Published in final edited form as:

Ann N Y Acad Sci. 2008 April ; 1127: 106–115. doi:10.1196/annals.1434.014.

Stem Cells and the Pathogenesis of Endometriosis

Isaac E. Sasson^a and Hugh S. Taylor^{a,b}

^a Department of Obstetrics, Gynecology, and Reproductive Sciences, Yale University School of Medicine, New Haven, Connecticut 06520, USA

^b Division of Reproductive Endocrinology, Yale University School of Medicine, New Haven, Connecticut 06520, USA

Abstract

Endometriosis is a common gynecological disorder that is defined by the presence of endometrial tissue outside the uterine cavity. This disease often results in extensive morbidity, including chronic pelvic pain and infertility. The pathogenesis of endometriosis is likely multifactorial, and extensive investigation has explored the role of genetics, environmental factors, and the immune system in predisposing patients to developing endometriosis. A series of recent publications have described the identification of endometrial stem/progenitor cells. Such cells have long been speculated to function in the cyclic regeneration of the endometrium during the menstrual cycle and in the pathogenesis of several gynecological disorders. This narrative review will (i) examine the evidence for endometrial stem cells, (ii) examine their potential role in the pathogenesis of endometriosis, and (iii) identify important unanswered questions with suggestions for future investigation.

Keywords

Endometrium; endometriosis; stem cells; bone marrow; uterus

Introduction

Endometriosis is a chronic benign gynecological disease characterized by the presence of endometrial glands and stroma outside the uterine cavity. The distribution of this ectopic tissue is most often within the pelvic peritoneum but can include the pelvic viscera, rectovaginal septum, pleura, abdominal wall, and, rarely, the brain. The consequences of endometriosis often include pelvic pain and infertility. The incidence of the disorder is between 6% and 10% of all women and 35%–50% of women with pelvic pain and infertility.^{1–5} The clinical manifestation of endometriosis and the presence of endometrial tissue outside the uterine cavity is probably the end point of a combination of several aberrant biological processes. These include retrograde menstruation in a woman with an improper immune response and a genetic predisposition to developing endometriotic lesions, possibly in the setting of an uncharacterized environmental exposure.^{6–15}

© 2008 New York Academy of Sciences.

Address for correspondence: Hugh S. Taylor, M.D., Division of Reproductive Endocrinology, Yale University School of Medicine, 333 Cedar St., P.O. Box 208063, New Haven, CT 06520-8063. Voice: +1-203-785-4005; fax: +1-203-785-7819. hugh.taylor@yale.edu.

Conflict of Interest

The authors declare no conflicts of interest.

Extensive investigations have been performed on the molecular biology required for establishment and survival of endometrial implants. Briefly, the mechanisms required include attachment of endometrial cells to the pelvic peritoneum, invasion into the mesothelium, and survival and proliferation of the ectopic endometrial cells. These data have been reviewed elsewhere¹⁴ but ultimately suggest that the development of endometriosis is probably a polygenetic disorder requiring alterations in multiple biological pathways for the establishment and proliferation of the disease state.^{6,9-12}

Microarray analysis has demonstrated that in patients with endometriosis the gene expression profile of ectopic endometrial implants differs from the gene expression profile of eutopic endometrium. Furthermore, the gene expression profile from eutopic endometrium of patients with endometriosis differs from that of unaffected controls.¹⁶⁻²⁰ Taken together, these data suggest that the presence of endometriotic implants can alter gene expression profiles within the eutopic endometrium and, by extension, the function of the eutopic endometrial tissue.

The origin of the ectopic endometrium has also been subject to extensive investigation, and several hypotheses have been proposed.¹⁴ The recent characterization of possible stem/progenitor cells in the endometrium may have identified the origin of ectopic endometrial tissue and the mechanism for the pathogenesis of endometriosis. In the following review, we will evaluate the evidence for endometrial stem cells and apply these data to the predominant hypotheses regarding the pathogenesis of endometriosis. Finally, we will identify important unanswered questions in the field with suggestions for future investigation.

Pathogenesis of Endometriosis

The origin of endometriotic implants and the pathogenesis of endometriosis has long been an area of active investigation. Multiple hypotheses have been explored, including retrograde menstruation, coelomic metaplasia, embryonic rest theory, and the lymphovascular metastasis theory.

Retrograde Menstruation Theory

This theory is the most widely accepted and postulates that endometriotic implants arise from retrograde menstruation of endometrial tissue through the fallopian tubes into the peritoneal cavity.^{15,21,22} Indeed, as early as 1938, women who underwent a laparotomy during menstruation were noted to have menstrual blood exuding from the fallopian tubes.²³ Laparoscopy performed on menstruating women has demonstrated retrograde menstruation in 76%–90% of women with patent fallopian tubes.^{15,24} Furthermore, women with endometriosis have larger volumes of retrograde menstrual flow than women without endometriosis.¹⁵ Women with endometriosis have an abnormal myometrial contraction pattern compared with unaffected women, who demonstrate a normal antegrade contraction pattern.²⁵ Women with müllerian anomalies and cervical or vaginal outlet obstruction are at an increased risk of developing endometriosis at an earlier age.²⁶⁻²⁸ Consistent with this observation, baboons whose cervixes had been ligated demonstrated a significant increase in the development of endometriosis.²⁹

Coelomic Metaplasia Theory

This theory proposes that endometriosis develops from metaplasia of the cells lining the visceral and abdominal peritoneum.³⁰ Some undetermined stimulus, either hormonal, infectious, or environmental, is believed to induce metaplastic changes in the peritoneal lining, resulting in endometrial implants.³¹ Support for this hypothesis lies in several lines of evidence. Embryologically, the thoracic, abdominal, and pelvic peritoneum and the

mullerian ducts are derived from the same cell lineage, the coelomic wall of the developing embryo. Clinically, this hypothesis would account for the rare occurrence of endometriosis in men,^{32,33} prepubescent and adolescent girls,^{34,35} distant ectopic sites such as catamelic endometriosis in the thoracic cavity^{36–38} and in women with a congenital absence of mullerian structures.²⁷ Conclusive proof for the coelomic metaplasia theory remains elusive despite these intriguing observations. Furthermore, if this were the primary etiology of endometriosis, then an increased incidence would be expected with aging, similar to metaplasia in other organs. Ultimately, proving the null hypothesis that coelomic metaplasia does not occur may be nearly impossible.

