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Basic mechanisms of vascularization in endometriosis and their clinical implications

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BACKGROUND: Vascularization is a major hallmark in the pathogenesis of endometriosis. An increasing number of studies suggests that multiple mechanisms contribute to the vascularization of endometriotic lesions, including angiogenesis, vasculogenesis and inosculation.

OBJECTIVE AND RATIONALE: In this review, we provide an overview of the basic mechanisms of vascularization in endometriosis and give special emphasis on their future clinical implications in the diagnosis and therapy of the disease.

SEARCH METHODS: Literature searches were performed in PubMed for English articles with the key words 'endometriosis', 'endometriotic lesions', 'angiogenesis', 'vascularization', 'vasculogenesis', 'endothelial progenitor cells' and 'inosculation'. The searches included both animal and human studies. No restriction was set for the publication date.

OUTCOMES: The engraftment of endometriotic lesions is typically associated with angiogenesis, i.e. the formation of new blood vessels from pre-existing ones. This angiogenic process underlies the complex regulation by angiogenic growth factors and hormones, which activate intracellular pathways and associated signaling molecules. In addition, circulating endothelial progenitor cells (EPCs) are mobilized from the bone marrow and recruited into endometriotic lesions, where they are incorporated into the endothelium of newly developing microvessels, referred to as vasculogenesis. Finally, preformed microvessels in shed endometrial fragments inosculate with the surrounding host microvasculature, resulting in a rapid blood supply to the ectopic tissue. These vascularization modes offer different possibilities for the establishment of novel diagnostic and therapeutic approaches. Angiogenic growth factors and EPCs may serve as biomarkers for the

diagnosis and classification of endometriosis. Blood vessel formation and mature microvessels in endometriotic lesions may be targeted by means of anti-angiogenic compounds and vascular-disrupting agents.

WIDER IMPLICATIONS: The establishment of vascularization-based approaches in the management of endometriosis still represents a major challenge. For diagnostic purposes, reliable angiogenic and vasculogenic biomarker panels exhibiting a high sensitivity and specificity must be identified. For therapeutic purposes, novel compounds selectively targeting the vascularization of endometriotic lesions without inducing severe side effects are required. Recent progress in the field of endometriosis research indicates that these goals may be achieved in the near future.

Key words: endometriosis / vascularization / angiogenesis / vasculogenesis / endothelial progenitor cells / inosculation / estrogen / VEGF / peritoneal fluid / miRNA

Introduction

Endometriosis is a benign gynecological disease affecting 6–10% of all women of reproductive age and up to 50% of women with infertility (Giudice, 2010). Typical symptoms are chronic pelvic pain, congestive dysmenorrhea, heavy menstrual bleeding, deep dyspareunia and fatigue (Culley *et al.*, 2013). In many cases endometriosis patients look back on a long history of suffering due to insufficient diagnostic options and therapeutic procedures, which are frequently associated with multiple side effects and high recurrence rates (Guo, 2009; Bozdag, 2015; Simpson *et al.*, 2015). Hence, endometriosis does not only substantially impair the patients' quality of life (De Graaff *et al.*, 2013), but also imposes a considerable economic burden on the health care system (Simoens *et al.*, 2007).

Endometriosis is defined by the presence of endometriotic lesions in extrauterine locations, such as the pelvic peritoneum, the ovaries and the rectovaginal septum (Burney and Giudice, 2012). These lesions originate from endometrium, which consists of endometrial glands that are surrounded by a well-vascularized stroma (Groothuis *et al.*, 2005). In contrast to other tissue types, the endometrium undergoes highly dynamic changes during the menstrual cycle, which are associated with estrogen-driven angiogenesis in the proliferative phase and progesterone-driven vascular maturation in the secretory phase (Okada *et al.*, 2014). Accordingly, the vascularization of endometriotic lesions also underlies a complex regulation by female sex hormones. Furthermore, it is determined by the hypoxic state as well as the developmental stage of the ectopic endometrial tissue.

Dependent on their typical appearance during laparoscopy, endometriotic lesions are classified as red, black and white lesions. Red lesions exhibit the highest microvessel density and mitotic activity (Nisolle *et al.*, 1993; Fujishita *et al.*, 1999; Kuroda *et al.*, 2009). Moreover, they contain much higher fractions of immature microvessels when compared to black lesions (Matsuzaki *et al.*, 2001a). Accordingly, red lesions seem to be highly active and indicative for an early stage of the disease (Nisolle *et al.*, 1993; Kuroda *et al.*, 2009). In this stage the ectopic endometrial tissue rapidly establishes an own blood supply, which is the prerequisite for its successful engraftment and long-term survival (Becker and D'Amato, 2007; Laschke and Menger, 2007). Hence, endometriosis is associated with the up-regulation of angiogenic factors in the serum and peritoneal fluid of the patients (Taylor *et al.*, 2002; Bourlev *et al.*, 2006a). This stimulates the formation of new blood vessels within endometriotic lesions and the surrounding peritoneum (May and Becker, 2008; Taylor *et al.*, 2009; Kuroda *et al.*, 2010; Rocha *et al.*, 2013). Taken together, these findings indicate that vascularization is a major hallmark in the pathogenesis of endometriosis, which represents a potential target for the development of future diagnostic and therapeutic strategies.

Recent evidence suggests that multiple mechanisms contribute to the vascularization of endometriotic lesions. These include angiogenesis and vasculogenesis as well as inosculation of preformed microvascular networks (Fig. 1). In this review, we provide a systematic overview of these processes in the context of endometriosis and particularly focus on their potential clinical implications in the diagnosis and therapy of the disease.

Methods

Literature searches were performed in PubMed for original and review articles written in the English language focusing on vascularization in endometriosis. The searches included the key words 'endometriosis' and 'endometriotic lesions', which were paired with the key words 'angiogenesis', 'vascularization', 'vasculogenesis', 'endothelial progenitor cells' and 'inosculation'. The searches included both animal and human studies. No restriction was set for the publication date.

Angiogenesis

Definition and biological process

Angiogenesis is defined as the development of new blood vessels from pre-existing ones (Chung *et al.*, 2010). It is initiated by angiogenic growth factors, such as vascular endothelial growth factor (VEGF), which activate the quiescent endothelial cells of a microvessel to release matrix metalloproteinases (MMPs) (Potente *et al.*, 2011). These proteolytic enzymes degrade the vessel's basement membrane. In addition, perivascular mural cells are stimulated to detach from the outer vessel wall by angiopoietin (Ang)-2 (Augustin *et al.*, 2009). In consequence, the endothelial cells migrate into the surrounding tissue, resulting in the formation of vascular buds and sprouts. The organization of these sprouts underlies the tight control of Notch signaling, which determines the cellular specification into tip and stalk cells (Phng and Gerhardt, 2009). Endothelial tip cells form multiple filopodia and, thus, guide the newly developing sprouts towards the angiogenic stimulus. They are followed by proliferating stalk cells, which form a vascular lumen and mediate sprout elongation. Finally, new blood-perfused vessel loops develop by interconnection of individual sprouts. In a last step, these vessels undergo maturation, which is characterized by the production of new extracellular matrix compounds and the recruitment of stabilizing mural cells (Potente *et al.*, 2011).

