Reprod Med Biol (2010) 9:9–16
DOI 10.1007/s12522-009-0032-y

REVIEW ARTICLE

Stem/progenitor cells and the regeneration potentials in the human uterus

Tetsuo Maruyama

Received: 20 June 2009 / Accepted: 4 August 2009 / Published online: 26 August 2009

Abstract The human uterus is unique in that it possesses the tremendous regenerative capacity required for cyclical regeneration and remodeling throughout a woman's reproductive life. Not only must the uterus rapidly enlarge to accommodate the developing fetus, the endometrium must also regenerate with each menstrual cycle. This plasticity of the reproductive system has recently been highlighted. My research group and collaborators showed that functional endometrial tissue could be regenerated from only a small number of singly dispersed human endometrial cells, transplanted beneath the kidney capsule of severely immunodeficient mice. This artificially generated endometrium resembles the natural endometrium, and contains human blood vessels that invade the mouse kidney parenchyma. Additionally, it mimics normal hormone-dependent changes including proliferation, differentiation, and tissue breakdown (menstruation). The regenerative capacity of endometrial cells makes them ideal candidates for tissue reconstitution, angiogenesis, and human–mouse chimeric vessel formation. The smooth muscle cells of the uterus (myometrium) share the plasticity of the endometrium. This is evidenced by their capacity for dramatic, repeatable, pregnancy-induced enlargement. Regeneration and remodeling in the female reproductive tract allude to the existence of endometrial and myometrial stem cell systems. We have recently isolated candidate populations of adult stem cells from both the human endometrium and myometrium. Characterization of these endometrial and myometrial cells, along with the study of the mechanisms controlling their regeneration, will improve the understanding of the physiology and pathophysiology of the female reproductive tract. Furthermore, myometrial and endometrial stem-like cells might also represent a novel source of biological material that could be used for the reconstruction of not only the human uterus but other organs as well.

Keywords Endometrium · Myometrium · Stem cell · Side population · Regeneration

T. Maruyama (✉)
Department of Obstetrics and Gynecology,
Keio University School of Medicine,
35, Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan
e-mail: tetsuo@sc.itc.keio.ac.jp

Introduction

Dramatic advances in stem cell biology and regenerative medicine have promoted not only the establishment of a research framework for the discovery of novel paradigms in cell lineage commitment, but also the development of therapeutics to be used in the treatment of diseases that result from abnormal cellular function and the destruction of tissue. The goal of the regenerative medicine-based therapies is to repair damaged and diseased tissues by providing replacement cells or factors that can restore tissue function. To date, embryonic stem cells, induced pluripotent cells, and adult stem cells have been considered as the candidate replacement cells to be used for regenerative medicine therapeutics [1, 2].

Adult stem cells, also known as somatic stem cells or tissue-specific stem cells, are undifferentiated cells, found throughout the body after embryonic development. They can multiply by cell division or self-renew indefinitely, and generate all the cell types of the organ from which they originate, potentially regenerating the entire organ from a few cells. Thus, the biological role of adult stem cells is believed to replenish dying cells and regenerate damaged

tissues, thereby leading to growth and maintenance of the corresponding organs and tissues. Hierarchy of adult stem cell differentiation is illustrated in Fig. 1.

The human uterus is unique in that it possesses the tremendous regenerative capacity required for cyclical regeneration and remodeling throughout a woman's reproductive life. Not only must the uterus rapidly enlarge to accommodate the developing fetus, the endometrium must also regenerate with each menstrual cycle. It is, therefore, rational to hypothesize that the existence of adult stem cell system(s) is deeply involved in the potential regeneration and remodeling capacities of the female reproductive tract.

Indeed, accumulating bodies of evidence have suggested that the human and mouse female reproductive tract possesses adult stem cells like various non-reproductive organs [3, 4].

This review briefly addresses what is currently understood regarding stem/progenitor cells and the regeneration of potentials in human uterus and presents our recently developed experimental model for the study of the process.

Regeneration of the human endometrium

The human endometrium undergoes cyclical changes including proliferation, differentiation, tissue breakdown, and shedding (menstruation) throughout a woman's reproductive life. The postovulatory rise in ovarian progesterone induces profound remodeling and differentiation of the estradiol-primed endometrium. After tissue breakdown and shedding of the differentiated endometrium (menstruation) that follows progesterone withdrawal, the endometrium is programmed to regrow under the influence of estrogen. The restructuring of the functional layer is critical to the development of a tissue ready for implantation or for menstruation [5].

