HISTOCHEMICAL STUDY OF NUCLEASE ACTIVITY IN CELLS OF TRANSPLANTABLE TUMORS AND THEIR METASTASES

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The distribution and activity of acid and neutral DNases and acid and alkaline RNases were studied by histochemical methods in cells of primary and metastatic tumor nodules of Walker's tumor and carcinoma PA (transplantable carcinoma of the kidney) in rats. Low intracellular nuclease activity was found in both transplantable tumors. While their localization in cells of the metastasize was the same, the activity of the enzymes was lower than in cells of the primary tumors. This is regarded as a sign of their more marked anaplasia.

A feature of progression of malignant tumors is their ability to metastasize. Although metastases mainly reproduce the morphological structure of the primary tumor, they often differ in their more rapid rate of growth and their greater resistance to chemotherapeutic agents; these differences may reflect differences in their cell metabolism, including their nucleic acid metabolism. No comparative investigations of the activity of enzymes involved in nucleic acid metabolism, namely the deoxyribonucleases (DNases) and ribonucleases (RNases), are described in the accessible literature.

The object of this investigation was to make a histochemical study of the distribution and activity of nucleases in the cells of primary tumors and their metastases, with special reference to Walker's tumor and carcinoma PA (transplantable carcinoma of the rat kidney), which differ in their histogenesis and the localization of the primary tumor and the metastases.

EXPERIMENTAL

Walker's tumor was inoculated into ablino rats subcutaneously in the region of the tail [5] and the development of metastases in the lungs was observed in 53% of cases. Carcinoma PA was implanted in the spleen [4] and metastases were found in the liver in 34% of animals.

Pieces of Walker's tumor growing subcutaneously in the tail were taken for study on the 31st-33rd day after transplantation (amputation of the tail) and metastases in the lungs were taken 12-13 days later at autopsy. Pieces of carcinoma PA, which was localized in the spleen, and its metastases in the liver were taken simultaneously on the 20th-21st day after inoculation. The tissue was fixed in cold calcium–formalin mixture for 12 h. In sections cut to a thickness of 10-12 μ on a freezing microtome activity of the following enzymes was studied: acid DNase pH 5.9 by Vorbrodt's method [13], acid RNase pH 5.9 by Vorbrodt's method in the modification of Gluzman and Shlyakhovenko [1], neutral DNase pH 7.2 and alkaline RNase pH 7.5 by the methods of Gluzman and Shlyakhovenko [1]. Control sections were incubated in medium without reaction substrate or with the addition of nuclease inhibitors. The intracellular localization and level of activity of the nucleases were estimated from the position, size, and intensity of staining of granules of lead sulfide, the end product of the histochemical reaction.

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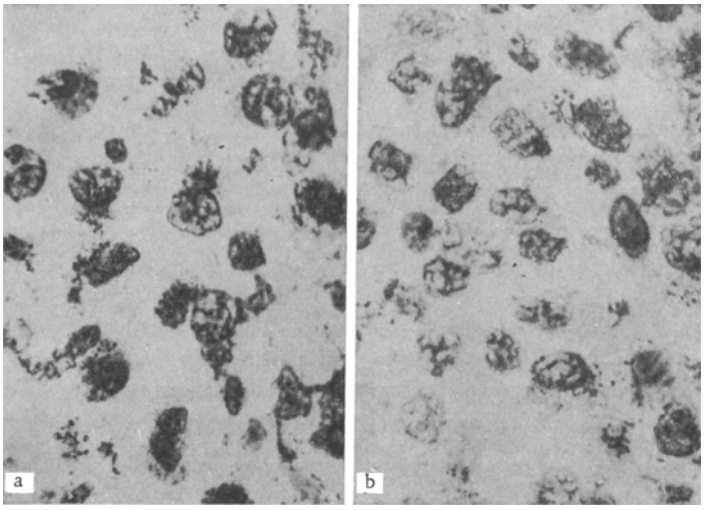

Fig. 1. Acid DNase activity in cells of primary carcinoma PA (a) and its metastasis (b). Vorbrodt's method, 320 ×.

