ONCOLOGY

Vascularization of Hepatocellular Carcinoma Tissue Depends on Its Differentiation Degree

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Quantitative characteristics of vessels in hepatocellular carcinoma and adjacent liver tissue were studied by morphometric methods after immunohistochemical staining for CD34 and CD105. The number of immunopositive vessels decreased with reduction of tumor histological differentiation degree.

Key Words: *angiogenesis; hepatocellular cancer; morphometry; CD34; CD105*

Neoangiogenesis is an important characteristic of biological atypia of tumors. The rate of endothelial cell proliferation in malignant tumor tissue in higher than in normal vessels by 20-2000 times [5,8]. Angiogenesis promotes tumor growth, invasion, and metastasizing [15].

The density of microvessels in the tumor is evaluated for predicting the course of renal [14], breast [2], and prostatic cancer [12]. Hepatocellular cancer (HCC) is a highly vascularized tumor. The index of microvessel density in its tissue is a prognostic marker of relapse development in primary cancer <5 cm in diameter [4].

We carried out a comparative morphometric study of vascularization of HCC varying by differentiation degree.

MATERIALS AND METHODS

We analyzed operation material from 22 patients (15 men and 7 women aged 17-72 years) with HCC treated at A. V. Vishnevsky Institute of Surgery in 2007-2009. In accordance with the recommendations of International Histological Classification of Hepatic Tumors [11], well-differentiated (5 patients), moderately differentiated (11 patients), poorly differentiated (4 patients), and undifferentiated (2 patients) HCC variants were distinguished.

Fragments of the tumor and adjacent liver tissue obtained during macroscopic examination were fixed in 10% neutral formalin. Histological studies were carried out on paraffin sections stained by hematoxylin and eosin. Blood vessels were detected by the immunohistochemical method using ready-to-use mouse monoclonal antibodies to CD34 (clone QBEnd/10) and rabbit polyclonal antibodies to CD105 using a polymeric detection system (Spring Bioscience). Preliminary antigen unmasking was carried out by boiling the specimens in citrate buffer (pH 6.0). Endogenous peroxidase was blocked with 0.3% H_2O_2 for 15 min. Hematoxylin served as the basic stain.

Morphometric analysis of preparations was carried out under a microscope with an Axio Imager M1 image analyzer system and AxioVision software (Carl Zeiss). The areas and perimeters of blood vessels and their number in a visual field were evaluated. These morphometric parameters were used to calculate the

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blood vessel shape factor and tissue vascularization degree (in percent). Quantitative data were statistically processed using Statistica 6.0 software.

RESULTS

Comparative immunohistochemical studies of the preparations showed different levels of CD34 and CD105 expression in HCC and adjacent tissue (Fig. 1), which was shown by morphometric characteristics of blood vessels (Tables 1, 2).

The mean area and perimeter of a vessel in well-differentiated HCC tissue in preparations stained for CD34 were 276.9 μ^2 and 72.5 μ (Table 1). The total number of all vessels in a visual field (35,600 μ^2) was 22.6. The summary areas and perimeters therefore reached 3320.8 μ^2 and 1250 μ, and tumor tissue vascularization was 9.4%.

With decreasing HCC differentiation degree (and hence, increasing malignancy), the numbers of blood vessels progressively decreased (Table 1) by 26.1 and 39.8% in moderately and undifferentiated forms, respectively, in comparison with well-differentiated tumor ($p<0.05$). Shifts in the mean parameters of a single vessel were opposite. The mean area of vessels was somewhat less in moderately and undifferentiated tumors and 12.6% higher than in well-differentiated HCC. The mean perimeter and vessel shape factor were higher in moderately and poorly differentiated forms and lower in undifferentiated HCC.

On the other hand, the summary areas of all vessels in a visual field increased by 11.7 and 20.4% in moderately and poorly differentiated cancer, respectively, while in undifferentiated cancer this value was lower than in well-differentiated HCC. Summary vascular perimeters in moderately differentiated tumors were virtually unchanged, while in poorly differentiated and undifferentiated HCC this value decreased progressively (by 13.4 and 23.5%, respectively). The tumor tissue vascularization indexes increased in moderately, poorly, and undifferentiated HCC.

A lesser number of blood vessels was detected in $CD105^+$ specimens (according to immunohistochemical findings), irrespective of tumor tissue differentiation degree. In well-differentiated HCC 12.5 vessels were seen in a visual field, i.e. by 44.7% lover that in preparations stained with antibodies to CD34 ($p<0.05$). The number of vessels in moderately differentiated cancer was higher and in poorly differentiated and undifferentiated HCC lower than in well-differentiated tumors.

