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Bone marrow-derived stem cells contribute to regeneration of the endometrium

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Stem cells are undifferentiated cells capable of self-renewal and differentiation into various cell lineages. Stem cells are responsible for the development of organs and regeneration of damaged tissues. The highly regenerative nature of the human endometrium during reproductive age suggests that stem cells play a critical role in endometrial physiology. Bone marrow-derived cells migrate to the uterus and participate in the healing and restoration of functionally or structurally damaged endometrium. This review summarizes recent research into the potential therapeutic effects of bone marrow-derived stem cells in conditions involving endometrial impairment.

Keywords: Bone marrow; Endometrium; Stem cells

Introduction

The human endometrium is a dynamic remodeling tissue that undergoes over 400 cycles of growth, differentiation, and shedding of endometrial cells during the reproductive period [1,2]. This physiology is mainly regulated by the effects of sex steroid hormones and other biological molecules, and is essential for implantation and pregnancy in mammals. The highly regenerative nature of the endometrium implies that stem cells are critically involved in endometrial physiology. Studies have implicated the endometrial stem cells that reside in the basal layer of the uterus. These cells are thought to be derived from endogenous somatic stem cells laid down during embryogenesis [3]. These endogenous stem cells facilitate the rapid replacement of the endometrial functionalis layer in each menstrual cycle and differentiate into endometrial cell types *in vitro* [1,4,5]. Non-

resident multipotent stem cells from the bone marrow (BM) migrate into the endometrium and contribute to reconstitution and uterine repair by releasing cytokines and through other mechanisms [6].

Stem cells are undifferentiated cells that possess unique capacities for self-renewal and differentiation into various cell lineages. They are responsible for the development of organs and tissues, as well as for the regeneration of damaged tissues. Adult stem cells, which have been identified in most adult tissues, are usually multipotent cells that can differentiate into one or more lineages. Adult BM is a well-known reservoir of stem cells, such as hematopoietic and mesenchymal stem cells (MSCs) and progenitor cells. Hematopoietic stem cells are capable of stepwise differentiation into specialized cells of the blood, including erythrocytes, thrombocytes, and lymphoid cells [7]. MSCs are defined by their plastic adherence; expression of CD73, CD90, and CD105 surface markers; lack of CD34, CD45, and HLA-DR [8]; and ability to differentiate into classic mesodermal lineages, which include osteoblasts, chondroblasts, adipocytes, and stromal cells [9].

Laboratory protocols and cell surface markers for the isolation and identification of BM-derived stem cells (BMDSCs) have been refined in numerous experimental studies. Moreover, BM transplantation has been utilized as a definitive therapy for some blood diseases or hematologic malignancies in clinical practice. Therefore, BMDSCs have become an attractive and important resource in regenerative medi-

cine. Herein, we review the current evidence and studies of the involvement of BMDSCs in endometrial physiology and the potential implications of these findings for endometrial pathologic conditions.

Bone-marrow derived cells in the uterus

The first evidence for the presence of BM-derived cells (BMDCs) in reproductive organs was the identification of donor-derived endometrial cells in the uterine tissue of women who had undergone HLA-mismatched, allogenic BM transplantation [10]. The engraftment rate of BMDCs into the endometrium varied from 0.2% to 48% for endometrial epithelial cells and from 0.3% to 52% for stromal cells, depending on the method of detection and time from BM transplantation [10,11]. In a murine model of male to female BM transplantation, localized endometrial cells that had been derived from donor male mice were identified in the uterus of the female mice, where the *SRY* gene and Y chromosome were detected by fluorescence *in situ* hybridization and immunofluorescence [12]. These findings provided further evidence that BMDCs migrate to the uterus, and they are potential endometrial progenitors that may be a source of reparative cells for the reproductive tract [6,10,12].

Studies of the biological roles of BMDCs have revealed that the recruitment of BMDCs into the endometrium is promoted more in conditions of endometrial injury or inflammation, as has been demonstrated in cases where injuries to other organs generated signals from the damaged tissue [13-15]. In a mouse model of ischemia-reperfusion injury to the uterus, the recruitment of BMDSCs to the endometrium was significantly increased (up to two-fold) after injury. This phenomenon was independent of estrous cycle, sex steroids, or treatment with granulocyte-colony stimulating factor (G-CSF) [6].