Embryonic Rest Theory

This theory proposes that the presence of cells of mullerian origin within the peritoneal cavity could be induced to form endometrial tissue when subjected to the appropriate stimuli.^{39,40} This hypothesis could account for the presence of endometriosis of the rectovaginal septum as well as in any location along the migration pathway of the embryonic mullerian system. Furthermore, this theory could account for the presence of rare endometriosis in men because the male embryo initially develops female-specific embryological structures that regress with activation of the male genome. This theory remains speculative, as it would require the assumption that these embryological rests persist to adulthood. Again, this theory remains unproven and purely hypothetical.

Lymphovascular Metastasis Theories

Sampson^{41,42} suggested that endometrial cells could spread to ectopic sites via lymphatic and hematogenous spread, accounting for the presence of endometriosis in distant sites outside the pelvis, including the brain, lung, lymph nodes, extremities, and the abdominal wall. Clinically, this hypothesis has been supported by the presence of endometrial tissue in the uterine vasculature, which has been documented in patients with adenomyosis.⁴² In addition, intravenous injection of endometrial tissue has been demonstrated to result in pulmonary endometriosis in the rabbit.³⁶ Although it is possible that some lymphovascular trafficking of endometrial cells contributes to the pathogenesis of endometriosis, this is not likely to be the primary mechanism of disease spread because the incidence of hepatic, pulmonary, and thoracic endometriosis is rare.^{37,38,43}

Stem Cells

Stem cells are undifferentiated cells that have the ability to self-renew as well as to produce more differentiated daughter cells.^{44,45} Broadly, stem cells can be divided into two categories: embryonic and adult. Embryonic stem cells are derived from blastocysts. Adult stem cells, derived from postembryonic cell lineages, have been described in a number of different organ systems and have been best characterized in the hematopoietic system.^{44,46–48}

Embryonic and adult stem cells are classified by their ability to differentiate into cells of different cell lineages. Totipotent stem cells are fully undifferentiated and able to generate all embryonic germ layers (endoderm, mesoderm, and ectoderm) as well as the extra-embryonic tissues (trophoblast, placenta, and extra-embryonic membranes). Pleuripotent stem cells lie along a spectrum of differentiation and can produce cells of all three germ layers, but not the extra-embryonic tissues. As stem cells undergo differentiation and their cell lineages become more restricted, they are described as multipotent because they can produce multiple cell types within the same germ cell lineage, or unipotent, differentiating into a single cell lineage.⁴⁹

Adult stem cells reside in an anatomic structure called the niche.⁵⁰ The stem cell niche is a microenvironment of surrounding support cells that signal to the stem cell population to maintain tissue homeostasis. The niche cells provide signals that maintain stem cells in an undifferentiated state, protecting them from differentiation, proliferation, and apoptotic cues. They also provide signals that balance the need for self-renewal with cues for proliferation and differentiation.^{50–52}

Stem cells asymmetrically divide to produce daughter cells, known as progenitor, or transient amplifying (TA), cells that begin the differentiation process.⁴⁴ TA cells undergo repetitive cycles of cell divisions to increase in number while progressively acquiring markers of the differentiated cell type; consequently, they lose the ability for self-renewal. The processes of differentiation, proliferation, and migration are often linked in multiple biological systems.⁵⁰ These have been best characterized for the intestinal and hair follicle stem cell niches.^{50,52} For example, as intestinal stem cells transition to progenitor/TA cells, they initially exhibit a dramatic amplification in number while physically migrating up the intestinal crypt. When they reach the top of the crypt, they differentiate and cohesively function in the villous structure.^{51,53}

Evidence for Endometrial Stem/Progenitor Cells

Multiple lines of evidence suggest that a similar mechanism of tissue regeneration may exist in the cycling endometrium.^{44,54}

Structure of the Endometrium

The endometrial lining of the uterus displays a structure similar to that of the intestinal villous and may demonstrate a similar mechanism for regeneration. The endometrium is broadly composed of two cell types—the luminal and glandular epithelial cells and the supporting mesenchymal cells.⁵⁵ Broadly, the endometrium is composed of two cell types: the glandular epithelium and the supporting mesenchymal cells, which include stromal fibroblasts, the vasculature, and leukocytes. Functionally, the endometrium is composed of two layers—the outer functionalis layer and the inner basalis layer. The basalis layer rests on the muscular subendometrial myometrium. Embryologically, all of these components have a müllerian origin. The functionalis is composed of dense glandular tissue and a loose connective stroma, whereas the inner basalis layer contains primarily stroma, the base of the glands, leukocytes, and vasculature.^{55,56} During menses, the functionalis and a small portion of the basalis are shed with each cycle.⁵⁷

Characterization of Putative Stem Cells

Because stem cells are a rare cell type and phenotypically difficult to distinguish, multiple assays have been used to isolate and characterize adult stem cells. *In vitro* assays include those that demonstrate a cell's clonogenicity, proliferative and differentiation potentials, and phenotypic characteristics. *In vivo* assays include the gold standard tissue reconstitution assay and the demonstration of label-retaining cells (LRCs). Ultimately, defining a population of cells as stem cells requires a combination of multiple assays that demonstrate the properties consistent with stem cell features: self-renewal and the ability to differentiate into a mature, functioning cell type.

