ONCOLOGY

Ultrastructural Criteria of Malignancy of Adrenal Medullary Tumor

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Histological analysis and electron microscopy of 12 benign pheochromocytomas and 9 malignant pheochromoblastomas showed that there are no reliable histological differences between cells of benign and malignant tumor of the adrenal medulla. The ratio of ultrastructurally differentiated and undifferentiated cells in the tumor can reflect their maturity; the more ultrastructurally undifferentiated and less differentiated cells in the tumor, the higher is the malignant potential of this tumor.

Key Words: *pheochromocytoma; pheochromoblastoma; prediction; survival; differential diagnosis; electron microscopy*

Pheochromocytomas are endocrine cell tumors of the APUD system [2,5,12]. Their histogenesis is linked with the nerve tube crest cells, which determines the development of both endocrine and neurogenic tumors in the adrenal medulla, as well as tumors of mixed composition. Mature endocrine (chromaffine) adrenal cells secrete catecholamines (dopamine, norepinephrine, and epinephrine). Developing medullary tumors can be hormonally active and hormonally inert. Clinical diagnosis of the latter tumors is difficult because they are not associated with hormonal disorders and are detected incidentally or when they reach a certain size. According to some authors, about 10% pheochromocytomas are malignant (pheochromoblastomas), 10% are located outside the adrenal, 10% are bilateral or multiple, 10% are detected in children, and 10% are associated with hereditary diseases. Pheochromocytomas differ by their clinical course and prognosis. Morphological analysis and especially differential diagnosis and evaluation of their malignancy are very difficult [1]. The size of tumors, their cell and nuclear polymorphism, pronounced discomplectation of cell structures, growth through the capsule and invasion into vessels are not always associated with a clinically malignant course. The most reliable sign of malignancy is the presence of metastases [12].

The purpose of our study was to determine electron microscopic signs of malignancy of hormonally inactive tumors of the adrenal medulla.

MATERIALS AND METHODS

Twelve benign adenomas of the adrenal medulla (pheochromocytomas) and 9 malignant tumors (pheochromoblastomas) were examined histologically and under an electron microscope. All patients were operated at the clinical departments of Institute of Clinical Oncology, N. N. Blokhin Cancer Research Center. Material for histological analysis and electron microscopy was treated routinely. Electron microscopic analysis was carried out in accordance with ultrastructural classi-

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fication of human tumors [3,6]. Ultrastructurally differentiated (containing numerous endocrine granules in the cytoplasm) and undifferentiated (low number or absence of endocrine granules) cells were counted. Five tumor fragments (blocks) collected from different parts of the tumor were examined in each case. Semithin and ultrathin sections were prepared. Five grids from each block were examined, up to 100 cells were counted in each block (a total of 500 cells per case).

RESULTS

The mean diameter of pheochromoblastomas was 14.7 cm (6-30 cm). All tumors were well encapsulated, the adjacent adrenal tissue was usually compressed and atrophic. Tumor tissue at the site of section was gray-pinkish or reddish with foci of hyperemia or previous hemorrhages. Histological analysis showed that solid (diffuse) tumors were the most incident (Fig. 1, *a*), trabecular, alveolar, and pseudoacinar structures were only occasionally seen. The majority of tumors had mixed (more often solid) structure. Pronounced discomplectation of the above structures was often observed. Some tumors were completely discomplexated. Cell composition of the studied tumors was different in all cases and differs even within one tumor. Some cells were small, round, polygonal with fine granular cytoplasm, while others were large, polymorphic, sometimes oxyphilic, and looked like oncocytes. Some cells had light cytoplasm; large elongated giant cells were also seen. Basophilic cells were rare. Cell nuclei varied in shape, size, sometimes were ugly with invaginations and with high content of heterochromatin; cells with bubble-shaped or ring-like nuclei were sometimes seen. Nucleoli were often found (up to 2-4 and more in one nucleus). Pseudocytoplasmic inclusions were often found in large and ugly nuclei; multinuclear cells with 2-4 nuclei were observed in many cases. Invasions in blood vessels, sinuses, and penetration of tumor cells into the capsule of the tumor and/or adrenal (of different degree) were observed in virtually all cases, which gave grounds to classify these tumors as pheochromoblastomas. Mitoses in tumors were rare. Foci of necrosis, hemorrhages, and fibrosis in the tumors were often seen.