The mean area of vessels was lower than in $CD34^+$ specimens. The summary area of blood vessels per visual field and hence, vascularization degree was by 15-71% lower than $CD34^+$ specimens. The summary vascular perimeters in CD105-positive HCC were also

TABLE 1. Morphometric Characteristics of Vessels Expressing CD34 and CD105 in HCC Tissue Varying by Differentiation Degree (*M*±*m*)

Parameter	CD34				CD105			
	WD	MD	LD	U	WD	MD	LD	U
Number per visual field	22.6±1.8	16.7±1.1	13.7±0.9	13.6±0.9	12.5±1.8	16.0±1.2	6.4±0.5	6.7±0.8
Mean area, μ^2	276.9±22.8	270.1±23.5	311.8±26.2	274.0±22.4	244.7±20.8	216.6±17.1	290.9±22.7	161.2±14.8
Mean perimeter, μ	72.5±5.9	81.0±6.1	87.4±6.3	70.5±6.2	67.1±4.2	69.4±5.1	71.4±6.5	54.2±3.6
Summary area per visual field, μ^2	3320.8± 284.6	3694.4± 293.4	3457.6± 307.3	3694.6± 284.5	2445.4± 213.6	3371.0± 267.7	1638.2± 146.8	1069.6± 98.9
Summary perimeter per visual field, μ	1250.0± 92.8	1260.3± 103.6	1082.6± 84.7	956.0± 81.7	741.1± 62.5	1093.8± 83.5	443.7± 38.9	361.9± 33.6
Vascularization degree, %	9.4	11.1	9.9	10.4	5.9	9.5	4.6	3.0
Shape factor	23.0	25.0	25.7	18.1	23.6	24.3	20.2	18.2

Note. Here and in Table 2: WD: well-differentiated HCC; MD: moderately differentiated; LD: low differentiated; U: undifferentiated HCC.

