Nuclear Ploidy Is an Indicator of Malignancy Endocrine Pancreatic Tumor

E. A. Dubova, M. N. Podgornova, and A. I. Shchyogolev

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 149, No. 2, pp. 197-200, February, 2010
Original article submitted August 17, 2009

Comparative morphometric and densitometric studies of cell nuclei in endocrine tumors of various differentiation degree and endocrinocytes of Langerhans islet in the adjacent pancreatic tissue were carried out. Index of proliferative activity, aneuploidy coefficient, and histogram of nuclear DNA content are recommended as additional criteria for the diagnosis of endocrine tumor malignancy.

Key Words: *pancreas; ploidy; endocrine tumor*

Endocrine tumors of the pancreas are responsible for 1-2% of all pancreatic tumors [8]. According to WHO recommendations, the presence of metastases and/or growth into adjacent organs and tissues are absolute criteria of endocrine tumor malignancy [8]. Other signs of unfavorable prognosis are tumor size, presence of necrotic foci, great number of mitoses in tumor cells, invasion into the capsule, high index of tumor cell proliferation (Ki-67), high density of nuclei on histological sections, high nucleus/cytoplasm ratio, loss of chromosome 17p13 and 22q heterozygocity, detection of chorionic gonadotropin in tumor cells or absence of progesterone receptors in them [3,9,10].

Evaluation of tumor cell nuclear ploidy is a sufficiently accurate and objective morphological indicator of the degree of tumor malignancy [4,12].

We compared nuclear ploidy in pancreatic endocrine tumors of different malignancy.

MATERIALS AND METHODS

A retrospective analysis of operation material from 46 patients (35 women and 11 men aged 15-79 years) treated at A. V. Vishnevsky Institute of Surgery in 1999-2008 was carried out. Based on comprehensive morphological studies in accordance with the WHO recommendations [8], adenomas were diagnosed in 8 patients and tumors with indefinite malignancy potential (borderline tumors) in 19 patients. Pancreatic cancer was detected in 19 cases: well-differentiated (WDC) in 15 and poorly differentiated endocrine cancer (PDC) in 4 cases.

Fragments of the tumor and adjacent tissue were fixed in 10% neutral formalin. Histological study was carried out on 5-μ paraffin sections stained with hematoxylin and eosin. Nuclear area, perimeter, and DNA content in tumor cell and intact Langerhans islet endocrinocytes were evaluated on preparations stained after Feulgen using a Mecos C1 image analyzer. The shape factor, proliferative activity index (PAI), and nuclear aneuploidy coefficient were calculated from morphometric values [2]. The content of DNA was expressed in ploidy units (c), lymphocyte nucleus values served as the reference diploid set value. Proliferative activity index characterizes the increase in DNA content in tumor cell nuclei due to synthesis of genetic material, which surpasses the standard diploid level in the cell sample in general (2c). The aneuploidy coefficient reflects the proportion of nuclei with ploidy >4c (aneuploid nuclei) to the rest nuclei with ploidy ≤4c. The values were processed by methods of variation statistics.

Department of Pathological Anatomy, A. V. Vishnevsky Institute of Surgery, Moscow, Russia. ***Address for correspondence:*** shegolev@ixv.comcor.ru. A. I. Shchyogolev

0007-4888/10/1492-0236

RESULTS

Histological study of endocrine tumors in preparations stained with hematoxylin and eosin showed several variants of their structure: alveolar (Fig. 1, *a*), trabecular, glandular-like, solid, and pseudorosettes. Tumor cells were homogeneous with granular eosinophilic cytoplasm and central round or oval nuclei. In the majority of cases nuclear cells of benign and borderline tumors contained large granules of heterochromatin ("salt with pepper"). Comprehensive morphological study of the operation material revealed metastases into local lymph nodes, liver, or growth into the adjacent cells in 19 patients, and endocrine cancer was diagnosed in these cases. WDC had primarily trabecular solid structure. Poorly differentiated forms consisted of nests and layers of cells with scanty cytoplasm and hyperchromatic nuclei and mitotic cells (Fig. 1, *b*).

Comparative morphometric and densitometric analysis of nuclei in intact Langerhans islet endocrinocytes and pancreatic tumor cells are summed up in Table 1. Tumor cell nuclei were larger and had longer perimeters in comparison with normal endocrinocytes in all cases, the degree of this enlargement increasing with the decrease in tumor differentiation degree. The maximum areas and perimeters of the nuclei surpassing the corresponding parameters of normal endocrinocytes by 54.7 and 27.2% ($p<0.05$) were observed in PDC. The areas and perimeters of cell nuclei in the borderline and well-differentiated endocrine carcinomas virtually did not differ, while the values in adenomas did not differ statistically from those in normal cells ($p>0.05$). These morphological features of cell nuclei impede their use for differential diagnosis. Estimated values of nuclear shape factor in endocrine tumors of different differentiation degree also did not differ statistically between each other and from the values of Langerhans islet normal endocrinocytes ($p>0.05$).