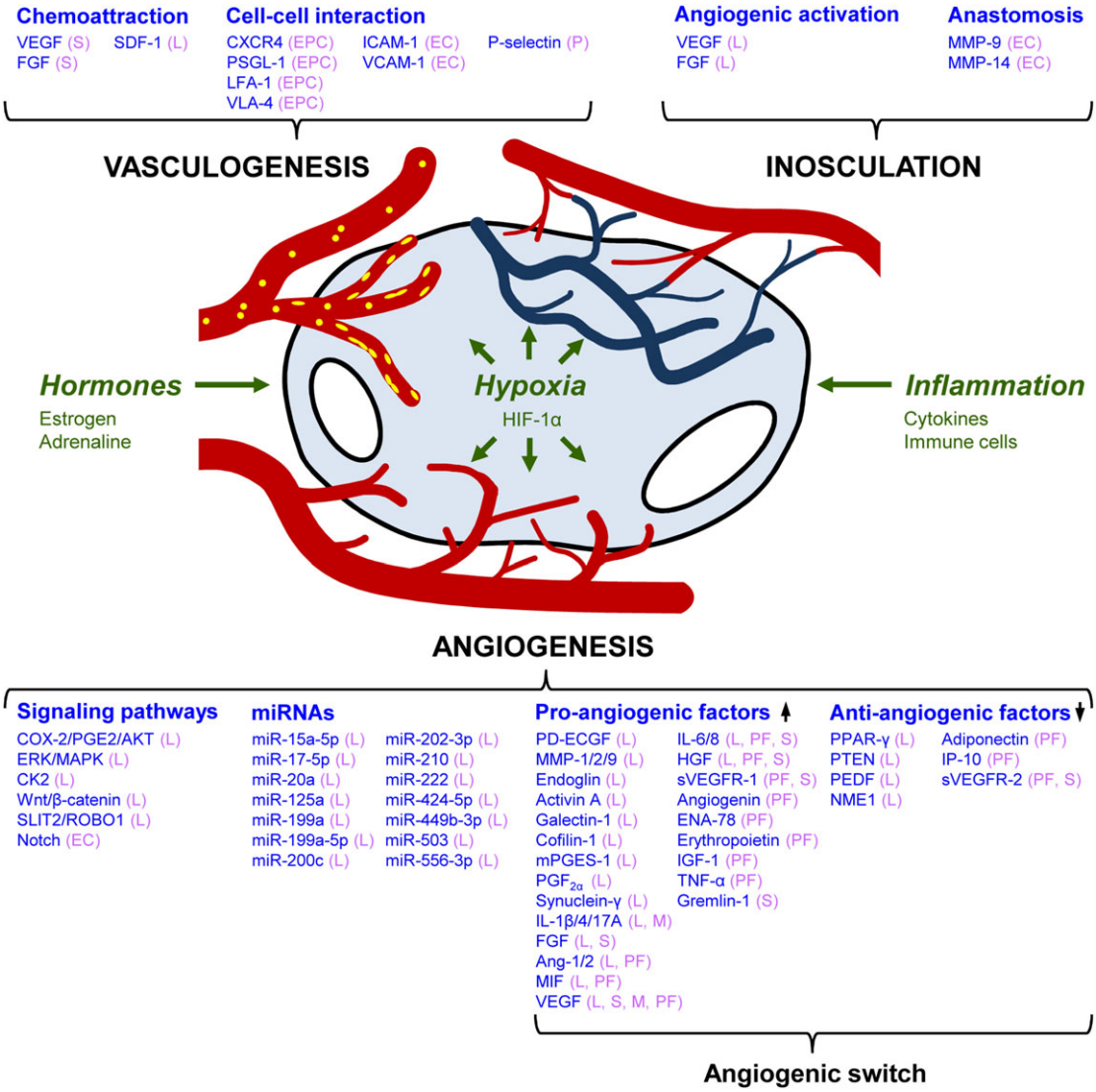

Figure 1 Basic modes of vascularization in endometriosis, i.e. angiogenesis (development of new blood vessels from pre-existing ones), vasculogenesis (incorporation of circulating EPCs from the bone marrow into the microvascular endothelium of newly developing microvessels) and inosculation (interconnection of individual blood vessels or entire microvascular networks with each other), as well as important molecular players and signaling mechanisms (purple brackets indicate their localization: EC, endothelial cell; EPC, endothelial progenitor cell; L, lesion/endometrial cells; M, macrophage; P, platelet; PF, peritoneal fluid; S, serum) that are involved in their regulation. Hypoxia, hormones and inflammation essentially stimulate all three modes of vascularization. Light blue, endometriotic lesion; dark blue, native microvessels of the lesion; red, microvessels of the surrounding host tissue; yellow = EPCs.

Driven by the hope that targeting this process is an efficient strategy for the treatment of growing tumors (Folkman, 1971), sprouting angiogenesis is by now the most thoroughly investigated mechanism of blood vessel formation. Accordingly, the majority of studies in the field of endometriosis research has also focused on this mode of vascularization.

Regulation

The regulation of angiogenesis in endometriosis shares many similarities to the mechanisms mediating the pathological angiogenesis of tumors and metastases. According to Sampson's implantation theory, endometriotic lesions develop from shed endometrial tissue fragments, which enter the peritoneal cavity by retrograde menstruation (Sampson, 1927). Hence, the ectopic endometrial tissue initially suffers from hypoxia comparable to the cells in the center of a growing tumor (Richard *et al.*, 1999; Becker *et al.*, 2008).

Hypoxia is one of the most potent stimuli for the up-regulation of angiogenic growth factors. It prevents the proteasomal degradation of hypoxia-inducible factor (HIF)-1α. This translocates into the nucleus, where it acts as a transcription factor for various genes, such as the gene encoding for VEGF (Becker *et al.*, 2008; Hsiao *et al.*,

2015). In line with these findings, Lu *et al.* (2014) detected a significantly higher expression of HIF-1α and VEGF in hypoxia-exposed human endometrial tissue when compared to hyperoxia- and normoxia-exposed samples. Subcutaneous implantation of these tissues in estrogen-stimulated ovariectomized severe combined immunodeficiency (SCID) mice resulted in an increased growth of the implants in the hypoxia group and a decreased growth of hyperoxic implants when compared to normoxic controls. High levels of HIF-1α are also detected in ovarian endometriomas (Goteri *et al.*, 2010; Filippi *et al.*, 2016; Yerlikaya *et al.*, 2016), which is associated with the up-regulation of VEGF mRNA expression (Filippi *et al.*, 2016). Hsiao *et al.* (2014) further found that hypoxia down-regulates dual specificity phosphatase-2 (DUSP2), which enhances the growth of endometriotic lesions by promoting interleukin (IL)-8-dependent angiogenesis. This may explain the observation of Fasciani *et al.* (2000, 2001) that levels of both VEGF and IL-8 are higher in the cystic fluid of ovarian endometriomas than in serous and follicular cysts.

The expression of VEGF has been extensively studied in different experimental endometriosis models and in tissue samples of endometriosis patients (Deguchi *et al.*, 2001; Gilabert-Estellés *et al.*, 2007; Xu *et al.*, 2013; Song *et al.*, 2014; Gonçalves *et al.*, 2015). Machado *et al.* (2010) reported that the expression of VEGF and its receptor VEGFR-1 (Flk-1) is significantly higher in rat peritoneal lesions when compared to eutopic endometrium. An up-regulation of VEGF mRNA was also observed in human endometrial biopsy samples, which were grafted on the chicken chorioallantoic membrane (CAM) (Kressin *et al.*, 2001). Clinical studies revealed that VEGF is particularly expressed in red endometriotic lesions (Donnez *et al.*, 1998; Novella-Maestre *et al.*, 2010), ovarian endometriomas with large cysts (Goteri *et al.*, 2004; Takehara *et al.*, 2004) and deeply infiltrating endometriosis affecting the rectum (Machado *et al.*, 2008). This indicates that VEGF expression correlates with the activity of endometriotic lesions and the stage of the disease.