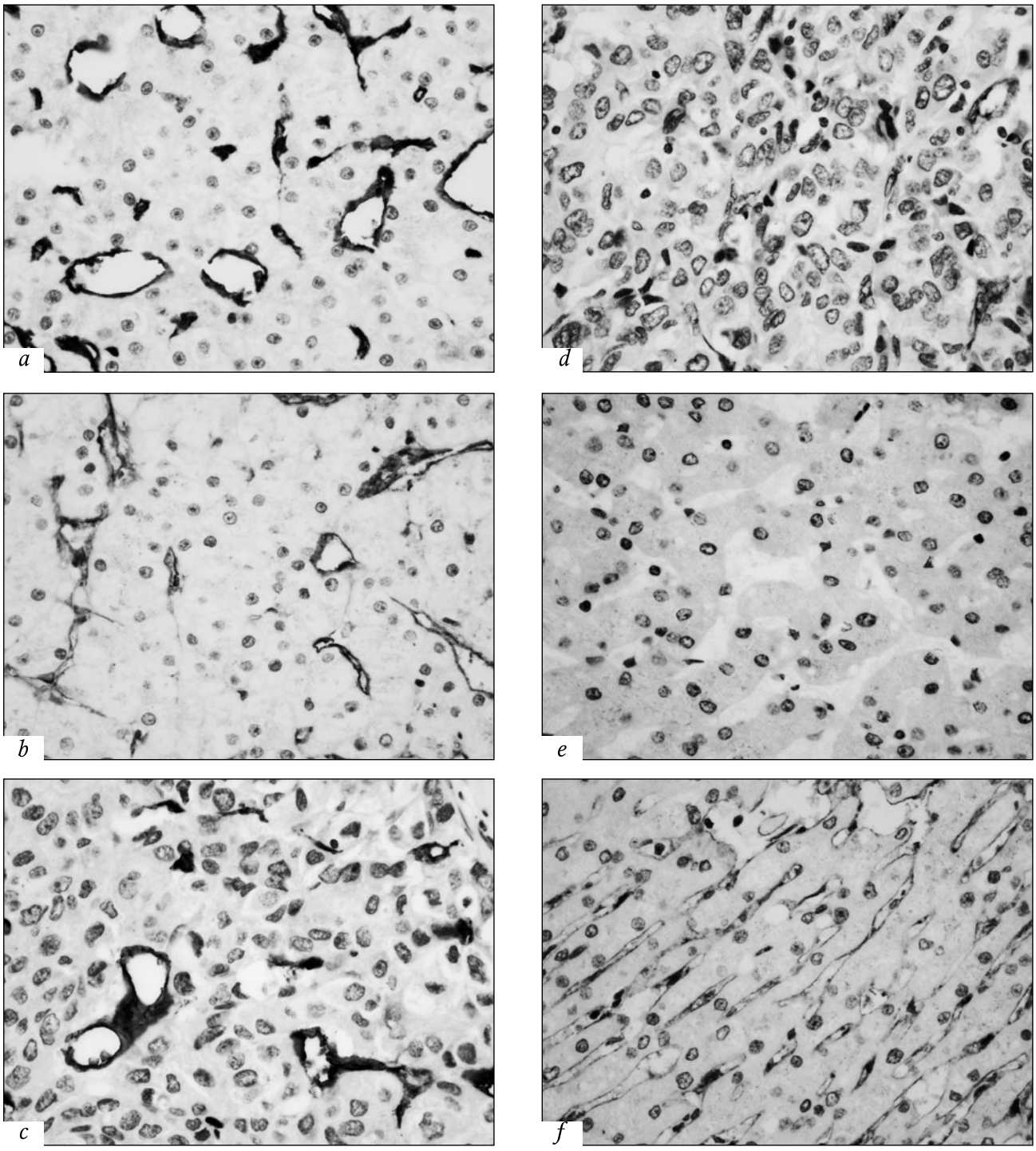

Fig. 1. Immunohistochemical characteristics of well- (*a*, *b*) and moderately differentiated (*c*, *d*) HCC and adjacent liver tissue (*e*, *f*). *a*, *c*) CD34 expression in vessels; *b*, *d*) CD105 in vessels; *e*) negative reaction with CD34; *f*) CD105 expression in sinusoids. Immunoperoxidase method, ×400.

13-62% lower. Importantly that the blood vessel shape factor for vessels expressing CD105 was virtually the same as for $CD34^+$ vessels.

Virtually no reaction to CD34 was detected in preparations of adjacent liver tissue (Fig. 1, *e*). Immunohistochemical detection of CD105 (Fig. 1, *f*) showed that the number of vessels (sinusoids) in the visual field depended on the degree of HCC differentiation (Table 2), but was almost always significantly higher than the parameters in tumor tissue: by 73.1% ($p<0.05$) in well-differentiated tumors and by 4.1 times ($p<0.01$) in poorly differentiated HCC. The mean size

TABLE 2. Morphometric Characteristics of Vessels Expressing CD105 in Liver Tissue Adjacent to HCC of Different Differentiation Degree (*M*±*m*)

Parameter	HCC form			
	WD	MD	PD	U
Number of vessels per visual field	32.8±1.6	27.7±1.5	26.0±1.5	23.7±1.6
Mean area, μ²	178.5±14.5	245.4±21.6	440.1±32.9	250.1±20.5
Mean perimeter, μ	68.7±6.2	81.0±6.9	103.9±9.5	77.5±6.1
Summary area per visual field, μ²	5788.6±416.6	6452.2±467.3	11422.9±897.7	5815.3±448.9
Summary perimeter per visual field, μ	2269.8±176.4	2154.8±190.5	2693.0±212.8	1804.6±159.4
Vascularization degree, %	16.3	18.3	32.1	16.3
Shape factor	26.2	27.3	24.5	24.0

of sinusoids was by 27.1% less than the area of vessels in well-differentiated cancer and by 13.3-55.1% higher in other HCC forms. The summary area of sinusoids per visual field and hence, degree of peritumor tissue vascularization was significantly (2-7-fold) higher than in HCC tissue.

Importantly, CD34 is a panendothelial marker reacting with the majority of endothelial cells, while CD105 is expressed in neoangiogenesis foci in growing blood vessels [10]. CD105 (endoglin) is a homodimeric membrane glycoprotein reacting with transforming growth factors β_1 and β_3 and stimulating endotheliocyte proliferation.

The lesser number of vessels expressing CD105 in HCC tissue was in line with published data. Intratumor density of vessels in $CD105^+$ preparations was less in comparison with $CD34^+$ specimens (50.8 *vs.* 125.6 per 0.74 mm² tissue) [7].

These changes seemed to be explained by tumor "age". Pronounced angiogenesis is characteristic of early stages of tumor development, but later it decreased. Tumor cell metabolism is changing towards anaerobic in the course of tumor development and growth [6]. Generally, a lesser oxygen consumption is characteristic of tumor tissues *vs.* normal ones [9]. This explains published data on lower density of intratumor vessels in larger tumors of more advanced stages of development [3]. We found that the number (per visual field) of vessels expressing CD34 or CD105 in tumors tended to decrease with decreasing tumor differentiation degree (*i.e.*, or increasing its malignancy). This was paralleled by reduction of the summary area of $CD105^+$ vessels and increase of the summary area of $CD34^+$ vessels. Thinning of processes and decrease in the numbers of mitochondria, profiles of cytoplasmic reticulum, and pinocytosis vacuoles were found in endotheliocytes [1]. Apoptosis processes were reduced in $CD105^+$ cells and their resistance to cytotoxic drugs was higher [13].

Hence, our data indicate a certain attenuation of neoangiogenesis with HCC progress and growth. These specific features of blood vessel growth should be taken into consideration when creating and prescribing antiangiogenic drugs to patients suffering from HCC.

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