Current Obstetrics and Gynecology Reports (2020) 9:7–14
https://doi.org/10.1007/s13669-020-00278-w

ENDOMETRIAL CANCER (G. SEL, SECTION EDITOR)

Endometrial Stem/Progenitor Cells

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Published online: 29 February 2020

Abstract

Purpose of Review To summarize endometrial stem/progenitor cell biology and endometrial cancer stem cells.

Recent Findings The subject is relatively new that only about 15 years ago, colony-forming units, as putative endometrial stem/progenitor cells, have been demonstrated in an endometrial sample. Earlier and some recent studies have justifiably focused on endometriosis as the serious gynecologic problem of women. However, on the side of endometrial stem cells research, many current studies are still working on in vitro methods, and the majority of the rest are on animal models. Reported cells as endometrial stem/progenitor cell populations have been heterogeneous such as endometrial mesenchymal stem cells, endometrial epithelial progenitor cells, and side population cells. After the recognition of putative endometrial stem cells, quite a few stem cell markers have been identified. Thereafter, cancer stem cell speculations have raised; in endometrial cancer, CD133+ and CXCR4+ cells, with potential cancer stem cell characteristics, have been shown to have higher antineoplastic resistance than that of other cell populations.

Summary Endometrial mesenchymal stem cells have plasticity characteristics and may differentiate into mature cells other than endometrium. On the other hand, bone marrow-derived stem cells can differentiate into endometrial cells. Since the endometrial stem/progenitor cells are easy to access, they can be an attractive source for regenerative medicine studies. Even though many markers have been identified for endometrial stem cells, more specific and sensitive marker combinations are yet to be defined for endometrial cancer stem cell in order to aim for more effective treatment approaches.

Keywords Endometrium · Stem cell · Progenitor cell · Endometrium cancer · Cancer stem cell

Introduction

Functional layer of endometrium unlike basal layer has cycles of proliferation/secretion and breakdown in response to the gonadal sex steroid hormones [1]. Throughout the reproductive ages, human endometrium undergoes over 400 menstruation and regeneration cycles suggesting a resemblance to hematopoietic tissue. Regeneration by self-renewal and differentiation to different mature cell types are unique property of stem cells, as in bone marrow [2]. In the last century, the high regenerative capacity of endometrium has led investigators to think about the presence of endometrial stem cells (ESC). Prianishnikov has suggested the ESC presence in the basal layer that was not undergoing desquamation during menstruation [3]. He proposed three types of endometrial cell as hormone unresponsive adult stem cells (ASC), estrogen responsive cells and both estrogen and progesterone responsive cells. Consecutively, research efforts have been focused on identifying the presumed stem cell in endometrial epithelium and stroma. Yet in this century, colony-forming units (CFUs) were demonstrated in human endometrial cells [4] and recently epithelial progenitor cell presence was hypothesized in the basal layer [5].

Endometrial Stem/Progenitor Cells

High proliferative capacity of endometrial stromal and epithelial cells can be considered as a first solid finding in ESC

This article is part of the Topical Collection on *Endometrial Cancer*

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survey [4, 6]. Approximately 0.2% of epithelial and 1.2% of stromal cells have the ability to form colonies that were purified from human endometrium [4, 6]. However, these colonies can be in different sizes and numbers and have different properties such as small colony-forming cells have limited self-renewal capacity and notable to differentiate [7], large colony-forming cells have the ability of self-renewal, epithelial types are able to differentiate into glandular epithelium, and stromal types can differentiate into mature cells that are alien to their natural environment [7].Yet, epithelial progenitor cell-specific markers were not identified on these colony-forming cells, even though studies have suggested that stage-specific embryonic antigen 1 (SSEA1 or CD15) and leucine-rich repeat containing G protein-coupled receptor 5 (LGR5) can be used as cell surface markers for endometrial epithelial stem cells, whether these cells have stem cell characteristics is not clear yet [8–10]. SSEA1(+) endometrial epithelial cells had greater telomerase activity and longer telomeres than SSEA1(−) epithelial cells in in vitro cultures studies, suggesting that human endometrial epithelial progenitor cells may be a subset of the SSEA1(+) population [10].

The large colony-forming stromal cells have typical mesenchymal stem cell (MSC) surface markers such as CD90, CD105, and CD73 [11]. While these and bone marrow (BM)-derived mesenchymal cells share similar characteristics under in vitro conditions [11, 12], large colony-forming stromal cells can be distinguished from hematopoietic cells by their surface markers [11]. These large colony-forming stromal cells also have markers similar to BM-derived perivascular endometrial MSCs, such as CD146, platelet-derived growth factor receptor-beta (PDGFR-β), and sushi domain containing 2 (SUSD2) [13–15].

