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The Role of Stem Cells in the Etiology and Pathophysiology of Endometriosis

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Abstract

Human endometrium is a dynamic organ that normally undergoes repetitive cyclic regeneration. To enable this rapid regeneration, it is not surprising that the endometrium contains a reservoir of progenitor stem cells. However, this pool of cells that allows the growth of the endometrium also allows for unrestrained growth that can reach beyond the endometrium. In this review, we will address the role of stem cells in endometriosis. Recent characterization of stem cell populations within human endometrium has opened the possibility of understanding their physiologic as well as their pathologic roles. While stem cells are critical to the cyclic regeneration of a healthy endometrium, we have shown that both endometrium-derived and bone marrow-derived stem cells can migrate to ectopic sites and contribute to the development of endometriosis. Furthermore, endometriosis interferes with the normal stem cell trafficking to the uterus that is necessary for endometrial growth and repair. Altered stem cell mobility and engraftment characterize this disease.

Keywords

endometrium; endometriosis; stem cells; inflammation

First described via microscopic observation by Karl von Rokitansky in 1860, symptoms of endometriosis have been noted in medical texts for thousands of years.[1,2] Endometriosis is an inflammatory, estrogen-dependent condition associated with pelvic pain and infertility.[3] This disease affects approximately 10% of reproductive-aged women and 20 to 50% of infertile women. Endometrial lesions are primarily located on the pelvic peritoneum and ovaries; although rare, endometriosis can also be found in the pericardium, pleura, lung parenchyma, and even the brain. Despite its frequency and impact on quality of life, our understanding of the pathogenesis of endometriosis remains incomplete.[4]

Endometriosis often goes undiagnosed for years. Dysmenorrhea and pelvic pain are frequently dismissed as normal variants. Diagnosis has been considered uncertain until proven by laparoscopy; however, this has only led to an unfortunate delay in treatment.[5–7]

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The average gap from the onset of symptoms to the diagnosis of endometriosis is between 3 and 11 years.[7] Endometriosis may also be asymptomatic, with up to 25% of women with the condition reporting no symptoms.[8] The delay in diagnosis typically results in more advanced disease. Once surgery is performed and putative endometriotic lesions have been located, biopsy is traditionally used to confirm the diagnosis. Sites of endometriosis have varied sizes and appearances, including dark blue, black, red, white, clear, yellow, and brown growths.[3,5,6] Owing to the varied presentation of disease, it can be missed at the time of surgery. Even after complete resection, endometriosis typically recurs and medical treatment should be used to prevent future disease.

Increased awareness of endometriosis symptoms as well as biomarkers of the disease should enable earlier diagnosis and treatment. Several biomarkers are under development.[9–11]

Although endometriosis is a benign condition, a study of the Swedish national inpatient register demonstrated an association between endometriosis and an increased risk for ovarian cancer (standardized incidence ratio = 1.9, 95% confidence interval: 1.3 to 2.8), hematopoietic cancer (1.4, 1.0 to 1.8), and breast cancer (1.3, 1.1 to 1.4).[12] A pooled case-control study in 2002 similarly demonstrated an elevated risk of ovarian cancer in women diagnosed with endometriosis (odds ratio = 1.73, 95% confidence interval: 1.10, 2.71).[13] It is unknown if these increased risks are due to the disease-state itself or other related complications. For example, endometriosis-related infertility may increase the risk for ovarian cancer given that pregnancy has a protective effect against ovarian cancer.[14] The association with these cancers accentuates the need for a better understanding of the pathophysiology of endometriosis.

