MORPHOLOGY AND PATHOMORPHOLOGY

Expression of Growth Factors in Endotheliocytes in Vascular Malformations

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The expression of growth factors and proliferation of endotheliocytes in vascular malformations were studied by immunohistochemical methods. The detected specific features of growth factor expression in the endothelium of venous and arteriovenous malformations seem to reflect the differences in the pathogenesis of these formations. High proliferative activity of the endothelium in angiodysplasias of both types can underlie the disease relapsing.

Key Words: *proliferation; vascular malformations; growth factors; endotheliocytes*

The pathogenesis of vascular malformations (angiodysplasias) is largely caused by disorders in normal interactions between the main mediators of angiogenesis. Despite numerous recent studies of molecular mechanisms of angiogenesis [13], the role of the key angiogenic cytokines and growth factors and their interactions in the development of some malformations remains little studied. For example, the mediator interactions in arteriovenous, venous, and mixed angiodysplasias is an interesting problem. The majority of scientists expect that the knowledge of the molecular bases of the pathogenesis of the major types of vascular malformations will promote the development of new target methods for the treatment of these diseases.

The immunohistochemical method, which can be used for target studies of expression of angiogenesis mediators and their receptors, is a prospective method of modern pathology. Results of immunohistochemical studies of vascular formations can be used for differential diagnosis of vascular malformations in hemangiomas,

We studied the expression of angiogenic mediators in endotheliocytes and the proliferative activity of these cells in arteriovenous and venous malformations.

MATERIALS AND METHODS

Operation material from 13 patients (7 women and 6 men) aged 22-67 years with peripheral vascular malformations has been analyzed. The patients were hospitalized at A. V. Vishnevskii Institute in 2004-2007. Arteriovenous malformations were diagnosed in 7 cases (4 women and 3 men), venous malformations in 6 cases (3 women and 3 men).

Tissue fragments were fixed in 10% neutral formalin. Histological studies were carried out on paraffin sections (5 μ) stained by hematoxylin and eosin. Immunohistochemical study of the expression of vascular endothelium growth factor (VEGF; DBC, 1:50), transforming growth factor β3 (TGF-β3; DBC, 1:100), fibroblast main growth fac-

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tor (β-FGF; BioGenex, 1:30), and Ki-67 proliferation marker (BioGenex, 1:30) was carried out by the immunoperoxidase method with poststaining of the nuclei by hematoxylin. The results of reaction with antibodies to growth factors were evaluated by a semiquantitative method in points by endotheliocyte cytoplasm staining intensity: 0: negative reaction; 1: weak reaction; 2: medium intense reaction; and 3: intense reaction. The content of Ki-67-positive cells was evaluated using MEKOS-C television image analyzer and expressed in percent of total number of endotheliocytes.

RESULTS

The specific features of pathological diagnosis of vascular malformations depend on their type, but relapsing is associated with emergence of a great variety of secondary changes, which impede the differential diagnosis [1,2].

Histological studies of arteriovenous malformations showed miscellaneous accumulations of arterial and venous capillaries 5-15 μ in diameter (Fig. 1, *a*). The arterial inner elastic membrane was characterized by uneven distribution of elastic fibers and their focal loss. This was paralleled by focal subintimal hyperplasia of smooth muscle cells and focal sclerosis of venous walls. Accumulations of compact fibrous connective tissue were seen at the periphery of angiodysplasias.

The histological picture of venous malformations was represented mainly by fine-wall caverns of different size and shape (Fig. 1, *b*). The thickness of these caverns' walls varied: the number of myocytes was low in thinned zones and higher than normally in thickened ones. Areas of compact fibrous connective tissue and hemorrhagic foci of different size were seen round venous malformations.

