Current understanding of somatic stem cells in leiomyoma formation

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Objective: To provide a detailed summary of current scientific knowledge of somatic stem cells (SSCs) in murine and human myometrium and their putative implication in leiomyoma formation, as well as to establish new therapeutic options.
Design: Pubmed and Scholar One manuscripts were used to identify the most relevant studies on SSCs and their implications in human myometrium and leiomyomas.
Setting: University research laboratory–affiliated infertility clinic.
Patient(s): Not applicable.
Intervention(s): Not applicable.
Main Outcome Measure(s): Not applicable.
Result(s): Despite numerous publications on SSCs, it was not until 2007 that scientific evidence based on the use of 5-bromo-2′-deoxyuridine (BrdU) and side population (SP) methods in murine and human myometrium were first published. Recently, it has been reported that SP cells are present in human leiomyomas; however, to date the pathogenesis of this benign tumor remains unclear. Besides many genetic/epigenetic alterations, changes to steroid hormones and growth factors may also be associated with the impaired function, proliferation, and differentiation of a subset of putative SSCs in human myometrium.
Conclusion(s): These findings open up new possibilities for understanding the origin of this benign tumor and help to develop new nonsurgical approaches for their management. (Fertil Steril® 2014;102:613–20. ©2014 by American Society for Reproductive Medicine.)
Key Words: Myometrium, side population, somatic stem cells, tumor-initiating cells, uterine leiomyomas

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During pregnancy, the human uterus undergoes a 500- to 1,000-fold increase in volume and a 24-fold increase in weight, which is mainly associated with the remarkable capacity of the myometrium to regenerate and remodel itself (1); based on this observation, it is reasonable to assume that the myometrium must be tightly regulated by a resident somatic stem cell (SSC) population (2, 3).

Diverse approaches have been used to identify SSCs in different organs and tissues, such as bone marrow (4, 5), mammary gland (6), skin (7), lung (8), dental pulp (9), and human uterus (10, 11). In this context, label-retaining and xenograft experiments with the use of the 5-bromo-2′-deoxyuridine (BrdU) technique suggest that the myometrium does indeed possess SSCs (Fig. 1). BrdU is a thymidine analogue that is incorporated into DNA during the replication phase of the mitotic cycle, thus labeling the newly formed cells. Thereafter, BrdU signals become diluted with each division cycle (known as the chase period), leaving only label-retaining cells (LRCs) still containing BrdU; these are thought to be SSCs because they divide at a very slow rate, which is one of the main features of SSCs (12).

In 2007, Szotek et al. identified the LRCs in β-catenin–deficient murine myometrium as fat cells, an observation that suggests that putative SSCs in human myometrium may also differentiate into adipocytes in the absence of β-catenin, and it has been postulated that these may give rise to lipoleiomyomas (13, 14). This event is probably related to the initiation and downstream regulation of various biologic processes by the Wnt/β-catenin pathway (15, 16). Wnt signaling is mediated via the cytoplasmic protein Dishevelled which inhibits the activity of the serine-threonine kinase glycogen synthase

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FIGURE 1

References	Main outcome	Model	Method used to isolated SSC	Source Tissue for isolated SSC
Szotek et al; 2007 Stem Cell	The objective of this study was to identify the mouse myometrial smooth muscle progenitor cell and its niche, defining the surface marker phenotype, and showing a functional response of these cells to normal myometrial cycling.	Mouse	5-bromo-2'-deoxyuridine (BrdU) technique/ Side Population(SP) technique	Myometrial cells
Ono et al; 2007 Proc Natl Acad Sci USA	Isolation and characterization of putative myometrial stem cells from the human uterus demonstrating that they possessed stem cell-like properties: an undifferentiated and quiescent phenotype, the potential for multidifferentiation, and the ability to reconstruct the myometrium *in vivo*.	Human	Side Population(SP) technique	Myometrial cells
Tanwar et al; Biol Reprod 2009	Study showing evidences that suggest the dysregulation, of WNT/beta-catenin signaling in putative progenitor myometrial/stem cells with pleiotropic effects on uterine function and tumorigenesis.	Mouse	_____	Myometrial cells
Chang et al; 2010 Reprod Sci	Comparative study between leiomyomas and normal myometrium by evaluating colony forming ability, mesenchymal stem cell markers by evaluating CD90 expression and side population (SP), and differentiation status by CD90 expression patterns after *in vitro* expansion.	Human	Side Population(SP) technique	Myometrial/fibroid cells
Maruyama et al; 2010 Reproduction	Review supporting the existence of uterine stem cell system to explain the physiological remodeling and regeneration of the human uterus and the associated pathogenesis.	Human	Side Population(SP) technique	Endometrial/ myometrial cells
Ono et al; 2010 Hum Reprod	Comparative expression of octamer-binding transcription factor 4 (OCT4) in the stem/progenitor cell population and whole human myometrial cells.	Human	Side Population(SP) technique	Myometrial cells
Zhou et al; 2011 Med Hypotheses	Role of hypoxia conditions as contributor to the pathogenesis of leiomyoma through aberrant estrogen pathway activation of myometrial stem cells.	Human	_____	Myometrial cells
Mas et al; 2012 Fertil Steril	Isolation and characterization of human leiomyoma SP cells as putative tumor-initiating cells and establishment of two leiomyoma SP lines.	Human	Side Population(SP) technique	Fibroid cells
Ono et al; 2012 Plos One	Isolation of leiomyoma-derived side population (LMSP) as responsible for cell proliferation and tumor growth.	Human	Side Population(SP) technique	Myometrial/fibroid cells
Maruyama et al; 2013 Semin Reprod Med	Review about current studies of mouse and human myometrial stem/progenitor cells and their contribution to leiomyoma formation.	Human/ mouse	5-bromo-2'-deoxyuridine (BrdU) / Side Population(SP) technique	Myometrial/fibroid cells

Summary of the most relevant scientific publications describing the presence of somatic stem cells (SSCs) in myometrium and leiomyomas.

Mas. Somatic stem cells in human leiomyoma. Fertil Steril 2014.

kinase 3. This inhibition leads to stabilization and accumulation of the soluble form of β-catenin (15, 16) and induces the accumulation of nonubiquitinated active β-catenin in the cytoplasm; from there it translocates into the nucleus. In the nucleus it functions as a transcription regulator which interacts with lymphoid enhancer/T-cell factor family members to regulate the transcription of target genes, as well as plays a key role in both stem cell self-renewal and their differentiation into smooth muscle cells (15–19).

In humans a candidate myometrial SSC population has also been isolated with the use of the side population (SP) technique, which is based on the ability of these cells to extrude the vital dye Hoechst 33342 through their ATP-binding cassette (ABC) membrane transporters. SSCs have a unique ability to pump this dye out via a membrane-bound ATP-binding cassette mechanism, which results in a low-fluorescence SP "tail" that can be detected by flow cytometry (20–22). Ono et al. were able to isolate SP cells from human myometrium with the use of this method (2), suggesting that these cells can functionally act as myometrial stem cells.

Recently, it has also been suggested that several myometrial pathologies, such as uterine leiomyomas, can be attributed to the dysregulation of these SSCs, or that they might be derived from committed cells that acquire stem-like features (23, 24). In fact, our group has recently isolated SP cells from human leiomyomas and tested their functional relevance in a xenotransplantation animal model, finding that these cells give rise to this benign tumor in vivo (25). However, although there are few articles published on this topic (26, 27), myometrial stem cells are still in the early phases of study and more information is needed to understand their relevance in myometrial physiology and pathology.