Nuclear Ploidy as an Indicator of Malignancy of Intraductal Pancreatic Papillary Mucinous Tumors

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We carried out comparative morphometric and densitometric analysis of cell nuclei in pancreatic intraductal papillary mucinous tumors. Proliferative activity values, aneuploidy coefficient, and histogram of nuclear DNA content are recommended as additional criteria for the diagnosis of tumor malignancy.

Key Words: *intraductal papillary mucinous tumor; ploidy; pancreas*

Intraductal papillary mucinous tumors (IPMT) of the pancreas constitute 0.5-9.8% of all exocrine pancreatic tumors [5]. The following tumors are distinguished in accordance with the International Histogenetic Classification of Pancreatic Tumors: benign (adenomas), borderline, and malignant (cancer) IPMT [8]. The degree of tumor cell dysplasia and atypia [3,10], epidermal growth factor and its receptor expression [14], telomere length and telomerase activity [9] are used as differential diagnostic criteria.

In addition to these, evaluation of tumor cell nuclear ploidy is a sufficiently clear-cut and objective morphological indicator of tumor malignancy [2].

We carried out a comparative study of nuclear ploidy in pancreatic IPMT of different malignancy.

MATERIALS AND METHODS

Operation material from 16 patients was analyzed. The patients (3 women and 13 men aged 36-71 years) were treated at A. V. Vishnevsky Institute of Surgery in 2004-2009. Comprehensive morphological studies were carried out in accordance with the WHO recommendations [10]. Adenomas were diagnosed in 3 patients, borderline tumors (with indefinite malignancy potential) in 4. Malignant tumors were detected in 9 cases, in 3 of these invasive cancer.

Fragments of the tumors with adjacent tissue were fixed in 10% neutral formalin. Histological studies were carried out on paraffin sections (5 μ) stained with hematoxylin and eosin. The areas and perimeters of the nuclei, DNA content in nuclei of tumor cells and intact Langerhans islet endocrinocytes were evaluated in preparations stained after Feulgen using a MECOS C1 television image analyzer. The nuclear shape factor, proliferative activity index (PAI), and aneuploidy coefficient (AC) [2] were calculated from morphometric parameters. DNA content was expressed in ploidy units (c). The lymphocyte nuclear values served as the diploid set reference. The PAI characterizes the increase of DNA content in tumor cell nuclei due to synthesis of genetic material, if this increase (total for a sampling of cells) surpasses the standard diploid level (2c). The AC reflects the proportion of the number of nuclei with ploidy higher than 4c (aneuploid nuclei) to the remaining nuclei with ploidy of 4c and lower. The numerical values were processed by parametric and nonparametric methods of variational statistics.

RESULTS

Involvement of the main pancreatic duct primarily near the head of the gland was detected in 13 cases, involvement of peripheral branches of the pancreatic duct in 3 cases.

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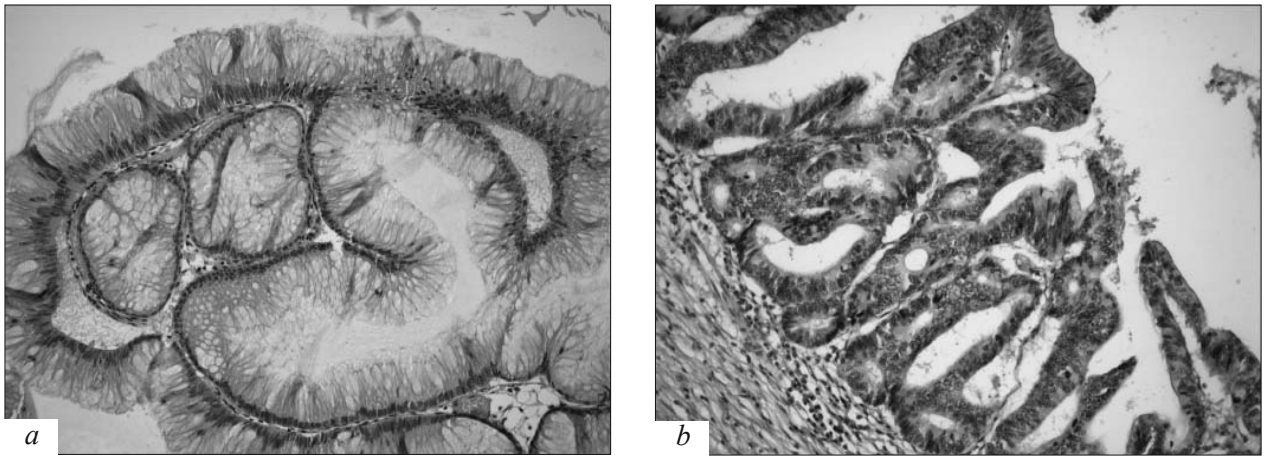

