Review

Stem cells: Are they the answer to the puzzling etiology of endometriosis?

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Summary. Endometriosis is a chronic benign disease characterized by the presence of abnormally located tissue resembling the endometrium with glands and stroma. This disease has a high degree of morbidity due to chronic pelvic pain and infertility. The disease is likely to be polygenic and multifactorial, but the exact pathogenic mechanisms are still not entirely clear. Recently, adult stem cells have been identified in several tissues, including the endometrium. These cells are probably involved in the regenerative ability of the endometrial cycle, and also in the pathogenesis of proliferative gynaecological diseases, such as endometriosis. The identification of stem cells in animal and human tissues is very complex and the putative stem cells are supposed to be found through several assays such as clonogenicity, label-retaining cells, “side-population” cells, undifferentiation markers, and cellular differentiation. Bone marrow-derived stem cells transplanted into humans and animals have also been identified in eutopic endometrium and endometriotic implants. This review evaluates the available evidence regarding stem/progenitor cells in the human endometrium and explores the possible involvement of these cells in the etiology of endometriosis.

Key words: Endometrium, Stem cells, Endometriosis, Etiology

Introduction

Endometriosis, one of the most common gynaecologic diseases, is a benign chronic disorder defined by abnormal growths of endometrial tissue in locations other than the uterine lining. It affects 10% to 15% of all reproductive-aged women (Giudice and Kao, 2004; Bulun, 2009). This ectopic tissue is most commonly observed in pelvic structures, such as ovaries, pelvic peritoneum, fallopian tubes, bladder, colon, rectovaginal septum, intestines, and sacrouterine ligaments (Carneiro et al., 2010). Although the classical symptoms of endometriosis include chronic pelvic pain and infertility, the clinical presentation is variable, with some women experiencing severe symptoms while others remain asymptomatic (Carneiro et al., 2010).

So far, several theories have been proposed to explain the pathogenesis of endometriosis. Briefly, one theory maintains that endometrial cells invade blood and lymphatic vessels and reach the peritoneum and/or other pelvic organs (Sampson, 1925), initiating an endometriotic lesion (Vigano et al., 2009; Candiani et al., 2010). To verify this hypothesis, Noel et al. (2008) have evaluated the expression of a sensitive marker, able to identify endothelial lymphatic vessels which have been extensively used to identify lymphovascular invasion in primary carcinomas (Noel et al., 2008). They have evaluated 26 samples of patients with intestinal endometriosis obtained after rectumsigmoid resection. Of these, 42.30% have presented positive staining for endothelium lymphatic vessels, confirming the involvement of lymphatic nodes in endometriotic areas (Noel et al., 2008). Although a possible explanation for the etiology of endometriosis, it cannot be the primary mechanism because the occurrence of endometriosis in distant sites from the pelvis is very rare (Olier and
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Harris, 1971; Schrodlt et al., 1980).

On the other hand, many studies have corroborated retrograde menstruation as an important mechanism for the etiology of endometriosis. Indeed, retrograde menstrual flow and abnormal myometrial contraction is found at a higher rate in women with endometriosis. In addition, women with Müllerian defects and cervical/vaginal obstruction are at an increased risk of developing endometriosis at an earlier age (Nunley and Kitchin, 1980; Olive and Henderson, 1987). Because only a portion of women with retrograde menstruation develop endometriosis, it is likely that other factors such as immunologic and peritoneal fluid abnormalities may be involved (Christodoulako et al., 2007; Bulun, 2009).

