Review

Women's Health

Stem cells in endometrium and endometriosis

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Endometriosis is a common chronic gynecological disease that is classically defined by the presence of endometrial stromal and glandular tissues outside the uterine cavity. Pelvic pain and infertility are the nonspecific but the most common symptoms of the disease; however, no currently definitive treatment has been developed since its pathogenesis has not been completely understood. Currently, none of the proposed conventional theories can explain all aspects of endometriosis. Recent evidence supports the presence of endometrial stem/progenitor cells and their possible involvement in endometrial regeneration and differentiation. The stem cell theory is a new hypothesis which may clarify the underlying pathophysiologic mechanisms of endometriosis. However, this theory could not only account for an alternative pathogenic mechanism of endometriosis but could also be involved in all conventional theories. This article will review the evidence for the presence of endometrial stem/progenitor cells, their possible sources and their possible involvement in the pathogenesis of endometriosis.

Keywords: chimerism • clonogenicity • endometriosis • endometrium • FISH • label-retaining cells • niche cells • side population cells • stem cells • xenograft

Stem cells

Stem cells (SCs) are rare undifferentiated cells that have the ability to self-renew as well as to produce more differentiated daughter cells. Embryonic and adult stem cells constitute the two main categories of SCs. Embryonic SCs are derived from blastocysts, whereas adult SCs are derived from postembryonic cell lineages and they have been described in several tissues and organ systems [1].

SCs can also be divided into various groups according to their differentiation capacity. Zygote is the representative of the totipotential SCs that are fully undifferentiated and able to generate all three embryonic germ layers (endoderm, mesoderm and ectoderm) as well as the extraembryonic tissues (trophoblasts, placenta and extraembryonic membranes). Embryonic SCs are pluripotent and are able to generate only cells of three germ layers but not the extraembryonic tissues. Adult SCs are multipotent and they have the ability to produce multiple cell types within the same germ layers.

Adult SCs (somatic SCs or tissue-specific SCs) are responsible for tissue regeneration and repair after damage and trauma encountered during life time. Since SCs do not have distinguishing morphological features and do not express specific markers, it is difficult to identify these rare cells in tissues and organs. However, functional properties such as high proliferative potential, substantial self-renewal capacity and ability to differentiating to other organ-/tissue-specific cell types are currently used to define adult SCs. Although adult SCs stay relatively in an undifferentiated and quiescence state, they have the ability of producing identical daughter cells. By the asymmetric cell division, an adult SC can produce an identical daughter cell and a more differentiated daughter cell, whereas by the symmetric cell division, it can produce two daughter SCs or two transit amplifying

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(TA) progenitors. TA cells have properties between SCs and end-stage-differentiated cells. They have limited proliferative potential, inability to self-renew and they undergo several rounds of cell division [2].

SCs are found in anatomic structures called niche. A SC niche consists of SCs, surrounding support cells, extracellular matrix and adhesive molecule. Signaling elements provided by these specific physiological microenvironments regulate adult SC fate decisions. Whether they maintain adult SCs in an undifferentiated state, protect them from differentiation, proliferation and apoptosis, or in the needs of the organism, they drive SCs to differentiate, proliferate and regenerate, thereby contributing to the structural and functional maintenance of the organs and tissues [2].

As it has been mentioned above, the lack of distinguishing morphological features and specific markers make it difficult to identify SCs. Therefore, *in vitro* and *in vivo* assays are used for the identification of SCs as well as endometrial SCs. *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 gold standard test and the demonstration of label-retaining cells (LRCs) [3].

