Ultrastructural Characteristics of Chemodectomas

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We present results of morphological study of chemodectoma samples obtained during surgery. Specific ultrastructural features and protein-synthesizing and proliferative activity of light and dark cells were demonstrated using data of electron radioautography.

Key Words: *immunohistochemistry; proliferation; radioautography; ultrastructure; chemodectoma*

Great diversity of cells of the diffuse endocrine system and their spreading in the human body in various organs and tissues (endocrine glands), including cell clusters (paraganlions) determine great variety of neuroendocrine tumors [6]. This diversity can explain complex morphological structure of these tumors and difficulties in evaluation of their malignancy [2]. Study of morphofunctional peculiarities of tumors is an important component in evaluation of their biological behavior and prognosis. This is particularly important for neuroendocrine tumors, especially for neck chemodectomas, which have no clear-cut microscopic signs of malignancy [5,9].

We studied morphofunctional peculiarities of carotid paraganglioma (chemodectoma), the most prevalent paraganglionic tumor of the neck.

MATERIALS AND METHODS

We analyzed tumor samples from 38 patients of A. V. Vishnevskii Institute of Surgery obtained during operations. The age of patients varied from 20 to 60 years, male to female ratio was 1:3.

Tissue samples were fixed in 10% neutral formalin. For histological study, paraffin sections (5 μ) were stained with hematoxylin and eosin. For immunohistochemical study, antibodies (DakoCytomation) against cytokeratin (LP34), vimentin (clone V9), synaptophysin (monoclonal), chromogranin A (polyclonal), neurospecific enolase (monoclonal, clone BBS/NC/VIH14), protein S-100 (polyclonal), and Ki-67 (monoclonal clone MIB-1) were used. The antigens were demasked using Target Retrieval Solution (Dako) by boiling on a water bath at 98°C for 35 min. After addition of primary antibodies, further processing was performed using LSAB2 System-HRP (Liqvid DAB; DakoCytomation). For evaluation of apoptosis, immunohistochemical reactions were carried out using monoclonal antibodies (Novocastra) to FAS, FAS-L, bcl-2, casp, DFF, p-53 in a working dilution. The sections with applied antibodies were incubated for 12 h at 4°C. For visualization, a universal Novostain Super ABC Kit (Novoñastra Laboratories Ltd.) was used. Poststaining with hematoxylin was performed on all preparations.

For electron radiography, tissue samples (1 mm) were incubated in medium 199 containing 100 μCi/ml ^{3}H-uridine (specific activity 26.0 Ci/mmol) or 20 μCi/ml ^{3}H-thymidine (specific activity 21.6 Ci/mmol) at 37-38°C for 1.5 h. The material was fixed with 2.5% glutaraldehyde and 1% OsO_4 and embedded in epon-araldite mixture. Radioautographs were prepared as described elsewhere [5]. The preparations were examined under light (Leitz) and electron (Phillips CM-10) microscopes.

RESULTS

Histological examination of preparations of chemodectomas stained with hematoxylin and eosin re-