Hence, the examined pheochromoblastomas were characterized by histological variability, discomplectation of cell structures, pronounced cellular and nuclear polymorphism, rare mitoses, as a rule invasion into blood vessels, sinuses, capsule of the tumor and/or adrenal, and necroses. However these morphological signs do not unambiguously indicate malignancy of pheochromoblastomas, because, according to published data, they do not always correlate with the development of relapses, metastases, clinical prognosis, and life span [1,5,11,12]. None of the patients had metastases before surgery. Four patients developed metastases after surgery and died because of disease progress after 2, 13, 14, and 118 months. The rest 5 patients were observed for 2-7 years without metastases and/or relapses.

Electron microscopy revealed several ultrastructural variants depending on the ratio of differentiated and undifferentiated cells in these tumors.

In one of 4 patients with metastases in different periods after surgery undifferentiated cells predominated (up to 70%) in the tumor. Solitary endocrine granules, mitochondria, endoplasmic reticulum structures were seen in their cytoplasm (Fig. 2, *a*). The patient (female) died 2 months after surgery from metastases and progress of the process. In 2 patients who developed metastases after surgery and died 13 and 14 months after it, respectively, electron microscopy showed up to 60% ultrastructurally undifferentiated cells in the tumors. One patient developed metastases 72 months after the operation, but he lived for 118 months and died from the disease progress. Ultrastructurally differentiated cells predominated (up to 80%) in the tumor of this patient; the cytoplasm of these cells contained numerous endocrine granules, mitochondria, and other organelles. Of 5 patients who are alive and have no metastases and relapses, the number of ultrastructurally differentiated cells was about 50% in 1 (living for 25 months). Ultrastructurally differentiated cells predominate (up to 60%) in 3 patients living for 24, 55, and 58 months, respectively. In one patient (female) living for 85 months without metastases and relapses the number of ultrastructurally differentiated cells in the tumor was about 80%. It should be emphasized that there was no correlation between the histological structure of pheochromoblastomas, their cellular and nuclear atypia, invasion, and ultrastructural characteristics. It seems that tumor size is to a certain measure prognostically significant parameter, but not an absolutely significant one, because the initial diameter of tumors in patients who died 13 and 14 months after the operation was 20.5 and 7.0 cm, respectively. On the other hand, the initial diameters of tumors in patients without disease progress after the intervention, observed for 25 and 85 months, was 10 and 12 cm, respectively.

Hence, the more ultrastructurally differentiated cells and less ultrastructurally undifferentiated cells in the pheochromoblastoma, the better is the prognosis, lower the probability of progress, metastases, and the longer life span of the patients. The predominance of ultrastructurally undifferentiated cells notably deteriorates the clinical course of pheochromoblastoma and, hence, its prognosis. It was shown previously for endocrine-cell tumors of the APUD system (lungs, gas-