In Vitro Assays

Clonogenicity is characterized as the ability of single cells to form colonies when plated at very low densities. This technique has classically been used to characterize populations of stem cells derived from multiple adult tissue types.^{58–62} Determination of the proliferative

potential of putative stem cells characterizes their self-renewal ability.^{58–60,63} Proliferative potential is assayed by determining the total number of population doublings of a single cell by serial passage until senescence. Adult stem cells will produce self-renewing daughters during colony expansion and thus will be passaged more times than a daughter cell with limited self-renewing potential. Assay of a putative stem cell's differentiation potential is performed by culturing cells in various media supplemented with specific growth factors and then determining the phenotypic properties of the cultured cells as well as by evaluating their gene expression patterns.^{64,65} Cells with greater potency have the ability to differentiate along multiple cell lineages.

In Vivo Assays

Tissue Reconstitution Assays

Tissue reconstitution assays comprise a series of experiments whereby the tissue of interest is reconstituted in a recipient animal by transplantation of donor stem cells.⁶⁶ This has been best characterized in the hematopoietic lineage in which mice, immunocompromised either by lethal irradiation or genetic manipulation, receive bone marrow–derived stem cells and can reconstitute a functioning immune system. In this assay, self-renewal is illustrated by harvesting bone marrow stem cells from the recipient and transplanting them and restoring immune function in a second immunocompromised mouse.⁶²

Some cell types cannot be readily transplanted back into their tissue of origin. In these cases, putative stem cells have been transplanted into regions of rich vasculature either subcutaneously or under the kidney capsule. Tissue explants are then examined for recapitulation of the differentiated cell type of interest as well as tissue morphology. These experiments are limited by the absence of the native stem cell niche and the inability of the explant to develop into a functional organ.

Label-Retaining Cells

The LRC technique is a pulse–chase experiment that can be used to identify a stem cell population *in vivo* by capitalizing on the quiescent state of stem/progenitor cells. Animals are treated with a pulse of the nucleotide analogue 5-bromo-2'-deoxyuridine (BrdU), which is incorporated into the DNA during cell division. Animals are then grown in the absence of BrdU for a prolonged chase period prior to being analyzed. In a population of rapidly dividing cells, the label is diluted after several cell divisions. However, in relatively quiescent cells, the BrdU label is retained. This has been shown to correlate with markers of somatic stem cell populations in multiple tissue types.^{67–72}

Stem Cell Markers

At present, no known markers are specific for the adult stem cell; however, this is an area of active research.^{63,73–76} Although markers are expressed on stem cells, this expression pattern is not limited to the stem cells, and the marker expression may persist despite differentiation along a specific lineage. Thus, stem cells are often phenotypically characterized by the expression of some lineage-specific marker and the absence of markers of a more differentiated cell type.

Identification of “side population” cells has been used to isolate and characterize somatic stem cells from multiple tissues.^{77–83} These cells have the ability to efflux Hoechst 33342 dye via the multi drug resistance (MDR) genes, such as the ABC transporter Abcg2/Brcp1.⁸⁴ Thus, the stem cells in this assay are defined as those cells expressing an MDR gene and, consequently, by the absence of Hoechst 33342 dye staining.

Evidence of Endometrial Stem/Progenitor Cells

Four recent functional analyses provide evidence of isolation and characterization of human endometrial stem/progenitor cells. These studies have employed clonogenic analyses, identification of a side population, and examination of proliferation and differentiation potentials. Additionally, LRCs were identified in the uterus using three model systems.

Clonogenic and Proliferation Assays

Chan *et al.*⁶¹ demonstrated clonogenicity of endometrial-derived cells by generating single-cell suspensions of epithelial and stromal cells from hysterectomy samples. When plated at clonal density, 0.22% of epithelial cells and 1.25% of stromal cells formed individual colonies in 15 days. Two types of colonies were generated by both epithelial and stromal cells: (i) large, densely packed colonies with greater than 4000 cells per colony with a high nuclear-to-cytoplasmic ratio and (ii) small, loosely packed colonies with cells that displayed a low nuclear-to-cytoplasmic ratio. These investigators hypothesized that the large colonies were derived from candidate endometrial stem/progenitor cells with a greater potential for self-renewal. Thus, more stem cells are generated within the colony and can produce a greater number of daughter cells in the colony. By contrast, the small colonies are presumably derived from TA cells that lack the ability for self-renewal and thus display a diminished proliferative potential.

Large putative stem/progenitor cell colonies were rare; occurring at 0.08% and 0.02% for epithelial and stromal cells, respectively. These colonies displayed a significantly greater self-renewal capability compared with the small, loose colonies that failed to serially clone and displayed limited proliferation potential. Serially passaged large colonies could undergo, on average, 30–32 population doublings prior to senescence, with rare colonies displaying greater than 90 population doublings.⁸⁵

Schwab *et al.*⁸⁶ performed a similar analysis of clonogenicity using samples collected from proliferative, secretory, and inactive endometrium. This work demonstrated that the frequency of clonogenic epithelial and stromal cells did not differ between phases of the menstrual cycle. Additionally, there was no significant change in the number of clonogenic cells isolated from inactive endometrium. Because inactive endometrium contains only a basalis layer and not an endometrium functionalis,⁸⁷ these data would suggest that putative endometrial stem/progenitor cells reside in the basalis layer and persist beyond menopause.

Identification of Side-Population Cells

Kato *et al.*⁸⁸ used the Hoechst 33342 dye efflux properties of stem cells to isolate and characterize side-population cells from the human endometrium. Side-population cells in this study demonstrated similar clonogenic frequencies as those isolated by Chan *et al.*⁶¹ and Schwab *et al.*⁸⁶ and demonstrated a long-term repopulating phenotype.