Evidence for the existence of endometrial SCs

Indirect evidence comes from the cyclic characteristics of the endometrium. The endometrium is broadly composed of two cell types; the epithelial cells (luminal and glandular) and supporting mesenchymal cells, which include stromal fibroblasts, endothelial cells and leukocytes. Functionally, the human uterine endometrium is divided into two layers: the outer functionalis and the inner basalis layer. Dense glandular tissue and a loose connective stroma comprise the functionalis layer, whereas the base of the glands, dense stroma, leukocytes and the spiral arterioles comprise the basalis layer. During each menses, almost whole of the functionalis and a small portion of the basalis layer discard with each cycle. However, following menstruation, the functionalis layer regenerates from the basalis layer under the influence of rising estrogen levels. Regeneration process is comprised of endometrial regrowth, angiogenesis and proliferation of endometrial stromal cells. Shedding and regeneration of the endometrial layer during menses and regeneration of the functional layer may be considered as an indirect evidence for the presence of endometrial stem/progenitor cells, which are found in the basalis layer.

In vitro assays

Evidence from cell cloning studies

Clonogenic activity defined as the ability of a single cell to form clones when this cell seeded in a culture medium with low cellular density. This assay is classically performed to characterized SC populations in adult tissues by identifying undifferentiation markers. First reports on the clonogenesity of endometrial cells came from Chan *et al.* studies [4]. This group showed clonogenesity of endometrial-derived cells by generating single-cell suspensions of epithelial and stromal cells from hysterectomy tissues. When plated at clonal density, 0.22% of epithelial and 1.25% of stromal cells formed individual colonies in 15 days. On the other hand, two types of colonies were generated by both epithelial and stromal cells: large and small colonies. The number of large colonies was lower than that of the smaller in both epithelial and stromal cells (0.08% of epithelial cells and 0.02% of stromal cells) and it has been postulated that they belong to SC population, which are rare among other more mature TA cells. These rare colonies showed a significantly greater self-renewal capacity compared with that of the small colonies. By contrast, authors postulated that the more common small colonies are presumably derived from TA cells that failed to self-renew and thus exhibit a diminished proliferative potential.

Following these first reports, the same group showed that cloning efficiency of endometrial epithelial and stromal cells also exists in the inactive endometrium of menopausal women and the levels were not significantly different from that of the active endometrium [5]. Since inactive endometrium does not contain functional layer, the results of this study suggest the presence of endometrial stem/progenitor cells in the basalis endometrium even in menopausal women.

Differentiation capacity of endometrial SCs

Differentiation capacity (plasticity) of endometrial stem/progenitor cells have been shown by several groups suggesting their potency to be used as a useful source in cell therapy and tissue engineering [6].

Recent studies demonstrated that endometrial stem/progenitor cells, when cultured in an appropriate differentiation medium, are multipotent and able to differentiate into endometrial glandular and stromal cells, adipocytes, chondrocytes, osteoblasts, cardiomyocytes and dopamine-producing neurons [7,8]. Interestingly, Wolff *et al.* showed that human endometrial derived SCs are highly inducible source of allogenic SCs that restore dopamin concentrations in an immunocompetent Parkinson's disease mouse model [8]. Santamaria *et al.* differentiated human endometrial stromal SCs into insulin secreting cells, and in a murine model these cells were injected into the kidney capsules of diabetic mice. Human insulin identified in serum and within 5 weeks blood glucose levels were stabilized in animals transplanted with dif-

ferentiated cells [9]. Endometrial cells from menstrual blood have been also shown to be able to differentiate into various cell lineages such as insulin producer cells, odontoblasts and neurons [10]. In a murine model of Duchenne muscular dystrophy, Cui *et al.* demonstrated that endometrial progenitor cells and menstrual blood-derived cells can transfer dystrophin into distrophied myocytes through cell fusion and transdifferentiation *in vitro* and *in vivo* [11].

Identification of side population cells

Another evidence for the existence of endometrial SCs is the identification of side population cells (SPc). These cells are a small fraction of cells found in various tissues that are highly enriched for SC activity [12,13]. SPc possess a unique ability to pump-out intracellular DNA-binding dye (Hoechst 33342) via ATP-binding cassette subfamily G member 2 (ABCG2), a plasma membrane transporter and a universal stem cell marker. SPc are separated by fluorescence-activated cell sorting and are determined as Hoechst understained or unstained cells [13]. Kato *et al.* established that 0.00–5.11% of the cells in the normal human endometrium constitute the SP fraction [12].

