The Role of the Microenvironment in Endometriosis: Parallels and Distinctions to Cancer

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Abstract

Phenotypes viewed as distinctive to cancer are often recapitulated in benign disease and consideration of these diseases can inform our understanding of the cancer microenvironment. Endometriosis is an estrogen-dependent inflammatory disease characterized by the presence of "metastatic" endometrium-like glands and stroma, together with hemosiderin and (often) fibrosis outside the uterine lumen. It is most often diagnosed as a result of pain and/or infertility and results in substantial economic and personal costs. However, in contrast to cancer it is typically not dysplastic and rarely causes death, though it increases the risk of several ovarian cancer subtypes. Like cancers, the disease is angiogenesis-dependent and genetic studies demonstrate that the VEGFR2 signaling axis plays a key role in the disease. In addition, molecular studies demonstrate that the immune/inflammatory milieu of endometriosis lesions is more similar to that of endometriosis-associated ovarian cancers (EAOCs) than it is to eutopic endometrium. This is consistent with the dysregulation of a host of immune/inflammatory cells and cytokines in disease tissue in ways that often resemble dysregulation observed in ovarian cancer. However, in contrast to EAOC, pain is often a key early symptom of endometriosis and can accompany even very small lesions. Another key contrast with cancers is the very limited range of medical treatments available. This is partially driven by the much more limited range of side effects that is acceptable for treatment of a non-life-threatening illness in women of childbearing age, but is also a function of the limited study of endometriosis pathophysiology that has occurred thus far.

Take-Home Lessons

- Endometriosis is an estrogen-dependent inflammatory disease that affects ~10% of women of childbearing age.
- Like cancer, endometriosis is proliferative, invasive, and metastatic.
- Endometriosis predisposes to "endometriosis associated ovarian cancers."
- Endometriosis shares key microenvironmental features with gynecologic malignancies, including activated angiogenesis and an altered immune/inflammatory milieu.
- Available treatments for endometriosis (NSAIDs, hormonal therapy, surgery) are frequently ineffective; new treatments are urgently needed.

Endometriosis is an estrogen-dependent gynecological disease characterized by the presence of endometrium-like glands, stroma, and hemosiderin in locations other than the lumen of the uterus. While endometriosis prevalence has not been clearly determined, the condition is estimated to affect ~10% of the general female population [1, 2], and is present in >50% of women and teenage girls with chronic pelvic pain and up to 50% of infertile women [3]. The annual costs of endometriosis in the USA have been estimated at $69.4 billion during the peridiagnostic period; however, this number is likely substantially higher once the ten years surrounding diagnosis are considered [4]. The disease incurs similar per-capita costs in Europe as well, emphasizing the high economic burden of the disease [5]. Current endometriosis therapies include medical and surgical options, but the success of these treatments is often limited and recurrence of symptoms is common [6]. Pain scores frequently return toward baseline levels after discontinuation of medication [7–9] and about half of patients report recurrence of pain by 12 months postoperatively [10].

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Endometrium as a Model of Cancer-Like Microenvironment

There are several microenvironmental alterations that are often described as key hallmarks of cancer. These include angiogenesis, immune dysregulation, inflammation, invasion, and metastasis [11]. Cancers are also often characterized by rapid cell growth. The intense study of malignancy over the last half century has sometimes obscured the extent to which non-cell-autonomous features of cancer are also exhibited by both normal physiology and benign pathologies. In this context a useful comparison can be made between cancer and the endometrium and the most common endometrial pathology, endometriosis (Fig. 28.1).

Endometriosis is most commonly characterized by infertility and/or chronic pain, especially in the pelvic or abdominal region [3]. The disease is associated with the growth of proliferative, invasive, endometrium-like tissue, often located on sites such as the ovaries, posterior cul-de-sac, or bladder. The best supported hypothesis for the origin of these lesions is Sampson's theory of retrograde menstruation [12]. It posits that endometriosis results when menstrual fluid flows through the Fallopian tubes into the abdominal and/or pelvic spaces where it seeds lesions. Endometriosis can lead to several cancers, but is not itself usually considered a precursor lesion. The fraction of endometriosis cases that lead to malignancy is relatively small (~1%). And though there are several "endometriosis-associated ovarian carcinomas" (EAOCs), the odds ratios for women with endometriosis being later diagnosed with these cancers is substantially smaller than with typical precursor lesions; for clear cell ovarian cancer (OR, 3.73), endometrioid ovarian cancer (OR, 2.32), and low-grade serous ovarian cancer (OR, 2.02) [13, 14]. Endometriosis is not usually dysplastic (though nuclear atypia is sometimes observed, especially in conjunction with EAOC).