Immunohistochemical study showed moderately elevated expression of TGF-β in endotheliocytes in all arteriovenous and venous malformations (Table 1). The level of TGF-β expression was higher in the arteriovenous malformation endothelium than in venous one (Fig. 1, *c*). The expression of VEGF in the venous and arteriovenous angiodysplasia endothelium was moderate and somewhat higher in the arteriovenous malformation endotheliocytes (Fig. 1, *d*). The expression of β-FGF in venous and arteriovenous malformation endotheliocytes was low or negligibly elevated, being higher in the venous malformation endothelium (Fig. 1, *e*). The endotheliocyte proliferation index of large and medium-sized vessels in arteriovenous and venous malformations was low (just solitary cells (0.1%) expressed Ki-67), while the proliferative activity of small vessels at the periphery of malformations was moderately elevated (2-4%; Fig. 1, *f*).

The TGF-β and its receptors (ALK1, ALK5, *etc.*) play an important role at different stages of angiogenesis, including vasculogenesis. During the early embryogenesis the expression of TGF-β is characteristic of many tissues, including endothelial and hemopoietic precursor cells. Inactivation of TGF-β_1 in mouse embryos leads to their death (in 50% heterozygotes and 25% homozygotes) at early stages of gestation [13]. Disorders in the vascularization and hemopoiesis of the yolk sac are directly responsible for the death of these embryos. Although the initial stages of mesodermal precursor cell differentiation into endotheliocytes run a normal course in the absence of TGF-β expression, the subsequent development of these cells with the formation of capillary-like tubules leads to the formation of abnormal vessels with defective walls. The absence of TGF-β-2 receptor in mice leads to development of similar abnormalities, which fact also proves the important role of the TGF-β — TGF-β receptor system in the formation of the integral vascular wall [13,15].

The role of the TGF-β system in the pathogenesis of vascular malformations remains not quite clear. It is involved in the development of angiodysplasias and their relapses after surgical interventions. The growth of cultured endotheliocytes from arteriovenous malformations does not change after addition of TGF-β, while the growth of normal endotheliocytes is inhibited by this factor and their apoptosis is induced. In addition, a moderate and in some cases a significant increase of expression of TGF-β_1 and its ALK5 receptor and their matrix RNA by endotheliocytes of the cerebral arteriovenous malformations was detected [3].

Analysis of the operation material showed moderately elevated expression of TGF-β in venous malformation endotheliocytes. Presumably, this indicates a slight impact of this factor for the pathogenesis of these angiodysplasias. The expression of this factor in arteriovenous malformation endotheliocytes was also moderately elevated, but in 3 cases it was high, which confirms the hypothesis on the probable involvement of TGF-β in the development and progress of the arteriovenous vascular abnormality.

The VEGF family is represented by several secretory glycoproteins (VEGF-A, VEGF-B, VEGF-C, VEGF-D, and VEGF-E). All members of the VEGF family bind to VEGF via tyrosine kinase receptors 1, 2, and 3 (VEGFRs). One of the most important regulators of *in vivo* angiogenesis is