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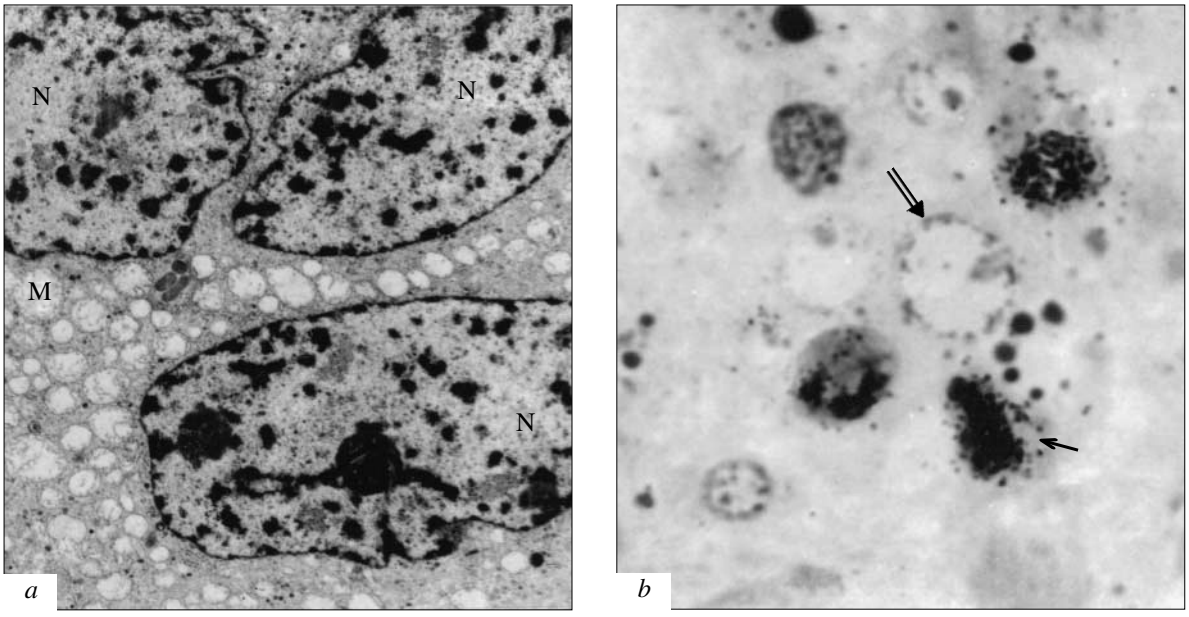

Fig. 1. Tumor cells and their protein-synthesizing activity. *a*) symplast of tumor cells; N: hypochromic nuclei with small heterochromatin lumps; M: mitochondria with destroyed matrix; ×7800; *b*) ^{3}H-uridine incorporation, RNA synthesis (black silver grains) in tumor cells. Intensive RNA synthesis in dark cells (arrow), absence of the label in light cells (double arrow). Semithin section. Toluidine blue staining, ×1000.

vealed two types of cells: dark and light (more abundant), which agrees with published data [7].

Electron microscopy showed that light cells usually have fine granular cytoplasm with numerous mitochondria of different size and shape, often with destructed cristae, numerous neurosecretory granules of different electron density and size, lysosomes, and vacuoles. Multinuclear cells (symplasts) were often seen (Fig. 1, *a*). These cells contained hypo- and hyperchromatic nuclei (depending on functional state of the cell) or large deformed nuclei with condensed chromatin and pronounced invagination of the nuclear membrane. Dark cells were less abundant. They were smaller and had dense and homogenous cytoplasm with poorly discernible organelles. These cells were characterized by high nucleus/cytoplasm ratio.

Radioautography showed that protein-synthesizing activity (determined by ^{3}H-uridine incorporation) was different in light and dark cells. Intensive RNA synthesis was usually observed in dark cells (Fig. 1, *b*). ^{3}H-Uridine incorporation into light cells was low or absent, which attested to the absence of protein-synthesizing activity in these cells. These cells often had signs of reversible or irreversible damage or were in a state of decomposition. Analysis of proliferative activity of tumor cells revealed mitoses only in few preparations of chemodectomas, which should be taken into account in histological diagnostics of tumors. ^{3}H-Thymidine incorporation (DNA synthesis) into tumor cells of parasympathetic paragangliomas reflecting their proliferation capacity was rarely seen and primarily into dark cells located in close proximity to the vascular wall (Fig. 2, *a*). Moreover, the presence of giant cells or symplasts (huge cytoplasm and several polymorphic nuclei) can be a result of increased mitotic activity of tumor cells and disturbed cytotomy, *i.e.* mitosis abnormality. Pathological mitoses are considered the main cause of tumor progression at all stages [8].

Rare detection of mitoses and ^{3}H-thymidine incorporation (DNA synthesis) can reflect cyclicity of mitotic activity of tumor cells typical of endocrine tumors, short duration of mitosis (~1% of the total duration of cell cycle), and long-term existence of parasympathetic paraganglioma cells in the period of relative rest (G_0 phase). Slow grow of the tumor and/or relapses after a long period after removal of chemodectomas, as well as the existence of cryptogenic metastases and/or delayed metastasizing can be explained by the presence of these resting cells and their subsequent proliferation [1].