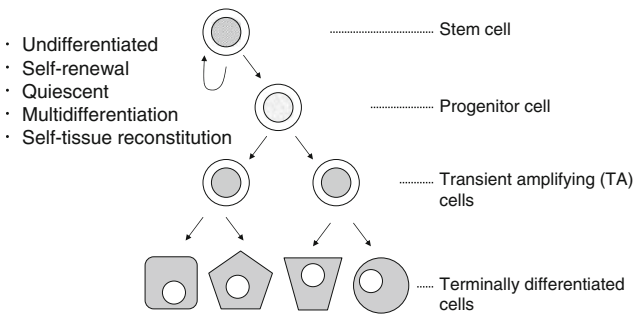

Fig. 1 Hierarchy of adult stem cell differentiation. The adult stem cell remains anchored within its niche and can undergo asymmetric cell division to renew itself and produce an immediate daughter/progenitor cell. This cell undergoes further division to produce cells that comprise the transient amplifying (TA) population. The TA cells rapidly proliferate to expand cell numbers and finally differentiate to produce many terminally differentiated functional cells with no capacity for proliferation

The regeneration processes are comprised of endometrial epithelial regrowth, angiogenesis, and proliferation of endometrial stromal cells. The remaining basal layer acts as a germinal compartment from which the different cell types grow and differentiate [6]. Regrowth is estrogen dominated; and for epithelial cells EGF, TGFα, and EGF receptor are all likely to be involved. Both TGFα and EGF compete for the EGF receptor; and both, along with platelet-derived growth factor (PDGF), are mitogens for epithelial cells from the basal layer [7]. Regrowth of the epithelium, beginning from the stumps of the glands, starts on menstrual day 2. The surface epithelium grows out of the cone-shaped gland edges, rapidly covering the luminal surface, two-thirds of which is covered by day 4. By day 6, epithelialization is complete [8]. Vessel growth is also important in the endometrium of menstruating species where the spiral arterioles are a characteristic feature. Endometrial angiogenesis and vessel remodeling are driven by a network of signaling molecules and receptors that include members of the vascular endothelial growth factor (VEGF) family, their splicing variants, fibroblast growth factors, angiopoietins, angiogenin, and the ephrins and their cognate receptors [9]. The specific roles of each of these factors in the endometrial angiogenesis-vessel remodeling cycle, however, remain to be elucidated.

Experimental model for endometrial regeneration and angiogenesis

The research on endometrial regeneration and angiogenesis is complicated by major species differences between the menstrual cycle in humans and primates and the estrus cycle in commonly studied rodent models. Although rodent models provide invaluable information, caution is required when translating information to the human menstrual cycle. Furthermore, studies for the molecular and cellular mechanisms for this process are limited by technical difficulties in reproducing menstrual tissue breakdown, shedding, and subsequent tissue regeneration in vitro. Despite the difficulties, the endometrium is an important model for studying physiological angiogenesis in adults well as angiogenesis in pathological conditions such as endometriosis [10, 11]. Endometriosis, one of the most common gynecological diseases, is characterized by the presence of functional endometrial-like tissue outside the uterine cavity. It is an estrogen-dependent disorder associated with substantial morbidity; however, the etiology and pathophysiology are not well elucidated [12, 13].

To study the physiology of the human endometrium and the pathogenesis of endometriosis, a variety of in vivo

animal models have been developed by using the transplantation of autologous or heterologous endometrial cells/tissues or endometriotic tissues [14–24]. The current in vivo models, however, do not completely satisfy the following requirements: (1) the transplanted human tissue must be quantitatively and characteristically uniform in each animal, (2) functional and morphological changes characteristic of human eutopic and/or ectopic endometrium should be reproduced, and (3) the transplant needs to be assessable for an extended period with noninvasive, real-time, and quantitative measures.

My research group and collaborators recently developed a novel mouse model that meets all of these requirements [25], which confirms the plasticity of endometrial cells. We have shown that a small number of singly dispersed human endometrial cells (SDECs), transplanted beneath the kidney capsule of severely immunodeficient NOD/SCID/γc null (NOG) mice, regenerates functional endometrial tissue [25]. This artificially generated endometrium resembles the natural endometrium and contains human blood vessels that invade the mouse kidney parenchyma. Interestingly, human-originated vasculature formed chimeric vessels with the host endothelium and functioned as a circulation system in the reconstructed endometrium. Additionally, it mimics normal hormone dependent changes including proliferation, differentiation (Fig. 2a), and tissue breakdown, i.e., menstruation (Fig. 2b).

