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Review: Human uterine stem/progenitor cells: Implications for uterine physiology and pathology

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ABSTRACT

The human uterus is composed of the endometrial lining and the myometrium. The endometrium, in particular the functionalis layer, regenerates and regresses with each menstrual cycle under hormonal control. A mouse xenograft model has been developed in which the functional changes of the endometrium are reproduced. The myometrium possesses similar plasticity, critical to permit the changes connected with uterine expansion and involution associated with pregnancy. Regeneration and remodeling in the uterus are likely achieved through endometrial and myometrial stem cell systems. Putative stem/progenitor cells in humans and rodents recently have been identified, isolated and characterized. Their roles in endometrial physiology and pathophysiology are presently under study. These stem/progenitor cells ultimately may provide a novel means by which to produce tissues and organs *in vitro* and *in vivo*.

1. Introduction

Adult stem cells or tissue-specific stem cells are undifferentiated cells which are retained following the completion of embryonic development [1,2]. They are capable of both self-renewal and asymmetric cell division, which results in some cells undergoing terminal differentiation. Somatic stem cells are critical for the maintenance of organ structure and function in that they are required for the replacement of apoptotic cells within organs and for tissue regeneration following damage [1,2].

Somatic stem cells have been identified, through unique cell surface markers, in a wide range of tissues and organs. In the case of the uterus, candidate populations of somatic stem cells have been isolated from the endometrium and myometrium with the "side population (SP) technique" [3,4], which identifies cells based on their ability to efflux Hoechst dye [5–7]. This article reviews current studies on endometrial and myometrial stem/progenitor cells particularly focusing on side population cells isolated from human endometrium and myometrium, discusses their possible roles in endometrial and myometrial physiology and addresses the role of these cells in the pathogenesis of disorders including endometriosis and leiomyomas.

2. Endometrial stem cells

The human endometrium undergoes cyclical breakdown and regeneration under the influence of estrogen and progesterone. Retrograde shedding and ectopic implantation of menstrual endometrial cells and tissue fragments outside of the uterus forms the basis of the implantation theory of endometriosis [8–10]. Exploitation of this theory has enabled *in vivo* modeling of the human endometrium.

Explanted single cell suspensions of human endometrial cells are sufficient to establish functional endometrial tissue in immunodeficient NOD/SCID/$\gamma_c$null (NOG) mouse xenograft models [11]. In this model, the endometrial tissue, which is established beneath the kidney capsule, recapitulates the morphological and functional changes of the menstrual cycle in response to treatment with estrogen and progesterone [11]. This observation illustrates the remarkable regenerative capacity of endometrial cells and alludes to the presence of endometrial stem cells and a unique system of angiogenesis. Indeed, it has been postulated that the endometrium contains such a pool of multipotent stem cells within the deep basalis layer from which endometrial cell component arises [12,13].

Recently, many groups including ours, through a variety of methods, have identified, isolated, and/or characterized putative endometrial stem/progenitor cells capable of pluripotent differentiation [4,10]. Endometrial side population (endoSP) cells are candidate stem cells that exhibit the potential for differentiation into glandular, stromal, endothelial and smooth muscle cells *in vitro* and

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in vivo. They are isolated based on the expression and function of a universal stem cell marker, the ATP-binding cassette sub-family G member 2 (ABCG2) [14]. These ABCG2$^+$ cells largely correspond to endSP cells. They reside in the vascular wall of endometrial small vessels of both the functional and basal layers, and have endothelial progenitor cell (EPC)-like properties [14]. It is plausible that the putative endometrial stem/progenitor cells within the side population may initially trigger neovascularization followed by propagation and differentiation into various cell components of the human endometrium [14]. As endSP cells are also found in the functional layer of the human endometrium [14], this layer may also contribute to endometrial renewal [3].

Stem cell characteristics differ based on the method used to isolate them, and are frequently not reproducible across laboratories. Endometrial side population cells reported in the literature differ with respect to the expression pattern of surface markers, clonal efficiency, preference for culture conditions, and localization in the eutopic normal endometrium [14–17]. Thus, it is unclear how many types of stem cells exist in the human endometrium and what role each plays. To achieve a consensus definition of the endometrial stem cell, our group has recently established a novel *in vivo* endometrial stem cell assay in which multipotential differentiation can be identified through cell tracking [18]. In this assay, endSP and non-endSP cells (namely endometrial main population cells, endMP cells) isolated from whole endometrial cells were infected with lentivirus to express tandem Tomato (TdTom), a red fluorescent protein. They were mixed with unlabeled whole endometrial cells and then transplanted under the kidney capsule of ovariectomized immunodeficient mice. These mice were treated with estradiol and progesterone for eight weeks and then subjected to excision of the kidneys. We found that all of the grafts reconstituted endometrium-like tissues under the kidney capsule. Immunofluorescence revealed that TdTom-positive cells were significantly more abundant in the glandular, stromal, and endothelial cells of the reconstituted endometrium in mice transplanted with TdTom-labeled endSP cells than those with TdTom-labeled endMP cells, indicating the *in vivo* multiple differentiation potentials of endSP. We postulate, therefore, that endSP cells are genuine endometrial stem/progenitor cells [18].