Other hypotheses support the idea that endometriosis develops from scattered embryonic rests or through spontaneous metaplasia of mesothelial cells of the pleura and peritoneum (Vigano et al., 2009; Candiani et al., 2010). The coelomic metaplasia theory is supported by the potential of coelomic epithelium to differentiate into several different histological cell types, and this theory is further supported by laboratory observation of this transformation (Matsuura et al., 1999). Another hypothesis that would reinforce the metaplastic theory was demonstrated by Anaf et al. (2000). They have found positive staining for smooth muscle cells in peritoneal endometriotic lesions, but negative staining for control peritoneum and eutopic endometrium samples of women with or without endometriosis which suggests a metaplastic phenomenon. The existence of smooth muscle cells in endometriotic lesions might be the result of a pluripotential ability of the secondary Müllerian system that might be capable of glandular and stromal endometrium differentiation but also smooth muscle cell differentiation. Another possibility would be stromal cells differentiating into peristomal smooth muscle cells (Anaf et al., 2000). Since mesothelial cells have the same embryologic origin as the müllerian ducts, the embryonic precursors of the endometrium, it is therefore plausible that spontaneous metaplasia of the mesothelium could be related to endometriosis. This mechanism is attractive because it explains the presence of endometriosis in most sites, and is probably involved in the genesis of endometriotic cysts originating from mesothelial invagination. The embryonic rests hypothesis proposes that müllerian cells inside the peritoneal cavity could be induced to form endometrial tissue when subjected to certain stimuli. The müllerianization theory states that cells with the potential to become endometrial are laid down in tracts during embryonic development and organogenesis. These tracts follow the female reproductive (Mullerian) tract as it migrates caudally at 8-10 weeks of embryonic life. Primitive endometrial cells become dislocated from the migrating uterus and act like seeds or stem cells. This theory is supported by foetal autopsy (Signorile et al., 2009, 2010). This mechanism could also explain the presence of endometriosis along the migration pathway of the embryonic müllerian system.

All of these mechanisms proposed to explain the etiology of endometriosis are controversial, and the disease is almost certainly multifactorial. Moreover, evidence of a familial component has been recognized, suggesting that the disease is inherited as a complex genetic trait, which is probably multigenic, in which the phenotype results from interactions between the genotype and environmental factors (Vigano et al., 2007). Finally, other factors that appear to play important roles in determining whether a woman will develop this disease include immunologic and reproductive factors, reproductive tract abnormalities and endocrine disrupters (Fraser, 2008). In any case, the cellular mechanisms required to initiate an endometriotic lesion include attachment of endometrial cells to the pelvic peritoneum, invasion into the mesothelium, and survival and proliferation of ectopic endometrial cells (Fraser, 2008). Recently, the identification of putative endometrial stem/progenitor cells (SPCs) has emerged as a possible origin of endometriotic lesions and a new mechanism for endometriosis etiology (Fraser, 2008; Sasson and Taylor, 2008).

Stem cells

Stem cell research has been raising considerable scientific and clinical interest because of the potential impact on prevention, diagnosis, and treatment of chronic and debilitating diseases (Mimeault and Batra, 2008). Stem cells are defined by their functional abilities-in particular, to self-renew or to produce daughter cells identical to the mother cell. In addition, these cells can differentiate into several kinds of specialized cells. Stem cells are present during the stages of embryonic development, but they can also be identified in certain adult tissues (Gargett, 2004). While stem cells derived from the zygote are totipotent, and therefore able to generate any kind of embryonic germ layer, as well as extra-embryonic tissue, adult stem cells are multipotent-that is, capable of producing only cells of the same germ lineage-or unipotent, which means able to form only one cellular type of the same lineage (Robey, 2000; Gargett, 2004).

Stem cells are found in anatomic structures called niches, which are microenvironments containing cells that send signals to maintain tissue homeostasis: self-renewal, proliferation, differentiation, and cellular apoptosis. When stem cells differentiate into progenitor cells, they acquire specific markers of more differentiated stages and lose the specific markers of undifferentiated phases (Li and Xie, 2005). Recently, these cells have been identified in several tissues and organs, including bone marrow, breast, prostate, brain, liver, and more recently, in the endometrium (Gargett, 2004; Chan et al., 2004).

Because stem cells are a “new cellular type” and difficult to characterize, several assays have been used and tested to identify them. In vitro assays include demonstration of the cell’s clonogenicity, differentiation
and proliferation potential, as well as phenotypic features. In vivo assays include tissue reconstitution as the ideal test, and the demonstration of label-retaining cells (LRCs). Therefore, in order to accurately characterize a population of stem cells, it is necessary to apply several assays with consistent methodologies, demonstrating the cell’s self-renewal capacity and its ability to differentiate into mature cells (van Os et al., 2004; Cervello et al., 2007).

Clonogenicity is characterized by the ability of one cell to form clones when this cell is in a culture medium with low cellular density. The assay is classically performed to characterize stem cell populations in adult tissue by identifying undifferentiation markers. The proliferative potential of putative stem cells, which is determined by their capacity of self-renewal, may also be demonstrated in an in vitro assay. To assess this potential, the cell is cultured with specific growth factors and its clonal expansion observed. As the cell differentiates, it gradually loses its proliferative potential when compared to the mother cell (van Os et al., 2004).

