Immunohistochemical Characteristics of Desmoid Tumors

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A comparative morphological study of primary and relapsing desmoid tumors was carried out. Immunohistochemical analysis showed that the progress of desmoid tumors in the form of relapses was paralleled by an increase in the counts of immunopositive cells and intensity of β-catenin and cycloxygenase-2 expression.

Key Words: *desmoid; immunohistochemistry; relapse*

According to modern international histogenetic classification of soft tissue tumors [10], desmoids-type fibromatosis (synonyms: desmoid, abdominal fibromatosis, abdominal desmoid, desmoid tumor, desmoid fibroma, desmoid fibromatosis) are referred to a group of fibroplastic and myofibroblastic tumors with intermediate potential of biological behavior.

Desmoids constitute less than 0.03% tumors in general and are characterized by infiltrative growth into the adjacent tissues, development of local relapses after surgical excision, and absence of metastases [8]. Due to these features some scientists refer desmoids to sarcomas of low malignancy [9]. Risk factors for desmoid tumors have been determined: female gender, presence of desmoid fibromas or colorectal polyps, and some hereditary mutations in relatives [7]. The molecular mechanisms explaining the development and specific behavior of these tumors remain unclear. In addition, the therapeutic methods used at present (surgical removal, chemotherapy, radiotherapy) are in fact ineffective. All these dictate the search for molecular markers for prediction of tumor behavior and development of appropriate targeted therapy.

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We carried out a comparative immunohistochemical study of primary and relapsing desmoid tumors.

MATERIALS AND METHODS

Comprehensive morphological studies of operation material from 16 patients with abdominal desmoid were carried out. The patients were treated in 1997-2008 at A. V. Vishnevsky Institute of Surgery. Three groups of patients were formed. Group 1 consisted of 11 patients (1 man and 10 women) aged 13-65 years operated on for primary desmoid tumors. Group 2 consisted of 3 patients aged 21-35 years operated for the first relapse of desmoid. Group 3 patients (2 women aged 21 and 28 years) were operated on for second or subsequent relapses.

Histological studies were carried out on paraffin sections stained with hematoxylin and eosin. Immunohistochemical studies were carried out by the standard method using mouse monoclonal antibodies to β-catenin (clone 17C2, 1:100; Novocastra), cycloxygenase-2 (COX-2; clone 4H12, 1:50; Novocastra), APC (Adenomatous Polyposis Coli Protein; clone EMM43, 1:20; Novocastra), survivin (clone 12C4, 1:100; Dako), Ki-67 (clone MIB-1, ready to use; Dako), and EnVision polymeric detection system (Dako). Preliminary antigen unmasking was carried out by boiling of the samples in citrate buffer (pH 6.0). Endogenous peroxidase was blocked with 0.3%

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H_2O_2 for 15 min. Harris' hematoxylin served as the background stain. The immunohistochemical reactions were evaluated by the percentage of cells with stained nuclei and cytoplasms and by staining intensity using a semiquantitative score: 0: no reaction; 1: weak reaction; 2: moderate reaction; and 3: manifest reaction. The proliferation level was evaluated by the percentage of tumor cells with Ki-67-positive nuclei.

The data were statistically processed by Statistica 6.0 software.

RESULTS

Macroscopically, the desmoid tumors are compact formations up to 41×32×12 cm without clear-cut border with the adjacent soft tissues and glossy grey-white or grey-yellow on section. More compact white sites making the section surface look trabecular were found in half of the cases.

Infiltrative growth into the adjacent soft tissues (striated muscle tissue and fat) was found on histological preparations stained with hematoxylin and eosin. Tumor tissue was presented by monomorphic elongated spindle cells with blurred borders and normochromatic nuclei with one to three nucleoli. No mitosis figures were seen. Tumor cells were separated by bundles of collagen fibrils and fibroblasts between them. A moderate number of blood vessels were seen in tumor tissue. Foci of excessive mucus discharge were found. Fragments of previously implanted mesh endoprostheses and suture material were found in relapsing desmoid fibroma tissue. The density of tumor cells slightly varied in different cases. However, the cell counts calculated per visual field were much the same in primary and relapsing desmoids ($p>0.05$). The tissue cellularity was 37.8±2.9 in primary tumors and 36.1±3.2 in relapses.