SP-based cell isolation is, however, limited in terms of extreme expensiveness of keeping and using ultraviolet (UV) laser-equipped flow cytometry, cell damage caused by UV exposure, and toxicity of Hoechst dye [7]. Thus, to overcome these obstacles and to facilitate possible future clinical use, it is urgently necessary to identify surface markers to permit selection of the endometrial stem/progenitor cell population.

Indeed, Gargett's group has identified endometrial mesenchymal stem cell-specific surface markers such as CD146, CD140b/PDGFR-β, and W5C5 [19,20]. We have investigated the expression of CD146, CD140b, and W5C5 in endSP cells [18]. We found that there was no difference in the percentages of W5C5-positive cells, purportedly putative human endometrial mesenchymal stem-like cells [20], between endSP and endMP fractions. In contrast, CD140b$^+$CD146$^+$ cells, reportedly putative human endometrial mesenchymal stem-like cells [19], were significantly more abundant in the endSP fraction than in endMP cells [18] substantiating the stem/progenitor cell property of endSP cells.

Endometriosis is endometrium or endometrium-like tissues located outside the uterine cavity. It is frequently associated with a variety of symptoms including dysmenorrhea and dyspareunia [8,9]. Theoretical explanations of endometriosis include retrograde menstruation, lymphatic and vascular metastasis, iatrogenic direct implantation, coelomic metaplasia, embryonic rest, and mesenchymal cell differentiation (induction) [10]. Each theory, however, does not in itself account for all types of endometriotic lesions, implying multiple mechanisms [10]. In light of the role of endometrial stem cells in eutopic endometrial regeneration and differentiation [3,4], a novel mechanism for the origin of endometriotic lesions is that they arise from translocated endometrial stem/progenitor cells [3,4,10,21,22].

Gargett, Sasson and Taylor originally proposed a possible role for endometrial stem cells in the pathogenesis of endometriosis [21,22]. The identification of endSP cells by our group and others [14,17] further strengthens this concept of a "stem cell theory". Additionally, these observations suggest that the endSP also contains the endometriosis-initiating (EMI) cell as they possess the following properties. First, they are present within the functional layer which is translocated during retrograde menstruation (implantation theory). Second, they possess the capacity for attachment, migration and angiogenesis essential for survival at an ectopic site. Third, they possess the multiple differentiation potential to reconstitute the endometrium at an ectopic site. We have shown that endSP cells have all of these characteristics [14]. In addition to lending further credence to the stem cell theory of endometriosis, our endSP cell observations also provide support to the retrograde menstruation theory [10].

3. Myometrial stem/progenitor cells

The human uterus expands its volume up to 1000-fold and its weight more than 20-fold over the course of pregnancy. In humans and rodents, this growth is the result of both hyperplasia and hypertrophy [23]. The uterus involutes postpartum as a result of extensive myometrial cell apoptosis followed by regeneration in order to maintain organ integrity [24]. These processes may occur over 20 times throughout a woman's reproductive life. This regeneration is poorly understood; it is unknown if new smooth muscle cells arise from differentiated cells or from tissue-specific stem cells.

To investigate myometrial stem/progenitor cells, we isolated SP cells from non-pregnant human myometria from patients undergoing hysterectomy [25]. The tissue was mechanically and enzymatically digested to produce cell suspensions that were stained with Hoechst dye and subjected to flow cytometric sorting to isolate the minority fraction of myometrial SP cells (myoSP). The expression level of ABCG2 mRNA was significantly higher in the myoSP than in the main population of myometrial cells (myoMP) [25]. MyoSP also had very low expression levels of estrogen-receptor-α and progesterone receptor, as well as smooth muscle cell-specific markers such as calponin and smoothelin. These observations are consistent with an undifferentiated state [25]. About 98% of myoSP (but only 20% of myoMP) were in the G_0 phase of the cell cycle [25], a trait characteristic of hematopoietic and other tissue-specific stem cells. They were capable of multipotent differentiation when subject to various stimuli, and regenerated myometrium-like tissue when transplanted into both the non-pregnant and pregnant uterus in an immunodeficient mouse xenograft model. These findings were not observed in myoMP. Additionally, transcripts of octamer-binding transcription factor 4 (OCT4)/POU5F1, an embryonic stem cell marker, are more abundant in myoSP than in myoMP [26].

Interestingly, myoSP cells preferentially expand *in vitro* under 2% oxygen tension in comparison to a 20% O_2 environment [25]. These stem cells are well suited for growth in a hypoxic environment, as hypoxia is known to stimulate somatic stem cell growth [27]. This observation supports the hypothesis that myoSP cells are indeed somatic stem cells and likely contribute to uterine enlargement. Mechanical stretching of the uterine wall does result in hypoxia [28]. Therefore, it is plausible that pregnancy-induced mechanical stretching leading to hypoxia may promote the proliferation of myoSP and further uterine enlargement.

It is well known that hypoxia regulates the proliferation, differentiation and function of trophoblast and placenta [29]. Hypoxia