Expression of Adhesion Molecules and Cyclin D1 in Cells of Solid-Pseudopapillary Tumors of the Pancreas

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Disturbances in the expression of cell adhesion molecules in solid-pseudopapillary tumor cells were detected by immunohistochemical methods. Positive reaction to E-cadherin, catenins, and overexpression of cyclin D1 were found; these changes reflect peculiarities of morphological structure and biological behavior of the studied neoplasms.

Key Words: *cadherin; catenin; tumor; pancreas; cyclin*

Solid pseudopapillary tumors (CPPT) of the pancreas are considered to be rare neoplasms with unknown histogenesis. These neoplasms usually appear in young women and are characterized by relatively favorable prognosis [1,8]. According to current views, cell adhesion disorders and Wnt-signal pathway activation play a great role in oncogenesis of various tumors [6,14].

Here we studied expression of E-cadherin, catenin, and cyclin D1 in SPPT.

MATERIALS AND METHODS

The study was based on complex morphological investigation of surgical material from 15 patients of A. V. Vishnevskii Institute of Surgery in 1998-2008 (14 women aging 15-67 years, mean age 35.2 years, and one 61-year-old man). Repeated relapses were observed in a 50-year-old female; metastasis in the liver was found in a 49-year-old female.

Fragments of the tumor and surrounding pancreatic tissue were fixed in 10% neutral formalin. Histological investigation was carried out using 5-μ paraffin sections stained with hematoxylin and eosine. Immunohistochemical detection of the expression of E-cadherin (1:50; Novocastra), α-, β- and γ-catenins (1:50; Novocastra), and D1-cyclin (ready for use; LabVision) was carried out using standard peroxidase technique with nucleus post-staining with hematoxylin.

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RESULTS

Macroscopic examination showed that the tumor was located in the head, body, and tail of the pancreas in 7, 4, and 3 patients, respectively. The size of neoplasm varied from 2 to 13 cm (mean diameter 7.6 cm). All nodules had clear boundaries. In 9 cases, the tumor had solid structure on section and looked like yellowwhitish or pink-yellowish tissue with hemorrhagic foci. In 6 neoplasms, hemorrhagic foci and cavities filled with blood were found (cystic form of the tumor). Microscopic examination of SPPT samples revealed sites with solid and papillary structure. Pseudopapillae were presented by centrally located fibrous septum lined with monomorphic cubic cells without evidence of atypia (Fig. 1, *a*). These pseudopapillae were usually found in the central part of tumor node, while near the capsule the tumor had predominantly solid structure, similar to neuroendocrine tumors (Fig. 1, *b*).

Immunohistochemical investigation revealed abnormal expression of cell adhesion molecules (cadherin and catenins). The positive reaction of tumor cell nuclei to E-cadherin was observed in all cases (Fig. 2, *a*), while in normal pancreatic cells this marker was observed in cell membranes (Fig. 2, *b*).

Positive reaction to β-catenin was observed in both the cytoplasm and nuclei of tumor cells; moreover, it

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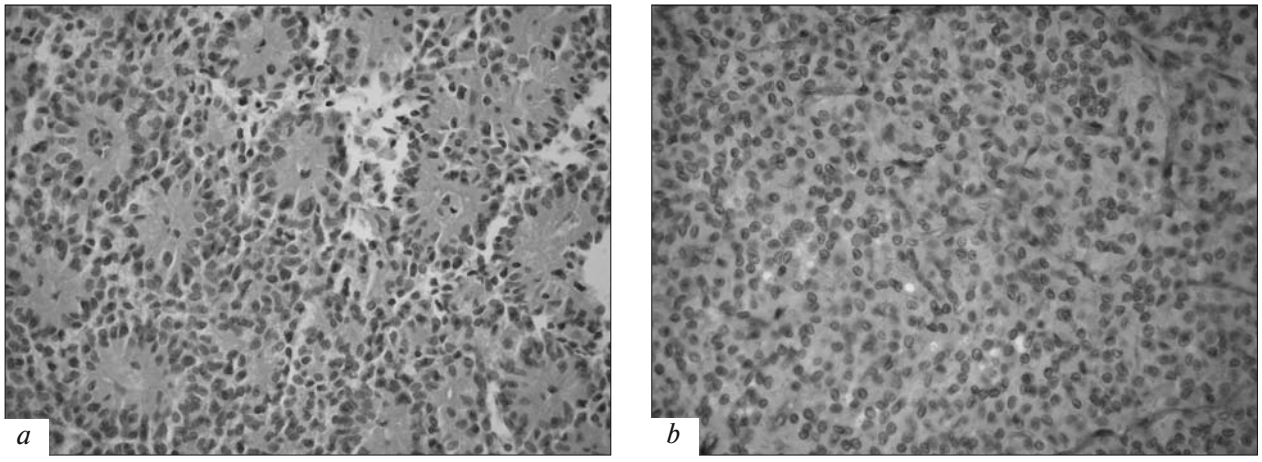