Besides VEGF, numerous other factors have been reported to promote angiogenesis in endometriosis. These include fibroblast growth factor (FGF) (Ferriani *et al.*, 1993), platelet-derived endothelial cell growth factor (PD-ECGF) (Fujimoto *et al.*, 1999), angiopoietin (Ang)-1/2 (Drenkhahn *et al.*, 2004; Hur *et al.*, 2006), MMP-1, MMP-2 and MMP-9 (Ria *et al.*, 2002; Wolber *et al.*, 2003; Li *et al.*, 2006; Juhasz-Böss *et al.*, 2010; Jana *et al.* 2016), endoglin (Kim *et al.*, 2001; Hayrabedyan *et al.*, 2005), activin A (Rocha *et al.*, 2012), galectin-1 (Bastón *et al.*, 2014), cofilin-1 (Xu *et al.*, 2010), microsomal prostaglandin E synthase (mPGES)-1 (Numao *et al.*, 2011), macrophage migration inhibitory factor (MIF) (Yang *et al.*, 2000; Carli *et al.*, 2009; Veillat *et al.*, 2010), IL-1β (Huang *et al.*, 2013), IL-4 (Ouyang *et al.*, 2010), IL-17A (Ahn *et al.*, 2015), $PGF_{2\alpha}$ (Ahmad *et al.*, 2015; Rakhila *et al.*, 2016a) and synuclein-γ (Edwards *et al.*, 2014). On the other hand, several factors, such as peroxisome proliferator-activated receptor (PPAR)-γ (Peeters *et al.*, 2005), pigment epithelium derived factor (PEDF) (Sun *et al.*, 2012; Fu *et al.*, 2013), protein tyrosine phosphatase (PTEN) (Lv *et al.*, 2016) and non-metastatic gene 23-H1 (NME1) (Chang *et al.*, 2013), have been shown to exert suppressive effects on endometrial angiogenesis. This continuously growing list of factors indicates that angiogenesis in endometriosis is not solely driven by a few specific mechanisms, but rather determined by the complex expression patterns of various molecular players mediating pro- and anti-angiogenic effects. Accordingly, it may be assumed that the balance hypothesis for the angiogenic switch, as it has originally been suggested for tumor angiogenesis (Hanahan and Folkman, 1996), is also applicable for endometriosis. The hypothesis postulates that the microvasculature of tissues is kept in a quiescent state under physiological conditions due to the balance of angiogenesis inducers and inhibitors. In contrast, non-physiological conditions, such as the retrograde menstruation of shed endometrial fragments into the peritoneal cavity, may increase the levels of angiogenesis inducers or reduce the levels of angiogenesis inhibitors. This dysbalance finally activates the angiogenic switch, resulting in the development of new blood vessels (Fig. 1).

The stimulation of endometrial and endometriotic cells leads to the activation of different intracellular pathways and associated signaling molecules. A key player in this signaling network is cyclooxygenase (COX)-2, the rate-limiting enzyme of PGE2 synthesis. There is a close correlation between COX-2 and VEGF expression in ovarian endometriomas (Ceyhan *et al.*, 2008). Moreover, COX-2 regulates MMP-2 activity in endometriotic lesions (Jana *et al.*, 2016). The expression of COX-2 underlies the control of p38 and extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK) (Carli *et al.*, 2009; Huang *et al.*, 2013). These kinases are activated by the pro-inflammatory cytokines MIF (Carli *et al.*, 2009) and IL-1β (Huang *et al.*, 2013), indicating a close link between inflammation and angiogenesis in endometriosis. Furthermore, they interact with other signaling cascades. For instance, Matsuzaki and Darcha (2015) observed an up-regulation of ERK expression in endometriotic stromal cells when AKT expression was decreased and vice versa. This important finding should be considered when developing novel treatment strategies for endometriosis, because such compensatory effects can cause therapy resistance.

Besides COX-2, protein kinase CK2 (Feng *et al.*, 2012), SLIT2/Roundabout (ROBO)1 (Guo *et al.*, 2013) and Wnt/β-catenin (de Mattos *et al.*, 2016) are also potential angiogenic regulators, which are extensively expressed in endometrial tissue. They are important for endothelial cell differentiation and function and, thus, are crucially involved in the development of new blood vessels under physiological and pathological conditions (Wang *et al.*, 2003; Dejana, 2010; Montenarh, 2014). Accordingly, Feng *et al.* (2012) detected a significantly reduced microvessel density within engrafting murine endometriotic lesions, which were treated with the CK2 inhibitor quinalizarin, when compared to vehicle-treated controls. Guo *et al.* (2013) cross-transplanted endometrial tissue within and between SLIT transgenic and wild-type mice. Using this elegant approach, they could demonstrate that SLIT2 overexpression increases the microvessel density and size of lesions in the peritoneal cavity of mice. Finally, de Mattos *et al.* (2016) observed a strong activation of the Wnt/β-catenin pathway in rat endometriotic lesions, as indicated by high levels of nuclear β-catenin.

In addition, Notch signaling has recently been shown to control angiogenic sprout formation in engrafting ectopic endometrial tissue (Körbel *et al.*, 2017). This process is stimulated by VEGF, which promotes the expression of membrane-bound Notch ligand Dll4 on individual microvascular endothelial cells (Lobov *et al.*, 2007). Consequently, they become non-proliferating tip cells that bind with Dll4 to Notch1 receptors on adjacent endothelial cells, inducing the cleavage of the Notch intracellular domain by a γ-secretase. This makes the neighboring cells less sensitive to VEGF stimulation by

down-regulation of VEGFR-2 expression and activates their proliferation (Gerhardt *et al.*, 2003). Accordingly, they become stalk cells and contribute to the growth of angiogenic sprouts. In line with these findings, Körbel *et al.* (2017) demonstrated that inhibition of Notch signaling by the γ-secretase inhibitor DAPT increases the number of angiogenic sprouts within newly developing murine endometriotic lesions.

A rapidly growing number of studies further suggests an important function of microRNAs (miRNAs) in the highly complex signaling network regulating angiogenesis in endometriosis (Teague *et al.*, 2010; Marí-Alexandre *et al.*, 2015). MiR-200c (Panda *et al.*, 2012), miR-15a-5p (Liu et al., 2016b; Yang *et al.*, 2016), miR-20a (Lin *et al.*, 2012), miR-199a (Dai *et al.*, 2015), miR-199a-5p (Hsu *et al.*, 2014), miR-210 (Okamoto *et al.*, 2015), miR-503 (Hirakawa *et al.*, 2016), miR-125a, miR-222, miR-17-5p (Ramón *et al.*, 2011) as well as miR-202-3p, miR-424-5p, miR449b-3p and miR-556-3p (Braza-Boïls *et al.*, 2014) have recently been reported to control the expression of angiogenic factors in eutopic endometrium and endometriotic lesions. Braza-Boïls *et al.* (2013, 2015) found that the peritoneal fluid from patients with endometriosis modulates the expression of such miRNAs in endometrial and endometriotic cell cultures. Hence, they suggested that this may be an important mechanism contributing to the angiogenic and proteolytic disequilibrium in the peritoneal cavity of endometriosis patients.

The production and release of angiogenic factors is not restricted to the ectopic endometrial tissue. In fact, endometriosis is typically associated with chronic inflammation, which is characterized by high pro-inflammatory cytokine levels and the activation of inflammatory cells in the peritoneal microenvironment (Taylor *et al.*, 1997). Accordingly, macrophages are a major source for VEGF in endometriosis (McLaren *et al.*, 1996a). They also release IL-1β, which stimulates the production of VEGF and IL-6 by stromal cells from endometriotic lesions (Lebovic *et al.*, 2000). Moreover, macrophages are alternatively activated in endometriotic lesions, which improves their pro-angiogenic capacity and promotes ectopic lesion growth (Bacci *et al.*, 2009). Capobianco *et al.* (2011) further found that Tie2-expressing macrophages (TEMs) infiltrate tissue areas surrounding newly formed endometriotic microvessels, where they maintain vessel viability by suppressing apoptotic cell death of endothelial cells. Additional inflammatory cells, which contribute to angiogenesis in endometriosis, are neutrophils (Lin *et al.*, 2006; Na *et al.*, 2006), dendritic cells (Fainaru *et al.*, 2008; Pencovich *et al.*, 2014) and regulatory T cells (Wang *et al.*, 2017).

Angiogenesis in endometriotic lesions is also markedly influenced by hormones. In line with the fact that endometriosis is an estrogen-dependent disease, vascularization of endometriotic lesions is stimulated by estrogen (Laschke *et al.*, 2005; Huang *et al.*, 2014; Zhao *et al.*, 2015; Zhang *et al.*, 2016), whereas progesterone suppresses blood vessel formation (Li *et al.*, 2016). Moreover, systemic adrenergic signaling induced by surgery or social psychogenic stress increases angiogenesis and accelerates growth of endometriotic lesions in mice (Long *et al.*, 2016a,b; Guo *et al.*, 2017). This raises the possibility that the progression of endometriosis may be positively influenced by stress-reducing measures.