On the other hand, proliferation index calculated from the count of Ki-67-immunopositive cells was 8.70±0.06. The index was lower in relapsing tumor tissue: by 27.6% in first relapses and by 33.3% in subsequent relapses in comparison with primary desmoid ($p<0.05$). The greater part of Ki-67-positive cells were located near the vessels (Fig. 1, *a*) and by the foci of infiltrative growth (Fig. 1, *b*).

The results of comparative immunohistochemical studies of preparations from primary and relapsing desmoids are presented in Table 1. Studies of β-catenin expression showed not only cytoplasmic, but also nuclear location of the reaction product (Fig. 1, *c*). Positive expression in nuclei and cytoplasm of primary tumors was detected in 69.7 and 85.8% cases, respectively (Fig. 2). In relapsing tumors, the incidence of immunopositive cells and cytoplasm increased progressively. In the first relapse, stained nuclei and cytoplasm were detected more often than in the primary tumor by 3.3 and 1.9% ($p>0.05$), respectively, while in subsequent relapses the corresponding values in-

TABLE 1. Immunohistochemical Characteristics of Primary and Relapsing Desmoid Tumors

Marker		Primary desmoid	Relapse	
			first	subsequent
β-Catenin	N_N, %	69.7±4.2	72.0±5.1	100
	E_N	1.25	1.68	2.32
	N_C, %	85.8±4.4	87.4±4.6	100
	E_C	1.19	1.6	2.11
Survivin	N_N, %	31.7±2.2	12.2±1.5	32.3±2.7
	E_N	0.44	0.17	0.52
	N_C, %	59.7±2.4	65.9±2.6	46.7±2.1
	E_C	0.76	0.91	0.47
COX-2	N_C, %	45.4±3.8	81.6±4.6	100
	E_N	0.54	1.32	1.57
APC	N_C, %	63.1±3.9	90.5±5.6	93.2±6.1
	E_C	2.21	2.09	2.18

Note. N_N: mean number of cells with immunopositive nuclei; N_C: mean number of cells with immunopositive cytoplasm; E_N, E_C: mean intensities (in points) of nuclei and cytoplasm staining, respectively.

creased by 43.5 and 16.6% ($p<0.05$), respectively. In the latter case, positive reaction was found in all cells. The intensity of the reaction varied, similarly as in the primary tumors. Weak activity of β-catenin predominated in primary tumors (nuclear and cytoplasmic expression score was 1.25 and 1.19, respectively). An increase of the mean expression level by 34.4 and 34.5% in the nuclei and cytoplasm, respectively, was found in primary relapse. In subsequent relapses, the expression levels increased by 85.6% in the nuclei and by 77.3% in the cytoplasm.

Positive reaction to APC in the cytoplasm with the mean expression of 2.21 points was found in 63.1% primary tumor specimens. Analysis of relapses showed

