Nuclear Ploidy and Intensity of Proliferation of Hepatocellular Cancer Cells

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Comparative morphometric and densitometric study of tumor cells and adjacent hepatocyte nuclei was carried out in hepatocellular cancer. Enlargement of the nuclei and increase in DNA content and index of proliferation of Ki-67 tumor cells inversely correlated with differentiation degree of hepatocellular cancer, which reflects the regularity of staged development of tumors.

Key Words: *hepatocellular cancer; ploidy; proliferation*

The tasks of morphological diagnosis of primary hepatic cancer are to rule out its metastasizing, identify histogenesis, and evaluate the differentiation degree. Histogenesis of the tumor is verified by immunohistochemical detection of specific markers of hepatocytes and cholangiocytes [4,14]. In order to evaluate the differentiation degree, it is recommended to count cells positively reacting to Ki-67 or evaluate the level of nuclear ploidy [2,5].

We compared the nuclear ploidy and level of tumor cell proliferation in hepatocellular cancer of different degree of differentiation.

MATERIALS AND METHODS

Operation material from 17 patients with hepatocellular cancer (7 women and 10 men) aged 18-66 years, observed at A. V. Vishnevsky Institute of Surgery in 2007-2008, was analyzed.

Tissue fragments were fixed in 10% neutral formalin. Histological study was carried out on paraffin sections (5 μ) stained with hematoxylin and eosin. The areas and perimeters of the nuclei and their DNA content in tumor cells and adjacent hepatocytes were evaluated using a Mecos C1 television image analyzer on preparations stained after Feulgen. The shape factor was calculated from morphometric values; the index of proliferative activity (IPA) and nuclear aneuploidy coefficient (AC) [2] were estimated. The content of DNA was expressed in ploidy units (c). The IPA characterizes the increase in DNA content in tumor cell nuclei at the expense of synthesis of the genetic material, the summary content of which in the cell sample surpasses the standard diploid level (2c). AC reflects the proportion between the content of nuclei with more than 4c ploidy (aneuploid nuclei) to nuclei with ploidy of 4c and lower. The level of tumor cell proliferation was evaluated by counting Ki-67-positive nuclei, detected by the immunoperoxidase method. The values were processed by methods of variation statistics.

RESULTS

Macroscopic examination of the operation material showed that the tumors were round formations 2-28 cm in diameter with well-discernible border. Dissected tissue was yellowish or whitish-yellowish.

Histological analysis of 6 preparations showed tumors presented by mainly trabeculas, this indicating well-differentiated cancer (Fig. 1, *a*). In 6 patients, glandular-like structures were presented by mainly acidophilic cytoplasm and hyperchromatic nuclei (moderately differentiated cancer; Fig. 1, *b*). Solid areas of large and sometimes giant hyperchromatic tumor

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cells without trabecular and glandular-like structures indicated poorly differentiated carcinoma (3 cases). In 2 patients, round tumor cells had basophilic cytoplasm and hyperchromatic nuclei occupying almost the entire tumor cells and forming no tissue structures of any kind; hence, undifferentiated hepatocellular cancer was diagnosed in these cases (Fig. 1, *c*).

Results of comparative morphometric and densitometric analysis of tumor cell nuclei and nuclei in visually intact hepatocytes from adjacent tissue are summed up in Table 1. Enlargement of the nuclear area and increase of perimeters were observed in all cases with hepatocellular cancer, the degree of the increase depending on the degree of tumor tissue differentiation. The maximum areas and perimeters of the nuclei surpassing the normal values by 105.5 and 50% ($p<0.05$), respectively, were observed in poorly differentiated form. These morphometric parameters reflect the presence of large and giant tumor cells and can be used for objective evaluation of cell atypia degree.