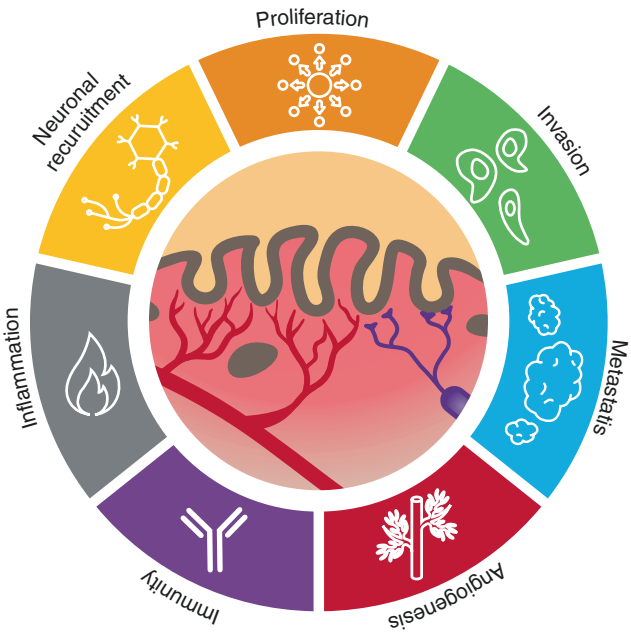

Fig. 28.1 Key Hallmarks of Endometriosis. Endometriosis shares multiple microenvironmental hallmarks of cancer, including: *Proliferation*; eutopic endometrium is highly proliferative and endometriosis tissue reflects this. Some normal differentiation is lost as endometriosis only rarely decidualizes. Importantly, the disease is highly responsive to steroid hormone manipulation. *Invasion*; essentially all lesions invade the mesothelium, with deep invasion into organ structures in a significant subset of women. *Metastasis*; metastatic tissue implants are produced without oncogenic transformation. Distal metastases are observed. *Angiogenesis*; robust angiogenesis defines lesion color. *Immunity*; dysfunctional innate immunity that fails to clear shed tissue and no longer reflects cyclic recruitment of immune cell types to lesions. *Inflammation*; lesions and surrounding tissue are characterized by ongoing sterile inflammation. *Neuronal recruitment* is dramatically increased in a subset of individuals, potentially contributing to lesion growth via neuroimmune communication

Importantly, although endometriosis is associated with individual cancer-associated mutations (in, e.g., KRAS, ARID1A, PIK3CA [15]), most lesions have no cancer-associated mutations and those that do have only a single cancer gene mutated. In addition, disease symptoms commonly manifest themselves in young women shortly after menarche, suggesting that the local microenvironment, rather than mutational processes (that take time) predominates in disease susceptibility. Thus, comparing endometriosis pathophysiology with that of EAOC can help differentiate mutation-driven processes from those that result from maladaptive results of normal biology.

The Endometrium Is an Extraordinarily Proliferative Tissue

The endometrium is the lining of the uterus and its major function is to enable embryo implantation, placenta formation, and gestation. It is composed of two layers, the basalis, a ~ 0.5 mm thick [16] layer of compact stromal tissue with "rhizome-like" horizontal glands [17, 18] that is not shed during menstruation. Overlying and arising from the basalis is the functionalis, an often spongy layer containing a characteristic stroma with vertical glands and an overlying luminal epithelium. This layer ranges in thickness from 0 to >8 mm in thickness, depending on the menstrual phase.

Menstruation is induced when a drop in progesterone causes the dense network of spiral arteries feeding the functionalist to constrict, resulting in tissue hypoxia/ischemia, apoptosis, and shedding of the vast majority, if not all, of the functionalis. The shedding process is followed by an extraordinarily rapid proliferation of endometrial epithelial and stromal cells, that rivals the fastest tumor growth rates. Within a few days of the onset of menstruation, epithelial cells from glands in the basalis and any residual functionalis proliferate and cover the newly denuded lumen of the endo-

metrium, forming a new luminal epithelium by the end of the menstrual phase. Then, during the proliferative phase, the endometrium rapidly expands, more than doubling in size in a week [19]. The proliferative phase ends with ovulation. The next phase is called the secretory phase and is named for the secretion by the endometrial glands of histotroph, which nourishes the developing embryo prior to establishment of the placenta. During this phase, modest continued glandular proliferation is accompanied by decidualization of the stroma and continued vascular proliferation in preparation for embryo implantation. If these steps do not occur, progesterone drops and the cycle repeats. In modern humans this can occur >400 times in a lifetime.

Dissemination/Colonization: Endometriosis as "Metastatic" Endometrium

Because the Fallopian tubes are open to the pelvic space, retrograde menstruation (flow of menstrual fluid through the Fallopian tubes, in addition to through the cervix) is common, being observed in ~90% of women. About 10% of women experience endometriosis, the presence of endometrium-like tissue in a location other than the uterine lumen [20]. The most widely accepted hypothesis for the origin of endometriosis is that it represents metastatic dissemination of eutopic endometrium via retrograde menstruation [12]. The retrograde menstruation hypothesis is supported by the observation that risk of endometriosis is increased by anything that is likely to increase the amount of menstrual tissue deposited in the pelvic space (earlier menarche, decreased cycle length, heavier flow, obstructed flow) [20, 21]. In bilateral endometrioma, lesions in a given patient typically do not share mutations [22], suggesting that the capacity for dissemination and implantation is not rate limiting in lesion generation. Importantly, endometriosis is not limited to the pelvic and abdominal spaces. Lesions have been reported throughout the body, including lungs, brain, etc. It is commonly assumed that lesions arrive at these locations via lymphatic or hematogenous spread, but this has not been clearly demonstrated. Thus, in addition to very rapid proliferation, endometrium exhibits the ability to metastasize. In the context of EAOC, and especially cancers that arise from endometriosis lesions, this means that the tissue has metastasized to a new location *before* oncogenic transformation.

Invasion in Endometriosis

In addition to high proliferative rates, endometriosis can also be invasive. (Adenomyosis, which consists of endometrium invading into the myometrium is typically considered a separate disease and will not be discussed here.) Endometriosis lesions are commonly divided into 3 types according to location and invasivity. Most endometriosis lesions are of the superficial peritoneal type and invade the mesothelium, but only exhibit shallow (<5 mm) invasion into surrounding tissue. Deep infiltrating endometriosis is most commonly found in the cul-de-sac, but can be found anywhere. Lesions of this type can invade deep into surrounding organs (bladder, bowel, etc.) complicating surgical removal and causing organ dysfunction. Finally, endometriomas consist of cysts of endometriosis tissue on the ovary. While not typically considered invasive, these lesions can grow to large sizes that compromise ovarian function.