It has been shown that endometrial SPc did not express endometrial epithelial (CD9) or stromal (CD13) cell differentiation markers in short-term cultures. However, in subsequent long-term Matrigel cultures, these markers have been shown to be expressed by SPc indicating a capacity to differentiate into $CD9^+$E-$cadherin^+$ gland-like organoids and $CD13^+$ stromal clusters [12].

Immunofluorescence staining of human cycling endometrium showed that $ABCG2^+$ cells are significantly higher in the early proliferative phase compared with that of the other cycle phases [14]. It has been also shown that they were distributed not only in the basalis but also in the functional layer of the endometrium and they were localized mainly in the perivascular region of these layers. Moreover, these cells also showed positive staining for PECAM1, which is an endothelial cell marker. These findings strongly suggest that SPc may have properties of endothelial progenitor-like cells and also play a role in angiogenesis [14].

In vivo assays

Tissue reconstitution assays

The ability of endometrial stem/progenitor cells to regenerate human endometrium has been demonstrated in xenograft models. In a tissue reconstitution assay conducted by Masuda *et al.*, singly dispersed human endometrial cells were xenotransplanted under the kidney capsule of severely immunocompromised mice [15]. In addition to the endometrium-like structures, hormone-dependent changes including proliferation, differentiation, tissue breakdown and bleeding were also observed in the reconstructed endometrium. Moreover, reconstructed endometrial tissue was positively stained with epithelial, stromal and also endothelial cell markers. The results of this study demonstrate that singly dispersed endometrial cells have the ability of tissue reconstitution, angiogenesis and human–mouse chimeric vessel formation. Furthermore, these data may provide new insight for both the mechanisms underlying physiological endometrial regeneration during menstrual cycle and the pathogenesis of endometriosis.

In another tissue reconstitution assay conducted by the same group, when freshly isolated human endometrial SPc were xenotransplanted under the kidney capsule of NOG mice, it has been observed that these cells contribute to the regeneration of the entire endometrium [14].

These studies not only demonstrate the presence of endometrial stem/progenitor cells but they also provide an experimental animal model for endometriosis, which is valuable in endometriosis research.

LRCs (evidence from mouse)

Although the structure and physiology of mouse endometrium is not exactly similar to that of human endometrium, this animal is generally used as an experimental model for endometriosis research. The mouse endometrium lacks an endometrial basalis layer, and the endometrium does not shed during menstruation but rather it is reabsorbed after the cycle. However, murine estrous cycle has characteristics similar to those of human menstrual cycle.

Epithelial and stromal LRCs have been identified as candidate adult SCs in mouse endometrium [16]. LRC technique relies on the basis of quiescent state of SCs. Since these cells stay in a relatively quiescent state, they have the ability of retaining 5-bromo-2'-deoxyuridine (BrdU), which is a DNA synthesis label, for a longer period than the active dividing cells. According to this technique, it has been shown that 3% of mouse endometrial epithelial cells after 56-day-chase period, and 6% of the endometrial stromal cells after 84-day chase period were LRCs [16]. On the other hand, Cervello *et al.* reported that 9% of stromal cells were LRCs after 49 days and this decrease to 1.7% after a 112-day chase period. However, no epithelial LRCs were identified even after a brief chase period of 21 days [17]. The different results of these studies are explained by the use of different microscopy.

In human endometrium, epithelial SCs are postulated to be located in the base of the glands in the basalis, while endometrial mesenchymal SC-like cells

are located near blood vessels in both the basalis and functionalis and express markers of perivascular cells such as PDGF-β receptor and CD146 [18]. On the other hand, LRCs which are the candidate epithelial and stromal stem/progenitor cells in mouse endometrium have been shown to be located in the luminal epithelium and nearly blood vessels at the endometrial-myometrial junction, respectively [1].

