Ultrastructural Characteristics of Solid Pseudopapillary Tumors of the Pancreas

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We carried out an electron microscopic study of solid pseudopapillary tumors of the pancreas (operation material) from 15 patients. Two cell types were distinguished. Type 1 cells had large oval nuclei, little changed mitochondria, and short fragments of the granular cytoplasmic reticulum. These cells formed mainly pseudopapillary structures around the vessels. Type 2 cells were characterized by pronounced nuclear polymorphism with specific "coffee bean" picture. The cytoplasm of these cells contained many large mitochondria with clarified matrix and often destroyed cristae; lipofuchsin granules were seen. The ultrastructural characteristics of the solid pseudopapillary tumor cells attest to their epithelial origin.

Key Words: *pancreas; solid pseudopapillary tumor; ultrastructure*

Solid pseudopapillary tumors (SPPT) are rare tumors responsible for 1-2% of all tumors of the exocrine part and about 5% of cystic tumors of the pancreas [7,9]. The biological behavior and histological characteristics of SPPT resemble those of the neuroendocrine tumors [4,6,8]. On the other hand, no hormone markers are detected by immunohistochemical methods [2,5]. Electron microscopy, an effective method of morphological studies, helps to identify the histogenesis and detect the specific diagnostic signs of the tumor in many cases [1,3].

We carried out an electron microscopic study of pancreatic SPPT.

MATERIALS AND METHODS

A complex morphological study of operation material from 15 patients with pancreatic SPPT (14 women aged 15-67 years, median 35.2 years, and 1 man aged 61) treated at A. V. Vishnevsky Institute of Surgery in 1998-2008 was carried out. One woman aged 50 years had repeated relapses of the tumor, another, aged 49 years, had metastasis in the liver.

The diagnosis of SPPT was morphologically verified by comprehensive histological, cytological, and immunohistochemical studies in accordance with WHO criteria [3]. Tissue specimens for electron microscopy were fixed in 2.5% glutaraldehyde, washed in phosphate buffer with sucrose, fixed in 1% osmium tetroxide, dehydrated in ascending ethanols, and embedded in araldite. The sections were prepared using LKB-V ultratome and examined under a Phillips-CM 10 microscope.

RESULTS

Macroscopic studies showed tumor location in the pancreatic head in 7 patients, in the pancreatic body in 4, and in its tail in 3 patients. Sites of solid and papillary structure were seen in preparations stained with hematoxylin and eosin. Pseudopapillae were presented by a central fibrous septa lined with monomorphic cuboid cells without signs of atypia (Fig. 1, *a*). Those pseudopapillae were usually seen in the central part

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of the tumor node. Closer to the capsule the tumor primarily consisted of solid sites resembling the neuroendocrine tumor structures (Fig. 1, *b*). Cell cytoplasms were transparent or slightly eosinophilic, with round or oval nuclei and rare mitoses. A characteristic sign was the presence of "coffee bean" nuclei. In common preparations those nuclei had a transverse hyperchromatic strip (Fig. 1, *c*).

Cytological studies of the material showed monomorphic cells with round nuclei and eosinophilic or foamy cytoplasm. A characteristic sign was the presence of phylloid structures (accumulations of monomorphic cells around small fibrous vascular formations; Fig. 1, *d*).

Immunohistochemical studies revealed positive reactions with antibodies to vimentin, α-1-antitrypsin, α-1-antichemotrypsin, CD56, and neuron-specific enolase in all SPPT specimens, and in 11 cases to cytokeratins 8 and 18. The nuclei of SPPT cells always expressed progesterone, cyclin D1, β-catenin, and E-cadherin receptors. The level of tumor cell proliferation evaluated by detection of Ki-67 varied from 0.2 to 8%.

Ultrastructural studies showed that all tumors had similar structure and were presented mainly by solid and papillary structures consisting of cells of two types. Type 1 cells mainly formed pseudopapillary structures (Fig. 2, *a*). They had large oval nuclei with small chromatin lumps in the nucleoplasm and along the nucleolemma. The cytoplasm of these cells was filled with small compact mitochondria with normal orientation of cristae, the granular cytoplasmic reticulum was presented by short tubular fragments (Fig. 2, *b*).

The vessels were mainly capillaries, around which the tumor cells were organized into pseudopapillary structures and were surrounded by collagen argirophilic fibers and fibrinoid masses. Endotheliocytes were functionally active. Numerous cytoplasmic processes were seen on their luminal and basal surfaces, presumably indicating high functional activity (increased absorption surface of the cell). Numerous micropinocytotic vesicles of different size were scattered along the entire cytoplasms of endothelial cells; some of these vesicles formed vesicular chains, others fused and formed vacuoles (Fig. 2, *c*).