Taken together, these findings demonstrate that angiogenesis in endometriosis underlies the complex regulation of numerous intra- and extra-cellular signaling molecules, which interact with each other and are influenced by local hypoxia and inflammatory stimuli within the specific microenvironment of the peritoneal cavity, as well as systemic hormone levels (Fig. 1). However, this regulation is even more complex considering the fact that different endometriosis phenotypes markedly differ in terms of their expression levels of genes associated with hypoxia and angiogenesis. For instance, Filippi *et al.* (2016) reported that ovarian endometrioma express high levels of HIF-1/2α, protease-activated receptor (PAR)-1/4 and VEGF, whereas deep infiltrating endometriosis does not show significantly different gene expression patterns when compared to endometrium from unaffected women. Moreover, in comparison to black and white peritoneal lesions, red lesions are typically characterized by the up-regulation of pro-angiogenic factors, such as VEGF and HGF, which is associated with an increased microvessel density and less fibrosis (Donnez *et al.*, 1998; Khan *et al.*, 2004). Hence, different subtypes of endometriotic lesions exhibit specific vascularization patterns. In reverse, it may be speculated that different vascularization patterns determine the development of ectopic endometrium into specific endometriosis phenotypes and, thus, actively contribute to the heterogeneity of the disease.

Clinical implications

The fundamental role of angiogenesis in the pathogenesis of endometriosis is reflected by the observation that the peritoneal fluid from endometriosis patients significantly increases the proliferation of endothelial cells (Sokolov *et al.*, 2005) and induces a strong vascular reaction in the CAM model (Oosterlynck *et al.*, 1993). This is due to the fact that the peritoneal fluid contains elevated concentrations of different angiogenesis-promoting factors (Table I), including VEGF (McLaren *et al.*, 1996b; Mahnke *et al.*, 2000; Bourlev *et al.*, 2006a; Kianpour *et al.*, 2013; Young *et al.*, 2015), soluble VEGF receptor (sVEGFR)-1 (Bourlev *et al.*, 2010), Ang-2 (Bourlev *et al.*, 2010), insulin-like growth factor (IGF)-1 (Sokolov *et al.*, 2005), erythropoietin (Matsuzaki *et al.*, 2001b), hepatocyte growth factor (HGF) (Zong *et al.*, 2003), MIF (Kats *et al.*, 2002), tumor necrosis factor (TNF)-α (Maas *et al.*, 2001), IL-6 (Mahnke *et al.*, 2000), IL-8 (Barcz *et al.*, 2002), angiogenin (Suzumori *et al.*, 2004a) and epithelial neutrophil-activating peptide (ENA)-78 (Suzumori *et al.*, 2004b). On the other hand, the peritoneal fluid of endometriosis patients also contains lower concentrations of the anti-angiogenic factors adiponectin (Takemura *et al.*, 2005), interferon-gamma-induced protein (IP)-10 (Yoshino *et al.*, 2003; Rakhila *et al.*, 2016b) and sVEGFR-2 (Bourlev *et al.*, 2010) (Table I). VEGF, ENA-78 and HGF levels are particularly high whereas IP-10 and adiponectin levels are low in women with advanced stages of the disease.

These findings imply the interesting possibility that pro- and anti-angiogenic factors may serve in the future as biomarkers for the diagnosis and classification of endometriosis or for the efficacy assessment of therapeutic approaches (May *et al.*, 2010). For this purpose, serum or urinary levels of VEGF (Bourlev *et al.*, 2006a, 2010; Wang *et al.*, 2009; Kopuz *et al.*, 2014; Vodolazkaia *et al.*, 2016), sVEGFR-1 (Cho *et al.*, 2007), FGF-2 (Bourlev *et al.*, 2006b), HIF-1α (Karakus *et al.*, 2016), Ang-2 (Bourlev *et al.*, 2010), HGF (Zong *et al.*, 2003), IL-8 (Barcz *et al.*, 2002) and gremlin-1 (Sha *et al.*, 2009) have been compared in patients with and without endometriosis (Table I). However, although promising differences between the study groups were detected, so far none of these factors has been clearly shown

Table I Studies reporting elevated levels of pro-angiogenic factors or decreased levels of anti-angiogenic factors in the peritoneal fluid, serum/plasma or urine of endometriosis patients.

Effect on angiogenesis	Factor	Analyzed fluid	Study
Pro-angiogenic (elevated levels)	Angiogenin	Peritoneal fluid	Suzumori *et al.* (2004a)
	ENA-78	Peritoneal fluid	Suzumori *et al.* (2004b)
	Erythropoietin	Peritoneal fluid	Matsuzaki *et al.* (2001b)
	FGF-2	Serum	Bourlev *et al.* (2006b)
	Gremlin-1	Serum	Sha *et al.* (2009)
	HGF	Peritoneal fluid	Zong *et al.* (2003)
		Serum	
	HIF-1α	Serum	Karakus *et al.* (2016)
	IGF-1	Peritoneal fluid	Sokolov *et al.* (2005)
	IL-6	Peritoneal fluid	Mahnke *et al.* (2000)
	IL-8	Peritoneal fluid	Barcz *et al.* (2002)
		Serum	
	MIF	Peritoneal fluid	Kats *et al.* (2002)
	sVEGFR-1	Peritoneal fluid	Bourlev *et al.* (2010)
		Serum	Bourlev *et al.* (2010)
			Cho *et al.* (2007)
		Urine	Cho *et al.* (2007)
	TNF-α	Peritoneal fluid	Maas *et al.* (2001)
	VEGF	Peritoneal fluid	Bourlev *et al.* (2006a)
			Bourlev *et al.* (2010)
			Kianpour *et al.* (2013)
			McLaren *et al.* (1996b)
			Mahnke *et al.* (2000)
			Wang *et al.* (2009)
			Young *et al.* (2015)
		Serum/plasma	Bourlev *et al.* (2006a)
			Bourlev *et al.* (2010)
			Kopuz *et al.* (2014)
			Vodolazkaia *et al.* (2016)
			Wang *et al.* (2009)
Anti-angiogenic (decreased levels)	Adiponectin	Peritoneal fluid	Takemura *et al.* (2005)
	IP-10	Peritoneal fluid	Yoshino *et al.* (2003)
			Rakhila *et al.* (2016b)
	sVEGFR-2	Peritoneal fluid	Bourlev *et al.* (2010)
		Serum	

to be of clinical use due to insufficient sensitivity and specificity. This may be partly explained by too low numbers or heterogeneous disease stages of the enrolled patients. In addition, it may not be expedient to focus on only one of these factors. As suggested by May *et al.* (2010), it is much more reasonable to develop a reliable diagnostic tool for endometriosis by combining a panel of different biomarkers. This is a realistic goal for the near future considering the impressive progress in genomics, proteomics and metabolomics. Such a tool may not only be useful for diagnostic means but also to assess the risk of developing endometriosis or adenomyosis. Several genetic polymorphisms of VEGF and FGF have already been identified, and may be associated with these diseases (Bhanoori *et al.*, 2005; Gentilini *et al.*, 2008; Kang et al., 2009, 2010; Li *et al.*, 2013; Cardoso *et al.*, 2017).

In line with the famous concept 'Fighting cancer by attacking its blood supply' postulated by Judah Folkman (1996), angiogenesis has also been proposed as a promising target for gene therapy (Dabrosin *et al.*, 2002; Ma and He, 2014; Wang *et al.*, 2014) or pharmacological treatment of endometriosis (Nap *et al.*, 2004; Becker and D'Amato, 2007; Van Langendonckt *et al.*, 2008; Liu et al., 2016a). Accordingly, numerous compounds with anti-angiogenic activity have been evaluated in pre-clinical endometriosis studies, as summarized in detail in a previous review (Laschke and Menger, 2012a). Briefly, they include growth factor inhibitors (Ricci *et al.*, 2011), endogenous angiogenesis inhibitors (Becker *et al.*, 2006), fumagillin analogues (Nap *et al.*, 2005), statins (Bruner-Tran *et al.*, 2009), COX-2 inhibitors (Laschke *et al.*, 2007), phytochemicals (Rudzitis-Auth *et al.*, 2013), immunomodulators

(Laschke *et al.*, 2006a), dopamine agonists (Novella-Maestre *et al.*, 2009), PPAR agonists (Nenicu *et al.*, 2014) and anti-hormonal drugs (Katayama *et al.*, 2010). Most of these compounds have been shown to reduce the microvessel density of endometriotic lesions in different animal models, which was associated with lower numbers of engrafted lesions or a suppressed lesion growth. These experimental findings indicate that anti-angiogenic approaches may indeed be useful to prevent the development of new lesions or the progression of the disease. Moreover, they may have direct beneficial effects on the pain symptoms of endometriosis patients. According to the concept of neuroangiogenesis (Asante and Taylor, 2011), blood vessels invading endometriotic lesions are accompanied by nerve fibers, which is regulated by estrogen-dependent SLIT/ROBO signaling (Greaves *et al.*, 2014a). These nerve fibers stimulate dorsal root neurons within the central nervous system, which increases the pain perception in endometriosis patients (Asante and Taylor, 2011). Based on this, Novella-Maestre *et al.* (2012) reported that treatment of human endometrial fragments in nude mice with the anti-angiogenic agent cabergoline diminishes not only blood vessel formation but also nerve fiber ingrowth in the ectopic tissue.