While the underlying cause of endometriosis has not been completely characterized, it is clear that heritability is involved.[5–7,15] A family history of the disease is a major risk factor; women with a diagnosed first-degree relative are about six times more likely to have endometriosis than women with no family history.[5] Increased exposure to menstruation, through either short cycles or long periods of menstrual flow, has also been associated with elevated risk.[16] Genome-wide association studies have failed to find any single gene that is responsible for this common disease; the etiology is likely multifactorial. Genetic, environmental, and epigenetic factors all contribute to this disease.[17]

The traditional theory for the etiology of endometriosis is that of Sampson.[2] Retrograde menstruation delivers endometrial cells to the peritoneal cavity where they implant and grow. This mechanism likely accounts for some peritoneal and ovarian endometriosis; however, it cannot account for the less common locations of endometriosis including remote areas that are not in communication with the peritoneal cavity. Some of these lesions may arise from hematogenous or lymphatic spread of endometrial cells; however, even this mechanism cannot explain endometriosis after hysterectomy or cases reported in men undergoing treatment for prostate cancer. As will be discussed later, it has become clear that some endometriosis can arise from stem cells. Progenitor cells that are deposited in the peritoneal cavity by retrograde menstruation likely lead to the most common forms of endometriosis. Remote endometriosis is likely derived from differentiation of multipotent stem cells originating in bone marrow and other sources. Stem cells continue to contribute to

all endometriosis independent of origin. Altered stem cell trafficking underlies the etiology and pathophysiology of this disease.

Stem Cell Populations within the Endometrium

Several types of stem cells contribute to the development, replenishment, and repair of tissues and organs. Stem cells have varied differentiation, clonogenic, and proliferative potential, and distinct origins. They have been isolated from embryonic blastocysts as well as adult tissues in many organs.[18] While embryonic stem cells are pluripotent, adult stem cells are often multipotent; they can differentiate into several but not all cell types. Some stem cells have a further restricted developmental potential and are termed *progenitor cells*; these cells give rise to cells of a single tissue or even to a single cell type. Multipotent and clonogenic stromal cells, termed *mesenchymal stem cells*, have been isolated from bone marrow, umbilical cord, periosteum, skeletal muscle, pancreas, dental pulp, adipose tissue, and endometrium.[19–23] Mesenchymal stem cells are defined by their plastic adherence, gene expression (CD73+, CD90+, CD105+, CD11b−, CD14−, CD19−, CD79α−, CD34−, CD45−, HLA-DR−), and ability to readily differentiate into chondrocytic, adipocytic, and osteocytic fates in vitro.[24]

The concept of stem cell populations with the endometrium was first proposed by Prianishnikov in 1978 due to the highly regenerative nature of the endometrium.[25] Many studies have been performed within the last decade to identify stem cell populations within endometrial tissue. The contribution of stem cells to endometrial regeneration was first described in 2004.[26,27] Both progenitor cells within the endometrium and multipotent cells from bone marrow were shown to contribute to endometrial growth. Distinct populations of cells from endometrial tissue with stem cell properties were identified; a small percentage of endometrial epithelial (0.22%) and stromal (1.25%) cells were shown to display clonogenicity after preparation of single cell suspensions from human endometrial tissue obtained during hysterectomy.[28,29]

The functionalis layer of the endometrium is shed in each menstrual cycle while the basalis layer remains intact. Schwab et al investigated the effects of the menstrual cycle on these stem cell populations in the functionalis and discovered no significant difference between proliferative, secretory, and inactive endometrium, suggesting that the basalis layer of the endometrium may be the main source of endometrial progenitor stem cells.[30] Further study demonstrated the mesenchymal stem cell character of these subpopulations through in vitro differentiation assays to chondrogenic, osteogenic, and adipogenic fates.[21,24,31,32]

Several populations of bone marrow-derived stem cells (BMDSCs) were also found in the endometrium; these were identified in bone marrow transplant recipients where the bone marrow origin of the cells could be clearly demonstrated.[26] We first reported that in women who had received single human leukocyte antigen (HLA) mismatched bone marrow transplantation during cancer treatment, 0.2 to 52% of endometrial stroma and epithelium was derived from donor tissue.[26] Using a mouse model, we confirmed that, following bone marrow transplantation from male mice, recipient female mice exhibited populations of Y-chromosome positive cells within their endometrial tissue, representing approximately 1 in