Differentiation Potential Assays

Three recent studies demonstrate the differentiation potential of putative endometrial stem cells. Kato *et al.*⁸⁸ used two markers of endometrial differentiation to illustrate that side-population cells can differentiate into endometrial epithelial and stromal cells, respectively. CD9 is a glycoprotein strongly expressed in glandular epithelium,⁸⁹ and CD13 is expressed on the surface of stromal cells.⁹⁰ The Hoechst 33342 dye-negative cells lack expression of CD9 and CD13. CD9[−]CD13[−] cells could be isolated and, after long-term culture, would differentiate either into CD9⁺CD13[−] gland-like structures consistent with an epithelial differentiation lineage or CD9[−]CD13⁺ stroma-like cells. Taken together, these data demonstrate that a side-population of cells that lack markers of epithelial or stromal

differentiation can be isolated and that these cells, when cultured, can differentiate along either stromal or epithelial lineages. Furthermore, these data suggest that a single stem/progenitor cell is capable of differentiating along both lineages.

Consistent with these data, endometrial stromal cells have been shown to differentiate into chondrocytes when cultured in a defined chondrogenic medium.⁹¹ Additionally, Gargett *et al.*^{54,85} showed that the large, putative stromal stem/progenitor cell colonies (described above) demonstrated multipotency by differentiating into four mesenchymal lineages—adipocytes, smooth muscle cells, chondrocytes, and osteoblasts—when cultured in the appropriate differentiating media. By contrast, the small, loose colonies with limited proliferative capacity failed to demonstrate similar differentiation capability, consistent with the hypothesis that these colonies are derived from TA cells with a restricted cell lineage.

Evidence from the Mouse

Model organisms often provide a surrogate for experiments that cannot be performed in humans. Extensive experimentation on the murine uterus has provided data on the molecular and cell biology underlying human gynecologic pathology. The murine estrous cycle has characteristics similar to those of the human menstrual cycle; however, the structure and physiology of the mouse endometrium differs from that of the human. The mouse lacks an endometrial basalis layer, and the endometrium in the mouse is not sloughed during menstruation but rather reabsorbed at the end of the cycle. This limits the interpretation of the data and the generalizability to human physiology and disease states. Nonetheless, extensive molecular, cellular, and genetic data can be obtained from experiments in this organism.

Uterine LRCs: Identification of Epithelial, Stromal, and Myometrial Stem Cells

Three recent papers have demonstrated the presence of LRCs in the mouse uterus.^{92–94} Furthermore, co-localization of tissue-specific markers as well as location of the LRCs within the tissue have been employed to classify the cells as epithelial, stromal, or myometrial stem/progenitor cells. Chan and Gargett⁹² found that LRCs composed approximately 3% of mouse endometrial epithelium after a 56-day chase period, and 6% of the of the endometrial stromal cells demonstrated LRC properties after an 84-day chase period. Cervello *et al.*⁹⁴ found that 9% of stromal cells were LRCs after 49 days and this decreased to 1.7% after a 112-day chase period; however, no epithelial LRCs were identified even after a brief chase period of 21 days. The pulse technique employed in these experiments was the same, although the microscopy used for detection was not identical.

Szotek *et al.*⁹³ identified LRCs in the stroma and myometrium that persisted after a 96-day chase. Epithelial LRCs were not identified in this study even after a 33-day chase; however, the pulse technique used in this study differed from that employed by Chen and Gargett,⁹² and thus, it is possible that the epithelial stem/progenitor cells were not initially labeled.

These studies employed an immunohistochemical analysis to exclude a hematopoietic source of the LRCs and to demonstrate the presence of stem/progenitor cell-specific markers.^{92–94} Furthermore, the functionality of the uterine LRCs was illustrated by hormonally manipulating animals with either estrogen or human chorionic gonadotropin and demonstrating that LRCs were induced to proliferate *in vivo* or differentiate *in vitro*.^{92,93}

Uterine Stem Cell Niche in the Mouse

Because of the disparity between murine and human endometrial structures, parallels cannot be drawn regarding a potential niche for the epithelial stem/progenitor cell. However, in the mouse, the majority of the stromal LRCs reside along the endometrial-myometrial border,

and 30% are localized to a perivascular locale, suggesting a possible niche for the stromal stem/progenitor cell.^{92,93} The myometrial LRCs reside along the periphery of the longitudinal muscle bundles⁹³ at a location similar to that of adult skeletal muscle satellite stem cells.⁹⁵

Bone Marrow as a Source for Endometrial Stem/Progenitor Cells

Fetal stem cells are speculated to persist in the adult uterus to replace the glandular epithelium and stroma that are shed with each menstrual cycle. Recent studies suggest that the bone marrow may be another source of endometrial stem cells. This hypothesis is appealing as bone marrow-derived cells (BMDCs) have been shown to circulate and to be able to differentiate into multiple cell types, including endothelial cells, hepatocytes, neurons, skin, cardiomyocytes, and gastrointestinal epithelium.^{96–102} Furthermore, endometrial ablation techniques have demonstrated a substantial failure rate with apparently normal endometrium returning to what appears to be a completely resected or ablated uterus. Thus, a nonendometrial source of stem cells could account for these observations.

Recent studies identified the presence of chimerism in the endometrial glands and stroma of four women who received single-antigen HLA-mismatched bone marrow transplants. These data suggest that bone marrow-derived stem cells contributed to the repopulation of the endometrium in these patients. The extent of chimerism ranged from 0.2%–48% and correlated with the length of time between transplantation and biopsy; however, the sample size in this analysis was too small to determine a clear time course for the repopulation of the uterus with bone marrow-derived stem cells. Nonetheless, donor cells localized to a focal area, suggesting local proliferation of a donor-derived stem cell. Co-localization of donor-derived stromal and epithelial cells would suggest a possible common stem cell ancestor for both of these cell types.¹⁰³

In this analysis, immunostaining for the pan-leukocyte marker CD45 was used to distinguish donor endometrial leukocytes from donor-derived endometrial epithelium and demonstrated that the observations were not a result of invasion of donor-derived leukocytes. Additionally, donor-derived endometrial cells expressed markers of a functioning, differentiated secretory endometrium. Fusion of BMDCs with native endometrial cells was excluded by examining cells with a DNA-complexing dye, which did not demonstrate an excess of nuclear material in donor-derived cells.¹⁰³