Thus, endometrial tissue and endometriosis exhibit many of the hallmarks typically associated with cancer. However, there are also important differences with EAOC. Among these is a lack of dysplasia and maintenance of apparently normal histology. And although stroma typically does not decidualize, differentiation in lesion glands is otherwise normal, in marked contrast to EAOC. Based on the appearance of a greater proportion of fibrotic lesions in older women, it is also likely that glandular cells are not immortal and can exhaust their proliferative potential in some cases.

The Microenvironment in Endometriosis

Notwithstanding these differences, it is clear that endometriosis and EAOC (and cancer, more broadly) share several key microenvironmental characteristics (Fig. 28.2). Lesions in both diseases are strongly angiogenic, with disease driven by VEGF and other angiogenic regulators. In the case of endometriosis, the angiogenic nature of the disease is emphasized by the strong genetic evidence that polymorphisms in angiogenic regulators affect disease susceptibility. Both diseases are also characterized by an ongoing sterile inflammatory response that is responsible for a significant fraction of disease pathophysiology and progression. It is likely that the inflammatory response is driven by release of damage-associated molecular patterns (DAMPs), including apoptotic and/or necrotic cell debris, as well as heme and other iron species. Differences in inflammatory state among diseases may reflect differences in the profile of DAMPs released by disease tissue (e.g., apoptotic, necrotic, and hematogenous debris), but co-clustering experiments show that there is considerable overlap among diseases. One important characteristic of endometriosis is the increased participation of sensory neurons, especially nociceptors, in the disease. These clearly contribute to the intense pain that can be an important feature of the disease. However, it is also likely that they participate in neuroimmune communication [23] that may support lesion growth. In the case of cancer, inflammatory effectors may also increase the mutational burden, thereby contributing to lesion progression. Each of these features of the endometriosis microenvironment is described in more detail below.