5,000 cells in the epithelium and 2 in 5,000 cells in the stroma.[33] This occurred over a short duration, while the enhanced numbers in humans likely reflect the longer interval from bone marrow transplant to assessment. Subsequently, similar studies have confirmed the bone marrow origin of endometrium.[33] Ikoma et al reported the in vivo differentiation of bone marrow–derived stem cells to an endometrial phenotype; endometrial biopsies from female recipients of male donor bone marrow contained Y-chromosome–positive cells, comprising 0.6 to 8.4% of glandular epithelial cells and 8.2 to 9.8% of stromal cells.[34] The majority of endometrial glands were chimeric, indicating that bone marrow–derived stem cells are able to differentiate in vivo into an endometrial glandular phenotype and contribute to the glands derived from the endogenous endometrial progenitor cells.[34] Bone marrow is a novel and unexpected source of uterine cells.

We additionally demonstrated that bone marrow–derived stem cells are recruited to sites of injury within the uterus. We produced ischemia reperfusion uterine injury in female mice after male bone marrow stem cell transplantation.[35,36] Injury was shown to increase recruitment of bone marrow cells to the stromal compartment by twofold when compared with sham surgery. Y-chromosome–positive bone marrow cells within the endometrium were analyzed for their gene expression, which indicated that they had differentiated to an endometrial phenotype due to the expression of vimentin and loss of CD45 expression.[36] Granulocyte colony-stimulating factor (G-CSF) is often used to increase bone marrow mobilization prior to transplant. It has been proposed as a means of mobilizing stem cells to the uterus. In our study, G-CSF treatment led to decreased BMDSC engraftment of the uterus after injury, likely by favoring mobilization of hematopoietic stem cells over the mesenchymal stem cells.[36] Bone marrow mobilization and stem cell engraftment of the uterus is a mechanism of endometrial repair in response to injury.

Endometrial Stem Cells and Regenerative Medicine

Some of the bone marrow–derived stem cells remain as multipotent stem cells in the endometrium with similar properties to those residing in bone marrow. We have shown that multipotent cells can be obtained from the uterus and used to derive multiple differentiated cell types. Indeed these cells can give rise to chondrocytes, neurons, and insulin producing cells useful in the treatment of Parkinson’s disease or diabetes.[23,37–39] Human endometrium-derived stem cells (EDSCs) were differentiated into insulin-producing cells that resemble pancreatic β cells.[37] These cells produced insulin in a glucose-dependent fashion. When transplanted into diabetic mice, these cells improved glucose control and reduced serious diabetic complications. Similarly, EDSCs were differentiated into dopamine-producing neuron-like cells. These cells have been transplanted into the brains of mice and nonhuman primates with Parkinson’s disease.[38,39] The brains of both mice and primates treated with EDSCs showed increased dopamine production compared with injection of control (culture medium).

EDSCs have furthermore been isolated from menstrual blood.[40] These cells have been shown to express CD9, CD29, CD41a, CD44, CD59, CD73, CD90, CD105, OCT-4, and hTERT (telomerase), but did not express CD14, CD34, CD38, CD45, CD133, and STRO-1. Their multipotent capacity has been demonstrated by in vitro studies demonstrating their

differentiation into endothelial, pancreatic, hepatic, cardiomyocytic, adipocytic, osteogenic, neurocytic, and respiratory epithelial fates.[38] EDSCs have additionally been shown to support angiogenesis by releasing growth factors such as platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), and matrix metalloproteinase (MMP).[40] A murine model of peripheral vascular disease was used to test the angiogenic potential of EDSCs in vivo. EDSCs were transplanted subcutaneously in mice following limb ischemia induced by ligation of the femoral artery and nerve excision.[41] Mice that received the EDSC transplant regained use of their limbs, while control mice experienced limb necrosis.[41] Endometrial cells obtained from menstrual blood have tremendous potential in the field of regenerative medicine.