Densitometric analysis of preparations showed that the mean nuclear ploidy of intact Langerhans islet endocrinocytes was 2.5c, which surpasses the diploid chromosome set. Presumably, these changes in islet cells adjacent to the tumor node are reactive. Nuclear ploidy in tumor cells surpassed that of normal endocrinocytes. Higher malignancy is associated with higher contents of DNA in tumor cell nuclei and reflects a known regularity, staged development of tumors [1]. The mean nuclear ploidy of benign

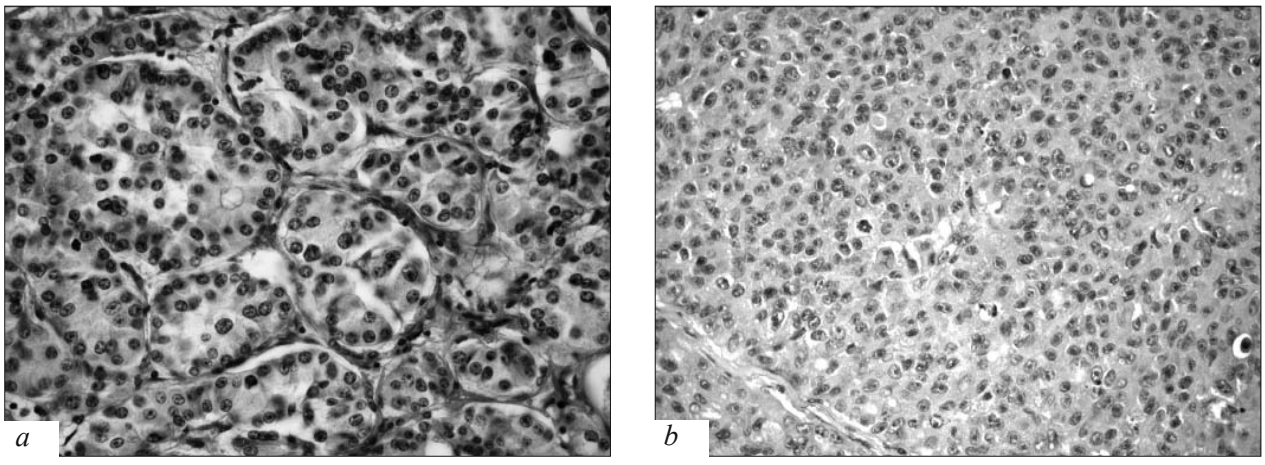

Fig. 1. Histological changes in pancreatic endocrine tumors. *a*) adenoma; *b*) PDC. Hematoxylin and eosin staining (×400).

TABLE 1. Morphometric and Ploidometric Characteristics of Langerhans Islet Endocrinocytes and Pancreatic Endocrine Tumor Cells of Different Differentiation Degree ($M\pm m$)

Tumor	Area, μ²	Perimeter, μ	Shape factor	Ploidy, c	PAI	Aneuploidy coefficient
Endocrinocytes	21.4±1.4	17.3±1.2	0.86±0.04	2.5	0.5	0
Adenoma	23.7±1.6	18.3±1.4	0.88±0.07	3.0	1.0	0.06
Borderline tumor	28.1±1.6	19.9±1.6	0.87±0.07	3.6	1.6	0.35
Well-differentiated endocrine cancer	28.0±1.7	19.8±1.8	0.88±0.07	4.0	2.0	0.49
Poorly differentiated endocrine cancer	33.1±2.3	22.0±1.8	0.84±0.07	4.1	2.1	0.61

tumor cells is 3c, while malignant cell nuclear ploidy corresponds to tetraploid cells. The HDC and PDC ploidy levels are virtually the same and hence, cannot be used for the differential diagnosis of endocrine cancer.

Estimated values of PAI and aneuploidy coefficient for tumor cells also surpassed the normal values (Table 1). Benign tumor cell PAI was 2-fold and of WDC cells 4-fold higher than that of normal endocrinocytes. Cell nuclear aneuploidy coefficient increases in parallel with the increase in endocrine tumor malignancy. Hence, changes in proliferative activity and nuclear aneuploidy to a certain measure reflect intensification of cell multiplication and transformation during oncogenesis, and presumably can be used for evaluation of endocrine tumor malignancy.