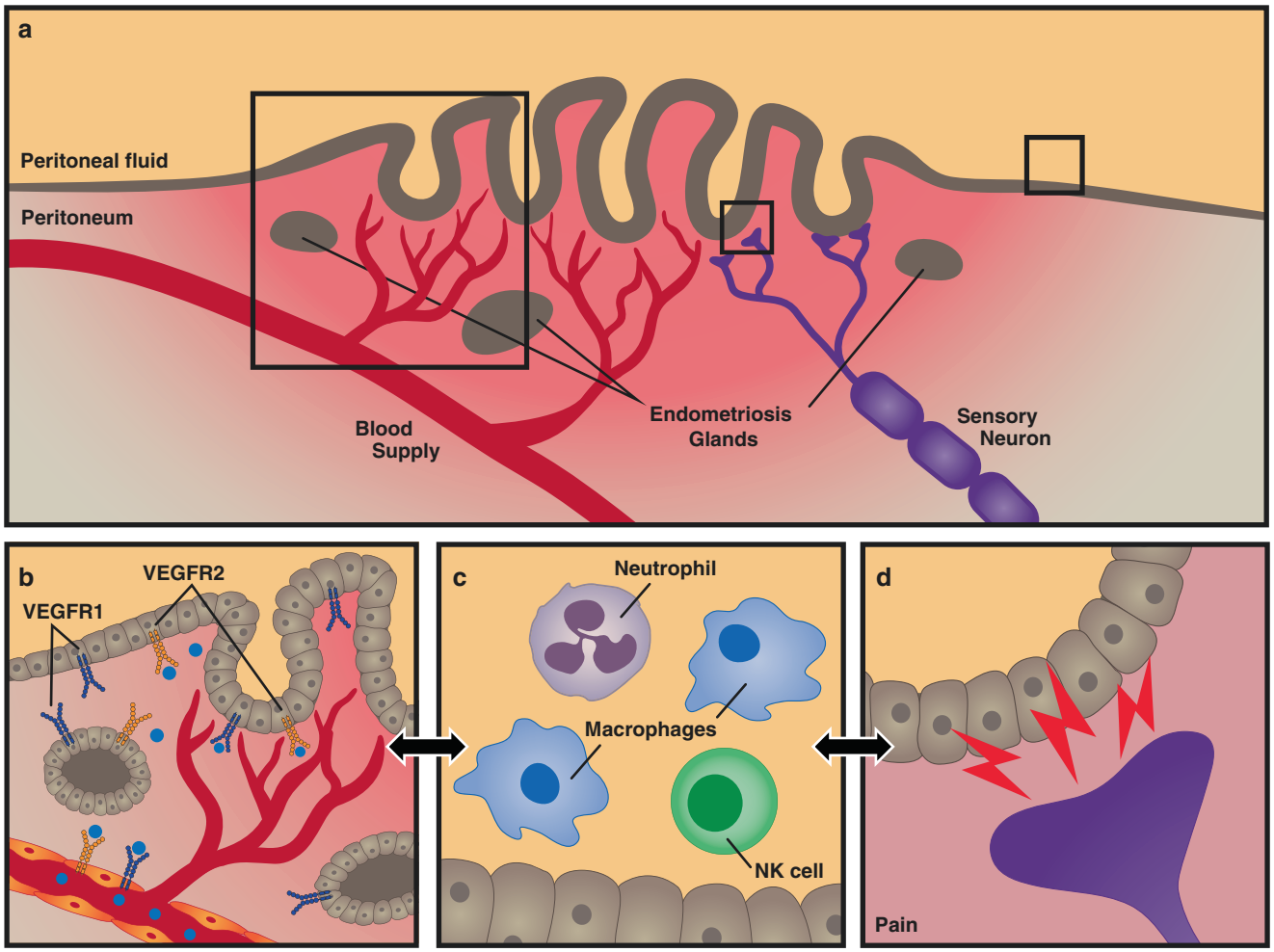

Fig. 28.2 Key pathalogical features of the endometriosis microenvironment. (**a**) Lesions consist of endometrium-like glands and stroma in an inflammatory milieu that is innervated and often highly angiogenic. (**b**) Angiogenesis is stimulated by a variety of growth factors, including VEGF, which is induced by local tissue hypoxia. VEGF signals not only through VEGF receptors on the endothelial surface, but VEGFR1 and VEGFR2 are also found on the surface of lesion tissue, suggesting direct support of lesion cells. (**c**) An altered immune and inflammatory milieu may contribute both to local inflammation and to angiogenic and neurogenic signaling without clearing lesion tissue. (**d**) Recruitment of neurites, especially from nociceptors is a common feature of endometriosis. It contributes to pain and may also be involved in neural immune communication that supports ongoing pathology

Angiogenesis and Endometriosis

The angiogenic response in cancers is described in detail in other chapters and will not be detailed here, except for a few comparisons. Rather, we will focus on the pro-angiogenic microenvironment in endometriosis. Ever since Dr. Judah Folkman proposed that tumors are angiogenesis-dependent and could be treated by antiangiogenic agents [24], the notion that this might apply to other pathologies was evident. The highly vascular nature of most endometriosis lesions, their irregular behavior, and their heterogeneous presentation made endometriosis a natural candidate for such a hypothesis.

VEGF and Angiogenesis

Vascular endothelial growth factor A (VEGF-A, also known as VEGF) is a member of the VEGF family of growth factors and is an important inducer of angiogenesis. VEGF-A signaling through VEGF receptor 2 (VEGFR2) plays an essential role during many important physiological processes such as embryonic development, organ remodeling, and wound healing [25–28]. VEGF-mediated angiogenesis also plays a key role in numerous pathologies where the formation of new blood vessels is required [29], including cancer tumor angiogenesis [30, 31]. VEGF is also implicated in the etiology of endometriosis. Importantly, polymorphisms in VEGFR2 are strongly associated with endometriosis risk

[32], underlining the importance of this pathway in disease establishment and progression.

Angiogenesis is the generation of new blood vessels from existing vessels. It is required for the generation or growth of new tissue beyond the oxygen diffusion distance, which is typically <1 mm in living tissue. Angiogenesis regulators include a long list of stimulators, such as VEGFs, basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF), hepatocyte growth factor (HGF), bone morphogenetic proteins BMP-9 and BMP-10, interleukins IL-6 and IL-8, angiopoietin-2, and lysophosphatidic acid. Normally, angiogenesis is inhibited by molecules including thrombospondin, angiopoietin-1, tissue inhibitors of metalloproteinases (TIMPs), and collagen fragments (e.g., endostatin, arresten, canstatin, etc.). Most research over the last two decades has focused on the VEGF-VEGFR2 axis, partially because in contrast with other angiogenesis stimulators, VEGF-A had few recognized non-angiogenic activities other than to increase the permeability of endothelial cells. VEGF-A is also dramatically upregulated upon hypoxia (via HIF1α) [33, 34], and thus consistently upregulated during tissue remodeling [35].

In mammals, the VEGF family of growth factors includes VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placental growth factor (PlGF). The best characterized is VEGF-A; its role in angiogenesis is well defined [25, 36]. VEGF family receptors include the tyrosine kinases VEGFR1, VEGFR2, VEGFR3, and the co-receptors NRP1 and NRP2 [37]. VEGF-A induces angiogenesis via VEGFR2 (either as a homodimer or as a heterodimer with other VEGF ligands), inducing complex intracellular signaling cascades resulting in endothelial cell responses such as proliferation, migration, survival, and permeability [38].

Aberrant expression of soluble VEGFR1 and VEGFR2 is associated with several pathologies [39–42]. Given the existence of multiple ligands and both membrane-bound and soluble receptors, measurement of a single factor can result in incomplete understanding as results can be confounded by other pathway members. Rather than a single factor controlling disease state, the net sum of all pro- and anti-angiogenic effectors is more likely to determine whether a given microenvironment supports lesion growth.

Genetic Associations Between Angiogenesis and Endometriosis

Genetic studies clearly link the VEGF-VEGFR2 pathway to endometriosis susceptibility. In GWAS, polymorphisms in VEGFR2 are associated with a ~10% difference in risk of disease [32], an effect that is stronger with increasing disease stage (and therefore, generally increased lesion burden). Candidate gene studies replicate this association [43, 44], and the absence of a cis-eQTL for VEGFR2 expression in whole uterine and other tissues [44] is consistent with altered expression of VEGFR2 in a minor cell population, such as endothelial cells.

Other angiogenesis regulators implicated in endometriosis susceptibility by GWAS include IL1A, CDKN2B-AS1, FN1, and ID4 [32]. Interleukin (IL)-1α promotes tumor growth, invasion, migration, and angiogenesis *in vitro* [45], and it also likely influences the immune/inflammatory microenvironment of lesions. The antisense RNA encoded by CDKN2B-AS1 is involved in the epigenetic silencing of the CDKN2B-CDKN2A cluster on chromosome 9 [46], which includes of tumor suppressors genes [47, 48] linked to multiple angiogenesis-dependent pathologies [49–51]. FN1 encodes fibronectin, an extracellular matrix protein that plays a key role in vessel growth by regulating cell adhesion, migration, and differentiation [52]. ID4 is a transcriptional regulator that affects multiple processes including angiogenesis [53–55].