To date, most research focusing on EDSCs has characterized cell populations by cell surface markers and in vitro tests of multipotency and clonogenicity.[20,23,28–30,32,42–44] Human EDSCs have been shown to express stem cell markers such as CD29, CD44, CD73, CD90, CD105, MSI1, and NOTCH1.[29,31] Generally, studies involving EDSCs primarily rely on cells isolated from the stroma, as it has been shown to be enriched for EDSCs compared with the epithelial cells.[28]

It has been proposed that the PDGF-Rβ+/CD146+ phenotype is a marker for human EDSCs, as double-positive cells exhibit multipotent capabilities and express mesenchymal stem cell markers such as CD44, CD29, CD73, CD90, and CD105.[45] Furthermore, it has been recently proposed that W5C5 is a potential single marker to identify human EDSC populations.[46]

The Role of Stem Cells in Endometriosis

While adult progenitor stem cells are responsible for normal regenerative capacity following menstruation, these same progenitor stem cells may also have an enhanced capacity to generate endometriosis. The dysregulation of endometrial stem cells has been proposed as a potential mechanism for seeding ectopic endometriotic lesions, perhaps in concert with the Sampson theory of retrograde menstruation.[4,29,47–52] When progenitor cells are shed at the time of menstruation, they can implant and generate endometrium in ectopic locations. The endometrium basalis has been proposed to contain endometrial stem cells suited for regenerating the endometrium functionalis after menstruation.[30] During menstruation, women with endometriosis shed more basalis than women without the condition, thereby potentially providing a means for tissue enriched with endometrial stem cells to migrate to ectopic sites.[53]

The progenitor stem cells reside in the uterus; however, less-committed mesenchymal stem cells may also travel from other tissues such as bone marrow to repopulate the progenitor population.[49] Mesenchymal stem cells are also involved in the pathogenesis of endometriosis and may be the principal source of endometriosis outside of the peritoneal cavity when they differentiate directly into endometriosis at ectopic locations.[49] Inappropriate differentiation of mesenchymal stem cells to endometrial cells likely accounts for endometriosis in remote locations. More broadly, ectopic differentiation of stem cells is a

novel mechanism of disease; perhaps diseases other than endometriosis are also caused by ectopic stem cell differentiation.

Our laboratory developed an experimental model in mice to determine the ability of extrauterine cells to engraft to endometriotic lesions.[35] Endometrium from wild-type mice was transplanted into the peritoneal cavities of hysterectomized LacZ transgenic mice to induce endometriosis. After 10 weeks, the ectopic lesions were excised and frozen sections were stained with 5-bromo-4-chloro-3-indolyl-β-d-galactoside (X-gal) to detect the presence of LacZ-expressing cells. LacZ-expressing cells constituted approximately 0.1% of stromal cells and 0.04% of epithelial cells, establishing the ability of extrauterine stem cells to contribute to endometriotic lesions.[35]

As described in nonhuman primate models, transplantation of normal endometrial cell samples can give rise to endometriosis.[54,55] Human endometrial mesenchymal stem cells were isolated from biopsies of eutopic and ectopic endometrium taken during surgical treatment of endometriosis.[56] While both cell types exhibited similar phenotypes, endometrium-derived mesenchymal stem cells from ectopic sites exhibited increased migration and proliferative capability during in vitro assays. Mesenchymal stem cells from ectopic and eutopic endometrium were also transplanted into immune-deficient mice to assess their invasive potential. In comparison with transplanted tissue scaffolds from eutopic endometrial mesenchymal stem cells, transplanted tissue scaffolds from ectopic endometrial mesenchymal stem cells demonstrated higher levels of invasiveness and vasculogenic potential.[56] This study provides powerful evidence for the stem cell origin of endometriosis by demonstrating the presence of invasive and proliferative endometrial stem cells within endometriotic lesions.

