The Implication of Angiogenesis in Endometriosis

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Abstract - Endometriosis is a common gynecological condition, associated with significant morbidity and having a strong social-economic impact. The widely accepted etiopathogenetic factor at the basis of endometriosis is the cyclical arrival of endometrial cells into the abdominal cavity through retrograde flux at menstruation. However, the endometriotic implants need neovascularization in their process of proliferation and invasion into ectopic sites within the host. Recent data suggest that the development and subsequent growth of endometriotic lesions is strongly influenced by the balance of local pro-antiangiogenic factors, a concept which is also widely accepted in tumor growth. In fact, various pro- and antiangiogenic molecules have been reported in the lesions and the peritoneal fluid of women. Out of all these molecules, VEGF proved to be the prototypical, most potent and most highly regulated endothelial cell mitogen among the angiogenic proteins synthesized by endometrial and endometriosis cells, as well as by the activated peritoneal macrophages and neutrophils. Thus, identifying the molecular mechanisms of angiogenesis and vasculogenesis in endometriotic lesions will lead to new, promising therapeutic approaches in this disease, such as antiangiogenic drugs.

Keywords - endometriosis, angiogenesis, VEGF

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I. Introduction

Endometriosis is a widely-met gynecological disorder characterized by the proliferation of endometrial glands and stroma outside the limits of the uterine cavity. The disease affects 5% to 10% of all reproductive-aged women and its prevalence reaches 20% to 50% in infertile women. Genetic factors proved to be involved in the likelihood of endometriosis; however, the mechanism of hereditary transmission is complex and likely multifactorial.[1, 2]

One of the most accepted causes of endometriosis is the dysregulated interaction between exfoliated menstrual endometrium and host tissue responses that enables ectopic lesions to implant and survive. However, as far as its pathophysiologic mechanism is concerned, it is now well-acknowledged that angiogenesis plays a key role in the implantation and development of endometriotic lesions. In normal eutopic endometrium, where prominent capillary growth takes place in the late proliferative and early-mid secretory phases of the cycle, vessel elongation is the predominant mechanism, whereas within endometriotic implants, this mechanism is not entirely known. [1, 3]

Similar to tumor metastases, endometriotic implants need neovascularization to develop and invade into ectopic sites within the host. The factors most probably involved in ectopic lesion growth via neovascularization prove to be a complex network of locally produced steroids, cytokines, oxygen free radicals, and possibly environmental toxins.[1]

The endometrial tissue, due to its strong stem cell populations and significant regenerative capabilities, is a rich source of proangiogenic factors. Among these, the vascular endothelial growth factor (VEGF) seems to be a critical vasculogenic regulator in endometriosis, enhancing capillaries recruitment toward the growing lesions.[1]

On the other hand, the endometrium has curious regenerative properties mostly accounted for by the presence of adult stem cells similar to mesenchymal stem cells (MSCs). While in the abdominal cavity, these MSCs may have the ability to proliferate, invade, and differentiate into endometrial cells, finally generating ectopic implants. Because steroid hormone receptors are only linked with differentiated endometrial cells, and not endometrial MSCs, the latter could be responsible for the high rate of persistence/recurrence of the disease following therapies that induce hypoestrogenism. MSCs-promoted angiogenesis itself could also be strongly involved, as the survival and proliferation of endometriotic tissue require the formation of new blood vessels. [4]

This review outlines the basic mechanisms of angiogenesis and vasculogenesis in the human eutopic endometrium and endometriotic implants.
II. Angiogenesis in Endometrium and in Endometriotic Implants

Though there still is the need for a comprehensive synthesis of the complex pathogenesis of this disease, so far endometriosis has been known to be a multifactorial disease in which the development and maintenance of endometriotic implants are tightly connected to their invasive capacity and angiogenic potential. [5]