Role of BMDSCs in animal models of endometrial injury

With an increased understanding of stem cell biology and accumulating evidence that BMDCs travel and engraft to the uterus, studies have explored the potential therapeutic effect of BMDSCs in various endometrial pathologies, such as Asherman syndrome (AS) and thin endometrium models. AS is an acquired pathologic condition characterized by fibrosis and intrauterine adhesion of the endometrial cavity following damage to the basal layer of the endometrium [16]. This condition is mostly caused by endometrial destruction due to trauma, infection, repeated or aggressive curettages, or endometritis [17]. Surgical adhesiolysis and supportive hormonal therapy have been clinically used to restore endometrial structure and function. The outcomes for embryo implantation and fertility vary depending on the endometrial regeneration capability in each patient [18]. Stem cell re-

cruitment and pregnancy outcomes were investigated in a reproducible murine model of AS [16]. After uterine injury was inflicted in female mice by scratching with needles, BMDSCs from male donor mice were transplanted to the females via the tail vein. In the control group, the females without uterine damage received normal saline. Three months after BM transplantation, Y chromosome-bearing $CD45^-$ cells comprised $<0.1\%$ of the total endometrial cells, although twice as many Y^+CD45^- cells were identified in mice with a damaged uterus as were found in the control mice, indicating that more engraftment of BMDSCs took place in cases of uterine injury. Improved fertility was noted, as nine of 10 BM-treated mice conceived compared to three of 10 control mice. Another study using a murine model of AS explored the engraftment of BMDSCs by assessing the proliferation of uterine tissue following uterine injury mimicking AS [18]. After injection of G-CSF to mobilize BMDSCs, $CD133^+$ BMDSCs were isolated from women with AS. CD133 is a recognized marker of immature hematopoietic and progenitor cells with high proliferative activity and circulating cells with endothelial regenerative capacity. BMDSCs were incubated with superparamagnetic iron oxide nanoparticles. Labeling the BMDSCs with the nanoparticle allowed the engraftment of the cells into the recipient mice uterus to be detected. The labeled BMDSCs were administered via either an intravenous or uterine injection to female mice with uterine injury. Engraftment of the $CD133^+$ BMDSCs was identified in the uterus of mice around the endometrial vessels, where the endometrial stem cell niche is located. Cell proliferation was significantly promoted, as evidenced by increased Ki-67 expression in both BMDSC-treated groups.

Several studies have addressed the effect of BMDSCs on the regeneration of endometrial tissue in models of thin endometrium. Poor endometrial growth or persistent thin endometrium during reproductive cycles is a pathologic condition that is strongly related to subfertility or recurrent implantation failure in women undergoing infertility treatment. Reported therapeutic modalities include low-dose aspirin, sildenafil, and G-CSF [19-22], but the outcomes remain inconclusive. In one study, thin endometrium was induced in rats by the instillation of ethanol into the uterine cavity, and then BMDSCs were transplanted into the experimental rats [23]. BMDSC treatment increased endometrial thickness and the number of capillaries and glands, and also modified the expression of various markers of integrin and leukemia inhibitor factor, which are involved in endometrial receptivity. A recent study demonstrated similar findings, in which systemic treatment with BMDCs significantly increased cellular proliferation and vascularization in uterine tissue in mice with induced thinning of the endometrium compared to controls [24]. In addition, a modest improvement of fertility outcomes was found in mice treated with BMDCs, together with favorable modifications of histological or biochemical markers of uterine receptivity.

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BMDSC transplantation for endometrial pathologies in clinical trials

A pilot cohort study sought to determine the effect of autologous cell therapy with CD133+ BMDSCs for patients with refractory AS and endometrial atrophy (EA) [25]. In BMDSCs, CD133+ is known as an endothelial progenitor cell marker that has been used safely in regenerative medicine with proven effects of the promotion of angiogenesis in refractory angina and improvement of segmental myocardial perfusion [26]. CD133+ BMDSCs were isolated from 16 patients (11 patients with refractory AS and five patients with EA) through peripheral blood apheresis after G-CSF injection for mobilization of BMDSCs. The cells were immediately injected through the uterine artery into the spiral arteriole in the basal layer via radiological procedures. After therapy, improved endometrial growth and restoration of menses were evident, with three patients achieving spontaneous pregnancy. The authors suggested that autologous cell therapy with BMDSCs might be a promising therapeutic approach for refractory AS and EA. Another study that included six patients with refractory AS in whom hysteroscopic surgery had failed examined the possibility of cell therapy [27]. Noncharacterized mononuclear stem cells were implanted in the subendometria zone using a needle. Improvements in endometrial thickness were evident at the 9-month posttreatment follow-up and menstruation resumed in five of the six patients.