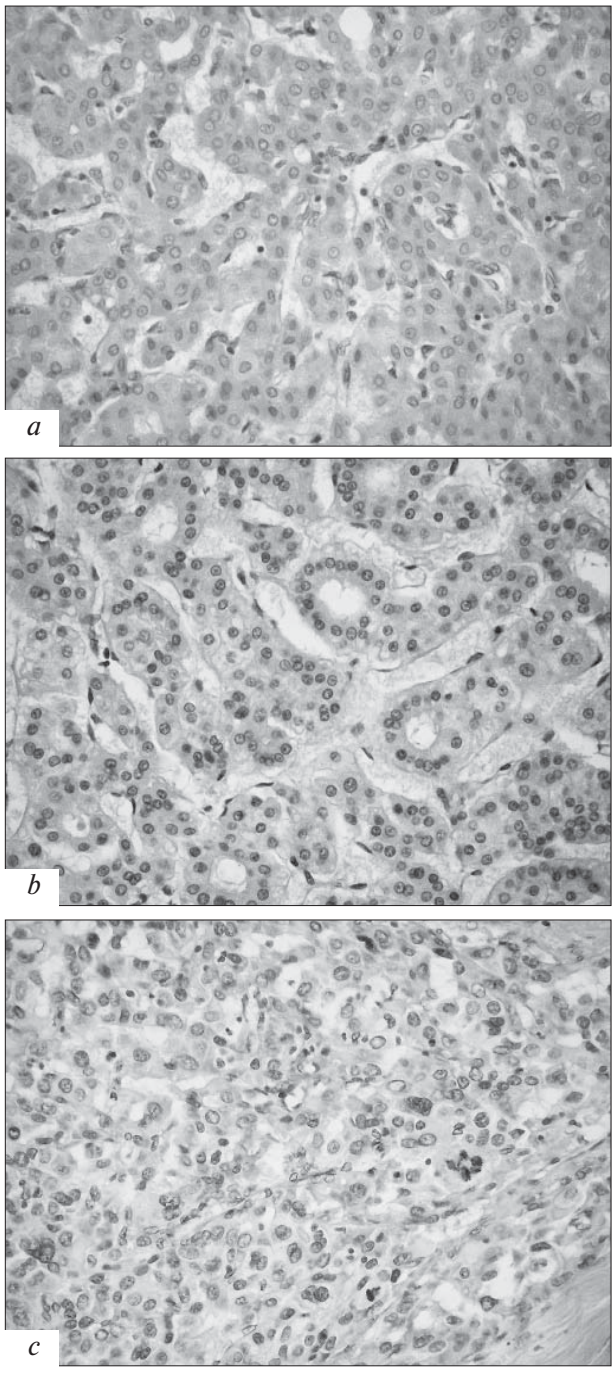

Fig. 1. Histological changes in well-differentiated (*a*), moderately differentiated (*b*), and undifferentiated (*c*) hepatocellular cancer. Hematoxylin and eosin staining, ×400.

Unfortunately, the nuclear area and perimeters less differ from the normal values in undifferentiated cancer (by 24.8 ($p<0.05$) and 16.3% ($p>0.05$), respectively). This is not so important for differential diagnosis in studies of histological preparations, because undifferentiated hepatocellular cancer is characterized by hyperchromatic cells without the typical trabecular structure of the organ. However, this indicates poor efficiency of morphometric analysis of cytological preparations collected by fine needle biopsy. Estimated values of tumor nucleus shape factor statistically did not differ from those of normal hepatocytes, and therefore, this criterion cannot be recommended for the diagnosis of hepatocellular cancer.

Densitometric analysis of the preparations showed that the mean nuclear ploidy of intact hepatocytes is 2.6c, which surpasses the diploid chromosome set. These changes in hepatocytes located near the tumor node are presumably caused by the compensatory adaptive processes. Hepatocellular cancer cell nuclear ploidy was higher than that of normal hepatocytes. The degree of excess inversely correlated with the tumor differentiation degree: the mean nuclear ploidy in well-differentiated cancer virtually corresponded to a tetraploid cell, while the ploidy level in undifferentiated cancer was 5.5c (Table 1).

Estimated IPA and AC values were also significantly higher than in health. The values increased with decreasing tumor differentiation degree. For example, IPA in well-differentiated cancer was 3-fold higher than in health, while in undifferentiated cancer it was 6-fold higher; AC was 2.6 and 15.9 times higher, respectively. These characteristics of proliferative activity and aneuploidy reflect significant intensification of tumor cell multiplication, as well as the degree and quality of cell changes in the course of carcinogenesis.

It is noteworthy that not only the mean ploidy values, but also cell distribution by ploidy levels are essential for the differential diagnosis. Histograms of DNA content in intact hepatocyte and hepatocellular cancer cell nuclei (Fig. 2) clearly show that the cancer histogram is shifted to the right at the expense of emergence of polyploid cells. Moreover, tetraploid cells predominate in well-, moderately, and poorly

TABLE 1. Morphometric and Ploidometric Characteristics of Hepatocyte and Tumor Cell Nuclei in Hepatocellular Cancer (HCC) of Different Differentiation Degree (*M*±*m*)

Diagnosis	Area, μ²	Perimeter, μ	Shape factor	Ploidy, c	IPA	AC	Ki-67, %
Normal values	32.7±2.8	21.4±1.2	0.88±0.04	2.6	0.6	0.14	0.5
Well-differentiated HCC	44.9±3.2	24.9±1.4	0.86±0.06	3.9	1.9	0.37	2.6
Moderately differentiated HCC	62.4±4.3	30.1±1.7	0.84±0.07	4.6	2.6	0.89	14.5
Poorly differentiated HCC	67.2±5.1	32.1±1.8	0.79±0.07	5.0	3.0	1.13	50.2
Undifferentiated HCC	40.8±2.7	24.9±1.6	0.81±0.07	5.5	3.5	2.23	9.8

differentiated forms, while 5c cells predominate in undifferentiated cancer.

