ORIGINAL ARTICLE: ENDOMETRIOSIS

Stanniocalcin-1 expression in normal human endometrium and dysregulation in endometriosis

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Objective: To determine expression of stanniocalcin-1 (STC1) in human endometrium with and without endometriosis and its regulation by steroid hormones.
Design: Laboratory study.
Setting: University.
Patient(s): Nineteen women with endometriosis and 33 control women.
Intervention(s): Endometrial biopsy and fluid sampling.
Main Outcome Measure(s): Analysis of early secretory (ESE) and midsecretory (MSE) endometrial secretomes from fertile women with the use of nano–liquid chromatography–dual mass spectrometry; real-time quantitative polymerase chain reaction, and immunohistochemistry for STC1 and its receptor calcium-sensing receptor (CASR) mRNA and proteins in endometrium with and without endometriosis; evaluation of STC1 and CASR mRNA expression in endometrial stromal fibroblasts (eSF) from women with and without endometriosis decidualized with the use of E_2P or 8-bromo-cyclic adenosine monophosphate (cAMP).
Result(s): STC1 protein was strongly up-regulated in MSE versus ESE in endometrial fluid of fertile women. STC1 mRNA significantly increased in MSE from women with, but not from those without, endometriosis, compared with proliferative endometrium or ESE, with no significant difference throughout the menstrual cycle between groups. STC1 mRNA in eSF from control women increased >230-fold on decidualization with the use of cAMP versus 45-fold from women with endometriosis, which was not seen on decidualization with E_2/P. CASR mRNA did not exhibit significant differences in any condition and was not expressed in isolated eSF. STC1 protein immunoexpression in eSF was significantly lower in women with endometriosis compared with control women.
Conclusion(s): STC1 protein is significantly up-regulated in MSE endometrial fluid and is dysregulated in eutopic endometrial tissue from women with endometriosis. It is likely regulated by cAMP and may be involved in the pathogenesis of decidualization defects. (Fertil Steril® 2016;■:■–■. ©2016 by American Society for Reproductive Medicine.)
Key Words: Stanniocalcin-1, human endometrium, endometriosis, stromal fibroblasts, decidualization, proteomics

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Human endometrium is a dynamic tissue that undergoes cyclic morphologic and molecular changes under a changing hormonal milieu and plays a central role in human implantation. Endometrial tissue in humans and other mammals is a target of extensive research with the goal of increasing understanding of its physiology and pathophysiology and improving treatment of gynecologic pathologies such as endometriosis,

adenomyosis, recurrent pregnancy loss, as well as implantation failure and unexplained infertility.

High-throughput "omics" studies of global gene expression profiling in human endometrium have identified stanniocalcin-1 (STC1) as an important player in normal and diseased endometrial functions. STC is expressed in pre-pregnancy endometrium and early pregnancy decidua in rats and pigs, is regulated by estrogen and progesterone, and has been suggested as an implantation marker in pig endometrium (1, 2). In humans, consistent midsecretory endometrial (MSE) STC1 gene expression was demonstrated in patients who conceived with the help of assisted reproductive technologies (3). STC1 was significantly up-regulated in microarray analysis of MSE of the normal menstrual cycle compared with early secretory endometrium (ESE) (4), and was down-regulated in microarray analysis of MSE from women with unexplained infertility versus fertile control women (5), suggesting a possible role in human endometrial receptivity and implantation. Placental expression of STC1 was documented in a well designed microarray study of women with pregnancy complications, which demonstrated increased STC1 in placenta and serum at term in women with pregnancy complications, particularly preeclampsia and small-for-gestational-age babies (6).

STC1 is a glycoprotein phosphorylated by protein kinase C (7), initially described in a bony fish, and produced by unique endocrine glands, the corpuscles of Stannius, eliciting antihypercalcemic and antihypophosphatemic responses in various tissues (8, 9). In mammals, STC1 is expressed in a wide variety of tissues, and interestingly is not detected in the circulation under normal conditions except during pregnancy (9–11), suggesting an autocrine/paracrine rather than an endocrine function. STC1 in mammals is not necessarily directly linked to calcium/phosphorus pathways, but rather is regulated by multiple factors (9). Its roles in calcium homeostasis, bone, and muscle formation, angiogenesis, and reproduction were demonstrated in experiments with transgenic mice that experienced growth retardation and small litter size (11, 12).