Mechanisms of stem cells for regeneration of damaged endometrium

MSCs are highly proliferative and can transdifferentiate into various nonhematopoietic cell types, such as muscle cells, neurons, and cardiomyocytes [13,28]. This differentiation capacity has been explored in models of uterine injury, which have revealed the differentiation of BMDCs engrafted in the uterus into an endometrial phenotype expressing vimentin and lacking CD45 expression [6,16]. On the contrary, a recent study failed to observe the transdifferentiation capacity of BMDCs in a mouse BM transplantation model [29]. Although the differentiation of stem cells into organ-specific cells is considered to be the principal rationale for stem cell therapy, the number of BMDCs that engraft into the uterus is low in various injury models, and they do not undergo clonal expansion to replace the entire endometrium [1,16,30-32]. Moreover, only a small number of transdifferentiated cells (< 0.1% of all tested cell types) can be detected in the injured organs after transplantation [7].

In addition to the multilineage differentiation capacity of MSCs, recent insights concerning the mechanisms of stem cell therapy have integrated the trophic role of BMDCs in the reconstitution of damaged tissues. The trophic effect of MSCs in tissue repair was first pro-

posed in 2006 [33]. The current understanding is that engrafted MSCs can modulate the activity of surrounding cells by means of cellular communication [34]. Indeed, BMDCs secrete a number of trophic factors that include chemokines, cytokines, and growth factors, which stimulate angiogenesis, immune modulation, and intrinsic stem/progenitor cells, in turn enhancing the endogenous repair system [7,35,36]. The therapeutic effect of MSCs may depend on their capacity to secrete soluble factors that promote several key biological activities [7,34].

More recently, the role of extracellular vesicles and membrane nanotubes in cell-to-cell communication have been discussed as additional mechanisms in stem cell research, and these possibilities may lead to the further development of new strategies and therapeutic tools for regenerative medicine [34].

Chemoattractants

MSCs are involved in immune processes in the innate immune system. They inhibit T cells from proliferating and differentiating into proinflammatory cells, and stimulate macrophage production [37]. In addition, MSCs release various trophic factors, including cytokines, chemokines, and chemoattractants, that enhance the mobilization of stem cells. Therefore, clarifying the effect and mechanisms of these substances in relation to stem cell therapy has become an important topic of research.

C-X-C motif chemokine 12 (CXCL12, also known as stromal derived factor-1) is produced from endometrial stromal cells. CXCL12 is the most potent chemoattractant that stimulates homing and migration of BMDSCs into damaged tissues [38,39]. This action is mediated by C-X-C chemokine receptor type 4 (CXCR4), a receptor for CXCL12, which is expressed on the surface of stem cells [40,41]. Endogenous CXCL12 expression is increased in response to various forms of damage, enhancing recruitment of BMDMSCs [42-44]. The effect of exogenous CXCL12 administration as a therapeutic model also has been investigated [17]. Female mice received BM transplantation using male mice expressing green fluorescent protein, and endometrial scratch injuries were created 2 weeks after BM transplantation. Mice were treated with recombinant CXCL12, AMD3100 (CXCR4 antagonist), or both. The injection of CXCL12 increased BMDSC engraftment into the damaged uterus, and this effect was significantly blocked by AMD3100. The authors concluded that CXCL12 augmentation induced stem cell engraftment into the uterus, reduced fibrosis formation with uterine function enhancement (litter size and decreased time to conceive), and possibly improved pregnancy in AS mice without supplemental BM transplantation. A recent study demonstrated a comparable effect of CXCL12 with BMDCs in mice with thin endometrium, observing significant improvements in the regenera-

tion of endometrial histology, expression of biochemical markers, and fertility outcomes in both treatment groups [24]. These lines of evidence indicate the potential therapeutic value of CXCL12 in stem cell-related therapy based on its biological action for stem cell mobilization, suggesting that it may serve as an alternative or replacement for stem cell transplantation [17].

Conclusion

Stem cell research and regenerative medicine are attractive subjects in gynecologic research that are attracting growing interest. Although cell therapy with BMDSCs remains experimental, with only a few ongoing clinical trials addressing their potency in gynecologic diseases, studies have consistently demonstrated promising effects of BMDSCs on functional or structural reconstruction in various conditions of endometrial impairment. BM was one of the first recognized sources of adult stem cells. The advantages of BM include relatively easy accessibility, with well-established basic scientific findings and technical methods for the manipulation of BMDSCs *in vivo* and *in vitro*. More data from clinical trials and scientific studies are needed to improve our understanding of the mechanisms related to stem cell therapy. This knowledge may lead to the acceptance and utilization of BMDSCs in clinical practice, such as in women with refractory endometrial conditions and/or related infertility concerns.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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