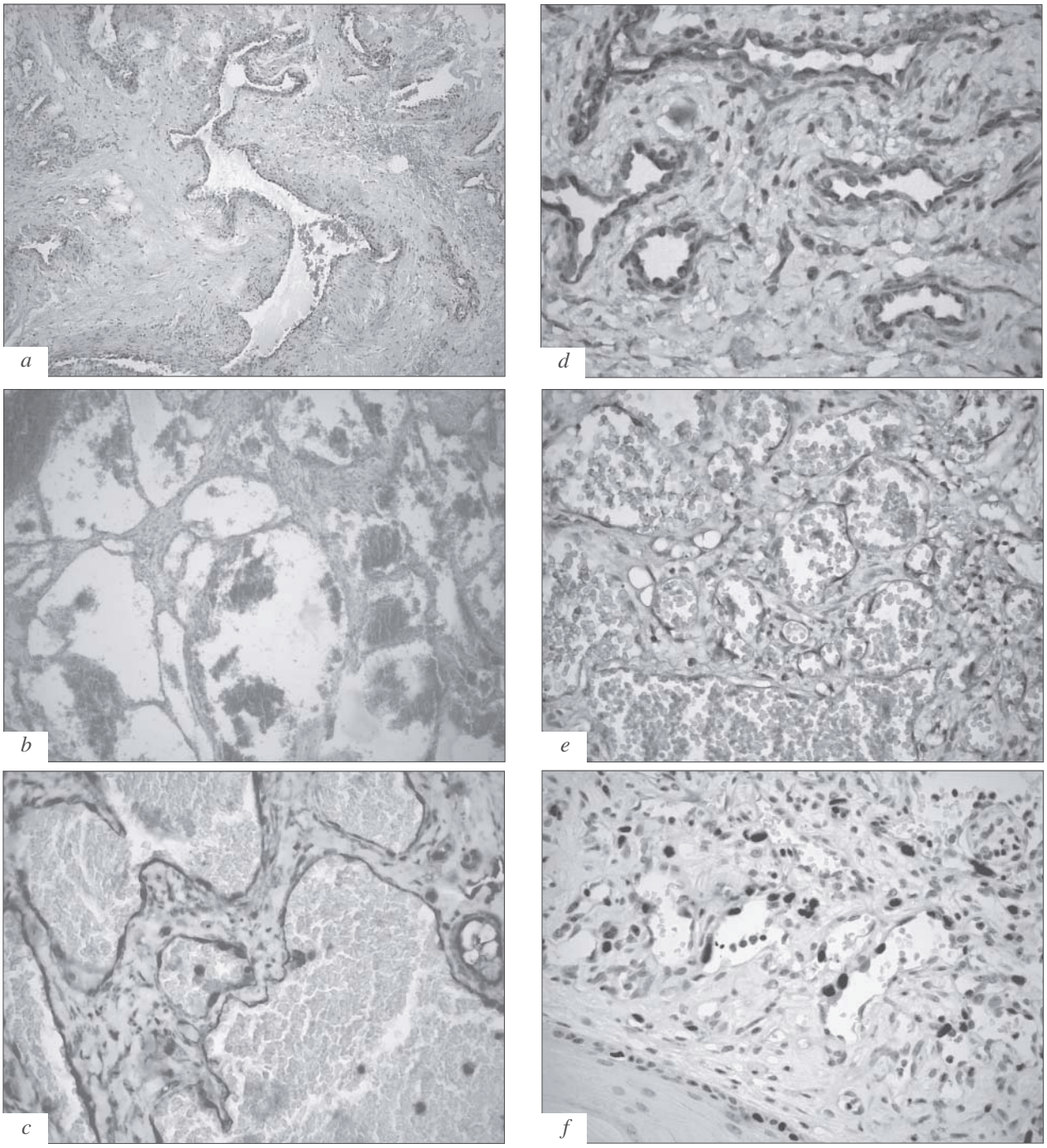

Fig. 1. Morphological characteristics of vascular malformations. *a*) arteriovenous malformation, represented by a large dysplastic artery with the adjacent small veins; *b*) venous malformation presenting as fine-walled caverns; *c*) high expression of TGF-β in venous malformation endotheliocytes; *d*) high expression of VEGF in arteriovenous malformation endotheliocytes; *e*) expression of β-FGF in venous malformation endotheliocytes; *f*) expression of Ki-67 in capillary endotheliocyte nuclei at the periphery of venous malformation. *a*, *b*) hematoxylin and eosin staining (×100); *c-f*) immunoperoxidase method (×400).

VEGF-A, particularly at the early stages of this process. Hyperexpression of VEGF-C and VEGF-D in transgenic mice leads to the formation of hyperplastic lymph vessels. By contrast, inhibition of these mediators arrests the growth of the lymph vessels [5].