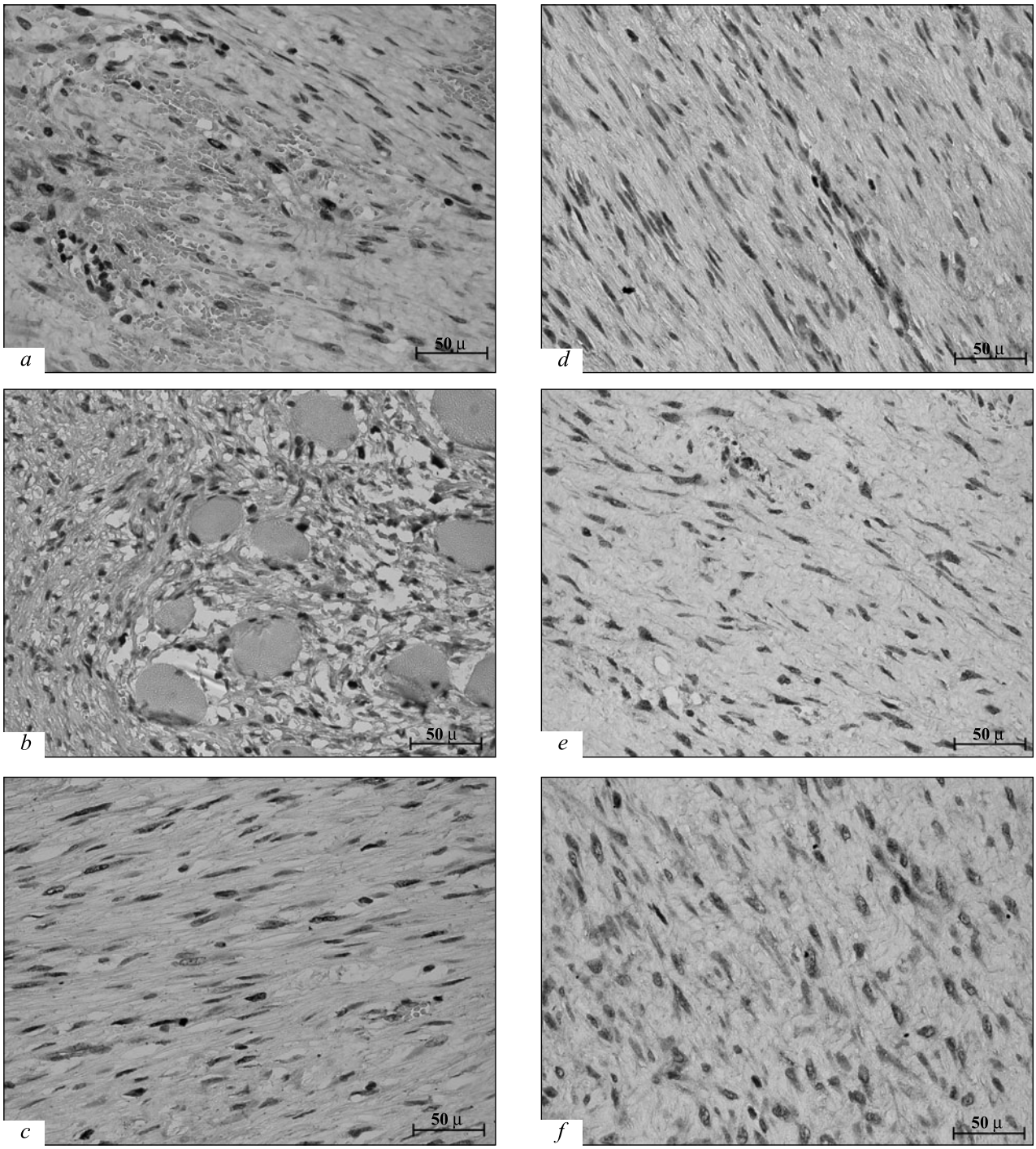

Fig. 1. Immunohistochemical characteristics of primary (*a*-*c*, *f*) and relapsing (*d*, *e*) desmoid tumors. Immunoperoxidase method (×400). *a*, *b*) Ki-67 expression; *c*) positive expression of β-catenin in tumor cell nuclei and cytoplasms; *d*) APC expression; *e*) COX-2 expression; *f*) survivin expression

a 43.4-47.7% increase ($p<0.05$) in the level of immunopositive cells, the staining intensity remaining virtually unchanged (Fig. 1, *d*).

Immunohistochemical study of COX-2 showed a positive reaction only in the cytoplasm. In primary tumor 45.4% cells were stained, while in relapsing tumor the content of immunopositive cells progressively increased. Their level increased by 79.7% ($p<0.05$) in first relapse tumor specimens and by 2.2 times ($p<0.01$) in subsequent relapses; positive reaction was found in all cells (Fig. 1, *e*). This was paralleled by an increase in the staining intensity: the expression level in primary and subsequent relapses was higher than in primary desmoids by 2.44 and 2.91 times, respectively.

The reaction to survivin was also positive in tumor cell nuclei and cytoplasm (Fig. 1, *f*). In primary desmoids, stained nuclei and cytoplasm were detected in 31.7 and 59.7% cases, the mean expression being somewhat higher in the cytoplasm (expression score 0.76 *vs.* 0.44 in the nuclei). In primary relapses the level of immunopositive reaction in the nuclei and their quantity decreased by 61.4 and 61.5%, respectively ($p<0.05$), in comparison with the values in primary nodules. On the other hand, the number of cells with immunopositive cytoplasm increased by 10.4% and the reaction intensity was higher by 19.7%.