SCs markers

Currently, no specific marker has been identified for endometrial SCs; however, this is an area of active research. Several markers are used in order to characterize undifferentiation stages. So far, only the following markers have been identified in endometrial cells: Oct-4, c-Kit, SALL4, telomerase, Musashi-1 and Notch-1 [19]. These three last markers were also shown in endometrial carcinoma and endometriosis [20]. Furthermore, endometriotic stromal cell clones obtained from endometriotic biopsies that have been cultured *in vitro* for a long time have presented SC characteristics and expressed *CD146*, *CD105*, *CD90*, *CD73*, *MS11*, *NOTCH-1* and *SOX-2* [21]. Several studies have reported these undifferentiation markers in human and animal endometrium, although some failed to identify them when different techniques were used [22].

Sources of endometrial SCs

Although the existence of endometrial SCs has been demonstrated, their origin still remains unclear. Fetal SCs were suggested to remain in the adult uterus, being able to regenerate the glandular and stromal epithelium that are shed with each menstrual cycle [3]. Likewise, fetal cells could remain outside the uterus, and establish ectopic endometrial implants.

However, recent studies revealed that circulating bone marrow-derived cells (BMDCs) are able to differentiate into several cell types including endothelial cells, hepatocytes, neurons, skin cells, cardiomyocytes and gastrointestinal epithelial cells [23,24,25]. Moreover, endometrial ablative techniques have demonstrated a substantial long-term failure rate and rarely result in complete long-term absence of endometrial tissue or abnormal endometrial bleeding. Therefore, a nonendometrial source of SCs could account for these observations. Additionally, women who conceived naturally or by assisted reproductive techniques after bone marrow (BM) transplantation, demonstrated that recipients' endometrium were functional and that the outcome of pregnancy after BM transplantation is likely to be successful [26].

In 2004, Taylor provided endometrial regeneration in four women who received single antigen HLA-mismatched BM transplants [27]. Donor-derived endometrial epithelial and stromal cells were detected in endometrial samples of recipients by RT-PCR and immunohistochemistry. The extent of chimerism was ranged from 0.2 to 48% for epithelial cells and 0.3 to 52% for stromal cells and correlated with the length of time between transplantation and biopsy.

Following this study, Du and Taylor, in a murine model, investigated the contribution of nonendometrial SCs to the endometrium [28]. In this study, female mice were lethally irradiated and subject to BM transplantation from male donor mice. After transplantation, it was observed that bone marrow-derived SCs engraft female mice endometrium. Both stromal and epithelial cells were derived from bone marrow origin. Although present in a small fraction (<0.01%), these cells could differentiate into endometrial epithelial cells.

In a case report, it has been shown that bone marrow-derived endothelial progenitors from male donor contribute to the formation of new blood vessels in the endometrium [29]. At the time of Cesarean section, it was shown that an average 14% of endometrial endothelial cells of bone marrow-transplanted patient were donor derived and 1 year later that figure was 10%.

Recently, Ikoma *et al.*, by using FISH targeting X or Y chromosomes, showed that BMDCs from male donors may generate endometrial glands in three female transplant recipients [30]. In this study, all recipients had donor-derived Y chromosome-positive endometrial cells, accounting for 0.6–8.4% of glandular epithelial cells and 8.2–9.8% of stromal cells. These studies demonstrated that donor-derived cells are capable of regenerating endometrium in recipients, even those of the opposite sex.

More recently, Cervello *et al.*, by using FISH, telomapping and SP method investigation, investigated the presence and contribution of BMDCs to the endometrium and endometrial SP of women who received BM transplantation from male donors [31]. In this study, donor-derived cells corresponding to chromosome Y-positive endometrial cells accounted for 1.7–2.6% of the total cell count. However, the extent of chimerism was ranged from 0.45 to 0.86% for epithelial cells and 1 to 1.83% for stromal cells, and was not correlated with the length of time between transplantation and biopsy (from 30 to 210 months). Moreover, it has been observed that XY donor-derived cells do not contribute to the SP of recipients and were not associated with SC niches assessed by telomapping technique. Authors, therefore, suggested that XY donor-derived cells of a BM origin may be considered as a limited exogenous source of transdifferentiated endometrial cells rather than a cyclic source of BMDCs.