The ideal test for stem cell characterization in vivo is the tissue reconstitution assay, a series of experiments in which the tissue of interest is reconstituted in a host animal after the transplantation of donor stem cells. In contrast, the LRCs technique identifies a stem cell population in quiescent state with 5-bromo-2’-deoxyuridine (BrdU) labelling. After that, the cells are analyzed for the presence of undifferentiation markers (Cervello et al., 2007).

The identification of specific markers of adult stem cells has been the goal of several studies because they are fundamental for the characterization of undifferentiated cells. So far, no specific marker has been identified. Although certain markers have been expressed by stem cells, none of them is considered specific for this cellular group. However, stem cells are phenotypically characterized by the expression of typical markers of progenitor lineage and by the absence of markers of more differentiated cell types (Cho et al., 2004; Forte et al., 2009).

Side-population (SP) cells are a rare subset of cells found in various tissues that are highly enriched for stem cell activity. The identification of SP cells is also used to isolate and characterize somatic stem cells of multiple tissues. During cellular cloning, these cells efflux antimitotic lipophilic dye, such as Hoechst dye, presenting the SP aspect at flow cytometry (Goodell et al., 1996; Hirschmann-Jax et al., 2004).

Endometrial Stem/Progenitor Cells (SPCs)

Endometrial tissue is known for its self-renewing capacity, allowing tissue regeneration during the estrous cycle in rodents and the menstrual cycle in humans. Recently, it has been postulated that the process of cellular regeneration may depend on stem cells located in specific niches of the endometrium. According to this model, these cells migrate to other places and generate groups of progenitor cells with the potential to differentiate into epithelial, stromal, and vascular cells when submitted to certain stimuli, such as high levels of estradiol. Several groups have investigated the presence of stem cells in human endometrium and in experimental models and their possible correlation with endometrial regeneration, menstrual cyclicity (Gargett, 2004; Du and Taylor, 2007; Gotte et al., 2008, 2009), besides future possible applications in gynaecology and regenerative medicine (Park et al., 2011) through use of samples obtained after little invasive techniques like transcervical biopsy previous to infertility treatments (Schuring et al., 2011).

Two groups presented in 2004 the initial evidence for the presence of stem cells in the endometrium. One group showed that the human endometrium contains a small group of cells with clonogenic capacity, comprised of a small percentage of epithelial cells (0.22%) and stromal cells (1.25%). The results demonstrated that these cell types have similar clonogenicity potential during menacme and throughout a woman’s life (Chan et al., 2004). Because inactive endometrium contains only basal cells, these data suggest that putative SPCs reside in this layer and persist even after menopause (Chan and Gargett, 2006). The other group suggested that bone marrow-derived cells (BMDCs) could be involved in the process of endometrium regeneration in transplanted patients (Taylor, 2004). The BMDCs have been shown to take on functions outside the hematopoietic system, and been shown to circulate and to be able to differentiate into multiple cell types (Taylor, 2004). These findings were further corroborated by studies using a murine model of bone marrow transplantation, demonstrating that endometrial cells can originate from male donor-derived bone marrow cells and suggest that nonuterine stem cells contribute to the regeneration of endometrial tissue (Du and Taylor, 2007).

The identification of label-retaining cells in the stroma and epithelium of mouse endometrium provided new evidence for the hypothesis that stem cells have an important role in endometrial renewal (Schwab et al., 2005). Moreover, the undifferentiation markers c-kit and Oct-4 were detected in mouse endometrial label-retaining cells (Cervello et al., 2007) and in endometriotic cells (Matthai et al., 2006; Pacchiarotti et al., 2011).

Furthermore, a study identified and characterized SP cells in the human endometrium, demonstrating that these cells are similar to SPCs in respect to self-renewal capacity and cellular differentiation (Kato et al., 2007; Wolff et al., 2007). Other studies have found that endometrial SPCs, when cultured in medium specifically formulated to promote cellular differentiation, are multipotent, and able to differentiate into endometrial glandular and stromal cells, adipocytes, leiomiocytes, chondrocytes, osteoblasts, cardiomyocytes, and dopamine-producing neurons (Kato et al., 2007; Wolff et al., 2011). The cardiomyocytes obtained from human uterine endometrium-derived mesenchymal cells and
menstrual blood-derived mesenchymal cells, showed the phenotype, efficiency, and physiological properties expected for the cellular group (Ikegami et al., 2010). The use of endometrial SPCs to differentiate into dopamine-producing neurons represents a future possibility for the treatment of Parkinson’s disease (Wolff et al., 2011).

