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Angiogenesis and pro-angiogenic factors in uterine fibroids — facts and myths

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Abstract: Uterine leiomyomata present major problem for females. Although they are benign tumors their frequency is associated with many symptoms like infertility, abdominal pain, menorrhagia. Authors based on their own morphological studies and review of the literature try to indicate main factors causing angiogenesis within leiomyomata and its influence on tumor growth. The strongest proangiogenic factor seems to be hypoxia, which stimulates up- and down-regulation of numerous genetically determined substances. Also mechanical pressure acting upon newly growing vessels is one of the factors which may determine formation of so called "vascular pseudocapsule" around the lesion.

Key words: uterine leiomyomata, microvessel density, angiogenesis.

Introduction

Angiogenesis is currently considered to be a very important factor controlling growth and ability to metastasize of malignant tumors [1]. Role of the angiogenesis in the growth of benign tumors still remains unclear. However if we consider the model of development of uterine fibroids based on adaptation of the vessels of normal vascular bed — their further regression and subsequent vascularization "de novo" — it may appear that angiogenesis plays key role also in the development of benign tumors. Formation of "vascular pseudocapsule" in the periphery of the fibroid requires undoubtedly involvement of pro-angiogenic factors. Although studies which consider angiogenic activity of uterine leiomyomata and surrounding myometrium, bring unanimous results by far.

It was proved that uterine fibroids are source of the whole palette of growth factors, which seem to be probable or to be angiogenic factors, too. Family of factors associated with uterine leiomyomata consists of: vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), platelet derived endothelial cells growth factor (PD-ECGF), epidermal growth factor (EGF), transforming growth factor (TGF alpha and beta), basic fibroblast growth factor (bFGF), insulin-like growth factor (IGF), adnenulomedullin (ADM) [2–12]. Some of mentioned factors, i.e. VEGF-A [6], bFGF [3, 11] and IGF-I [7] show increased expression in the uterine leiomyomata, comparing to the surrounding myometrium. Other factors, i.e. EGF [6], TGF-beta [13] show decreased expression in fibroids. It is generally assumed that VEGF and ADM show increased activity in uterine myomas [2]. From another hand however we have to cope with contrary reports. Lee and Novak described increased level of TGF-beta mRNA [10], while Vollenhoven *et al.* [14] did not show significant changes in expression of EGF and TGF-beta of fibroids and surrounding myometrium. Similar results were achieved by Harrisson-Woolrych *et al.* in EGF mRNA [9].

Material and methods

The study was carried out on 51 human uteri of females aged between 32–66 years, which died because of diseases not associated with the internal female genital tracts, obtained during autopsies. The study was approved by local Ethical Committee. The whole material was collected upon 12–22 hrs from the moment of decease. The protocol used was described in details in [14, 15]. Additionally 5 specimens were injected with water solution of acrylic emulsion (Liquitex R, Binney and Smith, USA) [16]. Thus obtained material was cut into slides, which after dehydration in rising concentrations of ethyl alcohol and immersed in methyl benzoesane were paraffinized and dyed with hematoxylin and eosin. The material was studied under light microscope (magnification 5–40x). Some of tissue blocs were deparaffinized and hydrated to perform immunohistochemical reaction for von Willebrandt factor.

Results and discussion

Most of proangiogenic factors are located in cytoplasm of smooth muscle cells — of both myometrium and cells of blood vessels. TGF-alpha, EGF and FGF-2 were observed also within endothelial cells, and IGF-1 in the fibroblasts of connective tissue [6]. It is interesting that bFGF is located mainly within intercellular matrix, and its relatively high level was associated with its condensation [11].

Growth factors present in fibroids may influent all its factors: smooth muscle cells, vascular endothelial cells and connective tissue fibroblasts. Their specific participation in angiogenesis hasn't been sufficiently elicited yet. It is interesting that so far any relationship between increased vascular density and level of VEGF expression hasn't been proved, and VEGF is considered to be the main factor which stimulates angiogenesis [17, 18]. That kind of positive correlation was observed according to PD-ECGF, TGF-alpha and ADM. Expression of this last factor correlates positively also with the endothelial cells proliferation index [2]. ADM, opposite to VEGF has limited mitogenic action, limited almost to endothelial cells only. Furthermore, it shows relatively wide influence on proliferation and growth of tumor cells, both malignant and benign, and may play key role in angiogenesis of uterine leiomyomata.

Regulation of angiogenic factors probably follows few complicated mechanisms. Genetic aberrations of chromosomes 6, 7, 12, and 14 may cause increased tolerance considering angiogenic growth factors or decreased ability of production or response to angiogenic factors with the growth of the tumor. Genetic failures leading to increased sensitivity to angiogenic inhibitors, i.e. angiostatin [19], may be responsible for clinical picture of necrosis within certain leiomyomata.

Factors which stimulate angiogenesis, i.e. VEGF and substances from group of FGF, prove frequently increased activity in hypoxia, tissue damage and growth of new tissues. ADM and VEGF prove certain similarities, because both these strong angiogenic factors are induced by hypoxia, and both increase vascular permeability. It has been well documented that on induction both these factors require hypoxia stadium [2]. It is postulated that similar to most of the neoplasms, uterine leiomyomata show decreased level of oxygen.

In our studies one could observe relatively dense network of vessels penetrating the lesions, and in the specimens injected with Liquitex R we could see compressed blood vessels — even not filled with dye which were visible by staining using von Willebrandt factor. These compressed by growing tumor masses vessels were useless — and such condition led to significant hypoxia, a strong proangiogenic condition.