The results of immunohistochemical analysis of removed tumor samples (Table 1) agree with published data [10] and attest to their neuroendocrine differentiation. Evaluation of mitotic activity of parasympathetic paraganglioma cells using cell proliferation marker Ki-67 showed that index of proliferation is equal to index of apoptosis (Table 1). These results attest to low proliferation capacity and low apoptosis intensity in this tumor, *i.e.* to the

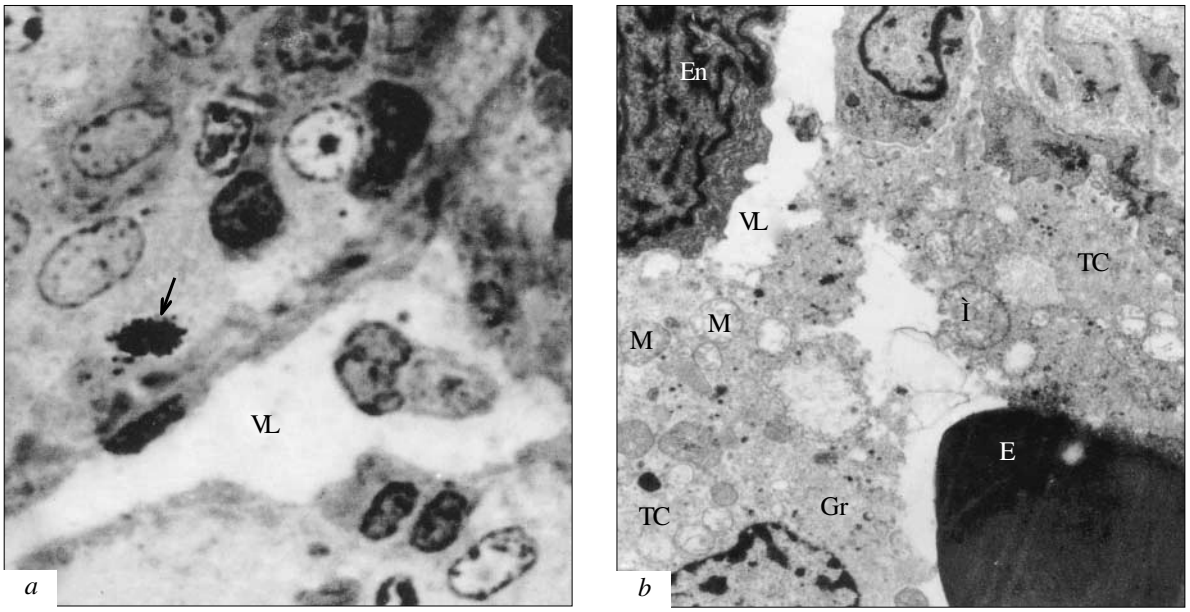

Fig. 2. Vessels in tumor tissue. *a*) ^{3}H-thymidine incorporation, DNA synthesis (black silver grains) in dark tumor cells (arrow) near the wall of destroyed vessels. Semithin section. Toluidine blue staining, ×1000; *b*) lumen of destroyed vessel (VL). E: erythrocyte; TC: tumor cell; M: mitochondria; Gr: neurosecretory granules; En (endotheliocyte). Electronogram, ×8400.

balance between these two opposite processes. Rare mitoses and/or their absence, low proliferation and apoptosis indexes, and the balance between these two processes can indicate the stage of progression of parasympathetic paragangliomas at the moment of their removal, rather than benign and malignant nature of the examined tumors.

Complex morphological examination of tumor samples obtained during surgery revealed invasion and presence of tumor cells in blood vessels (Fig. 2, *b*). Invasion and penetration of tumor cells into vessels (carcinemia) are prerequisites of tumor metastasizing. Moreover, analysis of chemodectomas revealed the phenomenon of malignant transformation of the stroma. It manifested in atypic structure of microvessel wall cells (endotheliocytes and pericytes), especially their nuclei, and high nucleus/cytoplasm ratio in these cells (Fig. 3, *a*). These cells were similar to tumor cells. Giant fibroblasts were seen (Fig. 3, *b*). It should be noted that hypertrophy of stromal cells was associated with intensive protein synthesis determined by ^{3}H-uridine incorporation. It can be hypothesized that these cells appear in the stroma as a tissue reaction to increasing hypoxia due to vascular destruction.