The STC receptor, calcium-sensing receptor (CASR), is a G-protein–coupled receptor initially identified in bovine parathyroid cells and expressed in wide variety of tissues, including ovaries and uterus in human and rodents (13–15). Its major physiologic role is thought to be maintenance of calcium homeostasis, including regulation of secretion, gene expression, cell proliferation, differentiation, and apoptosis (13, 16). CASR was found to mediate STC1 secretion in response to extracellular calcium fluctuation in fish (17). It is expressed in first-trimester and term human placenta (18, 19) and is induced during implantation and decidualization in rat uterus (15).

Based on the above, there are limited data on human endometrial STC1 expression, regulation, and signaling through its receptor. We therefore aimed to investigate expression of STC1 and its receptor in human endometrium and regulation of STC1 in human endometrial stromal fibroblasts (eSF) in women without and with endometriosis in an effort to determine potential roles for this protein/receptor complex in normal and abnormal endometrial function.

MATERIALS AND METHODS

Study Subjects and Materials

Endometrial samples were obtained through the University of California–San Francisco (UCSF) National Institutes of Health Human Endometrial Tissue and DNA Bank and from healthy volunteers at the Department of Obstetrics and Gynecology, Karolinska University Hospital, Huddinge, Sweden, and Tartu University Women's Clinic, Tartu, Estonia, with appropriate Institutional Review Board (UCSF) and Ethics Committee (Karolinska Institute and University of Tartu) approvals. Written informed consents were obtained from all participating subjects. Table 1 summarizes characteristics of all participating women and the techniques applied for analyzing the samples.

Endometrial secretome samples for proteomics analysis were collected from early secretory (ESE, LH+2) and midsecretory (MSE, LH+8) phase endometria during the same menstrual cycle from fertile healthy volunteers (n = 6; age 31 ± 1.5 y). The day of the LH surge (LH+0) was determined with a urinary ovulation prediction test (Kaigert), which is an accepted and validated method for predicting ovulation in the clinical and translational research setting; it is also patient friendly, because it is a noninvasive test applicable for self-use at home. However, being aware of substantial interpatient variability, we supported our endometrial dating with endometrial histology. Histology samples were collected for confirmation of endometrial dating according to the criteria of Noyes et al. (20) and were found to correspond to days 19–24 of the 28-day cycle.

Endometrial biopsies for polymerase chain reaction (PCR), immunohistochemistry (IHC), and cell culture experiments were obtained from subjects with and without endometriosis (n = 19 and n = 27, respectively). Menstrual cycle phase was assigned by the day of LH surge and endometrial histology as above. In addition, all samples used for mRNA expression analysis were assigned cycle phase by means of bioinformatics methods (21). Only samples where all evaluation criteria were in agreement were used in the study.

Control subjects (no endometriosis) were healthy women 35.6 ± 0.86 years of age (range 23–49 y) undergoing gynecologic surgery for pelvic pain (with no endometriosis found during laparoscopy) or management of fibroids, healthy volunteers without uterine pathology, or women undergoing laparoscopic tubal ligation. Control subjects had regular menstrual cycles, were not pregnant, had no history of endometriosis, and had not been on hormonal treatment for at least 3 months before tissue sampling.

Women with endometriosis participating in the study were 35.9 ± 1.65 years of age (range 22–49 y), were not pregnant, and did not use any hormonal medication for at least 3 months before surgery. The diagnosis of endometriosis was based on visualization of lesions during laparoscopy and confirmed by histology. Staging of endometriosis was defined according to the revised American Fertility Society classification system (22).

Out of 19 samples from women with endometriosis and 27 samples from control women (without endometriosis), 14 and 10 whole tissue samples, respectively, were used for real-time quantitative reverse-transcription (RT) PCR (n = 6