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Criteria for Predicting the Outcome of Pheochromocytoma by the Immunohistochemical and Electron Microscopic Findings

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Morphological examination of 22 functionally active adrenal pheochromocytomas was carried out. The content of catecholamine granules in tumor cells and in the number of sustentacular cells tended to decrease in metastasizing tumors. Electron microscopy showed two types of sustentacular cells and the possibility of their apoptotic death.

Key Words: *adrenal; pheochromocytoma; sustentacular cells; ultrastructure; immunohistochemistry*

Pheochromocytoma (PCC), a tumor arising from the adrenal medulla, is a rare tumor of neuroendocrine origin. PCC produces catecholamines. Epinephrine, norepinephrine, dopamine, and DOPA are also present in tumor tissue extracts, serum, and urine of patients with PCC. Morphological verification of PCC is based on immunohistochemical detection (IHC) of markers of neuroendocrine tumor cells and on their specific ultrastructure. Nonhormonal components of secretory granules (chromogranin A), neurofilament protein, enzymatic markers (neurospecific enolase), catecholamines, and regulatory peptides are the most practically valuable markers for verification of neuroendocrine origin of the tumor. Nonhormonal components of secretory granules can be also detected by Grimelius argirophilic staining. Cell composition of PCC is presented by chief cells (pheochromocytes) and supporting sustentacular cells. Electron microscopic detection of catecholamine granules is of particular importance for verification of tumors of the adrenal medulla [2,10]. Catecholamine granules in PCC cells (270 nm) are larger than in normal adrenal medullar cells (170 nm). Sustentacular cells, sometimes called glial-like cells, are elongated and have long thin processes. They are clearly seen under light and electron microscopes. IHC marker of these cells is S100 protein. Sustentacular cells express GFAP (glial fibrillar acid protein) [8]. Positive staining for proteins S100 and GFAP suggests the presence of two cell strains with different phenotypes: true sustentacular cells and potentially neuronal cells.

Early diagnosis of PCC is important for prevention of complications of arterial hypertension and because of the possibility of malignant degeneration of PCC in 8-10% cases. Difficulties in morphological verification of PCC are associated with its structural polymorphism and the absence of clear-cut histological and cytological signs specific for benign and malignant tumors.

Only the presence of metastases unambiguously indicates the malignant nature of PCC. Facultative signs of PCC malignancy are large size of the tumor (>5 cm), invasion into vessels and capsules, absence of hyalin granules in cell cytoplasm, decreased number of neuropeptides [5,11], high proliferative index (from IHC reaction to MIB-1) [3,7,9], and decreased number

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of sustentacular cells [3, 6]. Computer analysis detects significant differences in the size and "density" of tumor cell nuclei in clinically benign and metastatic tumors [4].

The aim of our study was to detect possible relationships between clinical "behavior" of adrenal PCC, tumor size, number of catecholamine granules in tumor cells, number and ultrastructural characteristics of sustentacular cells.

MATERIALS AND METHODS

Twenty-two functionally active adrenal PCC were examined (all patients were operated at Surgical Endocrinology Department (headed by Prof. A. P. Kalinin, Corresponding Member of Russian Academy of Medical Sciences) of M. F. Vladimirskii Moscow Regional Research and Clinical Institute). The tumors were clinically and morphologically benign in 15 cases, metastases to regional lymph nodes were detected during surgery in 2 cases, and in 5 cases tumors relapsed (three times in one patient, 10 years after surgery in another patient). The tumors were divided into 3 groups by their size: <5 cm (8 cases), 5-10 cm (3 cases), >10 cm (11 cases). The largest tumor was 23 cm in diameter.

Tumor fragments for histological and IHC studies were fixed in formalin and Bouin fluid. Paraffin sections were stained with hematoxylin and eosin, Sudan III for lipids, Grimelius argirophilic reaction was carried out, and IHC reaction was carried out by indirect PAP method using monoclonal antibodies to chromogranin A and protein S100 (Daco). The intensity of staining with monoclonal antibodies to chromogranin A was evaluated by a semiquantitative method. The density of protein S100-positive sustentacular cells was evaluated per unit of section area (the cells were counted using computer-assisted image analysis system). The results were statistically processed using Student's *t* test. Tumor tissue fragments for electron microscopy were fixed in 2.5% glutaraldehyde and postfixed in 1% osmium tetroxide; after standard treatment the preparations were embedded in eponaraldite. LKB ultratome and JEM 100-S electron microscope were used. The presence of catecholamine granules in tumor cells was evaluated by a semiquantitative method.