Many angiogenic factors produced by endometrial explants and/or the peritoneal cavity [10, 11, 26, 27] have been shown to enhance angiogenesis, presumably through recruitment of endothelial cells from the host tissue, leading to the acquisition of an adequate blood supply for the establishment and survival of endometriotic explants [28]. Several experiments employing immunodeficient mice implanted with human endometrial tissues have shown that endometriotic lesions derive their blood supply from the surrounding vascular network [21, 29, 30]. Furthermore, it has been demonstrated that native human-originated graft vessels disappear gradually, but instead host vessels invade during revascularization of endometrial explants [21, 30–32]. The predominance of human-derived vessels connected with mouse vessels as presented here suggests that endothelial cells or their progenitors derived from human endometrium have a unique angiogenic potential to migrate, invade, and form the vasculature in host tissue, even when originating from a different species. Interestingly, tumor endothelium has, at the molecular level, characteristics distinct from normal endothelial cells that may endow the tumor-derived endothelial cells with a unique angiogenic potential [33]. In support of this claim, Bussolati et al. [34] have reported that tumor-derived endothelial cells, but not normal endothelial cells, possess a unique neo-angiogenic ability to grow in immunodeficient

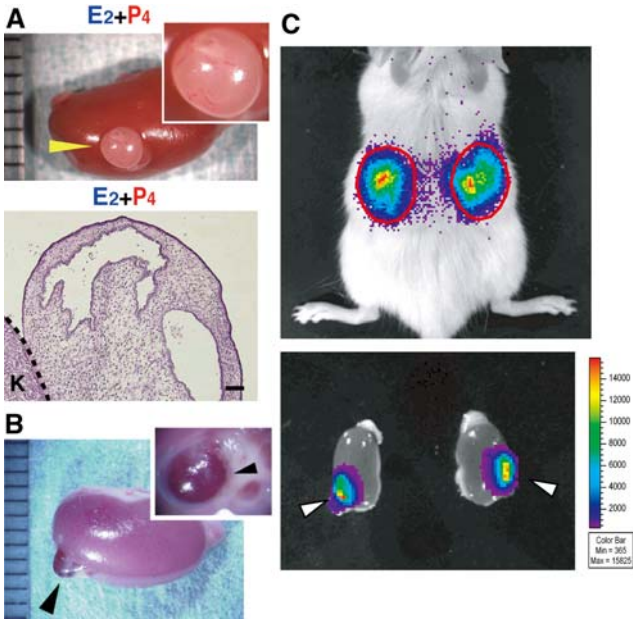

Fig. 2 Regeneration model of human endometrium. **a** Macroscopic and microscopic findings of the transplant site (*arrowhead*) in NOG mice 10 weeks after xenotransplantation. H&E staining was performed on the transplanted lesion of NOG mice treated with estradiol (E2) in combination with progesterone (E2+P4). Macroscopic findings of the transplant site (*arrows*) of a NOG mouse treated with cyclic E2+P4. The borders between the reconstituted tissue and the mouse kidney (K) are indicated by the *dotted lines*. *Bar* 100 μm. **b** Macroscopic findings of the transplanted site (*arrows*) of a NOG mouse treated with cyclic E2+P4. **c** Bioluminescence images of the endometrial reconstructs expressing luciferase in a ventrally positioned NOG mouse treated with E2 alone (*upper*) and its excised kidneys (*lower*). (Reproduced with permission from Masuda et al. [25])

mice and to form vascular structures linked with the mouse circulation. It is therefore conceivable that, in addition to the peritoneal environment [26], the angiogenic potential of the endometrial endothelial cells per se may be one of the critical determinants for the establishment and development of the endometriotic lesion. Anti-angiogenic therapy has been recently proposed as a potential alternative treatment for endometriosis [31, 35, 36]. This therapeutic strategy might be further strengthened by targeting endometrium- or endometriosis-specific angiogenesis.

A discrepancy between the high incidence of retrograde menstruation and the lower prevalence of endometriosis clearly suggests that there must be other factors which contribute to the pathogenesis of endometriosis [37]. There is substantial evidence that alterations in cell-mediated and humoral immunity, including impaired NK cell cytotoxicity, result in the inadequate removal of ectopic endometrial cells from the peritoneal cavity [37]. As NK cells play a critical role in the IL-12-mediated inhibition of angiogenesis [38], the lack of human endometrial graft-originated vessels in SCID and nude mice may be attributable to the

presence of functioning NK cells [21, 29–32]. In contrast, since NK cells are functionally incompetent in the NOG mouse [39], the human-originated neo-vascularization is allowed to take place. Taking these findings together, it is tempting to speculate that the main target of NK cells is endometrial endothelial cells or their progenitors, rather than stromal or glandular cells. In this context, NK cells may allow the attachment and ectopic implantation of retrograde menstrual fragments at an early stage; however, they may most often inhibit the subsequent angiogenesis, thereby eliminating such endometrial explants.

Bioluminescence imaging (BLI) recently has emerged as a useful tool for tumor, hematopoietic, and neural cell tracking studies in living animals [40, 41]. We assessed the dynamic state of the endometrial reconstructs derived from the genetically engineered SDECs by in vivo BLI. For this purpose, SDECs were infected with a lentivirus expressing a variant luciferase reporter gene prior to transplantation beneath the kidney capsule, on the dorsal side, in ovariectomized NOG mice. In vivo BLI revealed that the growth pattern of the reconstructed tissue derived from lentiviral-engineered cells could be assessed noninvasively, quantitatively, and sequentially, as determined by the magnitude of photon counts generated by the luciferase reaction [25] (Fig. 2c). Thus, combining the unique potential of SDECs together with NOG mice and lentivirus-mediated cell engineering, we present a novel animal model suitable for the study of endometrial physiology/pathophysiology and drug testing and gene target validation not only in endometrium-derived disorders, but also in various other types of neoplastic disease.