However, despite these promising experimental results, an anti-angiogenic therapy could not be implemented yet into the clinical routine treatment of endometriosis. This may be due to several reasons. Endometriosis is a heterogeneous disease with diverse types of lesions in different locations that markedly differ in terms of their tissue composition and vascularization (Jondet *et al.*, 2006). Anti-angiogenic compounds may mainly target early red lesions exhibiting a high angiogenic activity and many immature microvessels, whereas older black and white lesions may be resistant to this type of treatment. Hence, anti-angiogenic therapy may not be suitable as a monotherapy focusing on the pharmacological eradication of well-established endometriotic lesions in the peritoneal cavity. However, it could gain major importance in the prevention of new lesion formation after surgical removal, and, thus, help to reduce the high recurrence rates of surgical endometriosis therapy.

In addition, resistance to an anti-angiogenic therapy may be caused by the balanced cross-talk of different angiogenic factors and signaling pathways, compensating the inhibition of only one target (Matsuzaki and Darcha, 2015). This problem may be overcome by means of pleiotropic compounds, which simultaneously suppress different angiogenic mechanisms (Laschke *et al.*, 2011a), or by means of a combination therapy. For instance, Nenicu *et al.* (2017) treated murine endometriosis-like lesions with telmisartan, an angiotensin II type 1 receptor blocker and activator of PPAR-γ, together with the selective COX-2 inhibitor parecoxib. In contrast to the monotherapy with these compounds, this combination therapy inhibited both AKT and ERK signaling, resulting in a higher therapeutic efficiency as indicated by an enhanced regression of the lesions.

Finally, a further reason why the anti-angiogenic therapy has not yet made its way in the treatment of endometriosis comparable to the treatment of cancer is that women suffering from endometriosis are, in contrast to most cancer patients, in their reproductive age and may desire to have children. Fertility and pregnancy are crucially dependent on physiological angiogenesis in the ovary, uterus and placenta (Shimizu *et al.*, 2012). Therefore, anti-angiogenic compounds may be only acceptable for the short-term treatment of endometriosis patients and should not induce long-term side effects on the female reproductive organs. They should ideally inhibit blood vessel formation only in endometriotic lesions. This, however, requires the identification of highly selective, endometriosis-specific target molecules, which have not been identified so far. Hence, for the present it may be more realistic to develop an anti-angiogenic treatment modality for endometriosis patients with compounds, which have been shown to have a favorable safety profile and are already clinically approved for the therapy of other benign diseases, such as dopamine agonists (Delgado-Rosas *et al.*, 2011; Gómez *et al.*, 2011).

Vasculogenesis

Definition and biological process

Vasculogenesis has been originally defined as the de novo formation of blood vessels by differentiation and assembly of angioblastic progenitor cells during embryogenesis (Risau and Flamme, 1995). Meanwhile, it is well known that vasculogenesis also occurs in adults. This type of post-natal vasculogenesis is defined as the incorporation of circulating endothelial progenitor cells (EPCs) from the bone marrow into the microvascular endothelium of newly developing microvessels (Asahara *et al.*, 1999; Asahara and Kawamoto, 2004).

EPCs contribute to the formation of new blood vessels under various pathological conditions, such as tumor growth (Ding *et al.*, 2008), myocardial infarction (King and McDermott, 2014) and stroke (Ma *et al.*, 2015). However, they are also essential for the physiological vascularization of the regenerating endometrium during the menstrual cycle (Masuda *et al.*, 2007; Mints *et al.*, 2008; Demir *et al.*, 2010). In addition, EPCs are recruited in the microvasculature of endometriotic lesions (Laschke *et al.*, 2011b). The first proof of vasculogenesis in experimental endometriosis was provided by two independent studies in 2011. In these studies, endometriotic lesions were surgically induced in irradiated mice, which were reconstituted with bone marrow from green fluorescent protein $(GFP)^+$ mice (Becker *et al.*, 2011; Laschke *et al.*, 2011c). This GFP^+/GFP^- cross-over design allowed the immunohistochemical detection of GFP^+ EPCs in the engrafting endometriotic lesions, indicating that vasculogenesis is a relevant mode of vascularization in endometriosis (Fig. 2).

Regulation

EPCs are mobilized from the bone marrow into the circulation in response to high levels of chemoattractive factors in the blood (Heissig *et al.*, 2002; Rafii and Lyden, 2003). These factors include VEGF and FGF (Kalka *et al.*, 2000; Fontaine *et al.*, 2006), which are also increased in the serum of endometriosis patients (Bourlev *et al.*, 2006b; Kopuz *et al.*, 2014). The homing of circulating EPCs in endometriotic lesions is regulated by the interaction of their receptor chemokine receptor type (CXCR)4 with the small molecular weight chemokine stromal cell-derived factor (SDF)-1 (Laschke *et al.*, 2011c). Tissue hypoxia up-regulates HIF-1α-mediated SDF-1 expression (Ceradini *et al.*, 2004). Accordingly, tissue levels of SDF-1 are particularly high in early engrafting murine endometriotic lesions, which still exhibit an incomplete vascularization (Laschke *et al.*, 2011c). Increased SDF-1 gene and protein expression levels are also detected in different types of human endometriosis (Furuya *et al.*, 2007; Virani *et al.*, 2013). Blockade of the SDF-1/CXCR4 axis