Several markers are used in order to characterize undifferentiation stages (Table 1). So far, only the following markers have been identified in endometrial cells: Oct-4, c-Kit (Chan et al., 2004; Cho et al., 2004), SALL4 (Forte et al., 2009), telomerase, Musashi-1, and Notch-1 (Cho et al., 2004). In 2008, Gotte et al. identified these three last markers with a higher level of expression not only in endometrial cells, but also in endometrial carcinoma and human endometriosis (Gotte et al., 2008). The expression of pluripotential markers like SOX-2, Oct-4, KLF-4 and NANOG have also been recently demonstrated by Gotte et al. (Gotte et al., 2010), and the positive SOX-2 cells were significantly more present in the proliferative endometrium than in the secretory endometrium and less frequent in the secretory endometrium than in the endometriotic tissue. Besides that, endometriotic stromal cells clones obtained from endometriotic biopsies that have been cultured in vitro for a long time have presented stem cells characteristics and expressed CD146, CD105, CD90, CD73, MS11, NOTCH-1 and SOX-2 (Schuring et al., 2011). Many studies have reported these undifferentiation markers in human and animal endometrium (Goodell et al., 1996; Chan et al., 2004; Cho et al., 2004; Hirschmann-Jax et al., 2004; Cerello et al., 2007; Meng et al., 2007; Forte et al., 2009), although some failed to identify them when different techniques were used (Fraser, 2008, van Os et al., 2004).

### SPCs and the origin of endometriotic implants

Most hypotheses for the etiology of endometriosis are based on the principle that endometrium-derived cells are responsible for the formation of endometriotic implants. Nevertheless, new evidence has shown that endometrial SPCs may be a new mechanism for the pathogenesis of endometriosis (Gotte et al., 2008; Taylor, 2004).

Evidence suggests that fetal stem cells could remain in the adult uterus, being able to regenerate the glandular and stromal epithelium that are shed during the menstrual cycle (Padykula et al., 1989). Likewise, fetal cells could remain outside the uterus, generating endometrial ectopic implants (Taylor, 2004). However, the bone marrow could be an alternative source of endometrial SPCs. This latter hypothesis is based on the assumption that circulating BMDCs are able to differentiate into several cellular types, including endothelial cells, hepatocytes, neurons, skin cells, cardiomyocytes, and gastrointestinal epithelial cells. Additional evidence that BMDCs may be able to differentiate into endometrial cells and regenerate the endometrium follows increased bleeding recurrence rate after endometrial ablation of bone marrow-transplanted women (Taylor, 2004).

Recent studies have shown the presence of chimerism in glandular and stromal epithelium in the

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**Table 1. Undifferentiation markers in human eutopic endometrium and endometriotic lesions.**

<table>
<thead>
<tr>
<th>C-Kit</th>
<th>Oct-4</th>
<th>Notch-1</th>
<th>Musashi-1</th>
<th>Sox-2</th>
<th>Nanog</th>
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<tr>
<td>Osuga et al., 2000</td>
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<td></td>
<td>+ PCR(P.F.)</td>
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<tr>
<td>Elmore et al., 2001</td>
<td>+ IHC (P.E. and S.E.)</td>
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<tr>
<td>Cho et al., 2004</td>
<td>+ IHC (P.E., S.E. and P.)</td>
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<tr>
<td>Uzan et al., 2005</td>
<td>+ IHC</td>
<td>+ IHC</td>
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<td>Matthai et al., 2006</td>
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<td>+ PCR</td>
<td>+ IHC</td>
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<td>and IHC</td>
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<td>Cobellis et al., 2008</td>
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<td>+ IHC (P.E. and S.E.)</td>
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<tr>
<td>Gotte et al., 2008</td>
<td>+ IHC</td>
<td>+ IHC</td>
<td>+ PCR and IHC</td>
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<td>Forte et al., 2009</td>
<td>+ PCR and IHC</td>
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<td>Gotte et al., 2010</td>
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<td>Bentz et al., 2010</td>
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E.E.: Eutopic endometrium; E.L.: Endometriotic lesion; P.F.: Peritoneal fluid; P.E.: Proliferative endometrium; S.E.: Secretory endometrium; PCR: Polymerase chain reaction; IHC: Immunohistochemistry
endometrium of women who had received bone marrow transplantation (Taylor, 2004). These data suggest that BMDCs may contribute to endometrial regeneration in these patients. The percentage of chimerism ranged from 0.2 to 48% and was related to the time elapsed between the transplantation and endometrial biopsy. However, the minimum time required for endometrial regeneration by BMDCs could not be determined (Taylor, 2004).