Fig. 1. Histological changes in pancreatic IPMC. *a*) adenoma; *b*) cancer without signs of invasion. Hematoxylin and eosin staining, ×200.

Histological studies showed tumors presented by cylindrical mucin-producing cells forming papillary structures in 3 cases. These cells were characterized by basal nuclei and the presence of mucin in the apical part (Fig. 1, *a*). In 4 cases, papillary structures of various shapes were combined with cell stratification and signs of moderate dysplasia and focal atypia. These changes indicated borderline IPMT. The presence of severe dysplasia, nuclear polymorphism, and mitotic figures indicated intraductal papillary mucinous cancer. The papillae branched forming cribriform structures; virtually no mucin was detected in the cytoplasm (Fig. 1, *b*). Tumor growth into the adjacent pancreatic parenchyma was detected in 3 patients, and invasive cancer was diagnosed in them.

The results of comparative morphometric and densitometric analysis of cell nuclei in intact ducts and pancreatic IPMC are summed up in Table 1. Tumor cell nuclear size and perimeters were enlarged in the majority of IPMC cases in comparison with normal ductal cells. The maximum nuclear area and perimeter, surpassing the normal values by 74.0 and 53.4% ($p<0.05$), were observed in noninvasive cancer. The mean area of cell nuclei in benign tumors virtually did not differ from the normal values, while the parameters of borderline tumors were even 8.0% lower ($p>0.05$). In invasive cancer, the mean area of the nuclei was intermediate between the values in adenoma and noninvasive cancer. The mean nuclear perimeter in invasive cancer cells was the same as in adenoma. These morphometric characteristics of cell nuclei impede the use of these parameters for the differential diagnosis. Estimated values of IPMC cell nuclei shape factor in noninvasive cancer were significantly lower than the values for normal ductal cells ($p<0.05$).

Densitometric analysis of the preparations showed that the mean ploidy of intact pancreatic duct cell nuclei was 2.4c (higher than the diploid chromosome set; Table 1). These changes in the ductal cells seem to be reactive. The nuclear ploidy of tumor cells was higher than that of normal ductal cells. Higher malignancy was usually associated with higher content of DNA in tumor cell nuclei, thus reflecting the known regularity of staged development of tumors [1]. The mean nuclear ploidy for adenoma was 2.5c, for borderline tumors 3.0c. On the other hand, noninvasive cancer ploidy was 5.1c, while that of invasive cancer only 4.5c.

The PAI and AC values calculated for tumor cells also surpassed the reference values (Table 1).