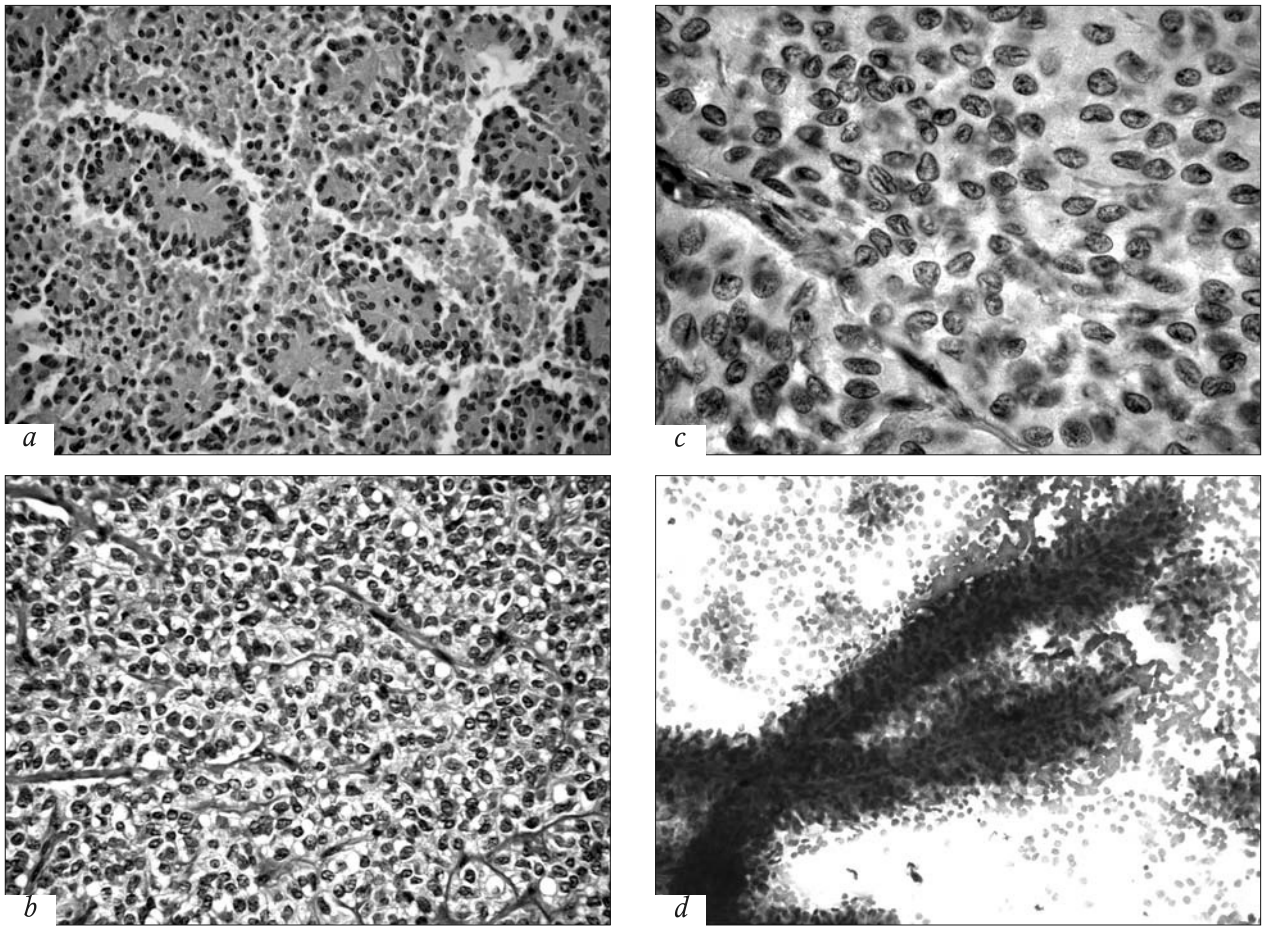

Fig. 1. Microscopic characteristics of SPPT. Staining by hematoxylin and eosin (*a-c*) and Azur-eosin (*d*). *a*) pseudopapillary structures, ×200; *b*) sites of solid structure of the tumor,×200; *c*) "coffee bean" nuclei, ×400; *d*) tumor tissue smear impression, ×100.

