

The elusive role of endometrial progenitor cells in immunoregulation and regenerative medicine: A brief review of current knowledge

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ABSTRACT

The human endometrium is a highly regenerative organ undergoing over 400 cycles of shedding and regeneration over a woman's lifetime. This massive regenerative capacity is thought to have a stem cell basis, with human endometrial stromal stem cells having already been extensively studied. Endometrial stem cells can be divided into three categories: endometrial epithelial stem/progenitor cells, CD140b+CD146+ or SUSD2+ endometrial mesenchymal stem cells (eMSCs), and side population cells (SPs). Endometrial stem/progenitor cells in the menstruation blood are defined as menstrual stem cells (MenSCs). Due to their abundant sources, excellent proliferation rate, and self-transplantation capabilities, MenSCs are ideal candidates for cell-based therapy in regenerative medicine, inflammation, and immune-related diseases. In this manuscript we review the available literature and outline the characteristics of endometrial stem/progenitor cells, summarizing their roles in immunoregulation, regenerative medicine and endometriosis, through which we provide some novel therapeutic strategies for reproductive and cancerous diseases.

Keywords: stem cells, endometrium, menstrual cycle, progenitor cells

INTRODUCTION

Menstrual shedding, and the subsequent repair of the functional layer of the endometrium, is a process unique to humans and higher-order primates [1]. The endometrium re-grows from a mere 1–2 mm thickness after menstrual shedding to 14 mm thickness in the secretory phase of the menstrual cycle and is able to completely regenerate after parturition, and in post-menopausal (PM) women, when exposed to estrogen replacement therapy [2]. This huge regenerative ability suggests that the en-

dometrium has a stem cell basis that supports the tissue maintenance/regrowth [1].

Endometrium can be divided into shallow and deep layers, based on its histologic structure [3]. The shallow layer is called functional layer, which experiences periodic changes of proliferation, secretion, and shedding, under the regulation of hormones. The deep layer is named as basal layer. The basal layer owns strong proliferation and repair abilities without falling off during the menstrual period but generates new functional layers [3]. The

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periodic endometrial regeneration implies the presence of stem/progenitor cells in the endometrium [1].

Endometrial stem/progenitor cell population is defined as CD140b⁺CD146⁺ or SUSD2⁺ endometrium-derived mesenchymal stem cells (eMSCs), endometrial epithelial stem/progenitor cells, and side population cells (SPs), whereas those derived from menstrual blood are called menstrual stem cells (MenSCs) [4].

In this manuscript, we collected the recent advances in the identification and characterization of adult stem/progenitor cells in female endometrium and summarized the cell-based therapy and immunoregulation of endometrial stem/progenitor cells. We also outlined the signaling pathways and molecular mechanisms involved in endometrial stem/progenitor cell populations.

ADULT STEM/PROGENITOR CELLS

The human endometrium is comprised of the functionalis and basalis layers. The functionalis is shed monthly with menstrual blood, due to changes in hormones and it is quickly reconstructed after menstruation [2]. Endometrial stem cells were initially thought to be located only in the basalis. During the menstrual phase, endometrial stem cells migrate to the functionalis in a stromal cell-derived factor-1 (SDF-1)/CXCR4 axis-dependent manner, contributing to the reconstruction of the endometrium [5]. The proliferating endometrium is thought to recruit endothelial cells to cover the new vasculature, and because SDF-1 is highly expressed in the proliferative phase, it is an ideal marker to study endothelial progenitor cell migration during menstruation. Mounting evidence has confirmed that there are stem cells in both the functionalis and basalis of the human endometrium (Figures 1 and 2) [4].

Three kinds of stem cells exist in the human endometrium: epithelial stem cells, mesenchymal stem cells, and endothelial stem cells. The subpopulation of endometrial stem cells that express CD146 and CD140b/PDGFR- β are the mesenchymal stem cells [4]. They are mainly located near small vessels in the functionalis and basalis, consistent with the conclusion mentioned above [5]. Endometrial stem cells can be obtained non-invasively from menstrual blood, and are referred to as menstrual blood-derived stem cells (MenSCs). MenSCs have an extremely high proliferative ability, and can maintain a relatively stable karyotype through 40 passages. The doubling time of MenSCs is approximately 20 hours, which is twice as fast as bone marrow-derived stem cells [6].

Endometrial MSC (mesenchymal stem/stromal cell) (eMSC) were first identified as clonogenic stro-

mal cells, comprising 1.3% of stromal fibroblasts harvested from hysterectomy tissue, which contains both functionalis and basalis endometrium. The large stromal colonies, comprised densely packed small cells with a fibroblast-like morphology and distinguishable from self-limiting small, sparsely packed colonies, likely originated from colony forming units-fibroblast (CFU-F) and do not vary in frequency during the menstrual cycle. This and their presence in postmenopausal endometrium indicate hormone independence of endometrial CFU-F [7].