The GWAS locus at 1p36.12 near the WNT gene may also be angiogenesis-linked. The Wnt4/β-catenin pathway regulates angiogenesis. But eQTL analyses does not support changes in WNT4 expression as mediating the effect [56] at this locus. While this may be another example of the insensitivity of eQTL analysis to differential gene expression in minor cell types such as endothelial cells, an eQTL for CDC42 (a Rho GTPase) was identified. CDC42 regulates multiple angiogenic cells processes, including cell migration [57]. It also increases VEGF-expression and promotes endothelial cell proliferation [58]. Strikingly, a significant fraction of genes identified by GWAS in both the 2017 study discussed here [32] and a more recent study currently available as only a preprint can play a role (either directly or indirectly) in regulating the local microenvironment.

Finally, candidate gene studies, including large meta-analysis associate VEGF-A polymorphisms with endometriosis [59–62]. The absence of signal in GWAS studies might be explained by stochastic effects; however, the nature of GWAS study design may also hamper detection. In GWAS, individual polymorphisms stand in for more complex haplotypes. As long as simple haplotype–phenotype associations are observed, this is effective; however, the VEGF gene exhibits a complex haplotype structure with no single polymorphism predicting changes in gene expression [60], violating key GWAS assumptions. Thus, when viewed together, the findings of candidate gene studies, combined with appropriate interpretation of GWAS results support the idea that genetically-mediated changes in the regulation of the VEGF signaling cause some individuals to generate a microenvironment more supportive of ectopic growth of endometrial tissue than others.

VEGF-A Expression in Endometriosis

Measurements of VEGF-A in tissues from endometriosis support a role for the protein in the disease; however, important caveats must be considered for all such studies [63, 64]. Key among these is the nature of controls. In contrast with genetic studies, identification and collection of appropriate control samples can be as challenging as disease tissue collection. Controls are most often collected from patients who need surgery for other indications (e.g., fibroids, infertility not related to endometriosis, etc.), which could result in false positives if VEGF-A were dysregulated in control samples. Best practice is to include controls from multiple indications, allowing Occam's razor to be applied to infer that the outlier is the disease where dysregulation occurs. However, since controls often have angiogenesis-dependent diseases, as well (e.g., fibroids), the studies outlined below may underestimate the role of VEGF-A in endometriosis.

A few studies report that VEGF-C is upregulated in endometriosis tissue [65–67], but many more studies have looked at VEGF-A. VEGF-A is upregulated in both epithelial and stromal cells [68–80] and peritoneal fluid (PF) [49, 50, 62–74] in endometriosis. In endometriosis patients, peritoneal fluid VEGF-A concentration varies with cycle phase [81], potentially as a result of regulation by 17β-estradiol [80, 82]. VEGF-A levels in PF also correlate with disease stage [83], though the direction of causality is not clear. Increased expression may enable lesion implantation and growth or increased lesion burden, but it may also be that VEGF release is caused by increased disease burden and associated inflammatory signals (e.g., VEGF-A release from neutrophils induced by IL-8 or TNFα [84]). Indeed, treatment of endometrial and endometriotic cell cultures with PF from endometriosis PF upregulates VEGF-A expression to a greater extent than treatment with control PF does [85]. Importantly, VEGF-A may not only act on the vasculature because endometriosis epithelial and stromal cells both express VEGFR1 and VEGFR2 [86].

There is striking visual heterogeneity in endometriosis lesion color, which is driven by the presence of blood vessels and blood breakdown products (e.g., hemosiderin) [87]. As might be anticipated, histology demonstrates that peritoneal red lesions are the most highly vascularized and the most mitotic [88], a result consistent with the fact that VEGF-A concentrations in the peritoneal fluid of women with endometriotic lesions are also the highest [89], demonstrating that this growth factor is angiogenically active [89].

In addition to ectopic tissue, the eutopic endometrium of women with endometriosis exhibits higher overall VEGF-A [90, 91] and greater VEGFR2 expression on blood vessels [75] when compared to eutopic endometrium in disease-free controls. However, in cultured endometrium there are no detectible differences between these two groups in either VEGF-A secretion or endothelial cell stimulation [92]. Thus, differences in the local microenvironment (e.g., menstruation-associated ischemia [93]) likely account for these differences.

Plasma VEGF-A concentrations are also correlated with endometriosis [94, 95], though it is important to take menstrual phase into account in such measurements; VEGF-A is highest during the menstruation phase [94, 95], likely as a result of tissue hypoxia. In contrast to plasma and peritoneal fluid, evidence that serum VEGF-A levels rise in endometriosis is less compelling [96–102]. The lack of consensus on the use of serum VEGF-A as a biomarker may be the result of the large number of underpowered studies on the hypothesis, the frequent use of comparators who themselves have angiogenesis-dependent diseases, dilution into the circulation, or the high variation in VEGF-A released when platelets degranulate. However, some studies do suggest a positive correlation [75, 103–107]. Overall, to the extent that it exists, the contribution of endometriosis to the overall variation in serum VEGF is small, while more substantial changes are observed locally and in plasma.

Other Angiogenesis Regulators

VEGF-A is only one of dozens of angiogenesis regulators that may affect endometriosis lesion implantation and growth, but others have received much less attention. Among those that have been studied, both HGF and its receptor (cMet) are upregulated in eutopic endometrium of patients vs. controls [108]. The observation that expression is highest in red peritoneal lesions [108] indicates that an important function of this overexpression is regulation of angiogenesis, but their involvement in cell migration suggests that they may also enable lesion establishment. RNA studies have also found that many other angiogenesis regulators are differentially expressed in the eutopic endometrium of advanced patients vs. unaffected controls, including VEGF-A, TNFRSF12A, RGCC, NR4A1, EREG, CYR61, and S100A7 [91].