A study with SUSD2(+) endometrial MSCs has observed their ability to form endometrial stroma around the renal parenchymal vessel walls of immune compromised mice [8]. Also, SUSD2(+) cell presence was reported in atrophic and estrogen-treated postmenopausal endometrial tissues [9].

During a regeneration process, BM-derived stem cells are lodged to support tissue homeostasis and to provide replacement cells for routine cell cycle [16, 17]. Interestingly, donor-derived endometrial BM cells have been identified in the endometrium of a recipient who had undergone HLA-mismatched allogenic BM transplantation [18]. The detection rates of these BM-derived endometrial epithelial and stromal cells are varied depending on the detection method and time from BM transplantation to sampling [4, 18].

Many stem cell markers are identified in epithelial, stromal, and perivascular cell populations of endometrial functional and basal layers [19]. Furthermore, some endometrial epithelial cells may show alterations such as spheroid formation, epithelial regeneration, and stromal cell differentiation [20].

In parallel to human research, animal studies in mice endometrium have also demonstrated quiescent stem-like cells named label-retaining cells (LRCs) that retain a label longer than mature cells [10, 21].

Environment of Stem/Progenitor Cells

Mechanisms behind stem cell survival and functional conservation have been a curiosity for researchers. The presumed ESCs are side population (SP) cells, endometrial MSCs and BM-derived MSCs [10].

Studies show that the niche, the environment of stem cells is very important for their survival and functional maintenance [16]. Historically, the "niche" hypothesis is proposed by Schofield to describe microenvironment of stem cells [22]. The endometrial MSCs reside in perivascular areas throughout the functionalis and basalis layers in both pre- and postmenopausal endometrial tissues [13–15]. These cells do not express estrogen receptor (ER), similar to LRCs of mice and SP and clonogenic cells in human endometrium, suggesting possible estrogen action via adjacent niche cells [5, 13, 23–25].

Investigations for regenerated endometrial epithelium lineage have demonstrated that stromal cells differentiate into endometrial surface epithelium but not basal epithelial glands [26, 27]. Functional endometrial layer fragments have been suggested to participate in endometrial regeneration process by overexpressing certain molecules that could facilitate ectopic and eutopic implantation [28].

Following parturition, human and mice endometrium regenerate alike, even though mice do not have menstrual cycles. Studies in mice showed that the transition from stromal mesenchymal cells to epithelial cells contributes to the postpartum regeneration of endometrial epithelium [29, 30]. The study findings from mice are consistent with those of humans, indicating the regeneration or differentiation of small stromal cells into or epithelial cells after menstruation [26, 27].

Epithelial progenitor cells, endometrial MSCs, and endothelial progenitor cells are thought to be human ESCs [31]. However, Evans et al. described human ESCs as endometrial MSCs, epithelial progenitor cells, and SP cells in state of endothelial progenitor cells [32].

Side Population Cells

The ASC presence and activity can be identified by phenotypic markers for SP cells [33]. These cells have been shown to be located around the blood vessels of basal and functional endometrial layers [34]. Approximately 2% of the endometrial cells consist of perivascular SP cells with migration and angiogenesis properties [35]. Epithelial and stromal SP cells show typical characteristics of MSCs in vitro and have some

level of telomerase activities between embryonic stem cells and mature cells [23].

The majority of SP cells obtained from fresh tissues stay in G0 phase of the cell cycle as in ASCs. However, they enter the G1 and G1/S/M phases when cultured, showing their clonogenic properties [33]. In a flow cytometry study, SP cells consisted of CD31+ endothelial cells (51%), CD326+ (EpCAM) epithelial cells (27%) and CD10+ or PDGFRb+ stromal cells (10–14%) [36]. SP cells can efflux the Hoechst dye, indicating the expression of ATP-binding cassette transporter (ABCG2/Brcp1) on their surfaces. Cells located in a similar location but unable to efflux Hoechst dyes are defined as main population (MP) cells [37]. Although SP cells were rich by CD146+ and PDGFR-b+ perivascular stromal cells, SUSD2+ cells are detected equally in both SP and MP cell populations [36].