In humans, previous work in our laboratory showing the ability of bone marrow–derived stem cells to contribute to endometriotic lesions and differentiate to stromal and epithelial phenotypes may provide further evidence for a novel theory of the origin of distal endometriosis and rare cases of endometriosis in men.[35,57–60] Endometriotic lesions have been found in areas in which retrograde menstruation is unlikely to reach, such as the brain, lung, and nose as the sole cause of endometriosis.[58–61] These cases also dispute the transition of peritoneal cells to an endometrial phenotype as the etiology of endometriosis, as these lesions are not in the peritoneum. Additionally, a few cases of endometriotic lesions in men have suggested that the traditional theories are not sufficient to explain the full etiology of endometriosis; men do not menstruate and do not have endometrial progenitor cells. These cases suggest that there must be another etiology of the endometriosis that occurs at sites distant from the pelvic cavity. As previously mentioned, our laboratory has demonstrated the presence of donor-derived bone marrow stem cells in the endometrium of women who received single-antigen HLA-mismatched bone marrow transplants.[26] These cells proliferated within the endometrium and were able to differentiate to an epithelial cell type. Fusion of bone marrow–derived stem cells with endometrial cells was eliminated as a possibility by the examination of cells with a DNA-complexing dye to indicate excess of nuclear material.[26] This model of bone marrow–derived stem cell contribution to endometrial tissue was again studied in mice; female mice were irradiated and received bone marrow transplants from male donor mice.[35] Rare populations of differentiated and localized

male donor–derived cells were found in the endometrium. Furthermore, our laboratory has described that sites of injury and inflammation within the uterus increase the recruitment of bone marrow–derived stem cells by twofold in a murine model.[36] Given that endometriosis is an inflammatory condition, bone marrow–derived stem cells are likely recruited to endometriotic lesions. We further studied this possibility and described the contribution of donor bone marrow–derived stem cells to experimentally induced endometriotic lesions in a mouse model, providing the proof of concept for the contribution of bone marrow–derived stem cells in the pathogenesis of endometriosis.[35]

In addition to mesenchymal stem cells, circulating endothelial progenitor cells (EPCs) contribute to the vascularization of endometriotic lesions.[62] A mouse model of experimental endometriosis was used to study blood levels of EPCs and mature circulating endothelial cells. 129/SvJ mice with experimentally induced endometriosis had higher concentrations of EPCs in their blood in comparison with mice that received sham surgery.[62] EPC concentrations also correlated with the size of endometriotic lesions. When mice were treated with an angiogenesis inhibitor, Lodamin, blood levels of EPCs dropped, as did lesion sizes. Endometriosis attracts EPCs to the lesions as a mechanism to provide a sufficient vascular supply.

In addition to EPCs, mesenchymal stem cells contribute to the vasculature of endometriosis. While human endometrium contains a small population of mesenchymal stem cells in the epithelial and stromal compartments, bone marrow provides an exogenous source of cells contributing to endometrial tissue. Bone marrow cells have been shown to contribute to endometrium and CD45+ bone marrow progenitor cells contribute to the formation of new blood vessels in the endometrium and endometriosis.[33,35,63]

Treatment of Endometriosis—Targeting the Stem Cell Contribution

In light of the stem cell origin of endometriosis, future medical treatments may look toward reducing migration of endometrial stem cells and bone marrow–derived stem cells to ectopic sites.[4] In a murine model of endometriosis, our laboratory has demonstrated that bazedoxifene (BZA), a selective estrogen receptor modulator, reduces bone marrow stem cell engraftment to ectopic sites, promoting engraftment to the eutopic endometrium.[64] BZA significantly reduced the mean size of implants, endometrial cell proliferation, and estrogen receptor α (ESR1) expression. The regression of endometriosis likely involved decreased estradiol-mediated stem cell recruitment and engraftment; this experiment demonstrated that estrogen is required for the recruitment of stem cells to the lesions.[65] Stem cell recruitment by the lesions contributes to the disease and blocking the stem cell contribution is a novel means of treating endometriosis. It is likely that antiestrogenic therapies for endometriosis function in part by blocking stem cell recruitment to the lesions.