Stem/progenitor cells in human endometrium

The concept that endometrial regeneration is mediated by endometrial stem/progenitor cells has been proposed for many years [6, 42, 43]. Indirect evidence has accumulated from proliferation studies, clinical observations, and the demonstration of gland monoclonality [3, 44]. Shedding of the endometrial functionalis layer at menstruation and its subsequent regeneration from the endometrial basalis suggests that the proliferation kinetics differ between the two layers [45], and that putative endometrial stem cells reside in the basalis. Since the endometrium is comprised of glands, surface epithelium and supportive stroma, it is plausible that there may exist both epithelial and stromal stem/progenitor cells responsible for the regenerative capacity of the endometrium [3, 7].

The first published evidence for the existence of endometrial stem/progenitor cells comes from a recent study that identified clonogenic human endometrial epithelial and stromal cells [7]. Clonogenicity, the ability of a single cell to initiate a colony of cells when cultured at extremely low seeding densities, is a classic in vitro stem/progenitor cell property. Clonogenicity has been demonstrated for many adult stem cell types [3, 46]. Human endometrial stromal cells are significantly more clonogenic than endometrial epithelial cells, although both cell types form large and small colonies [7]. Endometrial stromal cells demonstrate a trend for higher clonogenicity in the proliferative stage, whereas for epithelial cells, this occurs in the secretory stage [47]. Self-renewal or the ability to produce identical daughter stem cells is required to maintain the stem cell pool in tissues (Fig. 1). Asymmetric cell division is one mechanism for producing an identical daughter cell and more differentiated daughter. However, stem cells also undergo symmetric divisions either producing daughter stem cells or transit amplifying (TA) progenitors (Fig. 1). In summary, these studies identifying clonogenic epithelial and stromal cells suggest that two types of stem/progenitor cell exist in the human endometrium. They are present in different proportions, have differing growth factor requirements, and are likely to have specific niches in the endometrium. Current studies examining stem cell attributes of the rare epithelial and stromal cells have demonstrated their high proliferative potential as they undergo 30–32 population doublings before senescence or transformation [3]. Human endometrial clonogenic cells exhibit multilineage differentiation and similar to bone marrow and adipose tissue MSC differentiating into mesenchymal lineages; adipocytes, smooth muscle cells, chondrocytes and osteoblasts, in vitro [48]. This suggests that the rare endometrial clonogenic cells have characteristic properties of endometrial stem cells which are probably responsible for the remarkable, cyclical and regenerative capacity of human endometrium.

Side population (SP) cells are a small fraction of cells within tissues with characteristic dye-effluxing properties, due to the unique ability to efflux the DNA-binding dye Hoechst 33342 via the ATP-binding cassette transporter G2 (ABCG2) [49, 50]. They are detected by dual wavelength flow cytometric analysis after incubation with Hoechst 33342. SP cells have been isolated from various adult tissues, demonstrating that this phenotype may represent a common feature of adult stem cells [50]. The potential of SDECs for in vivo self-tissue regeneration [25] prompted us to hypothesize that SDECs may contain putative endometrial stem/progenitor cells. Taking advantage of the SP cell-based isolation method, my group and collaborators successfully and stably isolated endometrial SP (ESP) cells from SDECs (manuscript submitted). Several groups thus far have identified several endometrial cell subpopulations including clonogenic endometrial cells [7], ESP cells [51], CD146+PDGF-Rb+ stromal cells [48] and CD29+ CD73+CD90+ stromal cells [52] as candidates for

endometrial stem/progenitor cells, whose phenotypic and functional stem-like properties, however, have been characterized only in vitro. To address whether the stem cells were indeed responsible for the cyclical regeneration of the endometrium, we tested whether ESP reconstituted the endometrial tissue with stromal and glandular structures when xenotransplanted into NOG mice. We found that ESP cells reconstituted various endometrial tissue components or the entire endometrium with well-delineated glandular structures when transplanted under the kidney capsule of NOG mice (manuscript submitted).

One of the retrospective approaches for the study of adult stem/progenitor cell activity in the endometrium involves the analysis of methylation patterns in endometrial glands [53]. Epigenetic changes to DNA sequences, arising during cell division, encode a cellular history in individual glands. This history reflects the methylation patterns arising in resident stem/progenitor cells, since the changes are inherited in subsequent cell divisions and retained. In contrast, changes arising in more mature progeny are lost when these cells are shed. Mathematical models describing the methylation patterns observed in individual glands from cycling and atrophic human endometrium support the concept that an individual gland contains a stem cell niche. Within each niche is an unknown number of long-lived stem cells rather than a single stem cell [53]. Finally, gland diversity is maintained in the aging endometrium, which indicates that a reservoir of stem cells remains even when the endometrium is atrophic. This observation supports the data from the clonogenicity studies [47].

