Comparative Characteristics of Nuclear Ploidy of Cells in Endocrine and Solid Pseudopapillary Tumors of the Pancreas

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We carried out comparative morphometry and densitometry of cell nuclei in endocrine and solid pseudopapillary tumors and of exocrinocytes and endocrinocytes of the adjacent pancreatic tissue. The nuclear size, perimeters, and DNA content were higher in tumor cells. The index of proliferative activity, coefficient of aneuploidy, and histograms of DNA content in cell nuclei are recommended as additional criteria for differential diagnosis between endocrine and solid pseudopapillary tumors.

Key Words: *tumor; pancreas; ploidy; proliferation*

The endocrine (ET) and solid pseudopapillary tumors (SPPT) are rare pancreatic tumors constituting 1-2% tumors [5,12]. Comprehensive immunohistochemical studies (detection of specific markers) are needed for morphological and differential diagnosis of these tumors [3,6,9].

Evaluation of tumor cell nuclear ploidy [10] is a sufficiently reliable and objective method for morphological diagnosis of tumors; this characteristic reflects the regularities of the staged development of the tumor [1].

We compared nuclear ploidy of pancreatic ET and SPPT cells.

MATERIALS AND METHODS

A retrospective analysis of operation material from 18 patients treated at A. V. Vishnevsky Institute of Surgery in 1999-2008 was carried out. Pancreatic ET were diagnosed in 12 cases (11 women and 1 man aged 24-70 years; mean age 52 years). Pancreatic SPPT were detected in 6 patients (5 women and 1 man aged 18-67 years, mean age 41 year).

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Fragments of the tumor and adjacent tissue were fixed in 10% neutral formalin. Histological studies were carried out on paraffin sections (5 μ) stained with hematoxylin and eosin. In addition, preparations stained by Feulgen's method were examined. Nuclear area and perimeter and nuclear DNA content in tumor cells and cells of adjacent pancreatic tissue (acinar cells and Langerhans islet cells) were evaluated using a Mekos C1 TV image analyzer. The shape factor, index of proliferative activity, and coefficient nuclear aneuploidy [2] were calculated from morphometric and densitometric values. The content of DNA was expressed in ploidy units (c), the parameters of lymphocyte nuclei served as the diploid set standard. The index of proliferative activity characterizes the increase in DNA content in tumor cell nuclei at the expense of synthesis of genetic material, whose total content in the cell sample surpasses the standard diploid level (2 c). The aneuploidy coefficient reflects the proportion of nuclei with ploidy higher than 4 c (aneuploid nuclei) to nuclei with ploidy of 4 c and lower. The values were processed by methods of variation statistics.

RESULTS

Macroscopic examination of operation material showed that the ET were clearly separated from the

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glandular parenchyma. The tumors were 1-3.5 cm in diameter and were colored yellowish-white or pink-brownish on sections. The SPPT looked like solitary nodes of 1-6.5 cm, often fluctuating and well separated from the adjacent pancreatic parenchyma. Dissected tumor tissue was grayish-pink, with foci of necrosis and hemorrhages, with cystic formations filled with necrotic detritus.

Histological studies of ET preparations (mainly well-differentiated) showed several variants of their structure: solid, trabecular (Fig. 1, *a*), glandular-like, and pseudorosettes. Tumor cells were more or less homogeneous with granular eosinophilic cytoplasm or oval nucleus. The histological picture of SPPT (Fig. 1, *b*) in general resembled the picture of endocrine tumors. The tumors consisted of small monomorphic cells forming solid and papillary structures. Cell nuclei were round or oval, some nuclei looked like coffee beans. The cytoplasm was transparent or slightly eosinophilic. Papillary structures were detected usually in the center of the tumor node, while closer to the capsule the tumor was mainly presented by solid sites.

The results of comparative morphometric and densitometric analysis of the nuclei in ET and SPPT cells and of pancreatic intact exocrinocytes (acinocytes) and Langerhans islet cells (endocrinocytes) are presented (Table 1). The tumor cell nuclear areas and perimeters were higher in all ET and SPPT in comparison with acinar cells and endocrinocytes, which fact can be used for objective evaluation of the degree of cell atypia. The maximum nuclear size and perimeters, surpassing the corresponding values for normal exocrinocyte by 49.3 and 26.5%, respectively ($p<0.05$), were observed in SPPT. The nuclear areas and perimeters in ET and SPPT were similar, and hence, these parameters cannot be used for differential diagnosis. In turn, estimated values of tumor nucleus shape factor statistically did not differ from those of normal acinar cells and Langerhans islet endocrinocytes, which also precludes the use of this criterion for the diagnosis of these tumors.

Densitometric analysis of preparations showed that the mean levels of nuclear ploidy are 2.18 c for intact acinar cells and 2.21 c for Langerhans islet endocrinocytes, which slightly surpasses the diploid chromosome set and seems to reflect reactive changes in the pancreatic cells during tumor development. The ET and SPPT nuclear ploidy is higher than that of nontumorous cells: the mean nuclear ploidy for SPPT cells is 2.59 c, for ET cells 3.49 c.