Fig. 1. Pseudopapillary (*a*) and solid (*b*) sites of pancreatic SPPT. Heamotoxylin and eosin staining, ×400.

was more pronounced in the nuclei (Fig. 2, *c*). Dramatic decrease in the expression was found in 6 cases and absence of γ-catenin expression in 9 cases. Reaction to α-catenin was negative in all examined tumor samples. In normal acinar, ductal, and insular cells, marked expression of all studied catenin types was noted (Fig. 2, *d*). We also observed positive reaction to D1 cyclin in tumor cell nuclei (Fig. 2, *e*) and negative in surrounding pancreatic tissue in all SPPT cases (Fig. 2, *f*).

While explaining disturbances in the expression of the studied markers in SPPT cells, it should be noted that E-cadherin is a member of transmembrane glycoprotein family mediating Ca^{2+}-dependent homophilic intercellular adhesion. Intracellular domain of E-cadherin interacts with β- or γ-catenin, which in turn contacts with α-catenin providing attachment to actin cytoskeleton of the cell. As a result, stability of cell structure and intercellular contacts is attained [1,12]. Thus, we revealed abnormal expression of E-cadherin in nuclei of SPPT cells, but not on the membrane, which is possibly caused by mutations in E-cadherin gene [4]. These changes lead to disturbances in cell adhesion in these tumors.

The disturbances in β-catenin expression in SPPT cells observed in out study are consistent with published data [13,14]. Apart from the role in cadherin–cytoskeleton coupling, β-catenin plays a role in transduction of Wnt signal via its interaction with Tcf/Lef transcription factors and activation of β-catenin–Tcf/Lef-dependent gene transcription [9]. Free β-catenin undergoes rapid degradation, which provides regulation of Wnt signal pathway [5]. Due to mutations in the 3rd exon of β-catenin gene, it instead of providing phosphorylation processes forms β-catenin-Tcf/Lef-complexes accumulating in nuclei, which was confirmed by immunohistochemical assay.

In the nucleus, this complex activates a number of oncogenes, *e.g.* cyclin D1. This leads to overexpression of cyclin D1 in the neoplasm [11]. Indeed, we found overexpression of D1 cyclin in tumor cell nuclei in all cases. Our results agree with previous reports, where the frequency of overexpression of D1 cyclin in SPPT attained 70-100% [3,6,7,14]. The complex of cyclin D1 and cyclin-dependent kinases (CDK) is responsible for phosphorylation of retinoblastoma (Rb) protein. This leads to the release and activation of transcription factor E2F, activation of various genes, transition of the cell into S phase of cell cycle. These processes result in neoplasm growth and disease progression.

Thus, abnormal expression of E-cadherin and catenin and overexpression of cyclin D1 were observed in SPPT cells, which seems to be a result of mutations in different genes. Similar changes result in disturbances of intercellular adhesion with the formation of pseudopapillary structures in the tumor tissue and in neoplasm progression. At the same time, SPPT are characterized by slow growth and low metastasizing rate, which attests to the existence of factors and mechanisms inhibiting the above mentioned pathways. These assumptions require further investigations.