RESULTS

Pronounced polymorphism of tumor cells and variability of their combinations were clearly seen under a light microscope. Small cells with clear contours differing by eosinophilia of the cytoplasm were seen along with large light cells with poorly discernible

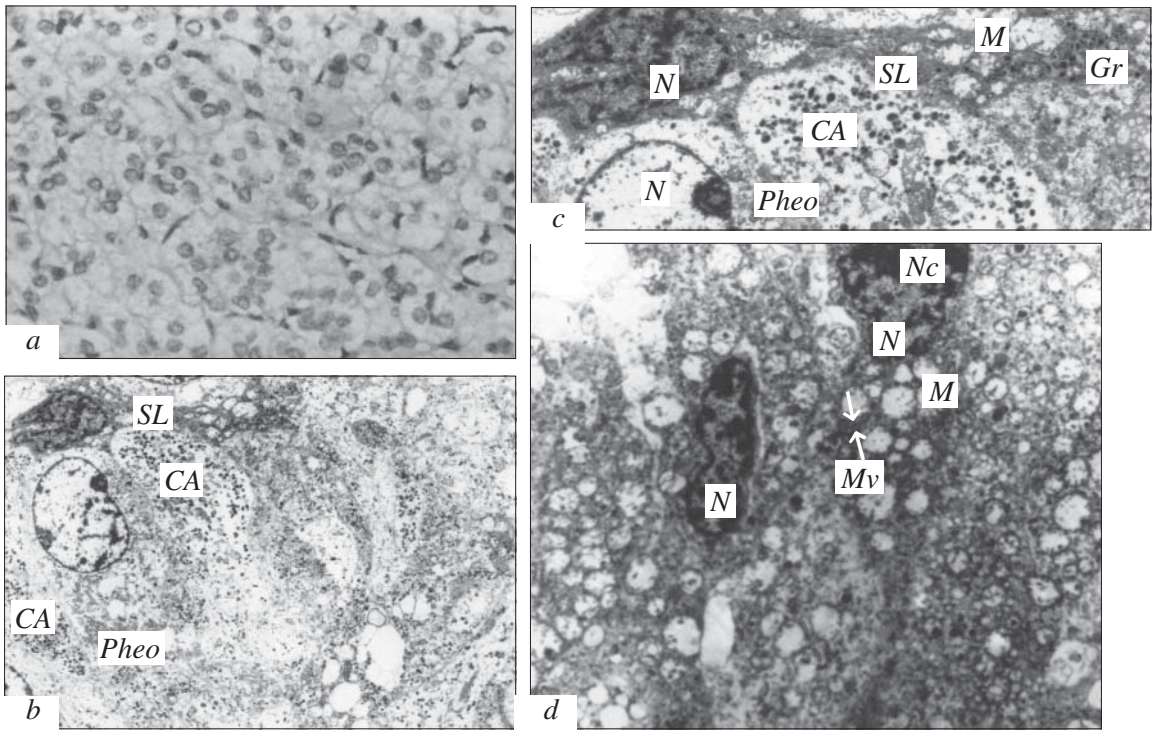

Fig. 1. Sustentacular cells of adrenal pheochromocytoma (PCC). *a*) protein S100-positive sustentacular cells in benign PCC. IHC reaction with antibodies to protein S100, ×125; *b*, *c*) sustentacular cells in PCC, ×4000, ×8800; *d*) apoptosis of sustentacular-like cell, ×6500; *Pheo*: pheochromocyte; *CA*; catecholamine granules; *SL*; sustentacular-like cells; *N*; nucleus; *M*: mitochondria; *Gr*: granules; *Nc*: nuclear chromatin; *Mv*: microvesicles (arrows).

contours. These cells contained round, elongated, giant, or irregularly shaped nuclei.

Ultrastructure of pheochromocytes is relatively stable. The most characteristic ultrastructural sign of tumor cells is the presence of osmiophilic granules. Granules usually interpreted as epinephrine-containing (round or elongated, of medium electron density, 100-300 nm in size) predominated in cells of all examined tumors. The number of catecholamine granules in the cytoplasm was minimum in patients with metastasizing tumors. All PCC contained chromogranin A (marker of chief cells), but the intensity of IHC staining correlated with tumor type. The most intense staining was observed in benign tumors, weaker staining in relapsing tumors, and poorly discernible staining was characteristic of clinically malignant tumors.

The number of sustentacular cells in benign PCC was higher than in malignant tumors, where these cells were not detected or were presented by solitary elements. The distribution of sustentacular cells in relapsing PCC varied (Fig. 1, *a*). The density per unit of section area was 5.4±0.4 in nonmetastasizing PCC, 3.3±0.3 in relapsing tumors, and 0.28±0.09/mm^2 in metastasizing PCC ($p \leq 0.001$).

By their ultrastructure the cells can be divided into two types. In type I cells the nucleus occupies the greater part of the cytoplasm, cell organelles are scanty, neurosecretory granules are absent.

Type 2 cells can be called sustentacular-like and their neuronal differentiation can be hypothesized. Their long and thick processes contain numerous mitochondria, small osmiophilic granules, and fibrillar material (Fig. 1, *b*, *c*). Changes corresponding to the initial stage of apoptosis were seen in some cells of this type [1,3]. The nuclei of these cells had irregular shape. Chromatin aggregation presented as large lumps arranged along the inner nuclear membrane. The cell cytoplasm was compact, microvesicles and edematous mitochondria with poorly expressed cristae were seen (Fig. 1, *d*).

Analysis revealed no correlation between PCC size and degree of cell differentiation.

It seems that the decrease in the number of cells expressing protein S100 in relapsing and metastasizing tumors can be partially attributed to their apoptic death.

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