Stem Cells of Human Endometrium: Trash to Treasure

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Background

Reproduction can be defined as the process by which an organism continues its species. The development of the normal female reproductive tract is a complex process. The paramesonephric ducts arise from the intermediate mesoderm, which are the precursors of the female reproductive organs that include uterus, fallopian tubes, cervix, and upper vagina [1]. The female reproductive system is designed to carry out several functions. It produces the female egg cells necessary for reproduction, called the ova or oocytes. The system is designed to transport the ova to the site of fertilization. Conception, the fertilization of an egg by a sperm, normally occurs in the fallopian tubes. The fertilized egg then gets implanted into the walls of the uterus, beginning the initial stages of pregnancy. If fertilization and/or implantation do not take place, the system is designed to menstruate (the monthly shedding of the uterine lining). In addition, the female reproductive system produces female sex hormones that maintain the reproductive cycle. Fallopian tubes are the passageways that egg cells enter after release from the ovaries. The Fallopian tubes lead to the uterus a muscular organ in the pelvic cavity. The inner lining, called the *endometrium*, thickens with blood and tissue in anticipation of a fertilized egg cell. If fertilization fails to occur, the endometrium degenerates and is shed in the process of menstruation. Based on its dynamic tissue remodeling during the menstrual cycle and pregnancy, it has been suggested that stem cells of the endometrium must possess a high regenerative potential [2, 3]. In this regard, the chapter provides an overview on endometrial stem cells with a special emphasis on its proliferation and multilineage differentiation potentials coupled with its in vivo therapeutic applications.

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Human Endometrium and Its Stem Cell Derivatives

The uterus is the largest female reproductive organ that plays a pivotal role in implantation and in the absence of pregnancy, menstruation.

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The innermost layer of the uterus is known as tunica mucosa, popularly termed as endometrium, opposed to the outer perimetrium and median myometrium. The uterus is the only organ whose lining is almost entirely expelled and reconstructed periodically, both phenomena taking place at each ovarian cycle. With the purpose of facilitating the periodic elimination of the endometrium that undergoes regression, shrinkage, and necrosis at the end of each cycle, the uterus also exhibits the unique peculiarity of physiological bleeding. The endometrium begins to reach full development at puberty and thereafter exhibits dramatic changes during each menstrual cycle. It undergoes further changes before, during, and after pregnancy, during the menopause, and in old age [3].

The endometrium is a simple columnar epithelium. It is divided into two zones: the inner functionalis which is adjacent to the uterine cavity and a deeper basalis layer which overlies the myometrium. The functionalis layer is shed each month with menstruation and is then regenerated from the basalis layer. The functionalis, comprising the upper two-third of the endometrium, is divided into stratum compactum and stratum spongiosum. The stratum compactum is a superficial thin layer nearest to the uterine cavity and contains the lining cells, necks of the uterine gland, and relatively dense stroma. The stratum spongiosum is the deeper part of functionalis composed of main portions of the uterine glands and accompanying blood vessels; the stromal cells are more loosely arranged and larger than in the stratum compactum. The lower basalis contains the basal region of the uterine glands, dense stroma (that remains relatively unaltered during the menstrual cycle), large blood vessel remains, and lymphoid aggregates. It serves as the germinal compartment for generating new functionalis each month [3–5]. It has been postulated that the niche of these adult stem or progenitor cells of the endometrium is the lower basalis. These stem or progenitor cells were also identified to be in the trophic endometrium of postmenopausal women [7, 8].

Accumulating evidence from the literature on the existence of epithelial and stromal/stem cells in endometrial tissue [6–9] has substantiated that it possesses a remarkable capacity for regeneration. Although endometrial tissue-derived stem cells are being reported, menstrual blood has become the most convenient source in the search for endometrial stem cells because collecting menstrual blood is easy and noninvasive and endometrial stem/progenitor cells are shed in menstrual blood [10–13]. For these reasons, reliable studies on menstrual blood-derived stem cells are in process. Furthermore, menstrual blood-derived stem cells demonstrate great promise for use in tissue repair and treatment of diseases, due to the plasticity and longevity of the cells. Menstrual blood has proven to be a unique and novel source of stromal stem cells from the endometrial functionalis. They have been identified through several in vitro and in vivo studies [12].