It has been suggested that the bone marrow is the potential source of human endometrial epithelial and stromal stem/progenitor cells [54]. Significant chimerism, ranging from 0.2 to 52%, is detectable in the endometrial glands and stroma of women who have received single-antigen, HLA mismatched, bone marrow transplants. This suggests that bone marrow stem cells contribute to endometrial regeneration in the setting of cellular turnover and inflammatory stimuli [54]. Most gland profiles are exclusively of the donor or host type, although there is some chimerism within individual glands. This suggests that not all are monoclonal, consistent with the methylation pattern data of individual glands [53]. In the case of women who received transplants as children, stem cells from their mothers may persist, as bidirectional cell trafficking occurs during pregnancy. Thus the ultimate source of endometrial stem/progenitor cells is currently uncertain. Neither is it known whether bone marrow-derived or fetal-derived cells regularly engraft the endometrium under normal physiological conditions or if they incorporate into endometrial tissue in the basalis, functionalis, or both regions.

Stem/progenitor cells in human myometrium

The human uterus, which is mainly composed of myometrial cells, displays considerable pregnancy-induced expansion multiple times. This process may be repeated over 20 times, throughout a woman's reproductive life. Both myometrial hyperplasia (an increase in cell number) and hypertrophy (an increase in cell size) are thought to contribute to the dramatic growth of the pregnant uterus in humans and rodents [55]. In the human, uterine growth during pregnancy is predominantly accomplished by stretch-induced myometrial hypertrophy. In the first weeks of pregnancy, however, significant myometrial hyperplasia also occurs, and is a more significant contributor to growth than hypertrophy [55, 56]. The pregnant rat uterus is similar. Myometrial hyperplasia is high during early gestation and decreases dramatically later, while myometrial hypertrophy is low at the beginning of pregnancy but increases considerably with gestational age [56]. I hypothesize that the myometrium harbors a population of stem cells with the potential to contribute to the enlargement of the pregnant uterus.

To prove my hypothesis, my group and collaborators isolated a side population of cells (myoSP), characterized by a distinct Hoechst dye efflux pattern, from non-pregnant human myometrium [57]. In brief, non-pregnant myometria were collected from consenting patients undergoing hysterectomy and mechanically and enzymatically digested. Dissociated myometrial cells were stained with Hoechst dye and subjected to flow cytometry sorting to isolate myometrial SP cells (myoSP) as well as the ESP. Flow cytometric analysis revealed that the stained cells contained a small fraction of SP cells. RT-PCR analysis showed that myoSP cells prominently and exclusively expressed ABCG2 mRNA, another characteristic of the SP phenotype. They differentiated into multilineage cell types in the differentiation-inducing media, and, when transplanted in the NOG mice, regenerated myometrium-like tissues (Fig. 3). These findings, as with the ESP, were not observed in the non side population cells (non-myoSP). We have demonstrated that myoSP cells exist in the human myometrium and that purified myoSP, but not the non-myoSP, meet the following adult stem cell criteria: quiescent cell cycle status and the potential for in vitro multidifferentiation and in vivo reconstitution of the original tissue [57]. Thus, we for the first time, isolated, cultured, and identified the myoSP as putative human myometrial stem cells.

Arango et al. reported that Müllerian duct mesenchyme-specific disruption of β-catenin resulted in a progressive turnover of the uterine myometrium to adipose tissue [58]. This supports the possibility that putative myometrial stem cells, with the potential for differentiation into adipocytes in the absence of β-catenin, may exist in the myometrium.