It is obvious that angiogenesis in the uterine fibroids may be influenced by ovarian steroids. Estrogens regulate expression of VEGF in the uterus at the transcription level [20]. In physiology they promote endometrial angiogenesis through regulation of VEGF expression in glandular cells and stroma [21]. Regulation of bFGF and its

receptors seems to be also dependent on the level of ovarian hormones [12]. PDGF and bFGF levels decrease in patients treated with agonists of gonadotropin releasing hormone (GnRH) [22, 23]. From another hand however in these patients nobody observed specific changes of microvessel density nor VEGF expression in fibroids [8, 24]. However the endothelial cells proliferation index of fibroids, myometrium and endometrium did not differ in particular phases of the menstruation cycle [2]. Xu *et al.* postulated that progesterone receptor modulator CDB-2914 down-regulates vascular endothelial growth factor, adrenomedullin and their receptors and modulates progesterone receptor content in cultured human uterine leiomyoma cells [25]. Also Catherino et al [26] indicate new potential targets for genetic researches.

In this context differences in vascular density observed between leiomyomata and the normal tissue may at least partially be result of different level of estrogen receptors (ER), which was shown in fibroids, comparing to the level of ER in regular myometrium [25, 27, 28]. The level of ER alpha RNA and immunoreactivity ER alpha are higher in leiomyomata than in surrounding myometrial tissue. Myometrial cells and in vitro cultured fibroid cells show increased expression of ER alpha receptors, and do not show changes in expression of ER beta. On the other hand endothelium of microvessels shows significant changes in expression of ER beta receptors [29]. Estrogens play vital role in survival of endothelial cells [30], so on this path they can stimulate changes which support or/and cause angiogenic response of microvessel endothelial cells, thus promoting growth of the new vessels within a tumor.

Despite expression of numerous proangiogenic factors within uterine leiomyomata, angiogenesis seems not to be specially outstanding during tumorigenesis, what is confirmed by relatively low vascular density (Fig. 1).

This hypothesis was lastly confirmed by studies of Weston *et al.*, which proved in the leiomyomata decreased expression of two factors promoting angiogenesis: connective tissue growth factor (CTGF) and cysteine rich factor inducing angiogenesis (CYR61), and also decreased expression of collagen 4 alpha2 (COL4A2), precursor of angiogenic inhibitor — canstatine. This is why fibroids comparing to the surrounding myometrium show antiangiogenic profile of gene expression.

For contrast, the most intense angiogenesis seems to occur at the border leiomyoma/myometrium, which leads to formation of vascular rich "pseudocapsule", visible especially in greater uterine leiomyomata. It might be a result of release of proangiogenic factors through stimulated by fibroid angiogenesis within surrounding myometrium. As a matter of fact we do not know, if the arrangement of the vessels in the central region of the lesion is resulted by physical pressure of the new vessels 'invading' the tumor or is it result of activity of the factors which inhibit angiogenesis in these parts of the tumor? This problem remains in the sphere of speculations, by far.

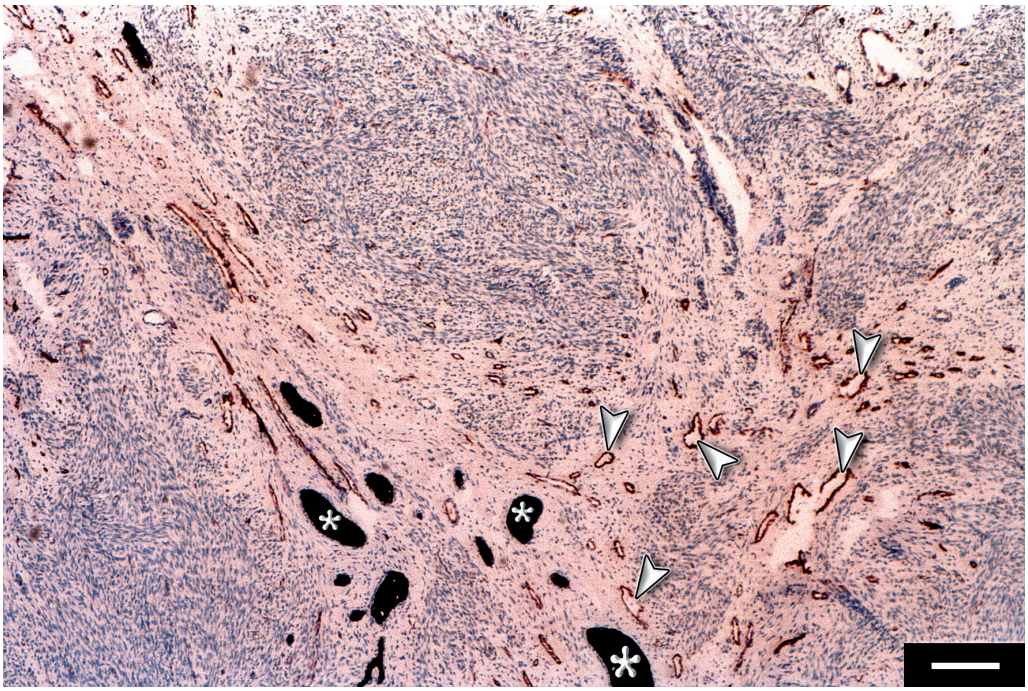

Fig. 1. Uterus of 43 years old female. Subserous leiomyoma injected through the arteries with water solution of Acrylic emulsion Liquitex R (Binney and Smith) (*). Immunostaining for von Willebrandt factor. Centrally visible numerous minute, not filled with emulsion vessels (arterioles and capillaries) (↑). Bar = 500 μm.

Conflict of interest

None declared.

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