Hence, the detected changes in the size and DNA content in tumor cell nuclei to a certain measure reflect the augmenting atypia and de-differentiation in hepatocellular cancer. These results are in good agreement with published data indicating that tumor malignancy increases with increasing nuclear area [3,11]. Some authors noted the largest nuclear areas in pleiomorphic form and the smallest in trabecular structure of hepatocellular cancer [13]. Moreover, it was showed that patients' life span inversely correlates with the size of tumor cell nuclei [12].

The content of DNA in tumor cell nuclei is a sign for differentiation between benign and malignant tumors of the liver [10], characterizing the staged pattern of tumor development [1]. Hypertriploid, tetraploid, and multiclone DNA histograms are detected in hepatocellular cancer [7]. According to some authors [9], the ploidy level is not only a diagnostic criterion, but also a sufficiently accurate prognostic factor for patients with hepatic cancer. Diploidy is associated with better survival than aneuploidy.

On the other hand, some authors [6,8] think that tumor cell proliferation index evaluated by immunoperoxidase method is a more accurate indicator of hepatic tumor differentiation. We analyzed proliferative activity by counting cells with nuclear Ki-67 positive immunohistochemical reaction. This protein detects proliferating cells at different phases of the cycle and, as the most accurate marker of proliferation, reflects the entire pool of dividing cells. The Ki-67 is a short-living protein, which is destroyed within 1-1.5 h after the start of its synthesis, and is therefore not accumulated and not detected in silent cells.

The proliferation index of visually intact hepatocytes was 0.5%. The content of proliferating cells was significantly higher in hepatocellular cancer preparations (Fig. 3, Table 1). The proliferation index in well-differentiated form 5.2-fold surpassed the normal. The maximum content (50.2%) of Ki-67-positive cells was detected in poorly differentiated form, which is in

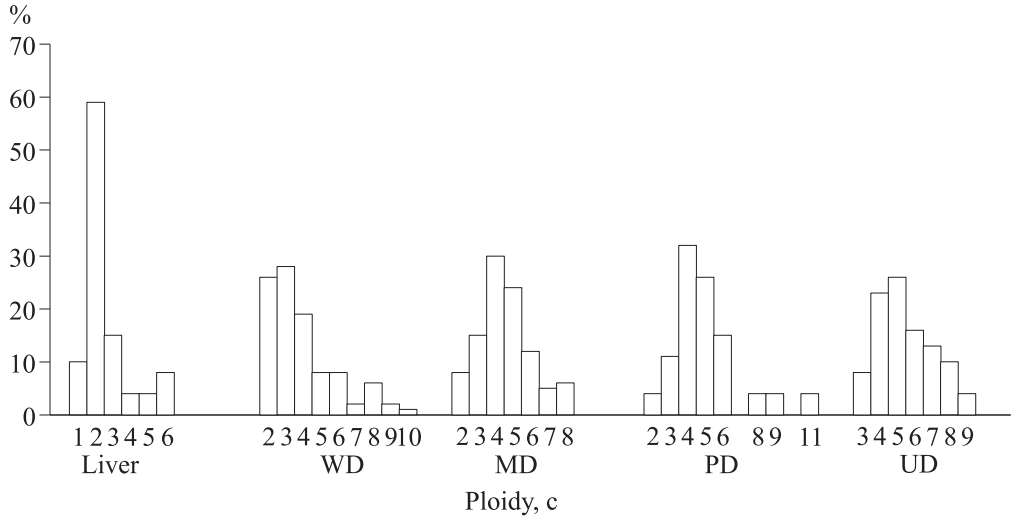

Fig. 2. Content of DNA in hepatocyte and hepatocellular cancer cell nuclei. WD: well-differentiated; MD: moderately differentiated; PD: poorly differentiated; UD: undifferentiated cancer.