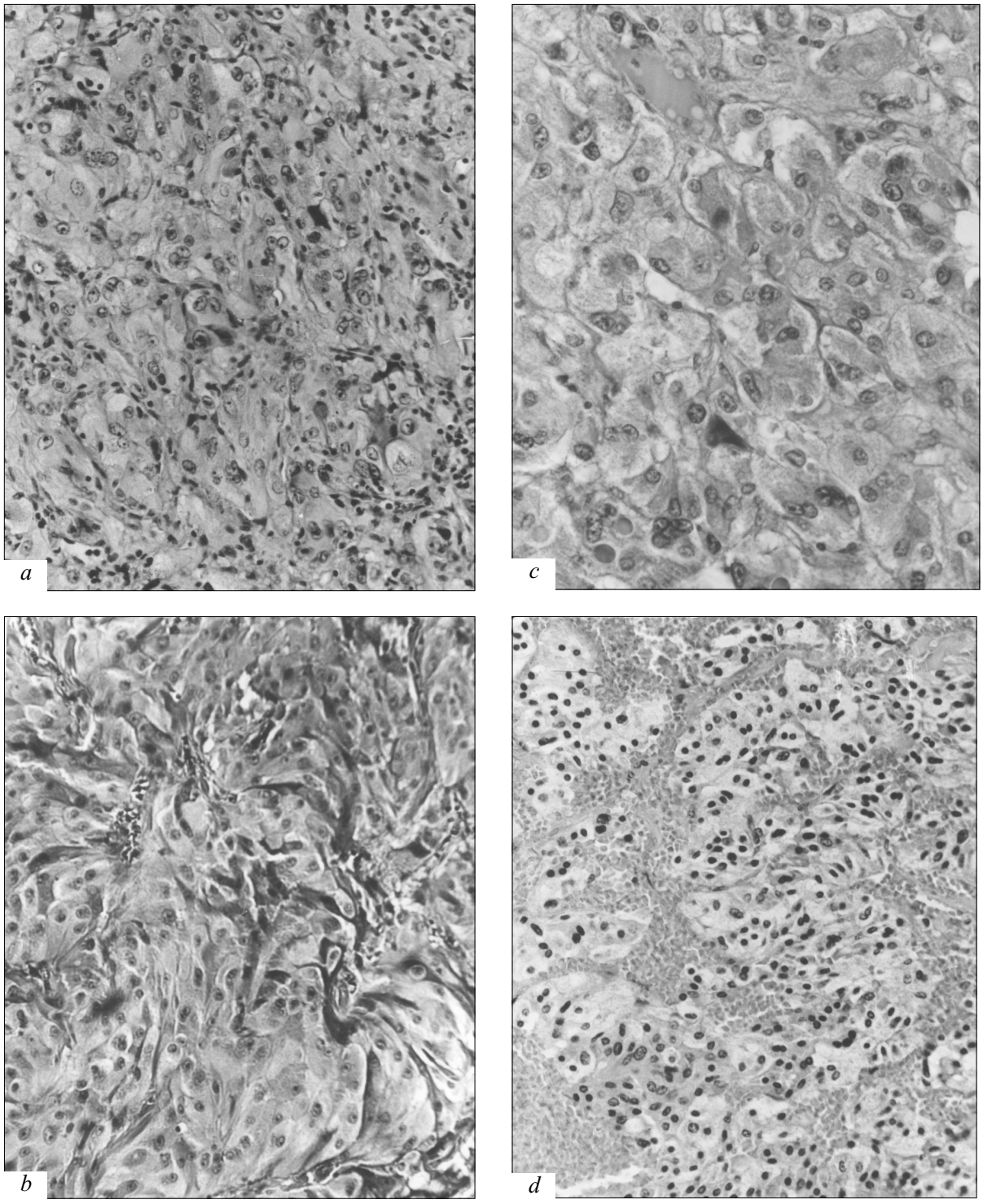

Fig. 1. Adrenal pheochromoblastoma (*a*, *b*) and pheochromocytoma (*c*, *d*). Hematoxylin and eosin staining. *a*) solid variant. Polymorphic cells with light cytoplasm and bubble-like nuclei. Each nucleus contains 1-2 nucleoli, ×250; *b*) sarcoma-like variant. Tumor cells are mainly elongated with dark cytoplasm and hyperchromatic nuclei, ×200; *c*) alveolar variant. Round or polygonal tumor cells with light vacuolated reticular cytoplasm. Nuclei are mostly eccentrical with nucleoli, ×400; *d*) solid alveolar variant with expressed vascular component. Tumor cells with light cytoplasm and hyperchromatic nuclei, the lacunas are filled with erythrocytes, ×200.

tropancreatoduodenal system, thyroid, and other organs) that the balance between ultrastructurally differentiated and undifferentiated cells reflects the degree of tumor malignancy [5,7,8,9-12]. The less ultrastructurally differentiated cells contains the tumor, the more malignant it is, and vice versa. These data are in