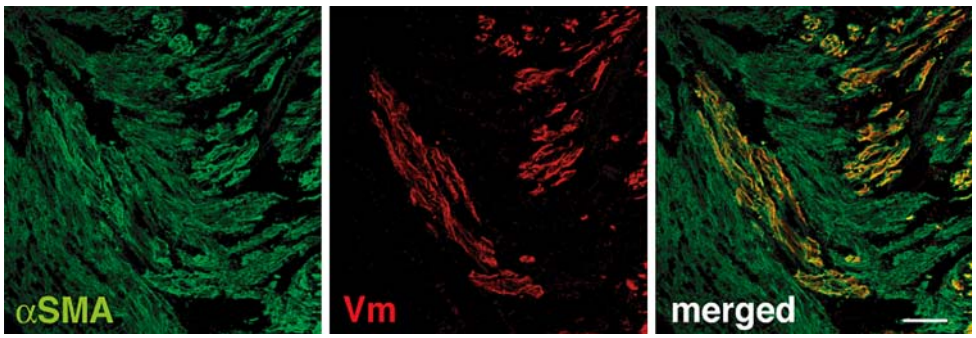

Fig. 3 In vivo reconstitution of myometrium from myoSP in the E2-treated uteri of NOG mice. NOG mice were ovariectomized and xenotransplanted with myoSP or myoMP into their uteri, s.c. implanted with an E2 pellet, and hysterectomized 10 weeks after transplantation. The excised uteri were subjected to immunofluorescence staining using antibodies against α-smooth muscle actin (αSMA; smooth muscle cell marker) and human vimentin (Vm), followed by DAPI staining. Note that mature human myometrial cells doubly positive for αSMA and Vm were found in 10 of the 16 uteri transplanted with myoSP but in none of the 16 myoMP-transplanted uteri. *Bar* 50 μm. (Reproduced with permission from Ono et al. [57])

These cells may give rise to lipoleiomyomas. Indeed, the same group has recently isolated a myometrial SP from the mouse uterus and provided evidence that this SP contains putative myometrial stem/progenitor cells derived from the Müllerian duct mesenchyme [59]. This finding supports our results on the enrichment of stem cells in myoSP in humans [57].

Concluding remarks

There are currently increasing bodies of evidence to conclude that rare populations of adult stem cells exist in the human myometrium and endometrium. The study of stem cells in the female reproductive tract, however, is still in its beginnings. Identification of definitive markers for both myometrial and endometrial stem cells is one of the most pressing needs. A thorough characterization of uterine stem cells is a prerequisite for understanding the complex mechanisms underlying the morphogenesis and physiological regeneration of the female reproductive tract and gynecological diseases including uterine cancer, hyperplasia, endometriosis, leiomyomas, and adenomyosis. Furthermore, these stem cells might also represent a novel source of biological material that could be used for the reconstruction of not only the human uterus but other organs as well.

Acknowledgments I thank members of my research group, Yasunori Yoshimura, Hideyuki Okano, and Yumi Matsuzaki for their generous assistance and collaboration with this project. This study was supported, in part, by Grants-in-Aid from the Japan Society for the Promotion of Science (to T.M., Y.Y), by a National Grant-in-Aid for the Establishment of High-Tech Research Center in a Private University (to T.M.), and by a Grant-in-Aid from the 21st Century Centers of Excellence program of the Ministry of Education, Science, and Culture of Japan at Keio University.

References

1. Passier R, van Laake LW, Mummery CL. Stem-cell-based therapy and lessons from the heart. Nature. 2008;453:322–9.
2. Yu J, Thomson JA. Pluripotent stem cell lines. Genes Dev. 2008;22:1987–97.
3. Gargett CE. Uterine stem cells: what is the evidence? Hum Reprod Update. 2007;13:87–101.
4. Ono M, Maruyama T, Yoshimura Y. Regeneration and adult stem cells in the human female reproductive tract. Stem Cells Cloning Adv Appl. 2008;1:23–9.
5. Maruyama T, Yoshimura Y. Molecular and cellular mechanisms for differentiation and regeneration of the uterine endometrium. Endocr J. 2008;55:795–810.
6. Padykula HA, Coles LG, Okulicz WC, Rapaport SI, McCracken JA, King NW Jr, et al. The basalis of the primate endometrium: a bifunctional germinal compartment. Biol Reprod. 1989;40:681–90.
7. Chan RW, Schwab KE, Gargett CE. Clonogenicity of human endometrial epithelial and stromal cells. Biol Reprod. 2004;70:1738–50.
8. Jabbour HN, Kelly RW, Fraser HM, Critchley HO. Endocrine regulation of menstruation. Endocr Rev. 2006;27:17–46.
9. Strauss III JF, Lessey BA: The structure, function, and evaluation of the female reproductive tract. In: Strauss III JF, Barbieri RL, editors. Yen and Jaffe's Reproductive Endocrinology. Physiology, Pathophysiology, and Clinical Management, 5th ed. Amsterdam: Elsevier Saunders; 2004. p. 255–305.
10. Donnez J, Smoes P, Gillerot S, Casanas-Roux F, Nisolle M. Vascular endothelial growth factor (VEGF) in endometriosis. Hum Reprod. 1998;13:1686–90.
11. Fujishita A, Hasuo A, Khan KN, Masuzaki H, Nakashima H, Ishimaru T. Immunohistochemical study of angiogenic factors in endometrium and endometriosis. Gynecol Obstet Invest. 1999;48(Suppl 1):36–44.
12. Giudice LC, Kao LC. Endometriosis. Lancet. 2004;364:1789–99.
13. Bulun SE. Endometriosis. N Engl J Med. 2009;360:268–79.
14. Zamah NM, Dodson MG, Stephens LC, Buttram VC Jr, Besch PK, Kaufman RH. Transplantation of normal and ectopic human endometrial tissue into athymic nude mice. Am J Obstet Gynecol. 1984;149:591–7.
15. Bergqvist A, Jeppsson S, Kullander S, Ljungberg O. Human uterine endometrium and endometriotic tissue transplanted into nude mice. Morphologic effects of various steroid hormones. Am J Pathol. 1985;121:337–41.