In an interesting study, SP and MP cells, labeled by a viral protein (TdTom+), were treated separately with unlabeled unfractionated endometrial cell suspensions and were planted under the kidney capsule of immunocompromised mice. Following the SP cell transfer, endometrial tissue formation has been observed. When analyzed, TdTom+ and vimentin+ cells contributed more to the stroma, TdTom+ and cytokeratin+ cells concentrated more in the epithelium, and TdTom+ and CD31+ cells made more contribution to endothelial formation than MP cells [36]. In other words, the contribution of SP cells in the formation of endometrium was high, but other cells have also contributed. SP cell is the predominant cell type in the formation of endometrial vascular and perivascular areas. SP cells have been observed to generate endothelial, epithelial, and stromal cells of endometrium in vivo and in vitro [38].

Plasticity

Endometrial MSCs may differentiate into chondrocytes [35], adipocytes [39], and hematopoietic [40] cells in vitro. They also can differentiate into endodermal cells such as pancreatic insulin and glucagon secreting cells. Interestingly, they were functional enough to lower the blood sugar levels in a model of diabetic-immunocompromised xenografted mice with the detection of human insulin in their sera [41]. These stem cells have also been shown to differentiate into hepatocyte-like cells in vitro and were able to secrete urea and to metabolize ammonium [42]. Similarly, they can differentiate into ectodermal cells as well. They differentiate into dopaminergic neurons and express neuronal stem cell markers and secrete tyrosine hydroxylase that converts tyrosine into L-dopa [43].Transplantation of these cells into the substantia nigra of Parkinson model mice led to a partial improvement in dopamine levels [43].

Endometrial Stem Cells in Menstrual Blood

In menstrual blood cultures, some cells found to display BM-derived MSC behavior [44]. Menstrual blood is mostly composed of mesenchymal cells, while epithelial cells are rarely seen. It has been suggested that epithelial cells may have been overlooked among many mesenchymal cells, since they were not expected in the menstrual blood, and the epithelial stem cells were thought to be located mostly in the basal layer [45]. These groups of cells in menstrual blood express telomerase reverse transcriptase (hTERT) and have telomerase activity similar to MSCs [46–48]. Since these cells have stem cell properties with plasticity, they have been shown to differentiate into adipogenic, chondrogenic, and osteogenic cells [31, 49] in addition to endodermal cells such as hepatocytes [50]. Thus, MSCs obtained from menstrual blood may have a potential to be used in future stem cell treatments [51, 52].

Bone Marrow-Induced Stem Cells in Endometrial Regeneration

Interestingly, inpatients who had BM transplantation donor cells were detected among their endometrium cells [53]. It has been shown that BM-derived stem cells can differentiate into various cell types including endometrial cells [46, 48]. BM contains hematopoietic, mesenchymal, and endothelial stem cells, as well as myeloid cells at various maturation stages. However, since myeloid and lymphoid cells come with the donor BM, it is important to distinguish these cells for the detection of endometrial cells differentiated from donor BM stem cells. These cells have been detected in cases which have had HLA incompatible BM transplantations [53]. Donor HLA carrying cells were detected in the endometrium of BM transplant recipients by real-time polymerase chain reaction (PCR) and immunohistochemical methods. Further evaluations showed that these donor cells in the endometrium consisted of 48% of epithelial cells and 52% of stromal cells.

Another interesting example is the detection of Y chromosome carrying cells in the recipient endometrium in cases with male BM donor [54]. However, these cells are only involved in endometrial regeneration process and do not contribute to endometrial SP population [51].

Animal studies have shown that endometrial trauma can expand stromal aggregation of BM-derived stem cells, but endometrial cyclic hormonal degeneration/regeneration is not effective enough for the collection of BM-derived ESCs [52].

Postmenopausal Endometrial Stem Cells in Postmenopausal Endometrium

In postmenopausal period, atrophic endometrium proliferates just like in perimenopause in response to estradiol replacement therapy. Stromal stem cells in this proliferated endometrium show similarities to that of premenopausal endometrium [9]. By means of endometrial vascularity, SUSD2(+) cells are thought to play a role in difference between the endometrium under estrogen effect and or not. On the other hand, the presence of SSEA1(+) cells has been shown universally in postmenopausal basal endometrium, but the role of these cells in postmenopausal regeneration is unknown.