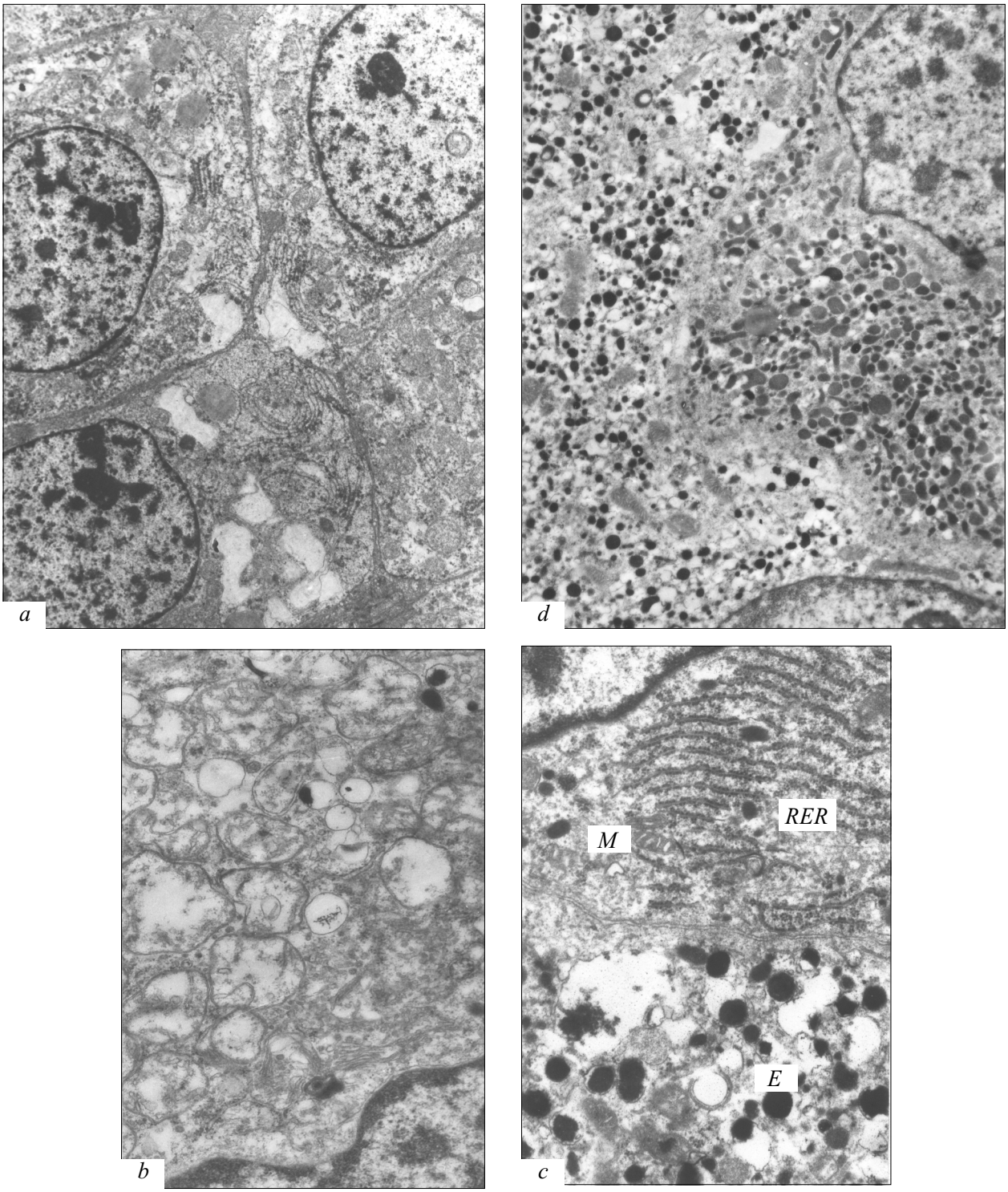

Fig. 2. Ultrastructure of adrenal pheochromoblastoma (*a*, *b*) and pheochromocytoma (*c*, *d*). Electronograms. *a*) ultrastructurally undifferentiated cells without endocrine granules. Ribosomes, solitary mitochondria, and fragments of rough endoplasmic reticulum in the cytoplasm, ×2500; *b*) fragment of an ultrastructurally undifferentiated cell with solitary endocrine granules and numerous mitochondria in the cytoplasm, ×40,000; *c*) fragments of two tumor cells. Electron-dense endocrine granules (*E*) in one cell. Parallel rows of rough endoplasmic reticulum (*RER*) and solitary mitochondria (*M*) in the other cell, ×20,000; *d*) numerous endocrine granules in the cytoplasm of tumor cells, ×5000.

line with the results of electron microscopic study of pheochromoblastomas, which possess different malignant potential depending, among other things, on their ultrastructure. Therefore, electron microscopy findings can be used as an additional marker of pheochromoblastoma malignancy.