Specific inactivation of VEGFR-1 gene leads to activation and differentiation of hemangioblasts, which, in turn, stimulates the growth of endotheliocyte-like cells and causes disorganization of the blood vessels [4]. Inactivation of VEGFR-2 gene leads to disorders in the formation of blood islets

TABLE 1. Immunohistochemical Characteristics of Endotheliocytes in Arteriovenous and Venous Malformations

Malformation type	Intensity of factor expression			
	VEGF	β-FGF	TGF-β	Ki-67, %
Arteriovenous	2.0	1.1	2.1	0.57
Venous	1.7	1.7	1.8	1.83

and embryonal blood vessels, causing embryonal death. Activation of VEGFR-2 stimulates the endotheliocyte proliferation and migration and increases the blood vessel permeability. The absence of VEGFR-3 gene causes embryonal death during early periods of gestation as a result of deficiency in the primary vascular network remodeling, which results in impossibility of normal development of the cardiovascular system before the formation of the lymph vessels [13].

At present the role of disorders in the VEGF—VEGFR system is most amply studied in vascular malformations of the CNS. Elevated (to a different degree) expression of VEGF family members (VEGF A, B, C, D) and their receptors (Flk-1, Flt-1, and Flt-4) was revealed in the endothelium of the cerebral arteriovenous malformations and the adjacent astrocytes [6].

The present study revealed a moderately elevated expression of VEGF in the endothelium of arteriovenous and venous malformations (somewhat higher in arteriovenous malformations). The expression of VEGF was the highest in the arterial endotheliocytes. These features of VEGF expression confirm its involvement in the pathogenesis of peripheral arteriovenous malformations, but this hypothesis can be confirmed and the role of EGF in the development of relapses and angiodysplasias of this kind be cleared out only after molecular genetic studies.

The FGF family is represented by 22 polypeptide compounds of similar structure. The majority of them are wide spectrum mitogens, stimulating such cell functions as migration, proliferation, and differentiation. The FGF family members play an important role in embryonal development, angiogenesis, vasculogenesis, and wound healing [10, 11]. All FGF family members mediate their effects on the cell through activation of the specific transmembrane tyrosine kinase receptors (FGFR1, FGFR2, FGFR3, and FGFR4). The FGF—FGFR2 system is involved in the regulation of endotheliocyte migration during neoangiogenesis [8,9]. The role of FGF system in the pathogenesis of vascular malformations is in fact not studied. We detected low or slightly elevated expression of β-FGF in endotheliocytes of the studied malformations. The expression was higher in the venous malformation endothelium, which can reflect their pathogenesis.

Two immunohistochemical markers (Ki-67 and PCNA) are now used for evaluation of cellular proliferative activity. Protein Ki-67 detects proliferating cells in different phases of the cycle and reflects the entire pool of dividing cells, being the most accurate marker of proliferation. It is a short-lived protein, destroyed within 1-1.5 h after the beginning of its synthesis, and hence, it is not accumulated and not detected in silent cells.

Moderate expression of PCNA and no expression of Ki-67 are detected in the arteriovenous malformation endotheliocytes in the dura mater [14]. This is explained by a longer half-life period of PCNA (about 20 h) and its expression (in contrast to Ki-67) in the cells in the G_0 phase of cell cycle. Moderate and in some cases high expression of Ki-67, correlating with liability of these formations to relapsing, has been demonstrated for cerebral arteriovenous malformations [7,12].

Our immunohistochemical studies of Ki-67 expression revealed a low proliferative activity of endotheliocytes in the peripheral venous and arteriovenous malformations. In some cases moderately elevated proliferative activity of the endothelium was detected in both types of malformations. We think that these patients are at a much higher risk of disease relapsing after surgical intervention.

Hence, the expression of growth factors in venous and arteriovenous malformation endotheliocytes reflect the differences in the morphogenesis and pathogenesis of these formations. High proliferative activity of the endothelium in malformations of both types can underlie the development of disease relapses.

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