Stem Cell Clinical Trials

The use of ESCs in regenerative medicine is promising. When compared to other stem cell sources, ESCs are easier to obtain from uterine cavity and menstrual blood. In a study, stromal cells obtained from human menstrual blood were injected into infarcted mouse cardiac tissue and resulted in differentiation to cardiomyocytes and improved cardiac function [31]. Similarly, in Duchenne muscular atrophy-induced mouse model, ESCs could differentiate into skeletal muscle cells [49]. In another example, menstrual blood stem cells have improved ovarian function and increased normal follicle development in cyclophosphamide-induced ovarian failure mouse model [55].

These stem cells, in addition to their cell differentiation capacity, affect their environment by secreting certain mediators. The MSCs exert anti-inflammatory effects by secreting mediators such as IL-10, prostaglandin E_2, TGF-β, and immunomodulatory functions and by suppressing T, B, and NK cell activities [46]. Endometrial MSCs were shown to reduce neural [18] and intestinal inflammation [56] in autoimmune encephalomyelitis and ulcerative colitis animal models, respectively. In an animal study, high aldehyde dehydrogenase (ALDH) producing 3D cultures of endometrioid cancer stem-like cells from clinical specimens showed a synergy with paclitaxel in blocking cancer proliferation when ALDH activity was inhibited [57]. It was shown that ESCs differentiate into insulin-producing cells (IPCs) using an in vitro 3D culture system [58]. In a clinical trial using autologous peripheral blood, CD133+ BM-derived stem cells have decreased the adhesion scores in patients with Asherman syndrome [59]. However, BM- and human menstrual blood-derived stem cell use in clinical practice still requires more investigations.

Endometrial Cancer Stem Cells

Human cancer cell autotransplantations or immunocompromised animal xenografts have demonstrated that initiation of tumor growth is not always possible [60–66]. Therefore, not all neoplastic cells within the tumor have a neoplastic capacity except putative cancer stem cells (CSC). The first evidence for the existence of CSC came from leukemia studies indicating that less than 1% of leukemia cell population comprised of CSC [62, 67, 68]. Even though it is still controversial, CSC theory is a challenging theory in understanding tumor biology and behavior.

CSCs resemble to ASCs since both of them express telomerase, having self-renewal and high proliferative potentials [19]. High telomerase activity is observed both in CSCs and metastatic cells [19]. CSCs are blamed for distant metastasis, recurrence, and resistance to nonsurgical therapies. The isolation of CSCs from the other cancer cells and from ASCs may be possible based on certain markers as previously hypothesized [69].

The first evidence for stem cell origin of endometrial cancer (EC) was demonstrated in a uterine carcinosarcoma-derived cell line study [70]. These highly proliferative clonal cells express markers similar to their origin tissues and their in vitro tumor phenotypes also suggest the stem cell origin in the eventuation of cancer. Around a decade after this study, it could be possible to isolate EC cells with CSC potential [71]. Isolated endometrial CSC can be passaged serially, initiate clones that can self-divide, and propagate tumors in vivo. Similar to ASC, approximately 1% of EC cells are able to initiate such as clone in vitro, even though more differentiated progenitor cells also contribute to the clonal formation. From such observations, it can be inferred that only a few cells within EC tissue can initiate colonies, supporting the CSC hypothesis.

Hubbard et al. further studied the tumorigenesis in all types (type I and II) and grades of EC. They transplanted human EC cells in different dilutions into mice kidney. Although not all dilutions have initiated tumor development, all grades and types of ECs have been observed [71]. The developed tumors showed the same parental histology and had ERa, PR, vimentin, and cytokeratin markers [71]. However, more EC cells were needed to induce tumorigenesis when Type I EC cells were xenografted subcutaneously [71, 72]. This was probably due to less blood supply of subcutaneous tissue than that of kidney [73]. On the other hand, the subcutaneously xenografted EC cells can be serially passaged up to five times in immunocompromised mice. These passaged cells maintain their original tumor morphology and phenotype and demonstrate CSC traits of self-renewal and differentiation [71, 72].

The CFU cells of EC showed self-renewal property even after several rounds of subcloning in vitro in all grades and types of EC, indicating that these cells have abilities like CSC

[71]. Besides, even secondary clones have expressed certain self-renewal genes such as BMI1, CTNNB1, SOX2, and NANOG. In the same study, self-renewal ability was associated with increased tumor aggressive behavior. Type II EC tumor cells seemed to initiate tumors more efficiently than Type I cells; however, an increasing self-renewal capacity of type II tumors was not associated with more aggressive behavior [71]. Since only certain EC cells can initiate tumors, this confirms the hypothesis of CSC origin in ECs [71]. Nonetheless, certain cell surface markers or their combination is still needed to isolate endometrial CSC and develop effective treatment strategies. Recently, it was reported that CD133 + and CXCR4+ cells may have some endometrial CSC characteristics, with higher antineoplastic resistance than that of other cell populations [74••]. It was speculated that leukocyte immunoglobulin-like receptor subfamily B member 2 (LILRB2) may be a functional stem cell marker for endometrial CSCs indicating their active statuses [75•]. Normal ASC with silenced tumor suppressor genes or mutated ASC were suggested to contribute to CSC formation [76].