The role of SCs in endometriosis

Endometriosis, classically described by the presence of endometrial glandular and stromal tissues outside the uterus, is still an enigmatic disorder. Pelvic pain and infertility are the nonspecific but the most common symptoms of the disease; however, no currently definitive treatment has been developed since its pathogenesis has not been completely understood.

Many theories have been proposed to explain the development and establishment of endometriosis; however, no single theory can explain all aspects of endometriosis. It has been suggested that retrograde menstruation is essential in the pathogenesis of peritoneal endometriosis, whereas the coelomic metaplasia and the embryonic rest theories may account for the pathogenesis of ovarian endometriomas and rectovaginal endometriosis, respectively. Lymphovascular metastasis theory suggests 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. All of these conventional theories separately or in combination may account for the pathogenesis of endometriosis. However, even the well-established retrograde menstruation theory has been questioned since it has been reported that it occurs as a physiological phenomenon in most of the reproductive age women. Currently, a growing body of evidence indicates that a combination of genetic, hormonal, immunological and environmental factors also seem to be involved in the pathogenesis of this enigmatic disorder [32,33].

The SC theory is a new hypothesis which may clarify the underlying pathophysiologic mechanisms of endometriosis. Endometriosis, adenomyosis, endometrial hyperplasia and cancer are gynecological diseases characterized by abnormal endometrial proliferation. It has been postulated that endometrial tissues containing endometrial stem/progenitor cells and/or their corresponding niche cells may be involved in the pathogenesis of these proliferative disorders [34].

Although no direct evidence for the role of endometrial stem/progenitor cells in the pathogenesis of endometriosis has been reported to date, regeneration of the functional layer from the basalis may be an indirect evidence for the presence of these cells, which are found in the basalis layer. It is well known that women with endometriosis have larger volumes of retrograde menstrual flow. Furthermore, human CD146$^+$ and PDGF-Rβ^+ mesenchymal stem-like cells are located perivascularly in both the functionalis and basalis layers of endometrium [18]. All these data suggest that peritoneal endometriotic implants may result from retrograde menstrual endometrial tissues containing endometrial stem/progenitor cells. Leyendecker *et al.* supported this hypothesis and demonstrated that in women with endometriosis, significantly more basalis layer was shed in the menstrual flow suggesting an increased number of stem/progenitor cells in this layer that can result in a propensity for endometriosis [35]. Furthermore, in the same study, it has been demonstrated that in the ectopic endometrium, cyclic expression patterns of the estrogen receptor, two isoforms of progesterone receptor and P450 aromatase were similar to that of the basalis layer of the eutopic endometrium and was out of the phase with the functional layer. Recently, Valentijn *et al.* showed that the expression pattern of SSEA-1 and nuclear SOX-9 are very similar and were largely confined to the basal epithelial cells in cycling endometrium and in the basalis-like postmenopausal endometria [36]. In the same study, it has been also showed that SSEA-1 and SOX-9 were also expressed by ectopic endometrial implants, supporting the notion that shed basalis layer contribute to the formation of these ectopic endometriotic lesions [36]. However, according to the current knowledge, it is not known whether SCs found in endometrium are abnormal and have increased capacity to implant and establish themselves as ectopic tissues, or that normal stem/progenitor cells find an abnormal peritoneum as a proper implantation site.

Additionally, as an indirect evidence for the role of SCs in the pathogenesis of endometriosis, several animal models including that of tissue reconstitution assays revealed that endometrium-derived cells have the capability of establishing endometriotic implants [14,15].

Another supporting evidence for the SC theory is the demonstration of the presence of ABCG2$^+$ SP cells in both the functionalis and basalis endometrium [14]. As it has been mentioned above, these cells are small subset of cells found in various tissues that are highly enriched for SC activity. Considering the low percentages of SP cells in endometrium, probability of initiating endometriotic lesions in a supportive microenvironment is also low. This might explain the aparent discrepency between the prevalanve of endometriosis and retrograde menstruation.