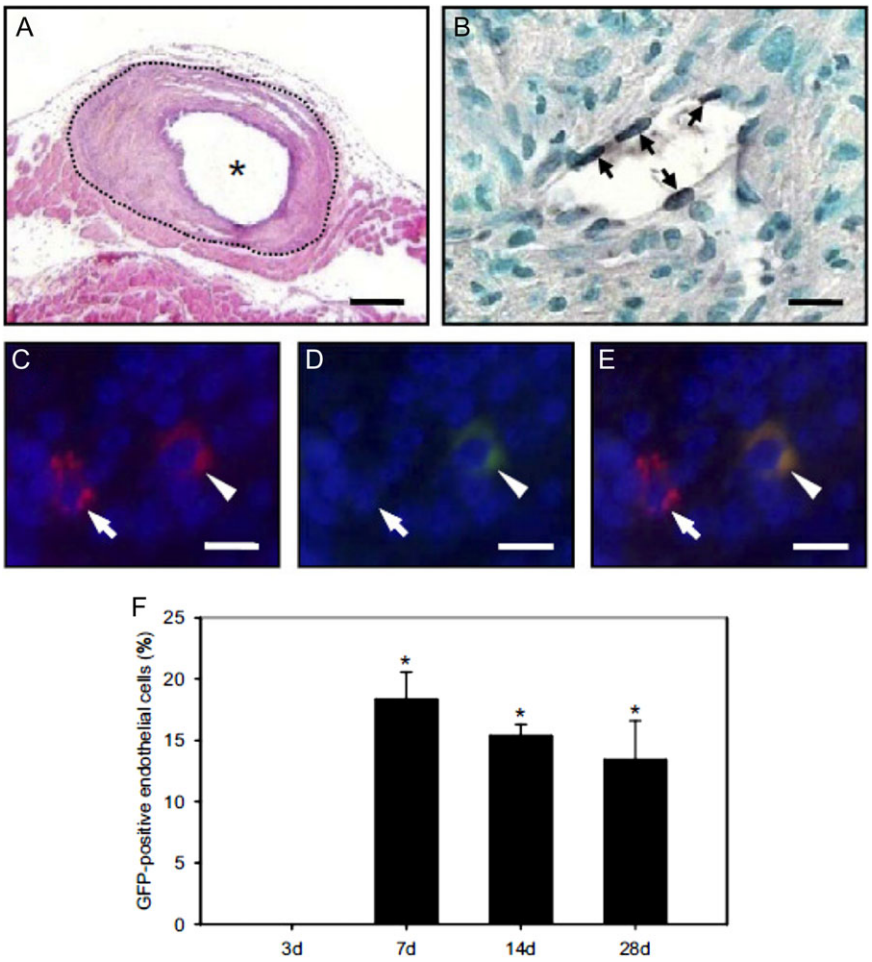

Figure 2 Experimental GFP$^+$/GFP$^-$ cross-over design for the immunohistochemical detection of GFP$^+$ EPCs in engrafting murine endometriotic lesions. (**A**) Hematoxylin–eosin-stained cross section of a peritoneal endometriotic lesion (borders marked by broken line) at Day 28 after fixation of an uterine tissue sample from a FVB/N mouse to the lateral abdominal wall of a Tie2-GFP bone marrow-transplanted animal. The lesion is characterized by a cyst-like dilated endometrial gland (asterisk), which is surrounded by a well-vascularized endometrial stroma. (**B**) Immunohistochemical detection of GFP$^+$ endothelial cells (arrows) of a blood vessel within an endometriotic lesion at Day 28, representing recruited bone marrow-derived EPCs. (**C**–**E**) Immunofluorescence microscopy of an endometriotic lesion at Day 14 after transplantation of an uterine tissue sample from a FVB/N mouse into the peritoneal cavity of a Tie2-GFP bone marrow-transplanted animal. Histological sections were stained with Hoechst to identify cell nuclei (C–E, blue), an antibody against the endothelial cell marker CD31 (**C**, **E**, red) and an antibody against GFP (**D**, **E**, green). **E** displays the merge of **C** and **D**. The EPC, which stains double positive for CD31/GFP (arrowheads), is next to a normal CD31-positive endothelial cell (arrows). Scale bars: 200 µm (**A**); 8 µm (**B**); 7 µm (**C**–**E**). (**F**) Number (percentage) of GFP$^+$ endothelial cells lining the wall of blood vessels in endometriotic lesions at Days 3, 7, 14, and 28 after fixation of uterine tissue samples from FVB/N mice to the lateral abdominal wall of Tie2-GFP bone marrow-transplanted animals. Data are given as the mean ± SEM. *$P < 0.05$ versus Day 3. The figure is reprinted from Laschke *et al.* (2011c); used with permission from Elsevier.

significantly reduces the number of recruited EPCs in endometriotic lesions, which are induced by transplantation of murine (Laschke *et al.*, 2011c) or human endometrium (Virani *et al.*, 2013) into the peritoneal cavity of recipient mice. This is associated with a lower microvessel density and survival of the ectopic endometrial tissue (Laschke *et al.*, 2011c; Virani *et al.*, 2013).

Vasculogenesis in endometriotic lesions may also be a hormonally regulated process. This has been indicated by a recent study demonstrating that the treatment of mice with β-estradiol 17-valerate promotes the incorporation of EPCs into the newly developing microvasculature of surgically induced endometriotic lesions (Rudzitis-Auth *et al.*, 2016). This observation can be explained by different estrogen effects. *In-vitro* analyses revealed that estrogen directly activates EPCs via estrogen receptor-α, which increases their proliferation, viability as well as their migratory and tube-forming activity (Strehlow *et al.*, 2003; Zhao *et al.*, 2008; Rudzitis-Auth *et al.*, 2016). Gene expression profile analyses of estrogen-treated murine endometriotic lesions further showed a strong up-regulation of the EPC-activating factor plasma kallikrein B1 (Rudzitis-Auth *et al.*, 2016), which stimulates EPC homing under inflammatory conditions by bradykinin-mediated up-regulation of chemokine receptor type-4 (Dai *et al.*, 2012). Moreover, several studies demonstrated that estrogen and phytoestrogens mobilize EPCs from

the bone marrow (Ruifrok *et al.*, 2009; Chan *et al.*, 2011). However, others could not detect increased levels of circulating EPCs in response to estrogen (Robb *et al.*, 2009; Webster *et al.*, 2013; Rudzitis-Auth *et al.*, 2016), but rather observed large variations of EPC levels between individual subjects (Webster *et al.*, 2013). Hence, additional studies are needed to clarify the influence of estrogen on EPC mobilization and to identify possible biases, which may have contributed to these contradictory findings.

Finally, it may be speculated that additional mechanisms mediate the recruitment of EPCs into endometriotic lesions, which, however, have not been investigated systematically so far. For instance, Lev *et al.* (2006) found that circulating EPCs can be captured from the bloodstream by activated platelets through interaction of their surface adhesion molecules P-selectin glycoprotein ligand (PSGL)-1 and P-selectin. In turn, pioneering work of Guo and coworkers showed that administration of soluble P-selectin to block PSGL-1 (Guo *et al.*, 2015) and different platelet depletion schemes (Guo *et al.*, 2016) reduces the size of developing murine endometriotic lesions. In line with the results of Lev *et al.* (2006), this was associated with a decreased lesion vascularization.

In addition, EPCs also express lymphocyte function-associated antigen (LFA)-1 and very late antigen (VLA)-4 on their surface (Duan *et al.*, 2006). Hence, they may directly bind to the endothelial ligands intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 (Duan *et al.*, 2006; Silverman *et al.*, 2007). These endothelial adhesion molecules are up-regulated in response to stimuli, which are typically associated with engrafting endometriotic lesions, such as progressively increasing microvascular shear stress (Laschke *et al.*, 2005) and inflammation (Jiang *et al.*, 2016).

Clinical implications

The finding that vasculogenesis is an important process in the vascularization of endometriotic lesions opens the door for the development of novel diagnostic and therapeutic tools for endometriosis patients. For this purpose, it may be expedient to clarify whether currently discussed EPC-based concepts developed in cardiovascular and oncology research can be transferred to endometriosis.

A common approach is the measurement of circulating EPC levels as biomarker for disease risk, activity and progression (Werner *et al.*, 2005; Zhang *et al.*, 2005). Becker *et al.* (2011) detected elevated levels of EPCs in the blood of 129/SvJ mice with surgically induced endometriotic lesions when compared to sham-operated control animals. The concentrations of EPCs positively correlated with the amount of endometriotic tissue within the peritoneal cavity and peaked in an early stage of lesion development. However, EPC levels were not increased in this endometriosis model when using C57BL/6 mice (Becker *et al.*, 2011; Laschke *et al.*, 2011c), which exhibit a markedly lower angiogenic activity than 129/SvJ mice (Rohan *et al.*, 2000). These findings indicate that the outcome of such analyses is crucially dependent on the used mouse strain. Moreover, they raise the important question of whether the results of experimental endometriosis studies are transferable to the human disease if contrasting effects are already observed within one species. It should be clear that animal models cannot completely mimic the pathogenesis of human endometriosis. On the other hand, due to the possibility of genetic manipulation, they represent powerful tools for the identification of novel molecular and cellular mechanisms of the disease in a controlled *in-vivo* setting. For this purpose, it is of utmost importance to continuously improve these models and to standardize experimental designs and end-points to increase their predictive power (Pullen *et al.*, 2011). Specific examples for such improvements are the establishment of humanized models of endometriosis by xenografting human tissue samples into immunodeficient mice (Bruner *et al.*, 1997; Hull *et al.*, 2008) or the establishment of a menstruating mouse model of endometriosis (Greaves *et al.*, 2014b). High-quality, clinically relevant data may be particularly generated by combining different *in-vitro* assays and *in-vivo* models that include human tissue (Greaves *et al.*, 2017). Nonetheless, finally the results from experimental endometriosis studies have to be confirmed in controlled randomized clinical trials.

Webster *et al.* (2013) performed the first clinical study to evaluate the usability of circulating EPCs as biomarker for endometriosis. They found that there was no significant difference in the mean EPC levels between endometriosis patients and healthy women. Additional subgroup analyses further showed no differences in the mean EPC levels in patients with different stages of the disease according to the revised ASRM classification system. However, the study did not consider different lesion types in individual patients. Hence, the authors speculated that an elevation of circulating EPCs may be possibly detected in women with newly developing red lesions exhibiting a high angiogenic activity (Webster *et al.*, 2013).