Endometriosis is very effective at recruiting stem cells. In these experiments, we noted that, without treatment, a far greater number of stem cells were recruited to the endometriosis than to the uterus.[64] Circulating bone marrow–derived mesenchymal stem cells are in limited supply. The ability of endometriosis to effectively recruit stem cells depletes the cells from the circulation and prevents them from reaching the uterus. Thus, we posit that ectopic

endometriosis implants seem to function as a “sponge,” attracting stem cells and preventing their migration to the uterus. Defects in endometrium of women with endometriosis may in part originate from the defective replenishment of endometrial stem cells. In our experimental model, BZA treatment restored stem cell recruitment to the uterus, to levels comparable to that of control groups without endometriosis.[64] The recruitment of stem cells to endometriosis is selective. Endometriosis is able to recruit more stem cells, but this ability can be suppressed using medical therapy. The eutopic endometrium maintains a basal level of stem cell recruitment even during endometrial repressive hormonal therapy.

We have also established that exposure to environmental toxins inhibits recruitment of bone marrow–derived stem cells to the uteri of mice.[66,67]

Our experiment employed human male mesenchymal stem cells (hMSC) in vitro and a mouse model of cigarette smoke exposure. After myeloablation, female mice received male murine bone marrow cells. Mice were exposed to room air or smoke from unfiltered cigarettes. Immunofluorescence and Y-chromosome fluorescence in situ hybridization (Y-FISH) was performed on uterine sections. After 4 weeks, the total number of Y-chromosome cells in the uterus was reduced by 68% in the smoke-exposed mice. Both leukocytes and bone marrow–derived endometrial stem cells were reduced by 60 and 73%, respectively. Differentiation of bone marrow–derived cells to endometrial epithelial cells was reduced by 84%. In vitro hMSCs were treated with 8-Br-cAMP to induce endometrial cell differentiation with or without cigarette smoke extract (CSE) and decidualization and assessed morphologically and by prolactin expression. hMSC treated with CSE failed to show cytological characteristics of decidualization. mRNA levels of the decidualization marker prolactin were decreased by 90% in CSE treated cells. Smoking inhibits recruitment of bone marrow–derived stem cells to the uterus and stem cell differentiation. Inhibition of stem cell recruitment may be a general mechanism by which smoking leads to long-term organ damage through inability to repair or regenerate multiple tissues. Endometrium is clearly one of the tissues that fails to engraft stem cells in response to tobacco smoke. These findings are consistent with the lowered risk of endometriosis that has been described among smokers.[68]

While we have described the untoward effects of stem cells in endometriosis, it is important to note that endometrial and/or bone marrow–derived stem cells play a critical role in normal physiology to regenerate the cyclic loss of the endometrium functionalis. There may be beneficial therapies to treat endometrial disease derived from the use of bone marrow stem cells. We have shown that treatment with bone marrow-derived stem cells can be used to treat Asherman syndrome (AS).[69] Initial studies in our laboratory employed a murine model of AS. To simulate AS, a uterine injury was created in a highly reproducible manner. Mice were randomly divided into two groups. In the injury group, a small incision was made in the uterine horn at the utero-tubal junction and the horn injured using a beveled glass pipette inserted two-thirds of the way through the lumen, rotated and withdrawn five times. In the control group, identical surgical incisions were created in the abdomen; however, neither horn was damaged. In half of the animals, bone marrow from male littermates was given after the injury to enhance the number of available reparative cells. Three months after the uterine horn injury, uteri were collected and evaluated by Y-chromosome FISH and