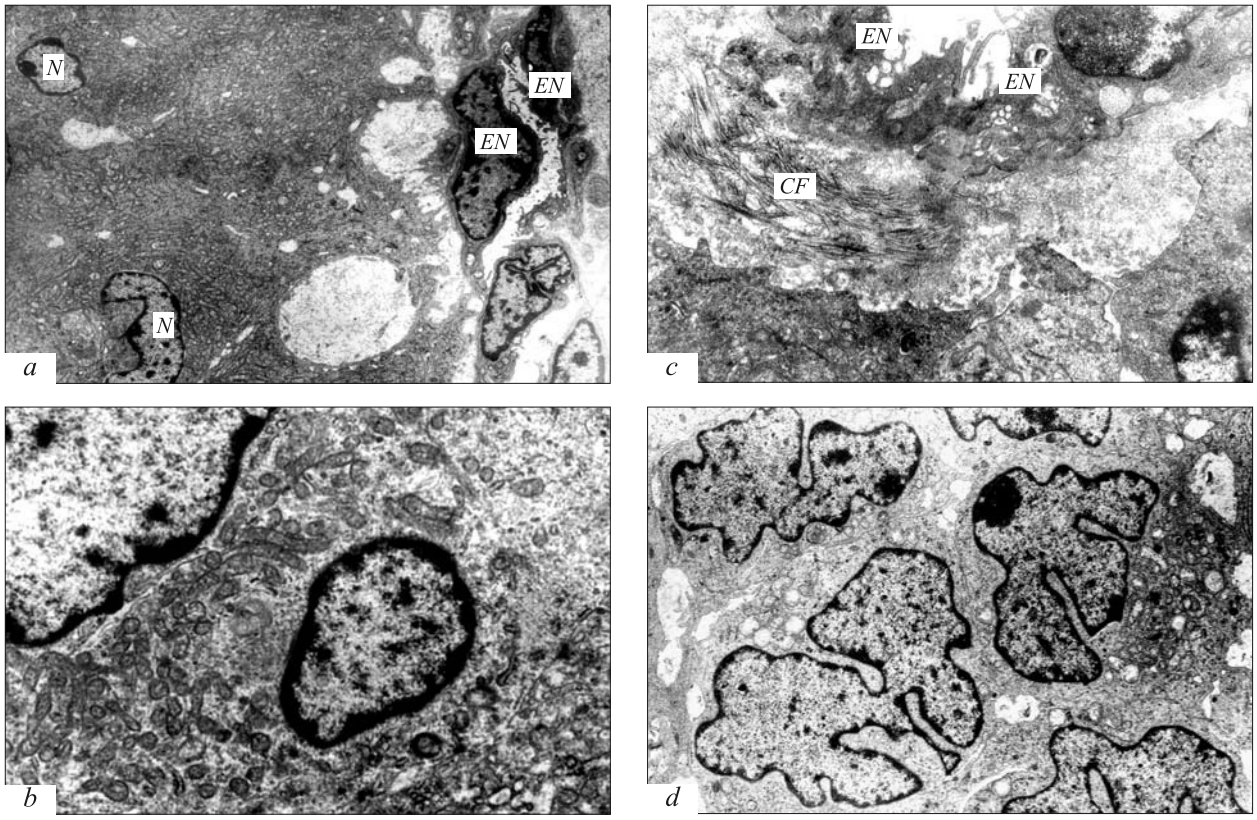

Fig. 2. Ultrastructural characteristics of SPPT. *a*) pseudopapillary structures of tumor, ×3500; *b*) tumor cell with oval nucleus, ×15,000; *c*) vascular wall, ×17,000; *d*) tumor cells with pronounced nuclear polymorphism, ×12,000. EN: endotheliocyte; N: tumor cell nucleus; CF: collagen fibers.

Type 2 tumor cells were larger and were characterized by pronounced nuclear polymorphism. These cells more often formed solid sites of the tumor and looked like coffee beans in histological preparations. Electron microscopy showed that these nuclei had a peculiar configuration (Fig. 2, *d*): they looked segmented with numerous invaginations looking like pouches containing elements of the cytoplasm. Chromatin was condensed in a narrow stripe along the nucleolemma and in lumps in the nucleoplasm. Nucleoli were clearly seen in some cells.

The cytoplasms of these cells had numerous large mitochondria with abnormal orientation of cristae and clarified matrix; they filled virtually the entire cytoplasm. Granular cytoplasmic reticulum was presented by short fragments, Golgi complex was poorly developed. Tumor cell bodies reacted between each other, forming desmosome contacts (accumulations of electron-dense substance on the cytoplasmic side of the plasmalemma) (Fig. 3, *a*). In addition, cell cytoplasms often contained numerous lipofuchsin granules, which attested to degenerative processes resulting from high activity of cells (Fig. 3, *b*). Contects between cells of these two types were often seen (Fig. 3, *c*).

Large vacuoles in the cytoplasm and signs of pronounced nuclear polymorphism were seen in some cells (Fig. 3, *d*). In addition, widening of the extracellular spaces and formation of bridge-like contacts were observed. Presumably, those intracellular destructive processes and changes in the tumor cell-cell interactions represented the initial stage of cystic degeneration of the tumor.