To develop an experimental system to explore the contribution of nonendometrial stem cells to the endometrium, female mice were lethally irradiated and subject to bone marrow transplantation from male donor mice. After transplantation, male donor-derived cells differentiated into endometrial epithelial cells, albeit rarely ($< 0.01\%$).¹⁰⁴

Stem/Progenitor Cells and the Origins of Endometriotic Implants

Identification of endometrial stem/progenitor cells and the possibility of bone marrow-derived stem cells contribute to the endometrium may illuminate the long-speculated origin of endometriotic implants. Furthermore, the phenotype of these stem/progenitor cells could account for the observations that drive all of the other hypotheses. Numerous experiments have demonstrated that endometrium-derived cells are capable of establishing endometriotic implants.^{14,105–107} However, several lines of experimental evidence suggest that endometrial stem/progenitor cells function in the development of endometriosis.

Leyendecker *et al.*¹⁰⁸ compared the expression pattern of the estrogen receptor, two isoforms of the progesterone receptor, and P450 aromatase in normal endometrium and ectopic endometriotic implants. With respect to these markers, the expression pattern of the

endometriotic implants mimicked the cyclic expression pattern of the basalis layer of the eutopic endometrium and was out of phase with the functionalis layer. In this study, significantly more basalis layer was shed in the menstrual flow of women with endometriosis compared with that of normal controls. In combination with the observations that the endometrium basalis contains endometrial/stem progenitor cells and that women with endometriosis have larger volumes of retrograde menstrual flow,¹⁵ these data suggest that endometriotic implants result from the retrograde menstruation of endometrial stem/progenitor cells.

To explore an alternative hypothesis that extrauterine stem/progenitor cells function in the pathogenesis of endometriosis, Du and Taylor¹⁰⁴ generated an experimental model to test whether extrauterine-derived cells could track to and populate endometriotic implants. Endometriosis was generated experimentally by ectopic wild-type endometrial implantation in the peritoneal cavity of hysterectomized LacZ transgenic mice. LacZ-expressing stem cells of extrauterine origin were incorporated into the endometriotic implants and were capable of differentiating along epithelial and stromal cell lineages at a frequency of 0.04% and 0.1%, respectively. Taken together, the data that female mice receiving bone marrow transplantation from male donors demonstrate genotypically male-derived cells incorporated into the endometrium¹⁰⁴ and that women receiving mismatched bone marrow transplantation display a similar phenotype,¹⁰³ suggest that bone marrow-derived stem cells may contribute to both normal tissue homeostasis and the pathogenesis of endometriosis.

Thus, the data suggest that endometrial stem/progenitor cells function in the development of endometriosis. Additionally, bone marrow-derived stem cells can target the uterus and differentiate into a functional endometrium and, experimentally, extrauterine stem cells can target endometriotic implants. Furthermore, the contribution of stem/progenitor cells to the pathogenesis of endometriosis could account for the observations that drive all theories of the cellular origin of ectopic endometriotic implants. Endometrium-derived stem/progenitor cells residing in the basalis layer can be shed through the fallopian tube to establish endometriotic implants, accounting for the findings that support the retrograde menstruation theory. Stem/progenitor cells derived from either the bone marrow, the endometrium, or an alternate source may be responsible for the observations that support the theory of coelomic metaplasia. Potential stem/progenitor cells that persist in the remnants of the mullerian system can form endometriotic implants and account for the embryonic rest theory. Finally, extrauterine stem/progenitor cells, derived from the bone marrow or an alternative source, are likely to travel to distant ectopic sites via the lymphovascular spaces.

Ultimately, to conclusively demonstrate the link between stem/progenitor cells and endometriosis, donor endometrial stem cells should repopulate a recipient endometrium, and these cells should be shown either to cause endometriosis and to track to endometriotic implants. Currently, multiple technical challenges limit the execution of these experiments. Furthermore, proving the null hypothesis that coelomic metaplasia, embryonic rest theory, and lymphatic and vascular metastasis does not occur may be nearly impossible.

Future Directions

Identification of putative endometrial stem cells presents numerous avenues for investigation.

- i. Understanding cell fate determination within the uterus. Specifically, this includes determining which cell lineages—epithelial, stromal, or myometrial—are generated by endometrial stem cells.

- ii. Identification of the endometrial stem cell niche along with a comprehensive analysis of the niche signaling pathways. These experiments can be aided by using other niche signaling pathways as guides.^{50,51} Multiple signaling pathways within the intestinal stem cell have been identified that regulate specific biological processes. At the cell surface, these pathways include wnt/frizzled, b-catenin, notch, hedgehog, the ephrins and their receptors, BMPs, and Noggin.^{50,51} Using the intestinal stem cell niche as a guide, these signaling pathways can be directly targeted in the endometrium to identify the signaling pathways responsible for linking signaling at the endometrial niche with processes of cell cycle control, cell fate specification, differentiation, and proliferation in the stem cell lineage. Additionally, efforts should be made to understand the role of the hormonal signaling components—estrogen and progesterone and their receptors—on endometrial stem cells and their niche. These experiments would be significantly enhanced by the development of *in vitro* systems to culture endometrial stem cells and an associated *in vitro* system to recapitulate the endometrial stem cell niche.
- iii. Elucidation of the role of endometrial stem cells and their niches in pathologic processes. What is the role of stem cells in endometriosis? Do endometriotic implant locations differ in their proclivity to originate from bone marrow-derived stem cells versus uterine-derived stem cells? For example, does retrograde menstruation of endometrial stem cells seed the pelvis, whereas hematologic dissemination can seed both the pelvis as well as distant sites that are not directly accessible to menstrual flow. Furthermore, does the primary defect in the endometriosis pathway lie in the stem cell, or does misregulation within the niche permit aberrant biological processes to function in the stem cell? Does ectopic stem cell differentiation function in the pathogenesis of other gynecological diseases, such as adenomyosis, chronic pelvic pain, and gynecological cancers?