Recently, Chan RW *et al.* similar to the uterine endometrial epithelial and stromal cells, identified cells with colony-forming activity, self-renewal capacity and multipotency in ovarian endometriotic cysts [37]. They demonstrated that purified epithelial and stromal cells isolated from ovarian endometriotic cysts formed large and small colony-forming units (CFU) in clonogenic assays. Overall, the clonogenic efficiency of endometriotic epithelial cells was 0.09 ± 0.02% with 0.04 ± 0.01% for large CFUs and 0.05 ± 0.01% for small CFUs. On the other hand, the mean total

clonogenic efficiency for endometriotic stromal cells was 0.13 ± 0.02% with 0.06 ± 0.01% for large CFUs and 0.07 ± 0.01% for small CFUs. The authors also showed that endometriotic stromal cells derived from large CFUs could differentiate into four mesenchymal lineages when cultured in the appropriate media, as determined by histochemical staining and RT-PCR of image specific markers. These findings also support the presence of SCs in endometriotic ovarian lesions. In some endometriosis lesions, it has been shown that epithelial cells are monoclonal, suggesting a single cell origin and possibility that the endometriosis initiating cell is an endometrial stem/progenitor cell. Moreover, some endometriotic lesions are polyclonal suggesting contamination with stromal cells, repeated seeding with cells from other sources such as BM, or from establishment of different fragments of shed endometrium containing several SCs [38]. However, careful analyses of microdissected ectopic endometrium have shown multiple monoclonal foci in endometriotic lesions [39].

Du and Taylor showed that as an extrauterine source of stem/progenitor cells, BMDCs may contribute in the pathogenesis of endometriosis [28]. In an experimental mice model, endometriosis was generated by ectopic wild-type endometrial implantation in the peritoneal cavity of hysterectomized LacZ transgenic mice and it has been shown that BMDCs of the hysterectomized mice incorporated into the ectopic endometriotic implant. The frequency of differentiation of BMDCs cells into epithelial and stromal cells was 0.004 and 0.1%, respectively.

Taken together, results of the experimental mice model studies which revealed that BMDCs from male donors may incorporate into the endometrium of the female recipients, and that women receiving mismatched or BM transplantation from male donors display a similar phenotype suggest that BMDCs may contribute both the normal tissue homeostasis and the pathogenesis of endometriosis [3,19,38].

The endometriotic implants derived from stem/progenitor cells could not only account for an alternative pathogenic mechanism of endometriosis but could also be involved in all conventional theories. Endometrium-derived stem/progenitor cells (containing SPc) residing in the basalis and functionalis layers can be shed through the fallopian tube to establish endometriotic implants, accounting for the findings that support the retrograde menstruation theory. The SC theory also supports the coelomic metaplasia theory that states that currently unidentified precursor cells in the mesothelium and/or müllerian duct remnants are capable of differentiation into endometrial cells under some specific stimulus. If the SC theory is widely interpreted, these putative precursor cells can be regarded as endometrial stem/progenitor cells. Furthermore, 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 [3,19,38].

The evidence of possible roles for SCs in the pathogenesis of endometriosis provides new potential therapeutic modalities for this enigmatic disease. It is well known that endometriosis is less frequent in cigarette smokers. On the basis of recent animal models, tobacco use has been shown to reduce SC recruitment both to eutopic and ectopic endometrium. However, pharmacological formulation of this treatment is not yet available [40].