Although the results of Webster *et al.* (2013) indicate that EPC levels are not an appropriate biomarker for all stages of endometriosis, they do not exclude the possibility that EPCs are recruited into the microvasculature of endometriotic lesions without a demonstrable effect on peripheral EPC levels (Laschke *et al.*, 2011c). In this case, EPCs may be alternatively used as carriers of contrast agents for the non-invasive diagnostic detection of endometriotic lesions within the peritoneal cavity. Although such an application is still fiction, promising experimental results already indicate that EPCs can be loaded with iron oxide nanoparticles, which allows their visualization by means of magnetic resonance imaging (Gazeau and Wilhelm, 2010). In addition, modified EPCs may also serve as shuttles for selective future gene therapy of endometriotic lesions, as currently being evaluated for the treatment of tumors (Chen *et al.*, 2014; Laurenzana *et al.*, 2016).

Inosculation

Definition and biological process

Inosculation is defined as the interconnection of individual blood vessels or entire microvascular networks with each other (Laschke and Menger, 2012b). This process contributes to the rapid onset of blood perfusion in tissue grafts, which already contain preformed microvessels, such as transplanted skin (Converse *et al.*, 1975) and bone (Rothenfluh *et al.*, 2004) as well as prevascularized bioartificial tissue constructs (Laschke and Menger, 2016). For this purpose, the grafts' preformed microvessels wrap around the microvessels of the surrounding host tissue and express high levels of MMP-14 and MMP-9 (Cheng *et al.*, 2011). This causes basement membrane and pericyte reorganization and localized disruption of the underlying host endothelium, resulting in the inflow of blood into the preformed microvascular network (Cheng *et al.*, 2011).

A common approach for the artificial induction of endometriotic lesions in non-menstruating rodents is the transplantation of isolated endometrial tissue fragments from the uterus of donor animals to ectopic sites (Grümmer, 2006). These fragments exhibit a physiological tissue architecture with fully functional microvascular networks at the time point of harvesting. Accordingly, their engraftment is associated with inosculation (Feng *et al.*, 2014). Considering Sampson's implantation theory, inosculation may not only be an important mechanism of vascularization under these experimental conditions, but also in clinical endometriosis. In line with this view, tissue integrity has been shown to be crucial for the ectopic implantation of human endometrium, whereas transplantation of dispersed endometrial cells from menstrual effluent does not result in lesion formation (Nap *et al.*, 2003).

Regulation

Inosculation is characterized by the close interaction of the preformed microvascular network within a tissue graft and the surrounding host microvasculature. Hypoxia-driven release of angiogenic growth factors from the graft stimulates the ingrowth of vascular sprouts from the host tissue, which internally inosculate with the preformed microvessels (Laschke *et al.*, 2009). On the other hand, the high angiogenic activity of the preformed microvascular network may also promote the outgrowth of blood vessels from the graft into the surrounding host tissue, where external inosculation is achieved (Laschke *et al.*, 2010). In line with these findings, Eggermont *et al.* (2005) observed that the revascularization of human endometrial implants in nude mice involves the disappearance of native graft vessels, which coincides with the invasion of the endometrial stroma by murine vessels. In contrast, Machado *et al.* (2014) found after transplantion of endometrial fragments from GFP^+ donor mice into the peritoneal cavity of GFP^- wild-type mice that most of the grafts' blood vessels consisted of GFP^+ cells. Intravital fluorescence microscopy of GFP^+ endometrial fragments in a mouse dorsal skinfold chamber model even demonstrated the outgrowth of GFP^+ vascular sprouts over time and their final inosculation in the GFP^- host tissue (Feng *et al.*, 2014).

Taken together, these results indicate that dependent on the experimental setting, both internal and external inosculation contribute to the vascularization of engrafting endometriotic lesions. Hence, these different vascularization modes may also determine the fate of menstruated endometrial fragments in women and, thus, the risk of developing endometriosis. In fact, it may be speculated that external inosculation particularly contributes to the rapid vascularization and successful engraftment of shed endometrial fragments in the peritoneal cavity. External inosculation, however, requires viable and well preserved microvascular networks within the fragments. Because the fragments solely survive by diffusion and suffer from hypoxia during retrograde menstruation through the Fallopian tubes, the survival of their microvasculature is crucially determined by the time period that they need to reach the peritoneal cavity. Hence, although not further analyzed so far, it may be hypothesized that uterine hyperperistalsis, as observed in endometriosis patients (Bulletti *et al.*, 2004; Leyendecker and Wildt, 2011), accelerates the transport of the endometrial fragments and, thus, essentially shortens this critical time period. Another prerequisite for external inosculation is a high sprouting angiogenic activity of the preformed microvessels within endometrial fragments. This may be supported by the fact that numerous signaling pathways, including COX-2 and Notch, are dysregulated in the eutopic endometrium of women with endometriosis (Su *et al.*, 2015; Logan *et al.*, 2018). Accordingly, excessive endometrial angiogenesis has been found in women with endometriosis when compared to healthy subjects (Healy *et al.*, 1998; Burlev *et al.*, 2005). The endometrium of endometriosis patients shows an up-regulated or dysregulated expression of several angiogenic factors, including VEGF (Hey-Cunningham *et al.*, 2013), Ang-1/2 (Hur *et al.*, 2006), tissue factor (Krikun *et al.*, 2008), pleiotrophin and midkine (Chung *et al.*, 2002) and contains more blood vessels expressing the cell adhesion molecule integrin αVβ3 (Healy *et al.*, 1998). In addition, it contains an increased number of proliferating endothelial cells (Wingfield *et al.*, 1995). In summary, these alterations may promote external inosculation and, thus, improve the capacity of endometrial tissue from women with endometriosis to proliferate, implant and grow within the peritoneal cavity (Fig. 3).

Clinical implications

The existence of preformed, mature microvessels in engrafting endometriotic lesions complicates the establishment of anti-angiogenic strategies for the treatment of endometriosis. Such microvessels are covered by pericytes, which stabilize their vascular wall and protect them against anti-VEGF therapies (Helfrich and Schadendorf, 2011). Therefore, approaches targeting the pericyte–endothelial cell interaction may be much more effective. In line with this view, selective blockade of VEGF by means of the small molecule tyrosine kinase inhibitor SU5416 only slightly reduces the microvessel density of murine endometriotic lesions

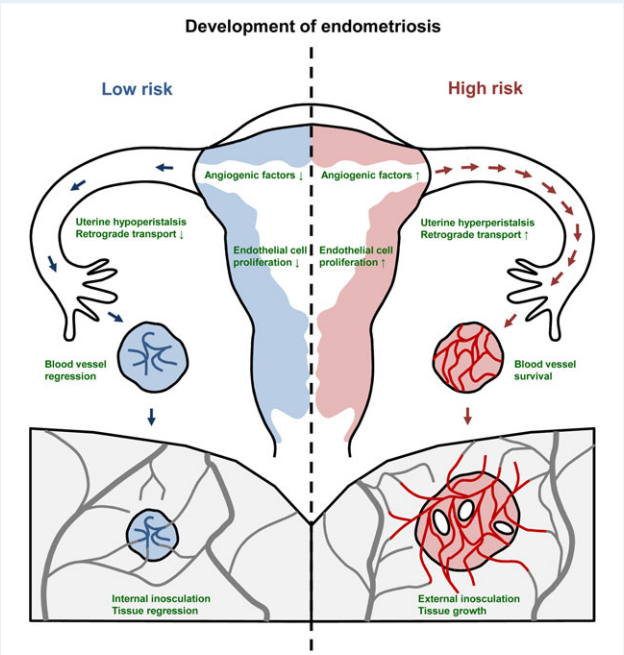

Figure 3 Basic mechanisms (marked in green), which may determine the fate of menstruated endometrial fragments in women and, thus, the risk of developing endometriosis.

when compared to vehicle-treated controls (Laschke *et al.*, 2006b) (Fig. 4). In contrast, combined inhibition of VEGF, FGF and platelet-derived growth factor (PDGF) by SU6668 significantly suppresses the vascularization of the lesions, which is associated with an inhibition of blood vessel maturation (Laschke *et al.*, 2006b) (Fig. 4).

