were frequently observed in hepatoma 22a cells (Fig. 3c). Sometimes cells with single large cytolysomes in which the acid phosphatase activity was either continuous or confined to discrete areas were found (Fig. 3d). A weak, diffuse reaction for acid phosphatase was observed in the cytoplasm of these cells and also in the microvilli (Fig. 3e). Acid phosphatase activity in the malignant cells of hepatoma 22a is fairly high, and in some lysosomes much higher than in normal hepatocytes.

Lysosomes could not be identified in sections not giving a reaction for acid phosphatase, or in the control sections (without substrate).

Some investigators [3, 5, 12-14, 17] describe an increase in the number of lysosomes and in their acid phosphatase activity in the cancer cells of certain experimental tumors. The results of the present investigation confirm the findings of most of these workers. The electron-histochemical study of the lysosomes also showed that, compared with normal hepatocytes of C3HA mice, in cells of hepatomas 46 and 22a a structural and functional reorganization of the lysosomes has taken place: their number is increased, their shape, size, and arrangement in the cells are changed, and their acid phosphatase activity is increased. In the cells of the undifferentiated hepatoma 22a the changes in the lysosomes were considerable. These changes evidently affected the properties of the lysosomal membranes, as is clear from the abnormal shape of the lysosomes and the liberation of the enzyme directly into the cytoplasm.

It must be emphasized that the tumor cells of hepatoma 46 and, in particular, of hepatoma 22a were heterogeneous with respect to the lysosomes which they contained. This phenomenon may be associated with the existence of different clones, with different stages of development of the tumor cells, and with certain other properties, especially the degree of differentiation of the cells. The evident connection between the state of the lysosomes and the degree of differentiation of the tumor cells may point to an important role of the lysosomes in the development of neoplasma.

It was difficult to identify the lysosomes in the tumor cells of hepatomas 46 and 22a of C3HA mice by means of the ordinary electron microscope, but the lysosomes were clearly visible after the reaction for acid phosphatase. One reason for this phenomenon was probably the nature of the ultrastructural organization of the malignant cells of the hepatomas 46 and 22a, in which the shape and size of the lysosomes were changed and, in addition, the endoplasmic reticulum had undergone considerable reorganization (especially in the hepatoma 22a): fragmentation and the formation of vesicles similar in shape and size to lysosomes but not containing acid phosphatase. Another possibility is that the low electron density of their matrix prevented detection of the lysosomes. Difficulty in finding the lysosomes of hepatoma 46 and 22a cells is also reported by Bresler and co-workers [1, 7]. They concluded that lysosomes are not present in the cells of hepatoma 22a but they are present, although rare, in the cells of hepatoma 46.

The results of the present investigation show that lysosomes can be demonstrated in the cells of hepatomas 46 and 22a only by the use of histochemical markers.

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