The mean diameter of pheochromocytomas was 7.9 cm (4-16 cm). The tumors were surrounded with a dense capsule, the tissue was pink-reddish at the site of dissection.

Histologically pheochromocytomas were characterized by trabecular, alveolar, solid, mixed, and discomplected structure (Fig. 1, *c*, *d*). Cell size and shape varied from round and polygonal to slightly elongated; giant ugly cells and multinuclear symplasts were rarely seen. The nuclei were mostly round, bubble-like, with 1-2 nuclei, sometimes the nuclei had invaginations and dispersed chromatin looking as a fine reticulum. The nuclei in giant cells were ugly, hyperchromatic. The cytoplasm was mainly oxyphilic; basophilic cells were rarely seen. Sometimes the cytoplasm was vacuolated. Mitoses were rarely observed. The vessels in the tumor were sinusoid, rarely cavernous. Necroses and hemorrhages were sometimes seen. In 6 cases penetration of tumor cells into blood vessels was observed, in one of these cases they grew into the tumor capsule.

Comparative histological analysis of pheochromocytomas and pheochromoblastomas showed that there were no permanent significant differences between them. Cellular and nuclear atypia, foci of necrosis and hemorrhages were seen in pheochromocytomas althought less frequently; invasions can also be observed. All this often impedes differential diagnosis and additional methods of investigation are required, for example, detection of expression of nucleolar organizer region-associated proteins [4,9,10] or electron microscopy.

Ultrastructurally differentiated cells with numerous endocrine granules in the cytoplasm predominated (up to 80%) in 6 pheochromocytomas not growing into the vessels and tumor capsule (Fig. 2, *c*, *d*). Of 6 cases with growth into vessels and capsule, the number of ultrastructurally differentiated cells was no more than 50% in 1 case and reached 80% in 3 other tumors. Hence, these 3 latter pheochromocytomas, despite the signs of invasion, can be regarded as sufficiently mature and a more favorable prognosis can be expected than in the case with only 50% differentiated cells. In 2 other cases of this group, in which the tumors corresponded to pheochromocytoma by histological criteria, 1 patient developed a relapse and metastases in the other adrenal, liver, and lungs 11 months after surgery. The other patient developed metastases 10 years after surgery. The number of ultrastructurally differentiated cells was about 30% in the first case and about 20% in the second.

Hence, from ultrastructural viewpoint (balance of differentiated and undifferentiated cells) pheochromocytomas can be characterized by different degree of maturity, which can be significant for the clinical course and outcome. This is well illustrated by 2 cases described above, when patients with histologically typical pheochromocytomas with up to 70-80% ultrastructurally undifferentiated cells developed relapses and multiple metastases several months after the intervention. We should like to emphasize that electron microscopy not only helps to evaluate the maturity of some tumors, but is widely used for the differential diagnosis. For example, it is sometimes difficult to differentiate anaplastic pheochromoblastomas from adrenocortical tumors [5]. Detection of endocrine tumor cells by electron microscopy unambiguously indicates neoplasms of the adrenal medulla.

Hence, we detected no clear-cut histological differences between benign (pheochromocytoma) and malignant (pheochromoblastoma) tumors growing from the adrenal medulla. More or less expressed cellular and nuclear polymorphism, atypia, discomplectation of histological structures, necroses, hemorrhages, signs of invasion into vessels and capsule can be seen in both types of tumors.

The balance of ultrastructurally differentiated and undifferentiated cells in adrenal medullary tumors can indicate the degree of their maturity; the more ultrastructurally undifferentiated cells and less ultrastructurally differentiated cells contains the tumor, the higher is its malignant potential.

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