TABLE 1. Immunohistochemical Characteristics of Chemodectomas

Marker	Location	
	carotid	vagal
Vimentin	+	+
Chromogranin A	+	+
Neurospecific enolase	+++	+++
Synaptophysin	+++	+++
Protein S-100	+++	+++
Lp-34 (pancytokeratin)	+	+
Ki-67, %	1	1
Index of apoptosis, %	1	1

Note. +: weak reaction; +++: strong reaction.

Thus, we revealed morphological peculiarities of chemodectomas, first of all, pronounced cellular and nuclear polymorphism and atypia, which can serve as criteria for gradation of the studied neoplasms. Structural peculiarities, high level of protein synthesis, and proliferation capacity of dark cells suggest that these cells are less differentiated than light cells. Moreover, according to published data [3], the percent ratio of light and dark tumor cells suggests that chemodectomas can be regarded as neoplasms with grade I-II cell anaplasia. The presence of tumor cells in vessels detected under electron microscope can be considered as a prerequisite for metastasizing. The phenomenon of malignant transformation of the stroma probably reflects the severity of tumor process and involvement of stromal cells into this process. All these signs are attributes of tumor progression determining its stage at the moment of the study. These characteristics can reflect peculiarities of biological behavior of not only parasympathetic paragangliomas, but also the entire group of paragangliomas.

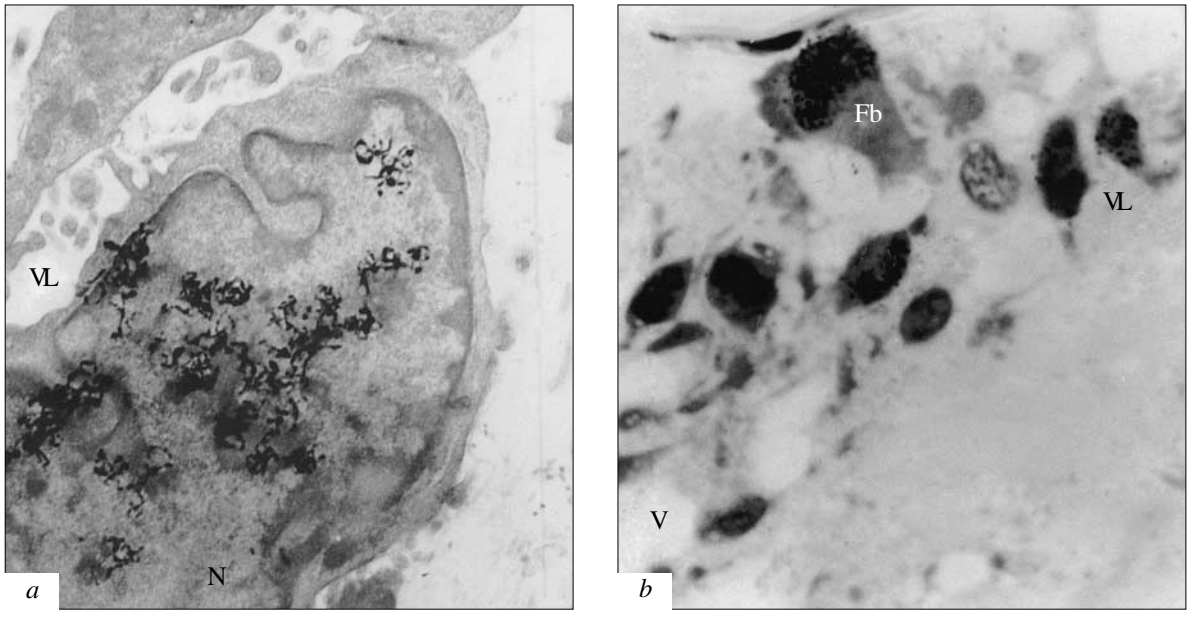

Fig. 3. Tumor stroma. *a*) capillary with pronounced functional activity of endotheliocyte; RNA is actively synthesized (^{3}H-uridine incorporation, black silver grains); the vessel lumen (VL) is narrowed, ×10,400; *b*) giant fibroblast (Fb) with intensive RNA synthesis in the nucleus (black silver grains) and vacuolated stroma (V) among chaotically scattered cells in the tumor tissue. Semithin section. Toluidine blue staining, ×1000.

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