As is true of VEGF-A measurements, the comparator tissue chosen can affect outcomes. Among the most common are other benign disease tissue and eutopic endometrium. For example, compared to benign cysts, VEGF and IL-8 are increased in the cyst fluid of both EAOC and endometrioma [109]. When ectopic and eutopic tissues are compared, additional regulators are differentially expressed, including inflammatory lipids such as prostaglandins. Prostaglandin F2α (PGF2α) is an angiogenesis stimulator and both the enzymes that synthesize it as well as its receptor are upregulated in peritoneal lesions [110]. Importantly, NSAIDs target this pathway. Since NSAIDs are known to be effective in treating endometriosis-associated pain in some women, this suggests that similar comparisons may identify additional drug targets. Thus, the observation that VEGFR2, HIF1A,

PDGFB, NRP1, EPH4B, and HGF are all upregulated in ectopic vs. eutopic tissue [111] may point toward additional therapeutic targets. Based on similar observations and in an effort to identify additional druggable targets, Lin et al. compared cell surface proteins expressed in endometriosis tissue vs. eutopic endometrium [112]. Overexpression of ANTXR2, an angiogenesis regulator [113], in endometriosis tissue vs. matched eutopic endometrium, was confirmed by qRT-PCR, western blot, and immunohistochemistry (n = 43, 42) [112]. Then, the antiangiogenic ANTXR2 inhibitor PGG [114] was found to inhibit lesion implantation and growth in a mouse model [112].

Immune/Inflammatory Microenvironment

Dysregulation of the immune/inflammatory system is an important driver of malignant transformation [13, 14]. Key cell types in this system are dynamically regulated in the eutopic endometrium throughout the menstrual cycle. During the secretory phase, macrophages, neutrophils, natural killer, and dendritic cells are increased and all but NK cells remain high during the menstrual phase. In contrast, increased T-cells are recruited during the proliferative phase. In lesion tissue, many of these cyclic fluctuations are lost or damped, indicating a (partial) loss of normal differentiation cues [115]. In addition, the sterile inflammation that contributes to both diseases is characterized by molecular changes, including upregulation of multiple immune and/or inflammatory mediators that themselves contribute to disease pathophysiology.

Immune/Inflammatory Cell Changes

In the endometrium, M1 macrophage polarization may decrease shedding thereby increasing lesion formation, but once in the ectopic location, the predominance of M2 macrophages likely contributes to lesion formation [115]. In the endometrium, (anti-inflammatory) M2 macrophages predominate in healthy women, while in endometriosis, M1 macrophages are more common. However, in ectopic lesions, M2 macrophages are commonly found to predominate and may play a role in lesion growth and/or maintenance [115, 116]. Also, in contrast to eutopic tissue, macrophages do not rise in the secretory phase in endometriosis, potentially contributing to the decrease in shedding in disease tissue [116, 117].

In lesions, a pro-inflammatory environment is generating by increased TNFα, IL-Iβ, and IL-6 [115–117], but notwithstanding their high activation state, macrophage phagocytic ability is decreased as a result of PGE2-mediated downregulation of CD36. Reversal of this effect may account for some of the effect of NSAIDs on disease pathology [116, 117].

NK cells normally protect the endometrium from infection. They also participate in blood vessel remodeling, increasing in the secretory phase in preparation for shedding at the end of that phase [115]. In endometriosis, the cytolytic activity of these cells decreases, both locally and, to a lesser extent, in the periphery [115–117]. Binding of NKG2D and c-type lectin-like NK cell receptor by MICA and MICB may decrease NK cell activity [116, 117]. NKG2D function may be further modulated by proteolysis as evidenced by increases in soluble NKG2D ligands in the peritoneal fluid of endometriosis patients allowing lesions to evade NK cell recognition [118]. This decrease may also be a consequence of high levels of IL-6, IL-10, IL-15, and TGF-β, as well as increased NKB1 and EB6 expression [116, 117].

In endometriosis patients, neutrophil numbers are increased in both the eutopic endometrium and the peritoneal cavity. Inasmuch as neutrophil depletion in a mouse model results in decreased lesion size, it is likely that these cells promote lesion growth. This may occur as a result of production of VEGF and IFN-γ [115, 117].

In endometriosis, dendritic cell maturation decreases and increased immature dendritic cells may contribute to neurogenesis and angiogenesis. The absence of mature dendritic cells may also decrease the efficacy of phagocytic clearing during menstruation, increasing the odds that any cells deposited in the pelvic space will survive long enough to implant [115].

In endometriosis, CD8+ T-cells increase vs. eutopic endometrium and the cyclic changes in T-cell number (increased in proliferative vs. secretory phases) are lost [115]. CD4+ cells increase to a greater extent resulting in an increased CD4+/CD8+ ratio. Importantly, the increased CD4+ cells are mostly Tregs and other anti-inflammatory subtypes [115, 116]. For example, in endometriosis Th17 cells are increased in ectopic lesions, peritoneal fluid, and peripheral blood, and their number correlates positively with disease stage [115, 117]. These changes may be a result of changes in cytokine patterns (see below). In the peritoneal fluid and peripheral blood of endometriosis patients, both Th1 (pro-inflammatory) and Th2 (anti-inflammatory) cytokines are increased with Th2 cytokines (e.g., IL-4 and IL-10) increased slightly more [115, 117].

Another potential modulator of T-cell function is B-cells. B lymphocyte stimulator (BLyS) is increased in endometriosis, suggests an induced state [115, 116], which may change CD4+ T-cell maturation via both ligand–receptor interaction and cytokine release [115, 117, 119]. Auto-antibodies are also a common feature of endometriosis [120].