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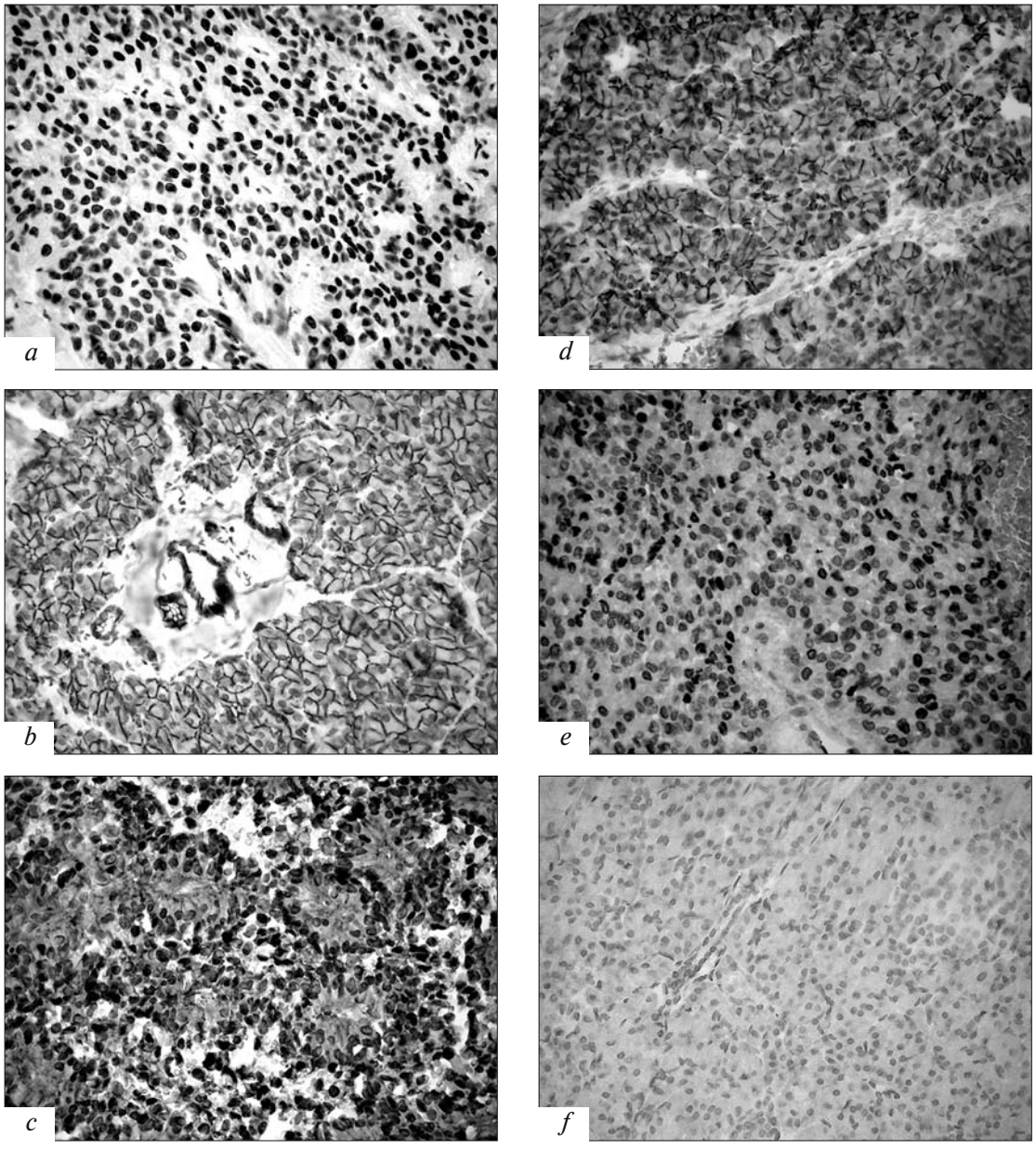

Fig. 2. Immunohistochemical characteristics of E-cadherin, β-catenin, and cyclin D1. Immunoperoxidase technique, ×400. *a*) localization of E-cadherin in nuclei of SPPT cells, *b*) localization of E-cadherin on the membrane of pancreatic cells, *c*) localization of β-catenin in nuclei and cytoplasm of tumor cells, *d*) localization of β-catenin on the membrane of pancreatic cells, *e*) overexpression of cyclin D1 in tumor cell nuclei, *f*) absence of D1 expression in pancreatic tissue.

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