simultaneously by IF using anti-CD45, anti-F4/80, anti-vimentin, and anti-cytokeratin. As expected, stem cells were recruited in response to traumatic injury to the endometrium. The cumulative pregnancy rate was dramatically improved by the bone marrow stem cell augmentation. In untreated controls, the cumulative pregnancy rate was nearly 100%. In the injury group, the pregnancy rate was approximately 30%. Bone marrow stem cell augmentation led to a cumulative pregnancy rate of nearly 90%. Normally, the limited supply of bone marrow-derived stem cells prevents recovery from catastrophic injury such as occurs in AS. Delivery of excess bone marrow-derived stem cells enables a better response to injury and improved healing. bone marrow-derived cells are a potential treatment for AS.

Case reports have described initial attempts at stem cell therapy for AS in humans. Six women with refractory AS that had failed standard treatments using hysteroscopic adhesiolysis were administered stem cells. Mononuclear stem cells were implanted in the subendometrial zone followed by exogenous oral estrogen therapy.[70–72] This resulted in improved endometrial thickness and five out of six patients resumed menstruation. While these are small studies and need to be independently verified, they indicate the potential use of endometrial stem/progenitor cells in reconstructing endometrial tissue in AS. Larger trials are currently in progress.

Stem Cell Migration between Endometriosis and Endometrium

Although it is clear that BMDSCs migrate to the uterus and endometriosis, stem cells are also capable of tremendous trafficking between locations. Our laboratory has identified the presence of a cell population that migrates from the endometriotic lesion into the uterus; these cells produce factors capable of altering uterine receptivity.[50] Specifically, to determine whether ectopic endometrial lesions contain stem cells that migrate to the uterus, we used the endometriosis mouse model transplanted with endometrial tissue from green fluorescent protein (GFP) transgenic or wild-type donors. PCR for the presence of GFP confirmed the absence of GFP in the control group and its presence in the uteri of mice in the GFP-endometriosis group. Immunofluorescence was used to locate the cells in the endometrium. Interestingly, GFP-expressing cells in the sample from the experimental group were mainly localized to the basal layer of the endometrium and never in the epithelial lining of the lumen or within the glands. Cells in the uterus that originated in ectopic lesions demonstrated a distinct gene expression profile compared with the eutopic endometrium. Following isolation by fluorescence-activated cell sorting, gene array, PCR, and immunofluorescence were conducted. The cells displayed increased expression of Snail1, Snail3, Goosecoid, and the downregulation of Zeb2. These genes are associated with epithelial-to-mesenchymal transition.[49] This is a process by which epithelial cells lose their polarity and are converted to a mesenchymal phenotype. These cells migrate as mesenchymal stem cells. After engraftment in the uterine stroma, these cells, all derived from the endometriosis, displayed activation of the Wnt signaling pathway indicating that they had taken on an epithelial identity; however, these cells were not located in the epithelium. These endometriosis-derived stem cells do not home to the appropriate location in the uterus. The cells were inappropriately localized to the stroma yet secreted signals typical of epithelial cells. Proper establishment of a gradient of signaling molecules between epithelium and stroma is essential for normal endometrial receptivity. The disincorporation

of stem cells in the eutopic endometrium disrupts stromal-epithelial signaling and leads to decreased endometrial receptivity in endometriosis patients.