The pathogenesis of endometriosis is probably multifactorial and extensive investigation has explored the role of genetics, environmental factors, and the immune system in predisposing patients to developing endometriosis. Identification of endometrial stem/progenitor cells and extrauterine stem/progenitor cells that target the uterus and endometriotic implants provides new insight into normal uterine physiology and the pathogenesis of endometriosis. Future research can use other stem cell systems as models to better characterize the molecular biology regulating endometrial stem/progenitor cells in normal physiology and multiple gynecological disorders.

Acknowledgments

The authors acknowledge Dr. Pinar H. Kodaman for her critical review of this manuscript.

References

1. Snedsky TE, Liu DT. Endometriosis: associations with menorrhagia, infertility, and oral contraceptives. *Int J Gynaecol Obstet.* 1980; 17:576.
2. Surrey ES, Schoolcraft WB. Management of endometriosis-associated infertility. *Obstet Gynecol Clinics North Am.* 2003; 30:208.
3. Houston DE. Evidence for the risk of pelvic endometriosis by age, race and socioeconomic status. *Epidemiol Rev.* 1984; 6:191.
4. Cramer, DW. Epidemiology of endometriosis in adolescents. In: Wilson, EA., editor. *Endometriosis*. Alan Liss; New York: 1987. p. 5-8.
5. Kuohung W, et al. Characteristics of patients with endometriosis in the United States and the United Kingdom. *Fertility Sterility.* 2002; 78:772.

6. Bischoff F, Simpson JL. Genetic basis of endometriosis. *Ann N Y Acad Sci.* 2004; 1034:284–299. [PubMed: 15731320]
7. Giudice LC, Kao LC. Endometriosis. *Lancet.* 2004; 364:1799.
8. Di W, Guo SW. The search for genetic variants predisposing women to endometriosis. *Curr Opin Obstet Gynecol.* 2007; 19:395–401. [PubMed: 17625425]
9. Bischoff FZ, Simpson JL. Heritability and molecular genetic studies of endometriosis. *Hum Reprod Update.* 2000; 6:44.
10. Campbell IG, Thomas EJ. Endometriosis: candidate genes. *Hum Reprod Update.* 2001; 7:15–20. [PubMed: 11212068]
11. Stefansson H, et al. Genetic factors contribute to the risk of developing endometriosis. *Hum Reprod.* 2002; 17:559.
12. Treloar S, et al. The International Endogene Study: a collection of families for genetic research in endometriosis. *Fertility Sterility.* 2002; 78:685.
13. Giudice LC. Infertility and the environment: the medical context. *Semin Reprod Med.* 2006; 24:129–133. [PubMed: 16804810]
14. Seli E, et al. Pathogenesis of endometriosis. *Obstet Gynecol Clinics North Am.* 2003; 30:61.
15. Halme J, et al. Retrograde menstruation in healthy women and in patients with endometriosis. *Obstet Gynecol.* 1984; 64:154.
16. Giudice LC. Genomics' role in understanding the pathogenesis of endometriosis. *Semin Reprod Med.* 2003; 21:119–124. [PubMed: 12917781]
17. Giudice LC. Application of functional genomics to primate endometrium: insights into biological processes. *Reprod Biol Endocrinol.* 2006; 4(Suppl 1):S4. [PubMed: 17118168]
18. Gogusev J, et al. Detection of DNA copy number changes in human endometriosis by comparative genomic hybridization. *Hum Genet.* 1999; 105:451.
19. Taylor RN, et al. Emerging role of genomics in endometriosis research. *Fertility Sterility.* 2002; 78:694–698.
20. Wu Y, et al. Genomic alterations in ectopic and eutopic endometria of women with endometriosis. *Gynecol Obstet Invest.* 2006; 62:148–159. [PubMed: 16679773]
21. Sampson JA. Ovarian hematomas of endometrial type (perforating hemorrhagic cyst of the ovary) and implantation adenomas of endometrial type. *Boston Med Surg J.* 1922; 186:446.
22. Sampson JA. Peritoneal endometriosis due to the menstrual dissemination of endometrial tissue into the peritoneal cavity. *Am J Obstet Gynecol.* 1927; 14:469.
23. Watkins RE. Uterine retrodisplacements, retrograde menstruation and endometriosis. *West J Surg Obstet Gynecol.* 1938; 46:494.
24. Liu DTY, Hitchcock A. Endometriosis: its association with retrograde menstruation, dysmenorrhoea and tubal pathology. *Br J Obstet Gynaecol.* 1986; 93:862.
25. Salamanca A, Beltran E. Subendometrial contractility in menstrual phase visualized by transvaginal sonography in patients with endometriosis. *Fertility Sterility.* 1995; 64:195.
26. Nunley WC Jr, Kitchin JD III. Congenital atresia of the uterine cervix with pelvic endometriosis. *Arch Surg.* 1980; 115:758.
27. Olive DL, Henderson DY. Endometriosis and mullerian anomalies. *Obstet Gynecol.* 1987; 69:415.
28. Sanfilippo JS, et al. Endometriosis in association with uterine anomaly. *Am J Obstet Gynecol.* 1986; 154:43.
29. D'Hooghe TM. Clinical relevance of the baboon as a model for the study of endometriosis. *Fertility Sterility.* 1997; 68:625.
30. Gruenwald P. Origin of endometriosis from the mesenchyme of the coelomic walls. *Am J Obstet Gynecol.* 1942; 44:474.
31. Gardner GH, et al. Endometriosis. *Obstet Gynecol.* 1953; 1:637.
32. Schrodt GR, et al. Endometriosis of the male urinary system: a case report. *J Urol.* 1980; 124:723.
33. Olikar AJ, Harris AE. Endometriosis of the bladder in a male patient. *J Urol.* 1971; 106:859.
34. Clark AH. Endometriosis in a young girl. *JAMA.* 1948; 136:690.
35. Schiffrin BS, et al. Teenage endometriosis. *Am J Obstet Gynecol.* 1973; 116:980.