The ability of BMDCs to regenerate normal endometrium and cause endometriosis was also demonstrated in a murine experimental model (Du and Taylor, 2007). In this study, female mice received ectopic endometrial grafts and male mouse-derived BMDCs, which could be traced by fluorescent in situ hybridization directed to the Y chromosome. Donor BMDCs were detected in both eutopic and ectopic endometrium, suggesting that BMDCs may not only contribute to maintaining endometrial homeostasis, but also to generating endometriosis (Du and Taylor, 2007).

Discussion and Conclusions

Endometriosis is a common gynaecological disorder, and although extensively studied its etiopathogenic mechanisms remain unclear. The current hypotheses fail to fully account for some of the endometriotic sites, for the occurrence of endometriosis in premenarchal and postmenopausal women, and for its rare occurrence in men (Bulun, 2009; Leyendecker et al., 2009).

The endometrium is one of the most dynamic mammal tissues; it has the capacity to regenerate glandular and stromal tissue during the oestrous/menstrual cycle. Some years ago it was suggested that the cell renewal process could be dependent on a small pool of multipotent endometrial stem cells (putative endometrial SPCs) and that under certain stimuli endometrial SPCs could differentiate into other cell types (Chan et al., 2004). It could be hypothesized that endometrial SPCs are involved in the pathogenesis of proliferative gynaecological diseases, such as endometriosis and endometrial carcinoma (Chan et al., 2004; Gargett, 2004; Fraser, 2008; Forte et al., 2009; Gargett et al., 2009).

The involvement of stem cells in the etiology of endometriosis is a new hypothesis that could shed some light on the puzzling mechanisms of this disease. Many studies have demonstrated the presence of adult stem cells in many tissues and organs, including the human and animal endometrium (Chan et al., 2004; Gargett, 2004; Fraser, 2008; Forte et al., 2009; Gargett et al., 2009). Moreover, SPCs were also detected in endometriotic implants and endometrial carcinoma (Uzan et al., 2005; Du and Taylor, 2007; Gotte et al., 2008; Fraser, 2008). The endometriotic implants derived from SPCs could not only account for an alternative etiopathogenic mechanism of endometriosis, but could also be involved in classic mechanisms such as retrograde menstruation and coelomic metaplasia (Fig. 1). It is therefore plausible to suggest that SPCs are involved in the pathogenesis of endometriosis. However, the required data are still scarce due to the technical limitations regarding stem cell research. Indeed, the
identification of SPCs in tissues is a very complex task as there is no single and specific technique fit for it. The characterization of SPCs is based on some of the following features: clonogenicity, label-retaining cells, SP aspect, markers of undifferentiation, capacity of tissue regeneration, cell differentiation (van Os et al., 2004) and/or using endometrial SPC genes signature, immunophenotyping and characteristic telomerase pattern (Cervello et al., 2010).

Recent studies identifying potential of BMDCs in regenerating normal endometrial tissue and generating ectopic tissues have corroborated the hypothesis that SPCs may be involved in the etiopathogenesis of endometriosis. The differentiation of BMDCs into endometrial cells has been demonstrated both in women receiving a bone marrow transplant and in animals submitted to experimental induction of endometriosis (Taylor, 2004; Du and Taylor, 2007).

Perhaps not a single mechanism but rather a combination of the mechanisms proposed may underlie the etiopathogenesis of endometriosis by acting synergistically, both in the origin and in the progression of this disease. Further research is necessary to settle whether or not stem cells play a role in the onset and maintenance of endometriosis and if so, by which mechanisms.

References


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