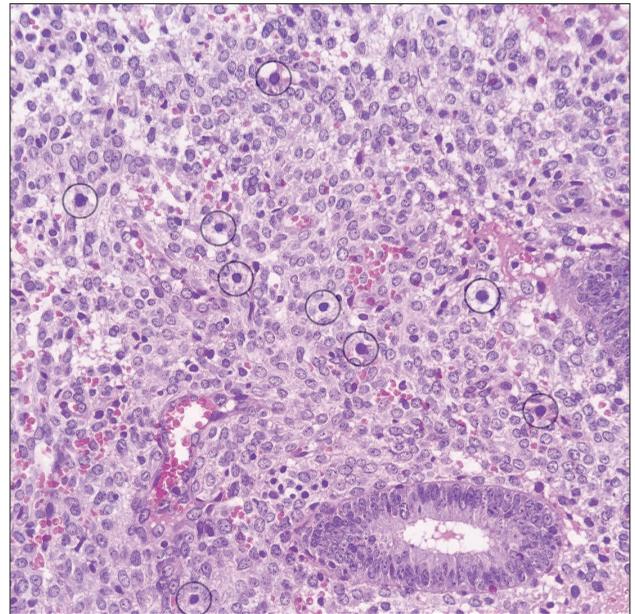


FIGURE 1. Histologic aspect of endometrial stem cells in standard stained slides (H.E., 400x) (personal collection of Tiberiu Augustin Georgescu)

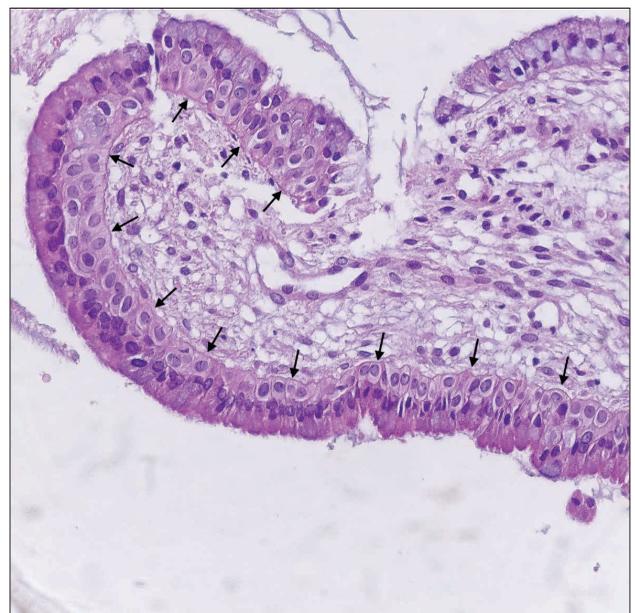


FIGURE 2. Histologic aspect of endocervical reserve cells in standard stained slides (H.E., 400x) (personal collection of Tiberiu Augustin Georgescu)

Menstrual blood is a readily accessible, non-invasive source of large numbers of endometrial stem/stromal cells (MenSC). Menstrual blood contains fragments of shedding endometrial tissue, which is cultured directly onto plastic similar to bmMSC. Adherent MenSC have typical stromal fibroblast morphology and rapidly propagate with a doubling time of 18-36 h. As for other adult stem cells, the lifespan of MenSC is relatively short in comparison with human embryonic stem cells (hESC) [8].

Cultured MenSC (menstrual blood stem/stromal cells) transdifferentiate across lineage boundaries into ectodermal (neurons and glia), and endodermal (hepatocyte) lineages [7]. Differentiation of MenSC into hepatocytes is dependent on the concentrations of hepatocyte growth factor (HGF), oncostatin M (OSM) and removal of serum from the induction medium [6]. Unfractionated MenSC have potential to differentiate into keratinocyte-like cells, generating epidermal lineage markers via co-culturing with keratinocytes derived from the foreskin of healthy newborns. MenSC were also induced to differentiate into keratinocyte-like cells in 3D culture with human foreskin-derived keratinocytes on a bilayer scaffold composed of amniotic membrane and silk fibroin [8].

ENDOMETRIAL STEM CELL-BASED THERAPY AND IMMUNOREGULATION

MenSCs contribute to tissue repair and reconstruction through a variety of mechanisms. MenSCs can directly differentiate into several different cell types, as previously mentioned. Hepatocyte growth factor (HGF), fibroblast growth factor-4 (FGF-4), and oncostatin M (OSM) could induce hepatocyte-like cell differentiation in MenSCs [9].