The relative content of cells with stained cytoplasms in subsequent relapses was lower than in primary tumor and in the first relapse by 21.8 and 29.1%, respectively. The level of immunopositive nuclei was virtually the same as in primary desmoid. The level of survivin expression in nuclei, in turn, was by 18.2% higher, while in the cytoplasm by 38.2% lower than in the primary tumor.

Hence, the progress of desmoid tumors in the form of relapses was associated with an increase in the count of immunopositive cells and intensity of β-catenin and COX-2 expression. These changes seemed to reflect molecular mechanisms of desmoid oncogenesis.

β-Catenin is a cadherin-binding protein involved in cell adhesion; in addition, it serves as transcription stimulant, when accumulated in the nucleus, and reacts with T-cell factor [14]. The level of β-catenin

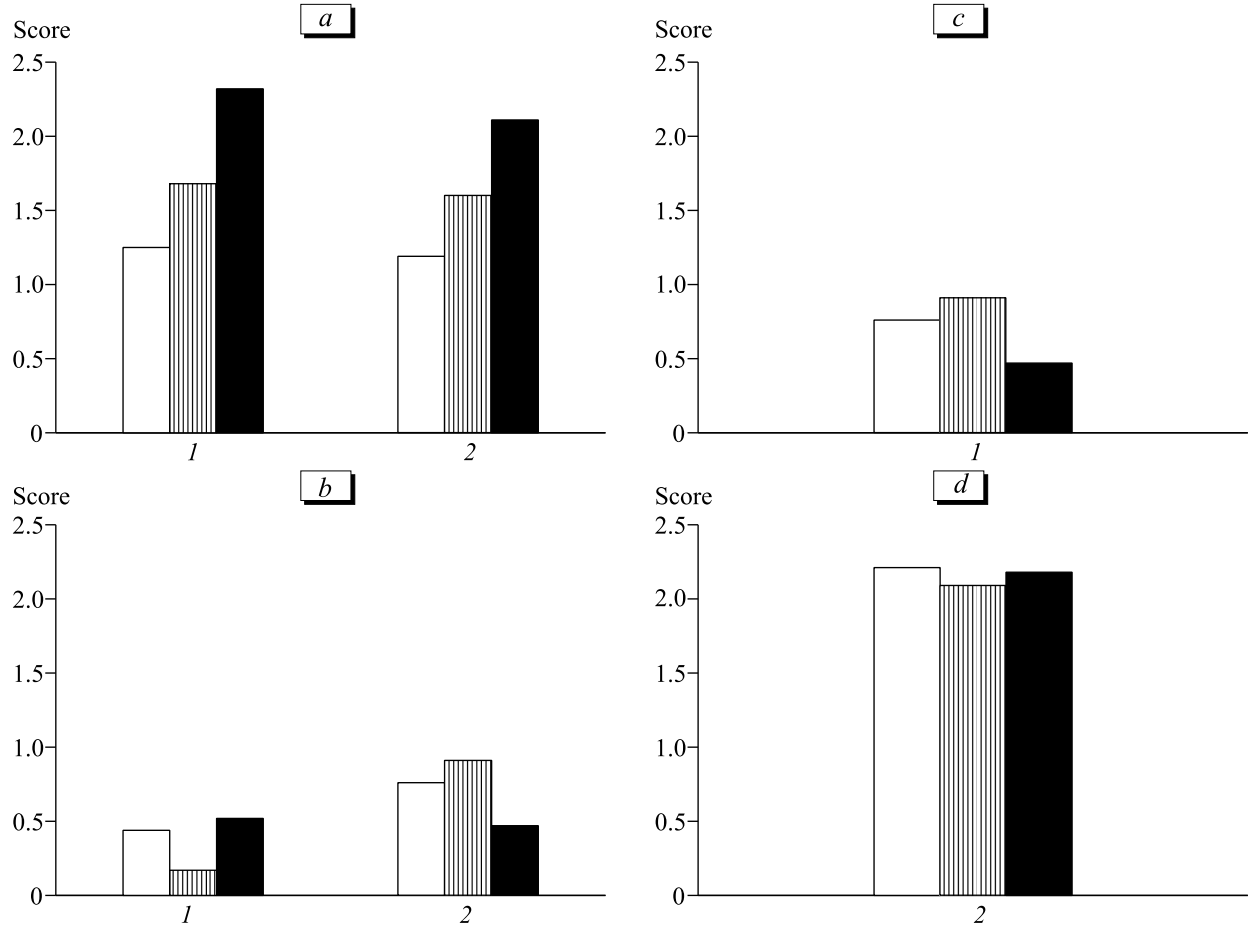

Fig. 2. Staining intensity (mean values) of primary and relapsing desmoid tumor cell nuclei (*1*) and cytoplasm (*2*). *a*) β-catenin; *b*) survivin; *c*) COX-2; *d*) APC. Open bars: group 1; hatched bars: group 2; dark bars: group 3.

in the cytoplasm is regulated by a multiprotein complex consisting of APC, axin/conductin, and glycogen synthase-kinase 3β (GSK-3β). Upon binding to this protein complex, β-catenin undergoes phosphorylation and subsequent degradation in proteosomes.

Mutations in exon 3 of *CTNNB1* gene encoding β-catenin sequences containing serine and threonine residues are detected in 87% sporadic desmoid tumors [2]. These mutations lead to stabilization of β-catenin, stimulate its accumulation in the nucleus and transcription activation of target genes, *e.g.* c-MYC, c-JUN, and cyclin D [11,14], thus promoting the formation of the pro-tumor phenotype [4,11]. Positive expression of β-catenin not only in the cytoplasm, but also in cell nuclei can be regarded as a morphological reflection of this process.

Mutations in the APC gene normally located in 5q21 also lead to disorders in the wnt/beta-catenine signal pathway. APC is a component of the molecular complex responsible for elimination and degradation of cytoplasmic β-catenin. APC is expressed in the cytoplasm of epithelial and mesenchymal cells. Its expression is confined to areas containing differentiated and non-dividing cells. Mutations in the APC gene contribute to the development of sporadic colorectal tumors and hereditary colorectal polyps, pancreatic, gastric, and esophageal cancer [7].