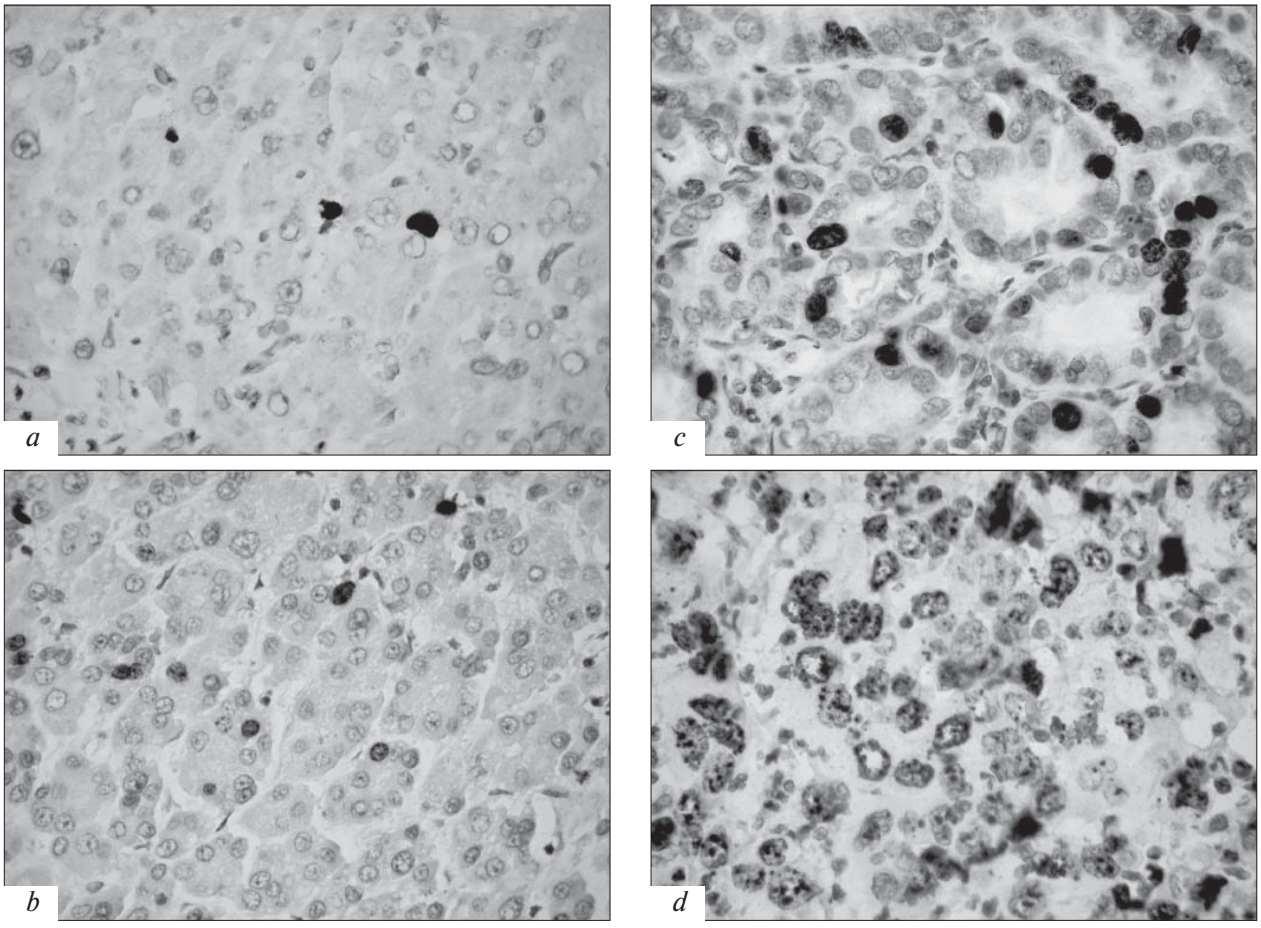

Fig. 3. Expression of Ki-67 in the nuclei of hepatocytes (*a*) and tumor cells of well-differentiated (*b*), moderately differentiated (*c*), and poorly differentiated (*d*) hepatocellular cancer. Immunoperoxidase method, ×630.

line with published data [7]. However, the proliferation index in undifferentiated cancer was lower than in moderately and poorly differentiated variants of hepatocellular cancer. These changes can be explained by starting predominance of cell damage and apoptosis processes in the undifferentiated cancer cells. However, we think that evaluation of proliferation index of tumor cell together with the results of histological and particularly ploidometric studies help to determine the degree of tumor differentiation with certainty.

Hence, hepatocellular cancer is characterized by enlargement of the nuclei, increase of nuclear ploidy, and increase in the number of proliferating cells. These changes to a certain measure reflect the regular staged pattern of tumor development. Evaluation of tumor cell proliferation index together with evaluation of ploidy level is an accurate diagnostic test indicating tumor differentiation, which should be used in practical pathology.

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