Hence, electron microscopy showed two cell types. Type 1 cells had large oval nuclei, little changed mitochondria, and short fragments of granular cytoplasmic reticulum; they formed mainly the pseudopapillary structures round the vessels. Type 2 cells were characterized by pronounced nuclear polymorphism with the formation of the specific "coffee bean" picture. Unfortunately, we failed to detect the relationship between the immunohistochemical reactions and ultrastructure of tumor cells. The only fact was more pronounced expression of α-1-antitrypsin in type 2 cells. Electron microscopic findings indicated that SPPT cells were mainly of epithelial origin.

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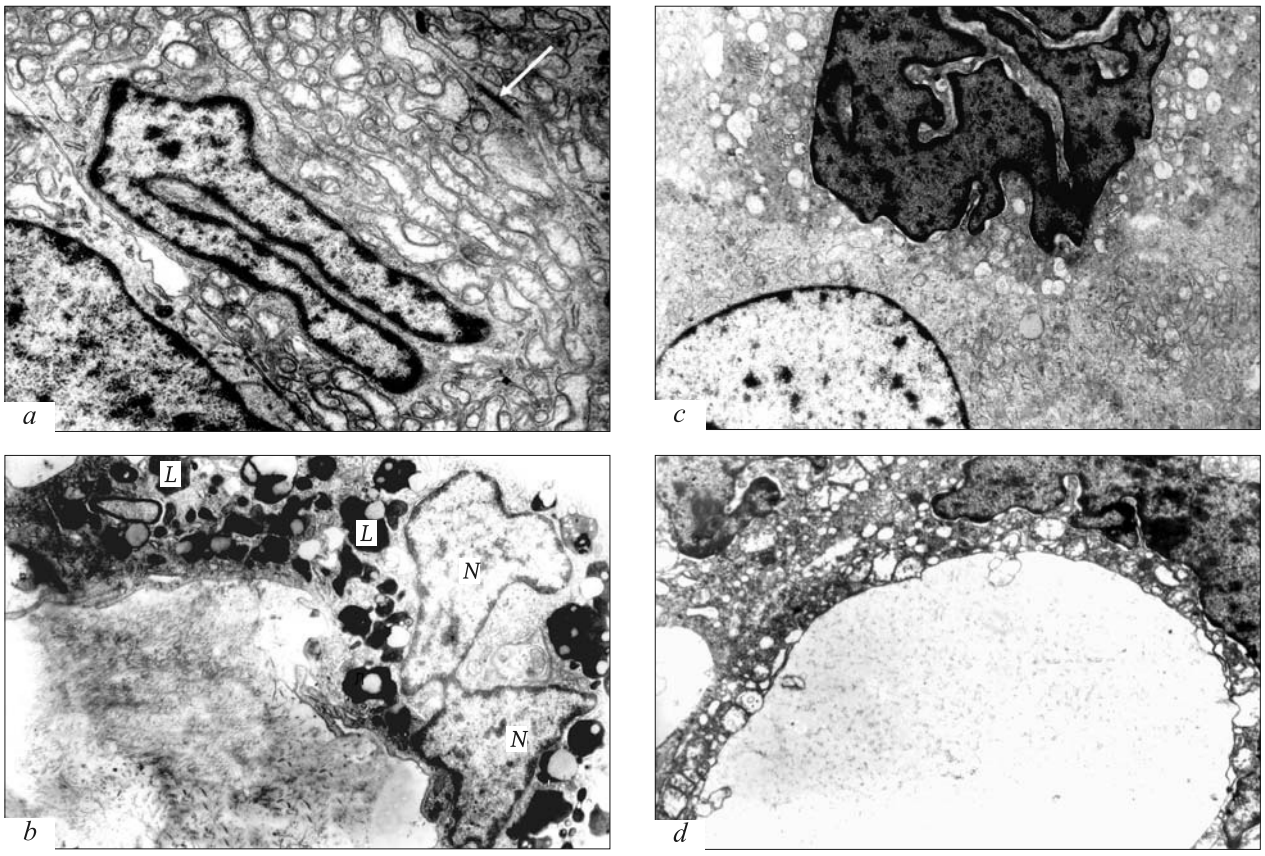

Fig. 3. Ultrastructural characteristics of SPPT. *a*) cell cytoplasm filled by large mitochondria (arrow shows cell-cell contact), ×18,000; *b*) tumor cell with lipofuchsin granules, ×12,000; *c*) two cell types, ×12,000; *d*) vacuolation of tumor cell cytoplasm, ×10,000. *N*: cell nucleus; *L*: lipofuchsin granules.

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