Its cause is the retrograde menstruation of endometrial cells which get fixed on peritoneal surfaces and induce an inflammatory response. The success of the ectopic implants is dependent on the other pathological processes such as neoangiogenesis, fibrosis, adhesion formation, avoidance of apoptosis, immune dysfunction, and neuronal infiltration. [5-10]

The establishment of the lesion is tightly connected to the angiogenic potential of both the endometrium and the peritoneal environment. [1, 11-13] as endometriotic lesions need an adequate blood supply to survive in their ectopic sites. [5]

The typical characteristic of endometriotic lesions is a dense vascularization that occurs during the angiogenesis process. [6, 11, 14] The process by which angiogenesis occurs within endometriotic implants is not known, but 3 general mechanisms have been proposed: sprouting, elongation, and intussusception. In normal eutopic (intrauterine) endometrium, it is vessel elongation, rather than branch point sprouting, that plays the most important role in rapid vessel growth during the proliferative phase. [3, 5] This corresponds to the time in the ovulatory cycle in which human endometrial VEGF messenger RNA (mRNA) reaches its maximum production. [1, 15] However, the precise mechanism in endometriosis lesions is yet to be fully assessed. [5] The development and maintenance of the disease is tightly related to the presence of blood vessels to the endometriotic lesions from pre-existing ones which assures the required oxygen and essential nutrient supply. [16] As neovascularization has been proved necessary for the survival of tumor implants larger than 2-3 mm³, endometriotic lesions recruit blood vessels by inducing angiogenesis. [12, 18] It has been confirmed that new capillaries are recruited from existing, adjacent peritoneal microvessels; [12] however, the derivation of new blood vessels from circulating endothelial progenitor cells (EPCs), the so-called “vasculogenesis,” also plays a major role in the pathogenesis of endometriosis. [14] The endometrium is a dynamic tissue in which populations of clonogenic epithelial and stromal stem cells requiring active cyclic angiogenesis can be identified. [19-21] Bone-marrow-derived EPCs can be found in developing endometriotic lesions. [22] These lesions show increased expression of factors and chemokines that are involved in EPC recruitment, such as hypoxia-inducible-factor- (HIF-) 1α and stromal-cell-derived-factor- (SDF-) 1. [14, 23] Additionally, the presence of hypoxia, endothelial injury, and inflammation and the expression of ER-α also play a role in the mobilization and recruitment of EPCs from the bone marrow into endometriotic lesions. [5, 14, 24-30] [5]

New blood vessels develop through a complex dynamic process, characterized by a coordinated sequence of humoral and cellular interactions. [31] When stimulated by angiogenic growth factors, the wall of mature blood vessels destabilizes because of the mural cells detachment and the degradation of the extracellular matrix, a vital step for the development of new vessels. Chen et al. (2004) [32] reported increased metalloproteinase-9 (MMP-9) levels and lower tissue inhibitor of MMPs-1 (TIMP-1) immunostaining in ectopic and eutopic endometrium which allow the endothelial cells to migrate into the surrounding interstitium. This triggers the formation of capillary buds and sprouts. [32] The endothelial cells located behind the migrating endothelium of the sprouts reproduce, determining a continuous increase of the length and the diameter of the newly-developing blood vessels. Last but not least, the new vessel wall is stabilized by the attachment of mural cells, including pericytes and smooth muscle cells and the production of extracellular matrix compounds. [18, 33]

Endometriotic lesions have the ability to generate cytokines and growth factors that regulate their proliferation and vascularization. Interleukin- (IL-) 1β, the dominant IL-1 secreted by activated peritoneal macrophages, is a major factor in the neovascularization of endometriotic lesions. [34, 35] Cultured human endometrial stromal cells (HESC) from women with endometriosis report increased secretion of IL-6 and IL-8. [36] The former is a potent multifunctional protein, which promotes endometrial cell proliferation [37] and angiogenesis [38]; it is highly produced in ectopic endometrial tissue and it can be found in significant concentrations in the peritoneal fluid of patients with endometriosis. [39] IL-8 is a proinflammatory cytokine that triggers the chemotaxis of neutrophils and highly stimulates angiogenesis. [5, 40, 41]