However, putative adult stem or progenitor cells that are responsible for the cyclical regeneration of the endometrial functionalis, every month, are thought to reside in the basalis region of the endometrium, as described earlier [4–8]. The study of these stem cells from the basalis is still in its infancy. Based on the dynamic tissue remodeling of the uterus, it has been suggested that adult stem cells must play a role in uterine tissue maintenance and function. Hence, a thorough characterization of the uterine/endometrial stem cells derived from the endometrial tissue biopsy of the inner lining of the uterus or from the intact uterus surgically removed in the treatment is equally important as that of studies on menstrual blood stem cells. Once a mechanical or functional characteristic platform has been constructed, it then becomes easier to understand the complex mechanisms underlying the morphogenesis and physiological generation of the female reproductive tract.

Marker Profiles

The concept that endometrial regeneration is mediated by endometrial stem/progenitor cells was proposed many years ago [5, 14]. Since then many evidences do arise for the existence of stem cells from endometrium. The

first published evidence for the existence of adult stem/progenitor cells in the human uterus identified in the endometrium is the clonogenic epithelial and stromal cells suggesting the presence of two types of adult stem/progenitor cells [15, 16].

Schwab and Gargett demonstrated the existence of endometrial stem cell identification through the characterization of perivascular markers CD146 and PDGF-Rβ. This study also reveals that CD146 and PDGF-R β cells were co-localized in both the functional and basal layer [17, 18]. They demonstrated that these perivascular markers enabled isolation of stromal cells from human endometrium which exhibit phenotypic and functional properties of MSC. They hypothesized that these endometrial MSC like cells may contribute to the cyclic regeneration of the endometrium and might play a vital role in cell-based therapies.

Various studies have examined the expression of stem cell markers in the human endometrium including our earlier published data [9, 19–22]. Dimitrov and his coworkers [3] analyzed the cell surface markers for the cultured endometrial stem cells derived from both functionalis and basalis layer of the endometrium for phenotypic expression. Hematopoietic stem cell markers including CD45, CD14, CD19, CD56/16, CD34, and CD3 showed a negative expression, whereas markers like CD29, CD73, and CD90 were stained positive, strongly suggesting the mesenchymal nature of the cells. Despite these citations, the identifications of biomarkers of endometrium are uncertain and hence research is underway. The discovery of those markers highlights the importance of the stem cell system in human reproduction and also demonstrates its therapeutic implications.

Besides the MSC-specific markers, Oct-4, a transcription factor and marker of human embryonic stem cells, and more recently of adult stem cells, is expressed in almost half of tested endometrial samples [9, 23]. The expression of OCT-4 suggests the existence of endometrial stem cells, lending further support to the hypothesis of endometrial regeneration by local stem cells in endometrial tissue. More Oct-4+ cells are observed during the proliferative stage; however, the identity and location of the Oct-4+ cells have not been reported [19]. Several general adult stem cell markers, including bcl-2, c-kit (CD117), and CD34, have also been identified in endometrial tissues [9]. The importance of these markers, however, cannot be determined since they are expressed in many more endometrial cells than the numbers of clonogenic or side population cells identified in functional studies [16, 20]. Apart from the markers specified above, our team could identify multitude of markers that are specific to heterogenous endometrial cells [21]. The wide specificity toward multitude of marker characteristics similar to bone marrow stem cells favors its application in therapeutics.

Multi-differentiation Potential

Extensive literatures exist to support endometrial stem cells as an additional source of curative stem cell therapeutics. To quote a few, its dynamics in coordinated functions of proliferation, differentiation, and menstrual shedding has been reported [5, 23]. Endometrial stem cells, unlike other postnatal adult stem cells, could retain its stemness and multi-differentiation potential even under extensive culture condition [unpublished data]. Several researchers demonstrated the ability of CD146+PDGFRb+ MSC-like cells [18] or clonogenic human endometrial stromal cells [2, 3] to differentiate into mesodermal origin such as adipocytes, osteocytes, smooth muscle cells, and chondrocytes [2, 3, 16, 24]. Masuda et al. demonstrated that the endometrial tissue-reconstituting cells also possess the ability to differentiate into endothelial cells [25]. It has been demonstrated that not only the endometrial stem cells but also the SP cells of the endometrium have the ability to differentiate into endothelial and smooth muscle cells [20, 26, 27]. It was reported that endometrial stem cells could effectively differentiate into muscular cells of urinary bladder using myogenic growth factors, thereby making endometrial stem cells ideal for bladder cell replacement therapies [24]. Besides, endometrial stem cells have also been

differentiated into megakaryocytes that produced functional platelets, thereby demonstrating its wide plasticity [28].