Side Population Cells as Endometrial Cancer Stem Cell

Friel et al. studied the presence of SP cells in EC cell lines and in four high-grade EC suspensions in investigating CSC [72]. Rare SP cells were observed in the AN3CA and Ishikawa cell lines (0.02% and 0.08%, respectively) but not in the SKUT-2, HEC1-A and HEC1-B EC cell lines, and in four high-grade EC cell suspensions. They have suggested that SP cells observed in AN3CA cell line have low proliferation stem cell properties, and their results were supported the hypothesis that EC tissues contain a subpopulation of tumor initiating cells. Also, paclitaxel resistance observed in SP cells implies the presence of drug transporters. In that study, SP cells have eventually reached their original SP/MP ratio, reflecting self-renewal and differentiation potential in SP fraction. Contrary to MP cells, SP cells from these cell lines and the four high-grade EC suspensions were able to initiate tumors when injected subcutaneously into NOD/SCID (non-obese diabetic/severe combined immunodeficiency mice). However, the presence of typical EC marker phenotypes such as ERa, PR, cytokeratin, and vimentin has not been studied in these resultant tumors. To conclude, their study adds another evidence for the CSC origin of ECs [72].

Endometrial Cancer Stem Cell Markers

Musashi-1, a protein expressed by epithelial progenitors in certain tissues, is associated with ASC self-renewal function [77, 78]. Cancer cells do not seem to survive without Musashi-1 [78]. Expression of this protein was also detected in EC samples, and it was associated with poor prognosis [77, 79, 80]. Whether the Musashi-1 expressing cells in EC are progenitor cells or CSC, it still needs to be investigated [5]. Thus, Musashi-1 expressing CSC may be considered as a possible therapeutic target [19]. Studies also showed the upregulation of SOX9 in EC and premalignant endometrial hyperplasia tissues, indicating that a possible progenitor ASC involvement in the process [81, 82].

High N-cadherin protein expression rates were reported in EC samples compared to normal controls [83]. However, E-cadherin expressions were not common in N-cadherin expressing cells, suggesting a switch between these types may be possible [83].

EC samples shows higher stromal Notch1 expression than endometrial hyperplasia and polyp samples [84], and the expressions of Numb are increased gradually parallel to the EC grade [85]. CD133 is among the first candidate markers for endometrial CSC; however, further experiments are needed to confirm this finding [86].

CSC as a Prognostic Marker

Investigations showed that there is a correlation between CSC markers and poor prognosis [63, 73, 87–89]. However, no correlation has been observed between the number of CSC and progression-free survival in breast cancer [90, 91]. It should be remembered that the cancer development, progression, metastasis, and recurrence are complex processes, and many other cells and mediators may have important roles [67, 72]. Therefore, it is essential to define precise markers to distinguish normal stem cells from CSCs and to develop novel therapeutic strategies. It was reported that reduced ER, CD44, and CD133 expressions are associated with poor prognosis in endometrioid endometrial adenocarcinoma [92, 93]. However, expressions of CD44 and CD133 in EC specimens suggest that these markers may participate in early-stage endometrial carcinogenesis and may be used in diagnosis [92].

Conclusion

Many studies on endometrial stem/progenitor cells are still occupied by investigating to standardize in vitro methods and animal models, leaving human clinical trials in early phases. With their high regenerative capacity and accessibility (i.e., from menstrual blood and/or by endometrial sampling), endometrial stem/progenitor cells may be a compelling source of stem cells in future regenerative medicine. Endometrial MSCs can differentiate into mature cells other than endometrial cells. Certain stem cell markers were identified for epithelial, stromal, and perivascular cell populations, both in the

functional and basal layers of endometrium. Certain EC cells carrying potential CSC properties could also be identified. However, a more explicit and sensitive marker or marker combinations are yet to be defined for endometrial CSC in order to aim for more effective treatment approaches.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Human and Animal Rights This article does not contain any studies with human or animal subjects performed by any of the authors.

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