CORRELATION BETWEEN THE ULTRASTRUCTURAL ORGANIZATION OF MAMMARY GLAND CANCER IN MICE AND ABILITY TO METASTASIZE

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A quantitative ultrastructural investigation of mouse mammary gland carcinoma and of its metastases in the lungs was undertaken. The surface area of the membranes of the endoplasmic reticulum and the number of bound ribosomes were found to be greater in tumors not giving rise to metastases than in tumors giving metastases in the experiments. Meanwhile the number of ribosomes combined into polysomes was greater in metastasizing tumors. Comparison of the tumors and their metastases revealed differences in the structure of the mitochondrial apparatus: The surface area of the mitochondrial cristae was greater in metastatic tumors. The results indicate that spreading metastases have a lower level of cell differentiation. Primary and metastatic foci are indistinguishable in their level in cell differentiation.

KEY WORDS: metastasization; morphometry; tumor cells; ultrastructure.

The study of the mechanisms of metastasization of malignant tumors demands investigation the possible connection between the ultrastructure of tumors and their ability to disseminate, and also a comparison of the fine structure of primary tumors and of their metastases. Data in the literature on correlation between the ability of tumors to metastasize and their ultrastructure deal mainly with the character of organization of intercellular contacts and of the microvessels of tumors [2, 4]. These investigations have shown that the process of metastasization is independent of the ultrastructure of tumor cells [4, 13]. A comparative study of tumors and their metastases has shown that the latter mainly repeat the structure of the primary tumors, differing only in their level of differentiation [5, 11]. However, in the investigations cited above the ultrastructure of the tumor cells was judged on the basis of qualitative analysis. Yet the results obtained by quantitative methods of investigation in morphology, that are nowadays widely used, show that morphometric analysis can reveal ultrastructural features that were not taken into consideration in the investigations described above [1, 8].

Consequently it was decided to undertake a comparative morphometric study of the ultrastructure of cells of spontaneous mouse mammary gland tumors and their metastases.

EXPERIMENTAL METHOD

Experiments were carried out on C3H mice aged 13-15 months. Two groups of spontaneous mammary gland tumors, 2 months after their appearance, were chosen for investigation: Group 1 consisted of five tumors with no metastases; group 2 of five tumors with metastases in the lungs. Additionally, five metastatic nodules of tumors of group 2 were investigated.

Material for light microscopy was fixed in 10% formalin solution and embedded in paraffin wax. Sections were stained with hematoxylin and eosin. Ultrathin sections were examined in the IEM 7A electron microscope. In each group 50 fields of the cytoplasm of different cells, chosen at random, were subjected to quantitative analysis. Primary morphometric characteristics were recorded on photographic prints with a final magnification of 27,000 times: the surface area of the mitochondrial cristae (S_V^{cr}); the surface area of the ergastoplasmic membranes (S_V^e); the number of ribosomes attached to one micron of the ergastoplasmic membranes (N_{PL}^e). On the basis of these parameters the coefficients of the level of morphological organization of the principal cell organelles were calculated: for the ergastoplasm

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TABLE 1. Morphometric Characteristics of Mammary Gland Carcinoma and Metastases as Reflected in Results of Electron-Microscopic Investigation (M ± m)

Material	S_v^{cr}	S_v^e	N_{pL}^e	k_f^e	K^e	k^p	k^e/k^p
Nonmetastasizing tumors	0,8±0,05	1.5±0,1	23±0,4	1±0,04	36±4	151±12	0,3±0,02
Metastasizing tumors	0,7±0,05	0,7±0,2	18±0,3	1±0,04	14±3	186±16	0,07±0,01
Metastases	1,1±0,06	0,6±0,1	18±0,2	1±0,05	13±2	191±15	0.07±0,01

(K^e), the polysome system (K^p), and the coefficient of fragmentation of the ergastoplasm (K_f^e). The ratio K^e/K^p also was studied. These coefficients are known to be the most objective indices of the morphological and functional state of cell organelles [8, 9].

EXPERIMENTAL RESULTS.

Histological investigation of the mouse mammary gland tumors showed that all the tumors (those giving and those not giving metastases) were similar in structure and belonged to the group of type A adenocarcinomas according to Dunn's classification [12]. The metastases in the lungs repeated the morphological structure of the primary tumors.

Electron-microscopic investigation of this material revealed the usual pattern of ultrastructural organization of the mouse mammary gland tumors. No differences could be found in fine structure between the groups of tumors studied and their metastases. Meanwhile the results of quantitative ultrastructural analysis revealed significant differences between the two groups of tumors and also between the tumors and their metastases. Comparison of tumors giving metastases and those which did not revealed differences primarily in the ultrastructural organization of the granular endoplasmic reticulum. Observations showed that the area of the membranes of the ergastoplasm in tumors not giving metastases was almost twice that of tumors which metastasized. The number of ribosomes attached to one micron of the ergastoplasmic membranes was significantly greater in the tumors of group 1. The coefficient of fragmentation, reflecting the mean length of the membranes of the endoplasmic reticulum, was the same in both groups. The results are evidence that the level of morphological organization of the ergastoplasm is considerably higher in nonmetastasizing tumors. Significant differences also were found in the organization of the polysome system. In tumors giving metastases, for instance, the number of ribosomes united into polysomes per square micron of cell cytoplasm was greater than in tumors not giving metastases.

Comparison of the results of the morphometric analysis of primary tumors and their metastases on the basis of indices such as the surface area of the ergastoplasmic membranes and the number of ribosomes showed no significant differences. Significant differences were found on comparison of the ultrastructural organization of the mitochondrial apparatus. As Table 1 shows, in cells of metastases the surface area of the mitochondrial cristae was significantly greater than in the tumor cells.

Morphometric analysis thus revealed significant differences in the ultrastructural organization of tumors giving metastases in these experiments and nonmetastasizing tumors, and also between the tumors themselves and their metastases. Differences in the structure of the groups of tumors studied are concerned with the ultrastructural organization of the granular endoplasmic reticulum and the polysomes, i.e., with the protein-synthesizing system of the cell [6]. In tumors giving metastases relative predominance of synthesis of nonspecific intracellular proteins, undertaken by polysomes, was observed. In the group of nonmetastasizing tumors more marked synthesis of specialized extracellular proteins, connected with the function of the ergastoplasm, took place. Considering that predominance of synthesis of nonspecific proteins destined for the internal needs of the cell is characteristic of tissues with a low level of differentiation [10, 14], it can be concluded that the group of metastasizing tumors has a lower level of cell differentiation and the biological features that go with it. The absence of any analogous data in the specialized literature can evidently be explained by the fact that the degree of differentiation of tumor cells at the ultrastructural level can be reliably detected only by means of quantitative methods of investigation.

Comparison of the primary tumors and their metastases showed no difference in the organization of the protein-synthesizing apparatus of the cells. This could indicate that

the level of differentiation of the cells in the metastases as a whole does not differ from that of the tumors. In some cases differences probably could arise, but their detection would require quantitative comparison of the individual tumor with its metastases.

Differences thus found in the ultrastructural organization of the mitochondrial apparatus (the greater surface area of the mitochondrial cristae in the metastases than in the primary tumors) may possibly be the morphological explanation of the distinguishing features between the energy metabolism of primary and metastatic foci. The character of metabolism in metastatic foci may be influenced by the character of metabolism of the affected organ [3, 7].

The results of this investigation are evidence of the need for comparative quantitative ultrastructural analysis in the study of metastasization of malignant tumors.

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