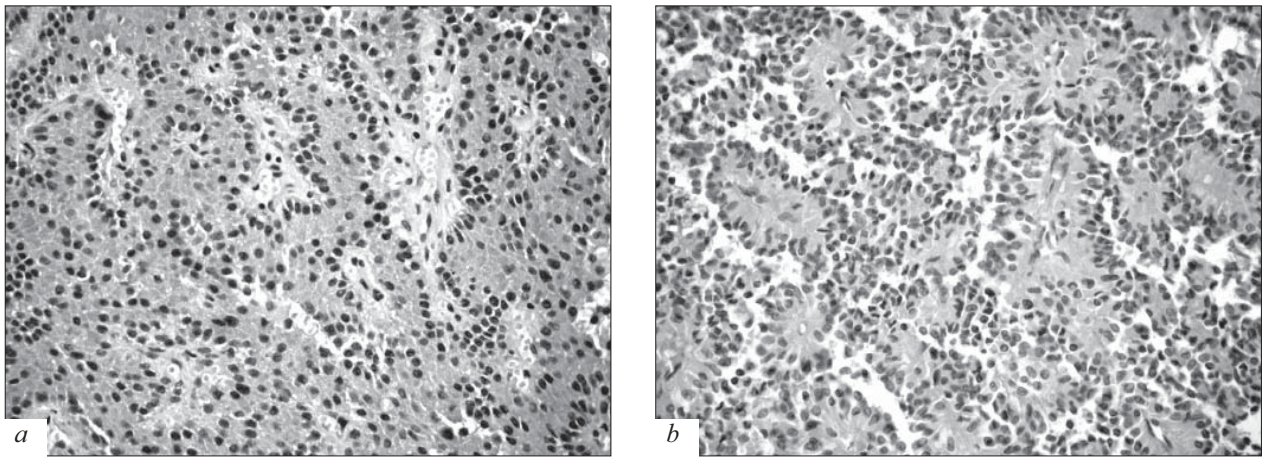

Fig. 1. Histological changes in pancreatic ET (*a*) and SPPT (*b*). Hematoxylin and eosin staining, ×400.

TABLE 1. Morphometric and Ploidometric Characteristics of the Nuclei in Pancreatic Acinar, Endocrine Cells, and ET and SPPT Cells ($M\pm m$)

Cells	Area, μ²	Perimeter, μ	Shape factor	Ploidy, c	PAI	AC
Acinar cells	20.46±1.5	16.65±0.7	0.91±0.06	2.18	0.18	0
Endocrinocytes	21.51±1.3	17.33±0.8	0.9±0.06	2.21	0.21	0
ET	29.2±1.9	20.39±0.8	0.87±0.07	3.49	1.49	0.78
SPPT	30.54±2.0	21.07±1.1	0.86±0.08	2.59	0.59	0.04

Note. PAI: proliferative activity index; AC: aneuploidy coefficient.

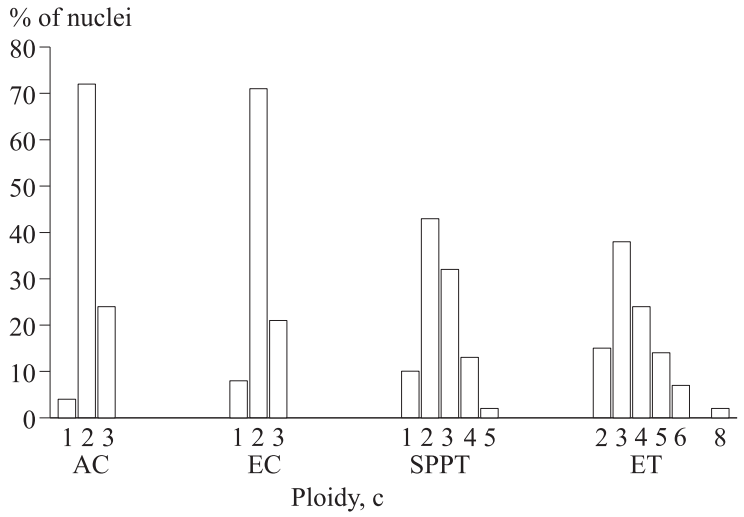

Fig. 2. Nuclear content of DNA in pancreatic acinar (AC), endocrine cells (EC), and SPPT and ET cells.

Estimated proliferative activity index and aneuploidy coefficient also surpassed the normal. Proliferative activity of SPPT more than 3-fold surpassed that of normal exocrinocytes; proliferative activity of ET cells 7-fold surpassed that of endocrinocytes. The aneuploidy coefficient of SPPT and ET cells was 0.04 and 0.78, respectively. These parameters of proliferative activity and aneuploidy reflected more intense multiplication of tumor cells, degree and quality of cell changes during oncogenesis, and can be used for differential diagnosis of ET and SPPT.

In addition to evaluation of the mean levels of tumor cell ploidy, we plotted histograms of DNA content in the nuclei of normal and tumor cells (Fig. 2). Diploid nuclei predominated in nontumor acinocytes and endocrinocytes. Diploid nuclei predominated in SPPT as well (43%), but there were also tri- (32%) and tetraploid nuclei (13%). Nuclei with 3 c ploidy predominated in ET (38%), but the histogram was clearly shifted to the right because of numerous nuclei with 4 c ploidy (24%) and appearance of nuclei with 5 c (15%) and even 8 c ploidy (2%).

Reports on DNA content in pancreatic ET are rare. The majority of authors [7,11] noted poor diagnostic significance of ploidometric study for differential diagnosis of benign and malignant ET. We also failed to detect reliable signs of ET malignancy. On the other hand, it is assumed that higher ploidy of tumor cells increases the risk of malignant degeneration of the tumor [4]. Our data on predominantly diploid nuclei in SPPT cells are in good agreement with published data [8].

However, our data indicate that some morphometric (nuclear area and perimeter) and densitometric parameters (proliferative activity index, aneuploidy coefficient) can be used for tumor detection in the pancreas. The index of proliferative activity, aneuploidy coefficient, and histograms of nuclear DNA content can be recommended as additional tests for the differential diagnosis of ET and SPPT.

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