Molecular Changes

At the molecular level, a macrophage-driven signature of peritoneal inflammation describes the peritoneal fluid of a significant subset of long-term endometriosis patients [121]. The core of this signature is defined by upregulation of IL-1β, IL1ra, IL-6 IL-8, IL-10, G-CSF, MCP-1, and RANTES; with HGF, IL-16, GROα, MIF, and MIG also contributing [121]. In patients with severe (stage III/IV) disease, IL-9, IL-4, IFN-γ, and TNFα are also increased [121]. The cytokine signature is associated with deep infiltrating disease [121], suggesting that inflammatory cells contribute to invasion.

Similar inflammatory processes likely contribute to EAOC, as well. In unsupervised clustering of immune transcriptome genes, most endometriosis tissue clusters with EAOC, demonstrating that the diseases often share a closely related immune microenvironment [13]. This environment is characterized, in part, by upregulation of elements of the complement cascade (C5, C7, CFD, CFB, CFH, and MASP1) [13, 122]. Likewise, IL-6 is increased in both endometriosis and EAOC [123, 124], and similarly, TNFα is increased in both diseases, both locally and systemically [123]. In the case of IL-10, there is a gradient of increased expression from endometriosis up to frank ovarian carcinoma [125]. This may be relevant to immune evasion in both diseases because IL-10 can increase HLA-G transcription [126]. HLA-G, in turn, can allow downregulation of HLA genes without inducing NK-mediated killing [127]. This may enable lesions to evade clearing by immune/inflammatory cells notwithstanding the presence of substantial DAMPs (in the case of endometriosis) as well as neoantigens (in case of EAOC). Increased HLA-G is, in fact observed in endometriosis [126], as well as in healthy eutopic endometrium during menstruation, suggesting that this is another example of disease hijacking normal processes.

CXCR3 is upregulated in both EAOC and endometriosis, while the fraction of (pro-inflammatory) CXCR3-expressing lymphocytes decreases modestly [128]. CXCR3 receptor and cognate CXC chemokines recruit a subset of T-cells and NK cells, thereby contributing to Th1-dependent T-cell responses [128]. This combination likely contributes to a response that is pro-inflammatory, but insufficient to clear disease tissue and thus prolonged, contributing to overall disease pathology. However, the extent of these changes can differ between malignant and benign disease, contributing to the former. For example, CXCR3B and its ligand CXCL4 are reduced in CCC compared to endometriosis [129]. The decrease in CXCR3B signaling decrease may reduce the effectiveness of any anti-tumor responses, thereby contributing to malignant transformation [109]. CXCR3 and CXC chemokines also inhibit angiogenesis [128]; for example, the CXCR3B ligand CXCL4 inhibits both VEGF and fibroblast growth factor, thereby reducing angiogenesis. In endometriosis, CD68 + macrophages express CXCL4 and CXCL4L1, but this gradually decreases in the transition zone to EAOC [130] likely contributing to a further increase in angiogenesis concomitant with oncogenic transformation and demonstrating another key distinction between the endometriosis and cancer microenvironments.

The Role of the Microenvironment in Endometriosis-Associated Pain

Pain is a key presenting symptom of endometriosis. Pain (dysmenorrhea, chronic pelvic and/or abdominal pain, dyspareunia, and dyschezia) correlates poorly with lesion type, size, anatomic location, or stage of disease [131, 132]. Women with endometriosis exhibit greater overall sensitivity to painful stimuli than women without disease [133–136], suggesting that systemic changes predispose to or result from the disease. In addition, several features of the local microenvironment correlate with pain. In rat models, increased NGF correlates with hyperalgesia [137] and both human endometriosis tissue and ectopic rat uterine tissue cause sensory nerve invasion [138]. In humans, TRPV1 staining in and around lesions is positively correlated with chronic pelvic pain, vs pain-free controls [139] and in painful endometriosis vs. pain-free woman having surgery for adnexal masses [140]. More generally, women with endometriosis-associated pain have nerve fiber densities 6 times higher than pain-free and disease-free controls, and there are functional differences in the types of neurons innervating both the eutopic endometrium and myometrium [141, 142].

Finally, it has long been known that there is correlation between angiogenic stimulation and innervation and endometriosis is no exception. In endometriosis, lesion microvessel density is correlated with pain [143, 144], and VEGF-A, acting through VEGFR1 has been shown to modulate pain in the context of cancer [30]. Thus, variation in the expression of angiogenesis stimulators has been suggested to explain variation in pelvic pain symptoms, including cyclicity and intensity [135]. However, in endometriosis expression of VEGF-A (tissue or plasma) is not correlated with pain [143, 144], suggesting that other angiogenesis drivers (e.g., NGF) play a larger role in driving pain.

Therapies Targeting the Microenvironment in Endometriosis Therapy

Endometriosis is an estrogen-dependent inflammatory disease and existing therapies leverage those characteristics. Early disease management strategies typically focus on

NSAIDs, which damp production of inflammatory lipids, such as prostaglandins. This strategy can reduce the initiating drive toward inflammation, but is less effective at resolving existing inflammation [145]. For the significant fraction of women for whom NSAIDs are insufficient to resolve symptoms, hormonal manipulations targeting the estrogen receptors are next pursued. However, such approaches are often unsuccessful and new approaches to medical therapy of endometriosis are urgently needed [146].