Recent studies revealed that ectopic endometrial mesenchymal SCs exhibit higher proliferative, migratory and pro-angiogenic potentials compared to that of the eutopic mesenchymal SCs from women with and without endometriosis [41,42]. Results of these studies strongly suggest that eutopic and ectopic mesenchymal SCs are substantially different in terms of functionality. Unlike to the fully differentiated endometrial cells, endometrial mesenchymal SCs do not express estrogen receptors and conventional hormonal therapies inducing hypoestrogenic state may not be sufficent to supress endometrial mesenchymal SC activity. Therefore, these cells are postulated to have pivotal roles in the persistence and recurrence of endometriosis. Treatment with sorafenib, a tyrosine kinase inhibitor and potent VEGF signaling suppressor has been shown to inhibit proliferative, migratory and angiogenic properties of ectopic endometrial mesenchymal SCs [42]. In a surgically induced endometriosis mice model, bevacizumab, a recombinant human monoclonal anti-VEGF antibody has been shown to induce a significant reduction in cell proliferation, increased apoptosis, and markedely decreased vascular density in transplanted tissue as well as striking reduction in VEGF levels in the peritoneal fluid [43].

In another mouse model of endometriosis, Sakr *et al*. demonstrated that a selective estrogen receptor modulator bazedoxifene administred with conjugated estrogens led to a dramatic regression of endometriotic lesions as well as a reduction in bone marrow-derived mesenchymal SC recruitment to the lesions and promoted mesenchymal SC engraftment in eutopic endometrium. Authors suggested that competition between ectopic and eutopic endometrium for a limited supply of SCs and depletion of normal SC flux to the uterus might be a novel mechanism by which endometriosis interferes with endometrial function and fertility [44].

Telomerase is a specialized reverse transcriptase that can prevent telomere shortening and thus, allows cells to overcome apoptosis acquiring immortal capacity. It

has been shown that endometriosis is associated with aberrant endometrial expression of telomerase and increased telomere length [45]. Furthermore, compared to heathy women longer telomere content in lymphocytes have been shown in women with endometriosis [46]. Since SCs may contribute to the pathogenesis of endometriosis, longer telomerase could be consistent with a SC origin of endometriosis. Therefore, downregulating telomerase activity may be a good candidate as a new therapeutic target for endometriosis.

Conclusion

Results of many recent studies provided strong evidences for the existence of endometrial SCs. Currently, a number of *in vivo* and *in vitro* labarotory assays are needed to identify endometrial SCs since they do not have distinguishing morphological features and do not express specific markers. The presence of mesenchymal stem-like cells and SP cells in both the functionalis and basalis endometrium suggest that endometrial SCs are likely involved in the monthly regeneration process of cycling endometrium. Moreover, the presence of endometrial SCs may provide a new concept for the pathogenesis of endometriosis. This new SC concept could not only account for an alternative pathogenic mechanism of endometriosis, but could also be involved in all previously proposed conventional theories. Further research on distinguishing SC characteristics, their niches and specific markers will reveal the exact roles of endometrial SCs in endometrial physiology and endometriosis.

Future perspective

The SC concept in the pathogenesis of endometriosis may also provide new therapeutic modalities for this enigmatic disease. It is well known that angiogenesis is essential for the development and maintenance of endometriosis lesions. Antiangiogenic therapies targeting angiogenic properties of ectopic endometrial SCs alone, or in combination with hormonal treatment might be a promising novel therapeutic option for endometriosis.

Furthermore, differentiation capacity of endometrial SCs may be a useful source for the treatment of several chronic debilitating diseases including Parkinson's disease, diabetes and Duchenne muscular dystrophy.

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Executive summary

- Many studies have recently provided strong evidence for the existence of endometrial stem cells (SCs).
- The presence of endometrial SCs, either from the residing cells in the endometrium, or tracking from bone marrow, supports their roles in both physiological and pathological processes.
- Physiologically, they are likely involved in the regeneration of cycling endometrium, whereas pathologically they may contribute to the pathogenesis of proliferative endometrial disease such as endometriosis.
- The SC theory, which is a new hypothesis could not only account for an alternative pathogenic mechanism of endometriosis but could also be involved in all conventional theories.
- As endometrial SCs become better characterized, their role in gynecological disorders associated with abnormal endometrial proliferation can be assessed.
- Furthermore, these cells may provide an immunologically matched source of multipotent SCs for tissue engineering and regenerative medicine in the future.

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