A possible alternative for the destruction of mature microvascular networks in endometriotic lesions are vascular-disrupting agents (VDAs) (Van Langendonckt *et al.*, 2008), which have emerged as a promising novel drug class for the treatment of tumors (Porcù *et al.*, 2014). VDAs destabilize the microtubular cytoskeleton of proliferating endothelial cells and increase the interstitial pressure by enhancing microvascular permeability (Tozer *et al.*, 2005). This causes rapid vessel collapse and shutdown of blood perfusion. Hence, VDAs are most effective in tumors that exhibit many proliferating endothelial cells and large numbers of immature microvessels (West and Price, 2004), similar to early red endometriotic lesions. However, even older black endometriotic lesions occassionally contain immature microvessels (Matsuzaki *et al.*, 2001a), most probably due to continuous microvascular remodeling.

In a proof-of-principle study Feng *et al.* (2013) treated engrafting murine endometriotic lesions with a single injection of combretastatin A4 phosphate (CA4P), which is the leading tubulin-binding VDA. This resulted in a short-term selective vessel collapse in the lesions without affecting the blood perfusion of the surrounding host tissue microvasculature. However, this short-term effect of CA4P did not affect the further development of the lesions, indicating that repetitive doses or different application schemes should be additionally tested to improve the therapeutic efficiency of this approach. As already

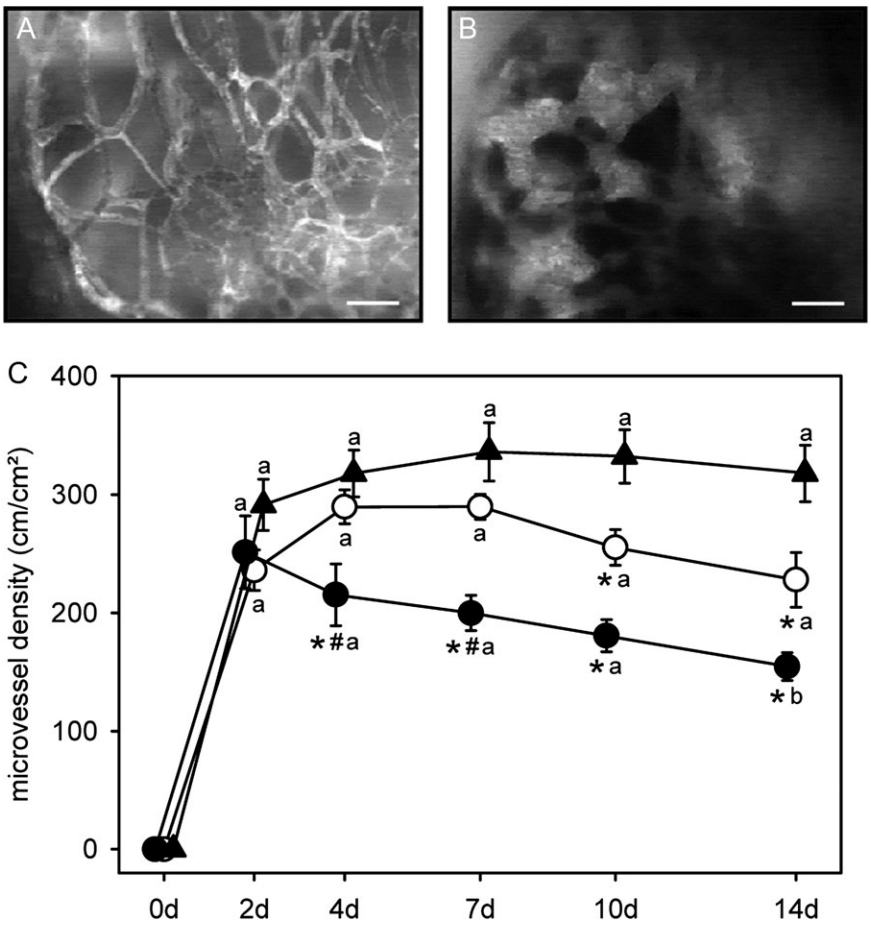

Figure 4 Suppression of vascularization in endometriotic lesions by combined inhibition of VEGF, FGF and PDGF. (**A**, **B**) Intravital fluorescence microscopy of the microangioarchitecture of endometriotic lesions at Day 14 after autologous transplantation of endometrial grafts into the dorsal skinfold chamber of a control (**A**) and a SU6668-treated (**B**) Syrian golden hamster. The anti-angiogenic effect of SU6668 is reflected in a decreased microvessel density of the newly formed microvascular network within the lesion (**B**). Moreover, the compound suppresses vessel maturation, as indicated by large vessel calibers and vessel wall irregularities (**B**). Blue-light epi-illumination with contrast enhancement by 5% FITC-labeled dextran 150 000 i.v. Scale bars: 75 µm. (**C**) Microvessel density (cm/cm^2) of endometriotic lesions in control (closed triangles), SU5416-treated (open circles) and SU6668-treated (closed circles) Syrian golden hamsters, as assessed by intravital fluorescence microscopy and computer-assisted image analysis. Data are given as the mean ± SEM. *$P < 0.05$ versus control animals; $^{\#}P < 0.05$ versus SU5416-treated animals; $^{a}P < 0.05$ versus Day 0 within each individual group; $^{b}P < 0.05$ versus Days 0 and 2 within each individual group. The figure is reprinted from Laschke *et al.* (2006b); used with permission from Oxford University Press.

Table II Potential future diagnostic and therapeutic strategies based on different vascularization modes in endometriosis.

Vascularization mode	Diagnostic strategies	Therapeutic strategies
Angiogenesis	Pro- and anti-angiogenic factors as biomarkers (peritoneal fluid, serum, urine) Gene polymorphism-based risk profiling	Application of anti-angiogenic compounds
Vasculogenesis	Levels of circulating EPCs as biomarker	Inhibition of EPC recruitment in endometriotic lesions
	EPCs as carriers for contrast agents during non-invasive imaging	EPCs as shuttles for selective gene therapy
Inosculation	Identification of uterus pathology (hypercontractility, increased angiogenic growth factor expression)	Application of compounds targeting endothelial-pericyte interaction Application of VDAs

discussed for anti-angiogenic compounds, it further has to be considered that the treatment of endometriosis with VDAs is only a realistic option in case of a tolerable side effect profile. With this claim in mind, Krikun *et al.* (2010) described an anomalous expression of tissue factor by endothelial cells in endometriotic lesions. The immunoconjugate molecule (Icon) binds with high affinity and specificity to this aberrant tissue factor, which induces a cytolytic immune response and vessel disruption (Hu and Garen, 2001). Accordingly, they further demonstrated in an athymic mouse model of endometriosis that Icon destroys endometriotic lesions by vascular disruption without apparent toxicity, reduced fertility or teratogenic effects (Krikun *et al.*, 2010).

Conclusions

Blood vessel formation is a major hallmark in the pathogenesis of endometriosis. The vascularization of endometriotic lesions is complex and involves angiogenesis, vasculogenesis and inosculation. Targeting these processes offers the possibility to develop novel strategies for the future diagnosis and therapy of endometriosis (Table II). However, essential challenges remain to achieve this goal. For diagnostic purposes, it will be necessary to identify reliable angiogenic and vasculogenic biomarkers or biomarker panels, which allow the identification of endometriosis with a high sensitivity and specificity. The establishment of novel therapeutic approaches is hampered by the heterogeneous nature of the disease with different lesion types that markedly differ in terms of their angiogenic activity and microvascular network composition. In addition, the safety requirements are high for the treatment of young women, who desire to have children. Hence, it will be necessary to identify novel compounds which selectively target the vascularization of endometriotic lesions without inducing severe side effects and affecting fertility or pregnancy. Recent progress in the field of endometriosis research indicates that these hurdles may be taken in the near future. This may offer more effective and satisfactory diagnostic and therapeutic solutions for patients suffering from this debilitating disease.

Authors' roles

M.W.L. identified the articles, drafted and revised the article. M.D.M. revised the article. Both authors approved the final version of the article.

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Conflicts of interest

The authors have nothing to declare.

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