Antiangiogenic agents are effect treatments for cancer [147, 148] and neovascular eye disease [149] and several lines of evidence indicate that direct targeting of the VEGF pathway can be successful in treating endometriosis. First, increased serum soluble VEGFR1 (sVEGFR1, a natural VEGF antagonist) was associated with lower rASRM stage of disease in a study comparing serum and urinary angiogenic factors among endometriosis patients [150]. Second, VEGF-A inhibitors (sVEGFR1 and anti-VEGF antibody) reduce lesion burden and microvessel density in mouse models of endometriosis [151]. Third, some drugs that have been used to treat endometriosis affect VEGF-A and angiogenesis. Danazol decreases serum VEGF-A levels to normal [152]; and gonadotropin-releasing hormone (GnRH) analog activity is partially mediated by VEGF-A-regulation. VEGF-A is a survival factor for endometrial epithelial cells [86] treated with leuprolide [153], which reduces VEGF-A production in culture [154–156]. *In vivo*, in a rat model as leuprolide reduces VEGF-A production concomitant with reduction in lesion size [157]. In humans, GnRH analog treatment reduces peritoneal fluid VEGF-A [83]. Thus, a portion of the effects of hormonal agents may be mediated by regulation of VEGF-A and angiogenesis in the lesion microenvironment.

In animal models, agents that target the VEGF-VEGFR2 axis reduce cell growth, microvessel density, and lesion size and number [158–171]. However, both anti-VEGF agents and small molecule antagonists of VEGFR2 kinase activity are classified as pregnancy category D agents, and thus not suitable for fertile women during their reproductive years. Nevertheless, regression of endometriosis lesions has been observed in patients treated for malignancy with small molecule inhibitors of VEGFR2 [172]. In addition, VEGFR2 may be targetable via non-teratogenic means. Dopamine-signaling through DRD2 inhibits angiogenesis by down-regulating VEGFR2 protein via endocytosis [173]. DRD2 is expressed in human endometriosis lesions, as well as eutopic endometrium [174], and the DRD2 agonists cabergoline and quinagolide inhibit angiogenesis in mice [175]. Cabergoline inhibits the growth of human endometrial tissue xenografts in mice [174] by reducing VEGFR2 activation and angiogenesis [176]. In humans, a small number of women with both hyperprolactinemia (which can be treated with quinagolide) and endometriosis were treated with quinagolide, which reduced the size of endometriosis lesions [177]. Since DRD2 agonists are generally considered to have no known risks during pregnancy, they may prove to be an effective means of disrupting VEGF-induced angiogenesis thereby treating endometriosis and clinical trials are currently underway to test this hypothesis.

Finally, agents targeting non-VEGF aspects of angiogenesis regulation should be considered. For example, in a mouse xenograft model, ABT-898 (an analog of the endogenous angiogenesis inhibitor thrombospondin-1) reduced endometriosis lesion vascularization and growth without affecting fertility or embryonic development [178, 179]. However, this agent is no longer in development, so alternative means of activating the Tsp-1 pathway would be needed to pursue this therapeutic avenue. In summary, existing therapeutics clearly target the abnormal microenvironment of endometriosis lesions and it is likely that identification and exploitation of new targets in this milieu will result in new therapeutics for this debilitating disease.

Concluding Remarks/Summary

Many phenotypes currently viewed as distinctive to cancer are recapitulated in benign disease, emphasizing the extent to which the normal milieu regulates the phenotype of both genotypically normal and malignant tissue. In the case of endometriosis, both eutopic tissue and disease lesions are characterized by rapid proliferation. Lesions exhibit a generally normal histology, with a modest decrease in cyclic differentiation. Lesions are less sensitive to progestin signals and do not typically decidualize. Cyclic recruitment of immune/inflammatory cell types is also absent. These differences suggest that a least some loss of differentiation that is evident in cancers may not result from genetic abnormalities; rather, loss of environmental signals may decrease tissue specialization.

Invasion and metastasis are often considered key hallmarks of cancer. However, endometriosis lesions exhibit both characteristics. By definition, endometriosis is metastatic, meaning that cancers that arise from metastatic lesions have metastasized *before* oncogenic transformation. Lesions also invade other structures, at least the mesothelium, but often deep into other organs. These observations demonstrate that neither invasion nor metastatic dissemination is unique to cancer. They also show that both processes proceed more readily than is sometimes imagined and do not require mutation. Nevertheless, the relative rarity of distal dissemination in endometriosis shows that there is some barrier to distal metastasis.

Blood and blood-derived hemosiderin play a key role in the wide variety of colors observed by surgeons in endometriosis. Angiogenesis is the key determinant of the vascularization of endometriosis lesions, and cancers as well. Both

diseases release a variety of angiogenesis stimulators, including VEGF. In the case of endometriosis, genetically regulated angiogenic responsiveness plays a key role in disease susceptibility. Multiple cancers are effectively treated by angiogenesis inhibitors, and there is emerging evidence that these drugs can effectively treat endometriosis. Importantly, such treatment is contraindicated with currently available direct VEGF and VEGFR2 antagonists because they are teratogenic. However, non-teratogenic antiangiogenic therapeutics can be expected to be highly effective.

Finally, it is clear that EAOC and endometriosis share important components of a sterile inflammatory microenvironment. Endometriosis is pro-inflammatory, but the innate immune system cannot clear disease tissue in much the same way that cancer has been described as a wound that does not heal. In endometriosis, there is growing evidence that this is supplemented by neuroimmune communication that further supports disease growth.

In summary, endometriosis and cancer share many microenvironmental changes and these changes support key hallmarks of both diseases. In the case of EAOC, these changes precede oncogenic transformation. A better understanding of pathophysiology of non-malignant disease may help understand mutation-driven processes and distinguish those from physiological responses to DAMPs, reactive iron, and hypoxia, thereby enabling improved targeting of the microenvironment in both cancer and endometriosis.

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