TABLE 1. Morphometric and Ploidometric Characteristics of the Nuclei in Pancreatic Normal Ductal Epithelium and Cells of IPMC of Different Differentiation Degree (*M*±*m*)

Diagnosis	Area, μ²	Perimeter, μ	Shape factor	Ploidy (c)	PAI	AC
Normal ducts	30.0±1.4	20.8±1.2	0.86±0.05	2.4	0.4	0
Adenoma	30.5±1.6	23.1±1.4	0.73±0.06	2.5	0.5	0
Borderline tumor	27.6±1.6	21.9±1.6	0.71±0.06	3.0	1.0	0.11
Noninvasive cancer	52.2±1.7	31.9±1.8	0.64±0.07	5.1	3.1	1.03
Invasive cancer	34.5±2.3	23.1±1.7	0.79±0.07	4.5	2.5	0.62

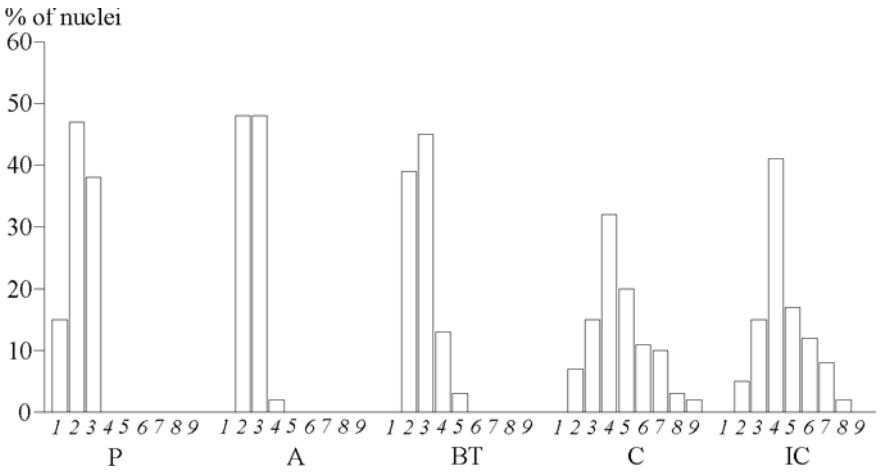

Fig. 2. Content of DNA in IPMC cell nuclei. P: normal pancreatic ducts; A: adenoma; BT: borderline tumor; C: noninvasive cancer; IC: invasive cancer.

Adenoma cell PAI was 25% higher than the values for intact pancreatic ducts and 2.5 times higher than the values in borderline tumors. The maximum PAI were observed in noninvasive cancer. No aneuploid nuclei were detected in benign tumors (AC=0). Ploidometric studies of borderline tumors characterized by signs of dysplasia detected aneuploid nuclei (AC=0.11). The maximum AC was found for noninvasive cancer cells. Invasive forms were characterized by lower AC. However, these changes in proliferative activity and nuclear aneuploidy seem to reflect to a certain measure more intensive proliferation and transformation of cells during oncogenesis and can be used for evaluation of IPMC malignancy.

Analysis of DNA content in normal and tumor cell nuclei yielded interesting results (Fig. 2). The greater part of cells in the intact pancreatic ducts had diploid nuclei, but 38% had a triple chromosome set. Adenoma cells had the same portions of diploid and triploid cells (48% each), and 2% nuclei were tetraploid. Nuclei with 3c ploidy predominated in borderline tumors. Tetraploid nuclei predominated in malignant tumors and the histograms were shifted to the right because of greater number of aneuploid nuclei.

Our results are in general in line with published data on ploidy changes in tumor cell nuclei. High levels of nuclear ploidy were detected in ductal adenocarcinoma cells in patients [4,13] and in animals with experimental pancreatic cancer [12]. Unfortunately, only few studies were focused on the analysis of DNA content in pancreatic IPMC. Some authors noted a diploid chromosome set in adenoma nuclei and detected aneuploid nuclei in carcinoma [11]. Others detected only diploid nuclei in all studied IPMC (benign, borderline, and malignant) [6].

Hence, morphometry (nuclear area and perimeter) and densitometry help to detect tumor cells. The PAI, AC, and histograms of nuclear DNA content can be recommended as additional tests for evaluating malignancy of endocrine tumors of the pancreas.

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