16. Zaino RJ, Satyaswaroop PG, Mortel R. Histologic response of normal human endometrium to steroid hormones in athymic mice. Hum Pathol. 1985;16:867–72.
17. Aoki D, Katsuki Y, Shimizu A, Kakinuma C, Nozawa S. Successful heterotransplantation of human endometrium in SCID mice. Obstet Gynecol. 1994;83:220–8.
18. Bruner KL, Matrisian LM, Rodgers WH, Gorstein F, Osteen KG. Suppression of matrix metalloproteinases inhibits establishment of ectopic lesions by human endometrium in nude mice. J Clin Invest. 1997;99:2851–7.
19. Awwad JT, Sayegh RA, Tao XJ, Hassan T, Awwad ST, Isaacson K. The SCID mouse: an experimental model for endometriosis. Hum Reprod. 1999;14:3107–11.
20. Tabibzadeh S, Miller S, Dodson WC, Satyaswaroop PG. An experimental model for the endometriosis in athymic mice. Front Biosci. 1999;4:C4–9.
21. Grümmer R, Schwarzer F, Bainczyk K, Hess-Stumpp H, Regidor PA, Schindler AE, et al. Peritoneal endometriosis: validation of an in vivo model. Hum Reprod. 2001;16:1736–43.
22. Greenberg LH, Slayden OD. Human endometriotic xenografts in immunodeficient RAG-2/gamma(c)KO mice. Am J Obstet Gynecol. 2004;190:1788–95.
23. Fortin M, Lepine M, Merlen Y, Thibeault I, Rancourt C, Gosselin D, et al. Quantitative assessment of human endometriotic tissue maintenance and regression in a noninvasive mouse model of endometriosis. Mol Ther. 2004;9:540–7.
24. Matsuura-Sawada R, Murakami T, Ozawa Y, Nabeshima H, Akahira J, Sato Y, et al. Reproduction of menstrual changes in transplanted human endometrial tissue in immunodeficient mice. Hum Reprod. 2005;20:1477–84.
25. Masuda H, Maruyama T, Hiratsu E, Yamane J, Iwanami A, Nagashima T, et al. Noninvasive and real-time assessment of reconstructed functional human endometrium in NOD/SCID/γ_c^{null} immunodeficient mice. Proc Natl Acad Sci U S A. 2007;104:1925–30.
26. Gazvani R, Templeton A. Peritoneal environment, cytokines and angiogenesis in the pathophysiology of endometriosis. Reproduction. 2002;123:217–26.
27. Fujimoto J, Sakaguchi H, Hirose R, Wen H, Tamaya T. Angiogenesis in endometriosis and angiogenic factors. Gynecol Obstet Invest. 1999;48(Suppl 1):14–20.
28. Taylor RN, Lebovic DI, Mueller MD. Angiogenic factors in endometriosis. Ann N Y Acad Sci. 2002;955:89–100. discussion 118, 396–406.
29. Nisolle M, Casanas-Roux F, Donnez J. Early-stage endometriosis: adhesion and growth of human menstrual endometrium in nude mice. Fertil Steril. 2000;74:306–12.
30. Bruner-Tran KL, Webster-Clair D, Osteen KG. Experimental endometriosis: the nude mouse as a xenographic host. Ann N Y Acad Sci. 2002;955:328–39.
31. Hull ML, Charnock-Jones DS, Chan CL, Bruner-Tran KL, Osteen KG, Tom BD, et al. Antiangiogenic agents are effective inhibitors of endometriosis. J Clin Endocrinol Metab. 2003;88:2889–99.
32. Eggermont J, Donnez J, Casanas-Roux F, Scholtes H, Van Langendonckt A. Time course of pelvic endometriotic lesion revascularization in a nude mouse model. Fertil Steril. 2005;84:492–9.
33. St Croix B, Rago C, Velculescu V, Traverso G, Romans KE, Montgomery E, et al. Genes expressed in human tumor endothelium. Science. 2000;289:1197–202.
34. Bussolati B, Deambrosis I, Russo S, Deregibus MC, Camussi G. Altered angiogenesis and survival in human tumor-derived endothelial cells. FASEB J. 2003;17:1159–61.
35. Dabrosin C, Gyorffy S, Margetts P, Ross C, Gauldie J. Therapeutic effect of angiostatin gene transfer in a murine model of endometriosis. Am J Pathol. 2002;161:909–18.
36. Nap AW, Griffioen AW, Dunselman GA, Bouma-Ter Steege JC, Thijssen VL, Evers JL, et al. Antiangiogenesis therapy for endometriosis. J Clin Endocrinol Metab. 2004;89:1089–95.
37. Seli E, Arici A. Endometriosis: interaction of immune and endocrine systems. Semin Reprod Med. 2003;21:135–44.