36. Hobbs JE, Bortnick AR. Endometriosis of the lung: an experimental and clinical study. *Am J Obstet Gynecol.* 1940; 40:843.
37. Cassina PC, et al. Catamenial hemoptysis: diagnosis with MRI. *Chest.* 1997; 111:1450. [PubMed: 9149612]
38. Van Schil PE, et al. Catamenial pneumothorax caused by thoracic endometriosis. *Ann Thorac Surg.* 1996; 62:586. [PubMed: 8694637]
39. Von Recklinghausen F. Adenomyomas and cystadenomas of the wall of the uterus and tube: their origin as remnants of the wolffian body. *Wien Klin Wochenschr.* 1896; 8:530.
40. Russell WW. Aberrant portions of the Mullerian duct found in an ovary. *Bull Johns Hopkins Hospital.* 1899; 10:10.
41. Sampson JA. Heterotopic or misplaced endometrial tissue. *Am J Obstet Gynecol.* 1925; 10:664.
42. Sampson JA. Metastatic or embolic endometriosis, due to the menstrual dissemination of endometrial tissue into the venous circulation. *Am J Pathol.* 1927; 3:109.
43. Reid GD, et al. Hepatic endometriosis: a case report and review of the literature. *Authorst N Z J Obstet Gynaecol.* 2003; 43:87–89.
44. Gargett CE. Review article: stem cells in human reproduction. *Reprod Sci.* 2007; 14:405–424. [PubMed: 17913960]
45. Bongso A, Richards M. History and perspective of stem cell research. *Best Pract Res Clin Obstet Gynaecol.* 2004; 18:842.
46. Shostak S. (Re)defining stem cells. *BioEssays.* 2006; 28:308.
47. Eckfeldt CE, et al. The molecular repertoire of the ‘almighty’ stem cell. *Nat Rev Mol Cell Biol.* 2005; 6:737.
48. Zhang J, et al. Identification of the haematopoietic stem cell niche and control of the niche size. *Nature.* 2003; 425:841. [PubMed: 14574413]
49. Trounson A. The production and directed differentiation of human embryonic stem cells. *Endocrine Rev.* 2006; 27:219.
50. Li L, Xie T. Stem cell niche: structure and function. *Annu Rev Cell Dev Biol.* 2005; 21:631.
51. Moore KA, Lemischka IR. Stem cells and their niches. *Science.* 2006; 311:1885.
52. Ohlstein B, et al. The stem cell niche: theme and variations. *Curr Opin Cell Biol.* 2004; 16:699.
53. Mills JC, Gordon JI. The intestinal stem cell niche: there grows the neighborhood. *Proc Natl Acad Sci USA.* 2001; 98:12336.
54. Gargett CE. Uterine stem cells: what is the evidence? *Human Reproduction Update.* 2007; 13:87–101. [PubMed: 16960017]
55. Spencer TE, et al. Comparative developmental biology of the mammalian uterus. *Curr Topics Dev Biol.* 2005; 68:122.
56. Padykula HA. Regeneration in the primate uterus: the role of stem cells. *Ann N Y Acad Sci.* 1991; 622:56.
57. Okulicz WC, et al. Zonal changes in proliferation in the rhesus endometrium during the late secretory phase and menses. *Proc Soc Exp Biol Med.* 1997; 214:138.
58. Pellegrini G, et al. Location and clonal analysis of stem cells and their differentiated progeny in the human ocular surface. *J Cell Biol.* 1999; 145:782.
59. Gronthos S, et al. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. *Proc Natl Acad Sci USA.* 2000; 97:13630.
60. Stingl J, et al. Purification and unique properties of mammary epithelial stem cells. *Nature.* 2006; 439:997.
61. Chan RWS, et al. Clonogenicity of human endometrial epithelial and stromal cells. *Biol Reprod.* 2004; 70:1738–1750. [PubMed: 14766732]
62. Van Os R, et al. Stem cell assays: something old, something new, something borrowed. *Stem Cells.* 2004; 22:1190.
63. Li A, et al. Identification and isolation of candidate human keratinocyte stem cells based on cell surface phenotype. *Proc Natl Acad Sci USA.* 1998; 95:3907.