In addition, intravenously injected MenSCs improved hyperglycemia significantly in mice with type I diabetes [10]. After being injected into the mice, most MenSCs migrated to the injured pancreas, finally locating near the pancreatic duct and islets. Interestingly, although the number of β cells increased after the injection of MenSCs, no differentiation of MenSCs was detected. Thus, MenSCs might stimulate endogenous pancreatic progenitor cell differentiation in a paracrine manner via the up-regulation of neurogenin [11].

MenSCs also have an immunomodulatory effect. Ulcerative colitis is a type of inflammatory bowel disease [12]. MenSCs showed an extensive immunomodulatory effect when injected into mice with colitis. They decreased the infiltration of inflammatory cells, including macrophages and NK cells, and modulated the number of immune cells [13]. In addition, MenSCs mediated the expression of multiple

cytokines. In the MenSC-treated mice, pro-inflammatory cytokines IL-2 and TNF- α decreased significantly, while anti-inflammatory factors IL-4 and IL-10 increased dramatically [11].

Recently it was shown that MenSC influence the humoral immune responses in a mouse model of heart transplantation by attenuating antibody responses [12]. MenSC injection 24 hours following allograft heart transplantation prolonged graft survival in recipient mice by rapidly reducing intra-graft deposition of donor-specific IgG and IgM antibodies and reducing donor-specific antibody secreting B cells. It remains to be investigated whether MenSC ameliorate autoantibody production in patients with antibody-mediated autoimmune diseases [13].

MenSC also have anti-microbial activity. In a cecal ligation and puncture mouse model of sepsis, the combination of MenSC and antibiotic improved the survival rate of affected animals (up to 95%) compared with control animals receiving antibiotic treatment alone [11]. The MenSC/antibiotic combination increased bacterial clearance from blood, and reduced the inflammatory cytokines IL-8, TNF, and MCP in peritoneal fluid, without loss of T and B lymphocytes [12].

Taken together, eMSC and MenSC have a range of effects on both arms of the innate and specific immune responses, however, the discrepancies between *in vitro* and *in vivo* findings and considerable variation between experimental models necessitate further investigation to identify the underlying mechanisms that orchestrate the cross-talk that MenSC and eMSC utilize to modulate the immune system [14,15].

The potential of eMSC in regenerative or reparative medicine has been explored in many directions [16-20], including a common women's gynecological disorder, pelvic organ prolapse [12]. An autologous approach has been investigated and tissue engineering constructs comprising eMSC and novel non-degradable mesh, degradable nanofiber mesh, and 3D bioprinted mesh/eMSC have been explored in both rodent and large animal ovine models [3].

Human and monkey endometrial stromal fibroblasts transdifferentiated into cells with morphological, chemical and electrical activity of dopaminergic neurons have been investigated in several animal models [7]. They engrafted and migrated to site of lesion in chemically induced mouse and green monkey models of Parkinson disease, differentiated to neuronal-like cells resulting in increased striatal dopamine and dopamine metabolite concentrations [11,16]. However, further studies on clinical outcomes such as the behavioral studies are warranted.

Type 1 diabetes mellitus is a clinical condition, which can benefit from islet-based cell transplantation [12]. The plasticity of human endometrial stro-

mal fibroblasts has been utilized to generate insulin-secreting cells [10]. These differentiated cells produced human insulin, decreased blood glucose levels and minimized diabetes-associated complications such as weight loss, dehydration, cataracts, delayed wound healing and sedative behavior in a mouse model of diabetes mellitus [12].

The reparative capacities of MenSCs were exploited to treat premature ovarian failure in mouse/rat models [9]. Intra-ovarian or intravenous injection of MenSCs reduced apoptosis and restored ovarian function, shown by expression of the ovarian markers, follicle-stimulating hormone receptor (Fshr), inhibin α/β , anti-Müllerian hormone (Amh), and rising plasma levels of the ovarian hormones, estrogen and progesterone [10]. Increased numbers of primary, mature and total ovarian follicle numbers were observed [2].

CONCLUSIONS

It is well established that endometrial stem cells have extensive applications in tissue repair and engineering.

Endometrial stem cells have the potential to become a powerful tool for both clinical diagnosis and therapeutics. Preliminary results from endometrial stem cell use in animal models of different diseases suggested a future for endometrial stem cell-based applications in the clinical setting. Further research is needed to advance the development of endometrial stem cells for clinical use.

Endometrial stem/progenitor cells in menstruation blood (MenSCs) are the most clinically accessible sources of stem cells with a great potential in the regenerative medicine and tissue engineering. The advantages of MenSCs are that they can be collected regularly and noninvasively. MenSCs are also promising candidates in the stem cell therapy for inflammation and immune-related diseases, and may play an immunosuppressive role in the regulation of the cell-mediated immunity and humoral immunity.

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