38. Yao L, Sgadari C, Furuke K, Bloom ET, Teruya-Feldstein J, Tosato G. Contribution of natural killer cells to inhibition of angiogenesis by interleukin-12. Blood. 1999;93:1612–21.
39. Ito M, Hiramatsu H, Kobayashi K, Suzue K, Kawahata M, Hioki K, et al. NOD/SCID/γ_c^{null} mouse: an excellent recipient mouse model for engraftment of human cells. Blood. 2002;100:3175–82.
40. Contag CH, Bachmann MH. Advances in in vivo bioluminescence imaging of gene expression. Annu Rev Biomed Eng. 2002;4:235–60.
41. Masuda H, Okano H, Maruyama T, Yoshimura Y, Okano H, Matsuzaki Y. In vivo imaging in humanized mice. Curr Top Microbiol Immunol. 2008;324:179–97.
42. Padykula HA. Regeneration in the primate uterus: the role of stem cells. Ann N Y Acad Sci. 1991;622:47–56.
43. Prianishnikov VA. On the concept of stem cell and a model of functional-morphological structure of the endometrium. Contraception. 1978;18:213–23.
44. Tanaka M, Kyo S, Kanaya T, Yatabe N, Nakamura M, Maida Y, et al. Evidence of the monoclonal composition of human endometrial epithelial glands and mosaic pattern of clonal distribution in luminal epithelium. Am J Pathol. 2003;163:295–301.
45. Brenner RM, Slayden OD, Rodgers WH, Critchley HO, Carroll R, Nie XJ, et al. Immunocytochemical assessment of mitotic activity with an antibody to phosphorylated histone H3 in the macaque and human endometrium. Hum Reprod. 2003;18:1185–93.
46. van Os R, Kamminga LM, de Haan G. Stem cell assays: something old, something new, something borrowed. Stem Cells. 2004;22:1181–90.
47. Schwab KE, Chan RW, Gargett CE. Putative stem cell activity of human endometrial epithelial and stromal cells during the menstrual cycle. Fertil Steril. 2005;84(Suppl 2):1124–30.
48. Schwab KE, Gargett CE. Co-expression of two perivascular cell markers isolates mesenchymal stem-like cells from human endometrium. Hum Reprod. 2007;22:2903–11.
49. Goodell MA, Brose K, Paradis G, Conner AS, Mulligan RC. Isolation and functional properties of murine hematopoietic stem cells that are replicating in vivo. J Exp Med. 1996;183:1797–806.
50. Challen GA, Little MH. A side order of stem cells: the SP phenotype. Stem Cells. 2006;24:3–12.
51. Kato K, Yoshimoto M, Kato K, Adachi S, Yamayoshi A, Arima T, et al. Characterization of side-population cells in human normal endometrium. Hum Reprod. 2007;22:1214–23.
52. Dimitrov R, Timeva T, Kyurkchiev D, Stamenova M, Shterev A, Kostova P, et al. Characterization of clonogenic stromal cells isolated from human endometrium. Reproduction. 2008;135:551–8.
53. Kim JY, Tavare S, Shibata D. Counting human somatic cell replications: methylation mirrors endometrial stem cell divisions. Proc Natl Acad Sci USA. 2005;102:17739–44.
54. Taylor HS. Endometrial cells derived from donor stem cells in bone marrow transplant recipients. JAMA. 2004;292:81–5.
55. Ramsey EM. Anatomy of the human uterus. In: Chard T, Grudzinskas JG, editors. The uterus. Cambridge: Cambridge University Press; 1994. p. 18–40.
56. Shynlova O, Oldenhof A, Dorogin A, Xu Q, Mu J, Nashman N, et al. Myometrial apoptosis: activation of the caspase cascade in the pregnant rat myometrium at midgestation. Biol Reprod. 2006;74:839–49.
57. Ono M, Maruyama T, Masuda H, Kajitani T, Nagashima T, Arase T, et al. Side population in human uterine myometrium displays

phenotypic and functional characteristics of myometrial stem cells. Proc Natl Acad Sci USA. 2007;104:18700–5.
58. Arango NA, Szotek PP, Manganaro TF, Oliva E, Donahoe PK, Teixeira J. Conditional deletion of beta-catenin in the mesenchyme of the developing mouse uterus results in a switch to adipogenesis in the myometrium. Dev Biol. 2005;288:276–83.
59. Szotek PP, Chang HL, Zhang L, Preffer F, Dombkowski D, Donahoe PK, et al. Adult mouse myometrial label-retaining cells divide in response to gonadotropin stimulation. Stem Cells. 2007;25:1317–25.