T-cell factor, in turn, causes transcription of such a target gene as COX-2 [5]. COX-2 is an inducible form of the COX enzyme family, its members regulating arachidonic acid transformation into prostaglandins that play the key role in many biological processes, including oncogenesis. Increased activity of this enzyme promotes tumor progress at the expense of apoptosis inhibition, angiogenesis and invasion stimulation, and modulation of cell proliferation via enhanced expression of growth factors, for example, platelet growth factor (PDGF) [3]. Our data indicate that desmoid relapse is characterized by considerably higher percentage of cells with positive expression of COX-2 and its more intense reaction.

Changes in survivin expression detected in our study in the primary and relapsing desmoids also seem to reflect disorders in tumor transformation of cells. Normally survivin expression is detected in developing embryonic and fetal tissues, while in an adult organism it has been found only in thymocytes, bone marrow $CD34^+$ stem cells, and colorectal epithelium [1]. On the other hand, it has been found in tissues of the majority of malignant tumors [6]. That is why survivin is assumed to be one of the most specific products of tumor genes.

Survivin is a member of apoptosis inhibitor family preventing cell death at the expense of the initiator and effector caspases. In cells it binds to mitotic spindle microtubules and blocks the development of mitochondrion-dependent apoptosis. In addition to its involvement in cell death regulation, survivin plays an important role in cell division. Alternative splicing of survivin gene leads to the appearance of numerous copies of this protein. The expression of this marker in tissue is associated with an increase in the number of PCNA-positive cells and correlates with the degree of sarcoma differentiation [13]. Irrespective of its intracellular location, the level of survivin expression directly correlates an unfavorable prognosis of the disease [12].

Hence, the characteristics of primary and relapsing desmoid cell immunophenotype to a certain measure reflect their tumor transformation and progress and can therefore be used as additional factors for disease prognosis.

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