Activin A, a growth factor member of the transforming growth factor β superfamily, is involved in the inflammation process and angiogenesis. [42-44] Activin A, which both originates from and targets the human endometrium, has the ability to modulate the expression and secretion of IL-8 and VEGF, from human endometrial stromal cells. [5, 45]
VEGF

As already mentioned, angiogenesis is under the control of numerous inducers and vascular endothelial growth factor (VEGF) represents the prototypical, most potent and most highly regulated endothelial cell mitogen. [5] It represents the main stimulus for angiogenesis and increased vessel permeability in endometriosis and plays a role in the progression of the disease [12, 18] as well as in delaying or reversing senescence of endothelial cells.

Although individually published studies showed inconclusive results, many researchers have reported that the rs3025039 (C>T) polymorphism of the VEGF gene increases the risk of endometriosis, but the rs699947 (A>C) and rs1570360 (G>A) polymorphisms might be protective factors for endometriosis. [46]

VEGF has been found in high concentration in the peritoneal fluid of women with moderate to severe endometriosis [11, 47-49], being secreted by activated peritoneal macrophages and neutrophils. [50] VEGF was identified in the epithelium and in the stromal cells of endometriotic implants, with a more visible expression in the epithelium. [15, 21] Endometriotic cells can also synthesize and secrete VEGF [5, 15] and this can be a result of the activation of pro-inflammatory cytokine IL-1β. [51, 52] Therefore, further research is needed to verify whether the VEGF level will change with the IL-1β treatment in ESC in vitro, and to fully assess its adjustment mechanism. [50] Endometriomas and red implants seem to be associated with the highest concentrations of VEGF. [5, 53, 54] Moreover, the peritoneal fluid concentrations of VEGF in patients with endometriosis go hand-in-hand with the stage of the disease. [15]

The following are among its effects: endothelial cell proliferation, migration, organization into tubules, and enhanced permeability, all of which participate in the angiogenic cascade[55] Estradiol enhances endometrial VEGF expression and its concentrations are linked to neovascularization and increased vascular permeability during the late proliferative phase. [56] There are cyclic changes in VEGF expression throughout menstrual cycle, the maximum level being reached during the secretory phase and menstruation. [5, 11, 15, 56]

COX-2 can also induce VEGF. [50] Moreover, research has proved [57-59] that IL-1β was followed by expression of the potent angiogenic factors VEGF and COX-2 in many malignant cases. These two are also known to play an important role in the angiogenesis of endometriosis (EMs). [50]

Similarly to these results, Huang et al. showed that IL-1β up-regulated the COX-2 expression by activating p38 MAPK pathway in human endometriosis stromal cells (ESC) in vitro. Moreover, VEGF level was also up-regulated by IL-1β treatment in human endometriosis stromal cell and the COX-2 inhibitor seemed to be involved in this process. [50]

VEGF binds to either of two tyrosine kinase receptors, the fm5-like tyrosine kinase (flt) and the kinase domain receptor (KDR or Flk-1). [60] There is also a connection between peritoneal endometriotic lesions with high proliferative activity, on the one hand and the high angiogenic activity, on the other, as reflected by the higher expression of VEGF-A in stroma and glandular epithelium and VEGFR-2 in blood vessels. [61] A recent study has proved that the vascular density and the expression of VEGF and its receptor VEGFR-2 (Flk-1) are significantly higher in the endometriosis which infiltrated deeply and affected the ovary, bladder and mainly the rectosigmoid, compared with the eutopic endometrium.[18]

Different from the eutopic endometrium, in a rat experimental model of peritoneal endometriosis, researchers identified higher levels of vascular density and the presence of VEGF and Flk-1 and MMP-9, and confirmed the angiogenic potential of these lesions. They also observed that the number of activated macrophages (ED-1 positive cells) increased in the endometriotic lesions, thus proving a positive correlation with VEGF. [18]

Additionally, Wang et al. (2005) [62] reported a higher Flk-1 expression in the endometriosis lesions of the peritoneal and abdominal wall, and this might be connected to neovascularization.