Analysis of histograms of DNA content in normal and tumor cell nuclei has revealed rather interesting characteristics of endocrine tumor cells (Fig. 2). The greater part of nontumorous endocrinocytes has diploid nuclei. Diploid nuclei predominate (34%) in adenoma cells as well, but there are also tetraploid nuclei (26%). Tetraploid nuclei predominate in borderline tumors. A shift of the histogram to the right due to accumulation of aneuploid nuclei is characteristic of malignant tumors. More pronounced changes are observed in poorly differentiated forms, which have 6c and higher ploidy (12 and 4%, respectively).

Our results are in general in line with published data. Unfortunately, reports about analysis of DNA content in pancreatic endocrine tumors are rare. Some authors noted poor diagnostic value of ploidometric studies for the differentiation between benign and malignant endocrine tumors [11,14]. Others think that an increase in the number of tumor cell polyploid nuclei correlates with worse prognosis [6]. Moreover, the number of aneuploid nuclei is higher in malignant tumors [13]. Nuclei with 3c and higher ploidy are more often detected in endocrine cancer preparations [7].

Hence, the use of morphometric (nuclear area and perimeter) and densitometric parameters helps to detect tumor cells. The PAI, aneuploidy coefficient, and histogram of DNA content in cell nuclei are recommended as additional criteria for evaluation of pancreatic endocrine tumor malignancy.

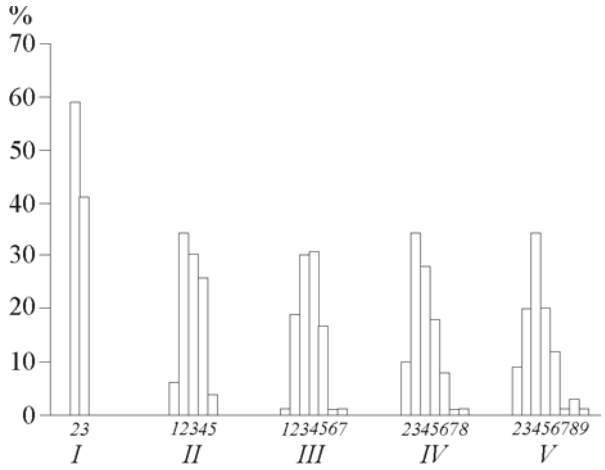

Fig. 2. Histograms of DNA content in the nuclei of Langerhans islet endocrinocytes and pancreatic endocrine tumor cells. *I*) Langerhans islets; *II*) adenoma; *III*) borderline tumor; *IV*) WDC; *V*) PDC. Abscissa: ploidy (c); ordinate: percentage of nuclei.

REFERENCES

1. G. G. Avtandilov, *The Law of Staged Development of Tumors* [in Russian], Moscow (2005).
2. G. G. Avtandilov, *Diagnostic Medical Ploidometry* [in Russian], Moscow (2006).
3. L. E. Gurevich, *Manual of Immunohistochemical Diagnosis of Human Tumors* [in Russian], Eds. S. V. Petrov and N. T. Raikhlin, Kazan (2004), pp. 76-92.
4. A. I. Shchyogolev and E. A. Dubova, *Byull. Eksp. Biol. Med.*, **148**, No. 9, 313-317 (2009).
5. A. I. Shchyogolev, E. A. Dubova, N. N. Shchyogoleva, and O. D. Mishnyov, *Pancreatic Endocrine Tumors* [in Russian], Moscow (2007).
6. K. A. Alanen, H. Joensuu, P. J. Klemi, *et al.*, *Am. J. Clin. Pathol.*, **93**, No. 6, 784-788 (1990).
7. T. Böttger, C. Siedl, and J. K. Seifert, *Oncology*, **54**, No. 4, 318-323 (1997).
8. P. U. Heitz, P. Komminoth, A. Perren, *et al.*, *Pathology and Genetics of Tumors of Endocrine Organs*, Eds. R. A. De Lellis *et al.*, Lyon (2004), pp. 177-182.
9. D. S. Klimstra, *Mod. Pathol.*, **20**, 94-112 (2007).
10. G. Klöppel, *Pathology*, **42**, 265 (2003).
11. T. H. Liu, H. R. Liu, L. N. Guo, and S. F. Ye, *Clin. Med. J.* (Engl.), **105**, No. 4, 271-276 (1992).
12. W. Mellin, *Path. Res. Pract.*, **186**, No. 1, 37-62 (1990).
13. F. Stipa, M. Arganini, M. Bibbo, *et al.*, *Surgery*, **102**, No. 6, 988-998 (1987).
14. T. Tomita, *Pancreas*, **12**, No. 1, 36-47 (1996).