64. Pittenger MF, et al. Multilineage potential of adult human mesenchymal stem cells. *Science*. 1999; 284:147. [PubMed: 10102815]
65. Zuk PA, et al. Human adipose tissue is a source of multipotent stem cells. *Mol Biol Cell*. 2002; 13:4295.
66. Joseph NM, Morrison SJ. Toward an understanding of the physiological function of mammalian stem cells. *Dev Cell*. 2005; 9:183.
67. Albert MR, et al. Murine epidermal label-retaining cells isolated by flow cytometry do not express the stem cell markers CD34, Sca-1, or Flk-1. *J Invest Dermatol*. 2001; 117:948.
68. Braun KM, Watt FM. Epidermal label-retaining cells: background and recent applications. *J Invest Dermatol Symp Proc*. 2004; 9:201.
69. Morris RJ, Potten CS. Slowly cycling (label-retaining) epidermal cells behave like clonogenic stem cells in vitro. *Cell Proliferation*. 1994; 27:289.
70. Kenney NJ, et al. Identification of stem cell units in the terminal end bud and duct of the mouse mammary gland. *J Biomed Biotechnol*. 2001; 2001:143.
71. Oliver JA, et al. The renal papilla is a niche for adult kidney stem cells. *J Clin Invest*. 2004; 114:804.
72. Morris RJ, Potten CS. Highly persistent label-retaining cells in the hair follicles of mice and their fate following induction of anagen. *J Invest Dermatol*. 1999; 112:475.
73. Uchida N, et al. Direct isolation of human central nervous system stem cells. *Proc Natl Acad Sci USA*. 2000; 97:14725.
74. Richardson GD, et al. CD133, a novel marker for human prostatic epithelial stem cells. *J Cell Sci*. 2004; 117:3545.
75. Xin L, et al. The Sca-1 cell surface marker enriches for a prostate-regenerating cell subpopulation that can initiate prostate tumorigenesis. *Proc Natl Acad Sci USA*. 2005; 102:6947.
76. Shackleton M, et al. Generation of a functional mammary gland from a single stem cell. *Nature*. 2006; 439:88.
77. Smalley MJ, Clarke RB. The mammary gland “side population”: a putative stem/progenitor cell marker? *J Mammary Gland Biol Neoplasia*. 2005; 10:47.
78. Behbod F, et al. Transcriptional profiling of mammary gland side population cells. *Stem Cells*. 2006; 24:1074.
79. Haraguchi N, et al. Characterization of a side population of cancer cells from human gastrointestinal system. *Stem Cells*. 2006; 24:513.
80. Patrawala L, et al. Side population is enriched in tumorigenic, stem-like cancer cells, whereas ABCG2 + and ABCG2 – cancer cells are similarly tumorigenic. *Cancer Res*. 2005; 65:6219.
81. Wulf GG, et al. A leukemic stem cell with intrinsic drug efflux capacity in acute myeloid leukemia. *Blood*. 2001; 98:1173.
82. Preffer FI, et al. Lineage-negative side-population (SP) cells with restricted hematopoietic capacity circulate in normal human adult blood: immunophenotypic and functional characterization. *Stem Cells*. 2002; 20:427.
83. Szotek PP, et al. Ovarian cancer side population de-fines cells with stem cell-like characteristics and Mullerian inhibiting substance responsiveness. *Proc Natl Acad Sci USA*. 2006; 103:11159.
84. Jonker JW, et al. Contribution of the ABC transporters Bcrp1 and Mdr1a/1b to the side population phenotype in mammary gland and bone marrow of mice. *Stem Cells*. 2005; 23:1065.
85. Gargett CE, et al. Characterising the stem cell activity of human endometrial cells. *Hum Reprod*. 2005; 20(Suppl 1):i95.
86. Schwab KE, et al. Putative stem cell activity of human endometrial epithelial and stromal cells during the menstrual cycle. *Fertility Sterility*. 2005; 84:1124–1130.
87. Sivridis E, Giatromanolaki A. New insights into the normal menstrual cycle-regulatory molecules. *Histol Histopathol*. 2004; 19:516.
88. Kato K, et al. Characterization of side-population cells in human normal endometrium. *Hum Reprod*. 2007; 22:1214–1223. [PubMed: 17283036]
89. Park KR, et al. CD9 is expressed on human endometrial epithelial cells in association with integrins $\alpha 6$, $\alpha 3$ and $\alpha 1$. *Mol Hum Reprod*. 2000; 6:257.

90. Seli E, et al. Expression of aminopeptidase N in human endometrium and regulation of its activity by estrogen. *Fertility Sterility*. 2001; 75:1176.
91. Wolff EF, et al. Demonstration of multipotent stem cells in the adult human endometrium by in vitro chondrogenesis. *Reprod Sci*. 2007; 14:524–533. [PubMed: 17959881]
92. Chan RWS, Gargett CE. Identification of label-retaining cells in mouse endometrium. *Stem Cells*. 2006; 24:1529–1538. [PubMed: 16456137]
93. Szotek PP, et al. Adult mouse myometrial label-retaining cells divide in response to gonadotropin stimulation. *Stem Cells*. 2007; 25:1317–1325. [PubMed: 17289934]
94. Cervello I, et al. Identification, characterization and co-localization of label retaining cell population in mice endometrium with typical undifferentiated markers. *Hum Reprod Adv Access*. 2006; 1:54.
95. Doyonnas R, et al. Hematopoietic contribution to skeletal muscle regeneration by myelomonocytic precursors. *Proc Natl Acad Sci USA*. 2004; 101:13512.
96. Blau HM, et al. The evolving concept of a stem cell: entity or function? *Cell*. 2001; 105:841.
97. Alison MR, et al. Hepatocytes from non-hepatic adult stem cells. *Nature*. 2000; 406:257. [PubMed: 10917519]
98. Theise ND, et al. Liver from bone marrow in humans. *Hepatology*. 2000; 32:16.
99. Mezey E, et al. Transplanted bone marrow generates new neurons in human brains. *Proc Natl Acad Sci USA*. 2003; 100:1369.
100. Korbiling M, et al. Hepatocytes and epithelial cells of donor origin in recipients of peripheral-blood stem cells. *N Engl J Med*. 2002; 346:746.
101. Quaini F, et al. Chimerism of the transplanted heart. *N Engl J Med*. 2002; 346:15.
102. Lagasse E, et al. Purified hematopoietic stem cells can differentiate into hepatocytes in vivo. *Nat Med*. 2000; 6:1234.
103. Taylor HS. Endometrial cells derived from donor stem cells in bone marrow transplant recipients. *J Am Med Assoc*. 2004; 292:85.
104. Du H, Taylor HS. Contribution of bone marrow-derived stem cells to endometrium and endometriosis. *Stem Cells*. 2007; 25:2082–2086. [PubMed: 17464086]
105. TeLinde RW, Scott RB. Experimental endometriosis. *Am J Obstet Gynecol*. 1950; 60:1173.
106. D’Hooghe TM, et al. Intrapelvic injection of menstrual endometrium causes endometriosis in baboons (*Papio cynocephalus* and *Papio anubis*). *Am J Obstet Gynecol*. 1995; 173:134. [PubMed: 7631670]
107. Ridley JH, Edwards IK. Experimental endometriosis in the human. *Am J Obstet Gynecol*. 1958; 76:790.
108. Leyendecker G, et al. Endometriosis results from the dislocation of basal endometrium. *Hum Reprod*. 2002; 17:2736.