Taken together, these results support the bidirectional movement of cells between eutopic and ectopic endometrial tissues. These cells likely undergo EMT, enabling this migration, and preferentially home to the basalis layer of the endometrium. The ectopic Wnt signaling likely distorts the epithelial-stromal dialog needed for optimal endometrial development and receptivity. These data suggest that defective trafficking of stem cells is an important mechanism for this disease.[49]

Mobilization and Homing of Stem Cells

The mobilization and homing of stem cells is regulated by chemokines, low molecular weight cytokines that attract various cell types via chemotactic mechanisms.[27,73–79] CXCR4 is a chemokine commonly expressed on the surface of stem cells that interacts with its ligand, CXCL12 (i.e., stromal-derived factor 1, SDF-1α). CXCL12 is produced by the cells of many tissues, including the stroma and epithelium of the endometrium, and is generally expressed at sites of inflammation and injury.[76] Variation in CXCL12 concentration creates a chemical gradient that directs the migration of stem cells.[76]

The CXCR4–CXCL12 axis has been well characterized in the pathogenesis of cancer where it acts in a paracrine manner, promoting cellular migration and invasion, as well as angiogenesis, in tissues that express CXCL12.[75] In cancer, binding of CXCL12 to CXCR4 leads to increased expression of matrix metalloproteinases, which are directly involved in facilitating metastasis through the degradation of extracellular matrix proteins.[75] The binding additionally promotes the development of new blood vessels by attracting endothelial cells and stimulating expression of VEGF.[75]

Our laboratory has recently characterized the role of the CXCR4–CXCL12 axis in communication between bone marrow–derived stem cells and human endometrial stromal cells in vitro.[80] Human endometrium–derived stromal cells, like many stromal tissues, produce CXCL12. Treatment with physiological concentrations of E2 (10^{-7} M) induced CXCL12 expression in stromal cells and CXCR4 expression in bone marrow–derived stem cells, respectively, thereby also enhancing the chemoattraction of bone marrow–derived stem cells to human endometrial stromal cells.[80] In a migration assay, bone marrow–derived stem cells migrated toward endometrial stromal cell-conditioned media. AMD3100, a CXCR4 antagonist, blocked this migration in a dose-dependent manner, demonstrating that CXCL12 is sufficient to induce migration of endometrial stem cells. Estrogen-driven recruitment of stem cells to endometrium is driven by endometrial production of CXCL12 and enhanced expression of the CXCR4 receptor in BMDSCs. These data identify a new mechanism that drives stem cell recruitment in endometriosis.

In patients with endometriosis, CXCL12–CXCR4 axis expression is increased in biopsies of human endometriotic lesions compared with healthy controls.[81] Interestingly, treatment with ovarian steroid hormones, estradiol and progesterone, decreased CXCL12 expression in normal endometrial stromal cells.[81] Given that hormone therapy is a commonly used

medical treatment for endometriosis to reduce pain and inflammation, these findings provide evidence that the effect of progestins may in part be mediated by decreased CXCL12 production leading to a reduction in stem cell recruitment.

Blocking of the CXCR4–CXCL12 axis, a primary pathway involved in the recruitment of bone marrow–derived cells, may have therapeutic utility in endometriosis. Inhibiting abnormal pathways of cell migration between the uterus, ectopic endometriotic lesions, and bone marrow may prevent stem cell recruitment and engraftment at ectopic sites.

Conclusion

Adult stem cells are found in numerous human organs including the endometrium, a tissue that is constantly regenerated during a woman's fertile years. The endometrium is rich in several stem cell populations. While these cells have a major role in endometrial physiology, regeneration, and repair, they also have a role in the generation of endometriosis. Recent studies have suggested that endometriosis may arise as the result of dislocated or aberrant stem cells, either from the endometrium or exogenous sources such as bone marrow. Bone marrow–derived stem cells have been shown to engraft eutopic endometrium as well as ectopic endometriotic lesions, taking on an endometrial phenotype. Furthermore, endometriotic lesions have been shown to contain populations of mesenchymal stem cell-like cells, similar to the mesenchymal stem cells present in the eutopic endometrium. These populations of stem cells likely play a role in the development and progression of endometriosis. These cells migrate, seeding new lesions as well as inappropriately incorporating into the eutopic endometrium contributing to infertility. Future treatments for endometriosis should address this mechanism of pathogenesis by inhibiting the inappropriate migration of stem cells.

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