VEGF is not only a potent pro-angiogenic factor but may also have a direct proliferative or anti-apoptotic effect on different cell types. [63] Bilotas et al. assessed the effect of VEGF and interleukin-1β (IL-1β) on the apoptosis caused by leuprolide acetate (LA) in endometrial epithelial cell cultures from patients with endometriosis thus confirming previous results that VEGF and IL-1β, besides a pro-angiogenic role, may also exert a protective role on endometriotic cells from undergoing apoptosis. [64]

Other proangiogenic factors

Other proangiogenic factors, namely IL-8 [36, 65-68], hepatocyte growth factor (HGF) [69, 70], erythropoietin [71], angiogenin [72], macrophage migration inhibitory factor [49], neutrophil-activating factor [73], and TNF-α [74, 75], all register high concentrations in the peritoneal fluid of patients with endometriosis. This proangiogenic environment is strengthened by reduced concentrations of
antiangiogenic factors, such as adiponectin [76] and interferon-gamma-induced protein 10 (IP-10) [77, 78], although the levels of the endogenous VEGF antagonist soluble Flt-1 proved to be higher in the pelvic fluid of endometriosis cases. [5, 79]

Hormones

Endometriosis is an estrogen-connected disease. Estrogen may stimulate angiogenesis by increasing VEGF expression. [55, 80] There are many ways in which estrogen accumulates in endometriosis[81], most of which are angiogenesis-dependent (e.g., the upregulation of aromatase, the enzyme involved in the conversion of the C_{19} hormones androstenedione and testosterone into estrone and estradiol. [82] This upregulation is caused by prostaglandins stimulation, particularly PGE_{2} [83] which also directly affects angiogenesis by increasing VEGF expression. [84] Cyclooxygenase-2 (COX-2) is also upregulated in the endometrium of endometriosis patients as compared to controls as well as in the endometriotic lesions themselves, which triggers the increase of PGE_{2} levels required for aromatase and VEGF stimulation. [85] VEGF itself has proved to enhance COX-2 expression at both transcriptional and post-transcriptional levels, producing a positive feedback loop. [86, 87] Additionally, IL-1β, which is also increased in endometriosis, has been demonstrated to upregulate COX-2 and PGE_{2} expression in endometrial stromal cells. Theoretically, this further stimulates aromatase. [86] [88]

The accumulation of estradiol in endometriotic lesions is also determined by an enzymatic defect in the enzyme that transforms the weaker estrogen estrone into estradiol and vice versa. 17 beta-hydroxysteroid dehydrogenase (17β-HSD) is identified in different tissues such as eutopic endometrium in two isoforms. The isoenzyme 17β-HSG type 2 involved in the conversion of estradiol to estrone is significantly downregulated, dysfunctional or even absent in endometriotic tissue, which triggers the accumulation of estradiol.[89]

III. Conclusions

Angiogenesis seems to be involved in endometriosis, a multifactorial and polygenic disease, characterized by the presence of the endometrium outside the uterine cavity. This disease has been widely recognized and investigated for many years. In spite of the fact that its underlying pathophysiology is poorly understood, it seems that new vessel formation has long been recognized as a feature of endometriosis. Due to this, recent studies have focused on identifying the role of vascularization as well as the roles of steroids, VEGF, cytokines, oxygen free radicals etc. in the pathogenesis of endometriosis. Further research is required, however, to fully investigate the molecular mechanisms of angiogenesis and vasculogenesis, and, most importantly, how they can be exploited in the treatment of endometriosis as angiogenesis inhibition has recently emerged as a new, promising therapeutic approach for the disease.

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The authors report no conflicts of interest.

References