It is a well-known fact that angiogenesis plays a key role in the reproductive processes such as embryo implantation and endometrial regeneration after menstruation. Evidence is reviewed for the hypothesis that the endometrium in women has a high capacity of cell proliferation and angiogenesis [29, 30]. Evidences show the angiogenic phenotype in human endometrium by their establishment of their ability to implant [31]. 3D cultures of human endometrial cells demonstrate its high capacity of cell proliferation and angiogenesis [32].

With its built-in angiogenic role throughout reproductive phase of women coupled with proven experimental records, it is clear that endometrial stem cells could serve a better tool for treating vascular disorders. Apart from its mesodermal and ectodermal differentiation potency, endometrial cells were also shown to possess endodermal differentiation via differentiating into insulin-producing cells [33]. The differentiated islets that form endometrium were able to produce insulin both in vitro and in vivo in a murine model [34], thus serving its further applicability in treating diabetes.

In Vivo Applications of Endometrial Stem Cells

Endometrial stromal cells possess wide range of advantages as opposed to other postnatal stem cells, to prove themselves as a valuable tool in cell-based therapies. They are as follows—easy to isolate, high accessibility, trash source, immunogenic, longer preservation, highly clonogenic with a higher multi-differentiation, and angiogenic potential—thereby serving as a better autologous/allogenic therapeutic tool in regenerative medicine. This is evidenced by several preclinical and clinical trials on endometrial stem cells in autologous/ allogenic transplantations. Some of its in vivo preclinical and clinical applications are discussed below.

With the dynamic cyclical regenerative and angiogenic potency of endometrial stem cells, there are proven record tracks on its efficacy in treating vascular disorders. The applications of bone marrow-derived cells for heart failures and its related diseases are enticing. This is due to its directed cardiomyocyte differentiation [35] and its ability to secrete angiogenic and trophic factors. However, angiogenic potency of bone marrow in patients with coronary artery disease is impaired, in part due to its deficiencies in the CXCR4 migration activity [36]. The relationship between angiogenesis and post myocardial infarct healing is well known. Endometrial stem cells could outweigh this obstacle and many other such hindrances, thereby entering the clinic in an efficient manner.

As stated above, endometrium undergoes rapid angiogenesis in a controlled manner every month. With this built-in potency, upregulated production of angiogenic factors including PDGF, EGF, and VEGF has been described both in the mouse and human endometrium [29, 30, 37]. Besides, administration of endometrial regenerative cells into a post myocardial infarct model showed recovery as compared to bone marrow cells. Furthermore, the cells were capable of functionally integrating with existing cardiomyocytes and exerted effects through direct differentiation [13]. The possibility of using endometrial cells for treating critical limb ischemia has been demonstrated in mouse model due to its high levels of growth factors and MMPs. Besides, as it possesses superior immunomodulatory potential, its off-the-shelf allogenic therapeutic application is well demonstrated [38]. The first report of clinical use of ERC involved four patients with multiple sclerosis who received both intrathecal and intravenous injections. No adverse events were reported at the time of last follow-up [38]. Similar lines of clinical trial were also demonstrated with muscular dystrophies and heart failures [39–41]. Studies reported no adverse events at time of last follow-ups of these cases. Besides these applications, insulin-secreting capacity of endometrial cells to functionally recover insulin inefficiency in vivo has also

been demonstrated. Preclinical murine model system treated for diabetes has restored its normoglycemic condition and produced insulin [31, 32].

Conclusion

Undoubtedly, it can be concluded that endometrial stem cells may become key players in treating various disorders because of their noninvasive mode of collection, ease of isolation, its enhanced clonogenecity, and multi-differentiation potentials. Furthermore, its off-the-shelf storage capacity and superior immunomodulatory property allow greater applicability for allogenic cell therapeutics. However, further investigations are warranted on use of endometrial tissue in cell-based therapies. Besides, studies on wide characteristics of putative endometrial stem cells that contribute to gynecological disorders might explore its applicability in targeted cell-based therapies for such reproductive disorders.

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