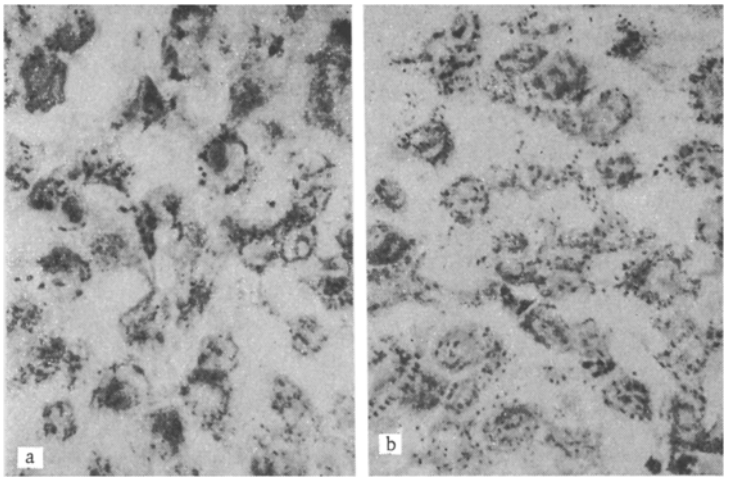

Fig. 2. Alkaline RNase activity in cells of primary carcinoma PA (a) and its metastasis (b). Method of Gluzman and Shlyakhovenko, 320 ×.

EXPERIMENTAL RESULTS

The reaction for acid DNase in the cells of carcinoma PA (Fig. 1) was marked by the presence of numerous small and medium-sized granules, compactly arranged in the perinuclear zone or scattered throughout the cytoplasm. A positive reaction was determined also in the structures of the nucleus: the chromatin, nucleoli, and nuclear membrane. The intracellular distribution of acid DNase in cells of the metastases was the same but the activity of the enzyme was somewhat lower.

Acid DNase activity in the cells of Walker's tumor was revealed as solitary brownish-black, medium-sized granules diffusely scattered in the cytoplasm; it was very weak in the nuclei. A weaker reaction for acid DNase was observed in cells of the metastases.

Neutral DNase was distributed only in the cytoplasm of the cells as small, brown granules, distributed diffusely or more compactly. Neutral DNase activity in cells of metastases of Walker's tumor was lower than in the primary tumor. The differences were less marked in the case of carcinoma PA.

Activity of alkaline and acid RNases in the cells of Walker's tumor and carcinoma PA (Fig. 2) was found mainly in the cytoplasm. In some cases the granules were grouped around the nucleus, and this was

a more characteristic feature of carcinoma PA. RNase activity in the cells of the metastases was less marked than in the cells of the corresponding primary tumors.

Nuclease activity in the cells of Walker's tumor and carcinoma PA detectable histochemically was much lower than in the epithelium of the convoluted tubules of the healthy rat kidney. The results agree with biochemical data indicating a low level of absence of nucleic acid depolymerase activity in experimental and human tumors [8, 9, 11] and also with the results of histochemical investigations both by Vorbrodt's method [2, 7, 14] and by the less-refined film substrate method [10].

Enzyme activity was lower in Walker's tumor than in carcinoma PA, but the histochemical differences between cells of the primary tumors and metastases were more marked. These differences may be explained by differences in the genesis and also the localization of the primary tumors and metastases and by their biological properties.

Judging from the histochemical results, the same pattern of intracellular distribution of nucleases is found in the metastases as in the primary tumors. The lower enzyme activity in cells of the metastases than of the primary tumors possibly provides for increased nucleic acid synthesis and more rapid growth. The biochemical investigations of Wannenmacher et al. [15] showed an inverse correlation between the rate of growth and increase in size of Walker's tumor and its level of RNase activity, whereas other investigators [6, 12] found increased nuclease activity during regression of malignant tumors.

The enzyme-histochemical properties of metastatic tumor cells discovered in this investigation can be regarded as evidence of their further anaplasia by comparison with primary tumor cells. Determination of the intracellular localization and activity of nucleases in tumors, in conjunction with the investigation of other enzyme systems [3], can thus be used to study the histogenesis, character of growth, and criteria of their malignancy.

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