Regulation of myeloid ecotropic viral integration site 1 and its expression in normal and abnormal endometrium

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Objective: To identify the expression profile and sex steroid regulation pattern of myeloid ecotropic viral integration site 1 (MEIS1) in endometrium.
Design: Molecular studies in human and animal tissue.
Setting: Reproductive medicine center of a university hospital.
Patient(s) and Animal(s): Women with normal menstrual cycles for male infertility and female infertility with endometriosis. Sexually mature female mice (Kunming White strain).
Intervention(s): Primary cultured endometrial stromal cells, Ishikawa cells, and oophorectomized mice were treated with sex steroid.
Main Outcome Measure(s): MEIS1 expression in the human endometrium during the menstrual cycle, mouse uterus during the peri-implantation period of pregnancy, and eutopic endometrium from patients with endometriosis was analyzed by immunohistochemistry staining and western blot. In addition, MEIS1 expression in response to sex steroid was examined both in vitro and in vivo by immunohistochemistry staining and western blot.
Result(s): MEIS1 expression was markedly increased in endometrium during the implantation period, and in decidualizing stromal cells in human endometrium and murine uterus. Steroid hormones increased MEIS1 expression in primary cultured endometrial stromal cells, Ishikawa cells, and endometrium of oophorectomized mice. The effects of estrogen and progesterone were more marked in oophorectomized mice and were additive. MEIS1 expression was significantly lower in eutopic endometrium compared with normal endometrium in the midsecretory stage.
Conclusion(s): MEIS1 is likely a key mediator between sex steroid and genes for uterine receptivity. Diminished endometrium MEIS1 expression may contribute to implantation failure in endometriosis. (Fertil Steril® 2014;102:856–63. ©2014 by American Society for Reproductive Medicine.)
Key Words: MEIS1, implantation, steroids, endometriosis

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Homeobox (Hox) genes are the key regulators that determine the patterning and segment identity along the anterior–posterior axis of the skeleton as well as a variety of organ systems (1). All Hox genes share a deoxyribonucleic acid (DNA) sequence, called the homeobox, that encodes a DNA binding motif referred to as the homeodomain. Hox genes are transcription factors that control the transcription of their target genes, which are essential for growth control and differentiation during embryogenesis as well as homeostasis (2).

Homeobox A10 (Hoxa10) has been reported to play an important role in embryonic implantation (3–7). Mice with a targeted mutation in the HOXA10 gene ovulate normally and produce normal embryos, but their uteri do not support the implantation of their own or wild-type embryos.

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Our previous results also showed that the HOXA10 pathways play a role in the cell cycle regulation of decidualization (8, 9). In humans, HOXA10 expression is up-regulated during the midluteal phase at the time of implantation. The expression of HOXA10 is altered by steroids, embryo culture fluid, and hydrosalpinges (10, 11).

HOX proteins usually act as a part of hetero-dimeric or hetero-trimeric complexes. It has been shown that the affinity and specificity of DNA binding by HOX proteins is indeed augmented by cofactor interactions. The three-amino-acid loop extension (TALE) family of homeodomain proteins is the most important group of HOX cofactors. The unique TALE motif allows TALE proteins to interact with other homeobox-containing proteins. The myeloid ecotropic viral integration site (MEIS) genes belong to the TALE homeobox family, which interact with HOXA9 and HOXA10 in several body systems.

MEIS proteins are not only the cofactors of other homeodomain-containing proteins, but also play multiple roles in development and disease processes (12–14). MEIS1 was first cloned from a site of viral integration in leukemias (15). In human acute myeloid leukemia, MEIS1 forms multimeric complexes with HOXA9, which binds to target sequences of DNA in order to activate gene transcription (16). Specifically, MEIS1 expression is vital for maintaining Hoxa9-immortalized myeloid cells in an undifferentiated and proliferating state, which is refractory to granulocyte-colony stimulating factor–induced terminal differentiation (17).

Among MEIS homeobox genes, only MEIS1 is expressed in human endometrium (18). MEIS1 expression in myometrium and endometrium was the highest in all 87 different normal human tissue types investigated (19). MEIS1, PBX2, and HOXA10 form trimers to activate or repress target gene expression (18). Our previous research suggested that blocking MEIS1 expression by small interfering ribonucleic acid (siRNA) in the mouse significantly reduces average implantation rates and decreases expression of integrin ß3 (19).

Although the expression of MEIS1 and its interaction with Hox transcription factors have been demonstrated in other systems, and MEIS1 down-regulation significantly reduces average implantation rates (19), the expression status of MEIS1 protein in the endometrium and its regulation by sex steroids have not been previously investigated. In the current study, the MEIS1 expression pattern was examined in normal human endometrium, pregnant mouse uterus, and eutopic endometrium from patients with endometriosis. Results confirmed that MEIS1 was expressed in response to sex steroid in the endometrium both in vitro and in vivo.

MATERIALS AND METHODS

Sample Collection, Preparation, and Biopsy

Endometrial tissues were isolated from women with normal menstrual cycles for male infertility and female infertility with endometriosis. Histological examination revealed normal endometrium. This study was approved by the Tongji Hospital Research and Ethics Committee, and informed consent was obtained before biopsy. Endometrial dating was determined by menstrual history and confirmed by histological examination based on the Noyes criteria (20) by a pathologist who was blinded for clinical outcome. One half of the tissue sample was immediately frozen in liquid nitrogen and stored at −72°C. The other half of the tissue was fixed in formalin, embedded in paraffin, cut into 5-μm sections, and mounted onto slides for immunostaining.

Cell Culture and Treatment

Tissues were washed twice in phenol red–free Dulbecco's modified Eagle's medium (DMEM)/F12 (Invitrogen Corporation), minced, and then enzymatically digested with 0.1% collagenase I (Sigma Chemical Co.) for 30 minutes at 37°C. After centrifugation at 400 g for 5 minutes, the pellet was resuspended in the maintenance medium, which consisted of DMEM/F12 and 10% charcoal-stripped fetal bovine serum (FBS) (Invitrogen Corporation). Endometrial stromal cells were separated from epithelial cells and cultured as previously described (21). The cells were passaged every 3 days and grown to confluence. An 80% confluent monolayer was maintained in serum-free media for 24 hours and subsequently treated with 17β-estradiol (E_2; 1×10^{-8} M; Sigma Chemical Co.) or medroxyprogesterone acetate (MPA; 1×10^{-8} M) (Sigma Chemical Co.) in phenol-free medium containing 2% charcoal-stripped FBS for 24 hours. Ishikawa cells were grown in the same medium and treated the same way as stromal cells.

Animal and Tissue Preparation

Female Kunming mice of reproductive age were oophorectomized and received E_2 and MPA alone, or as a combination injection after 2 weeks of rest. Mice received E_2 (100 ng/mouse) or MPA (2 mg/mouse) singly or in combination. All animal procedures were approved by the Institutional Animal Care and Use Committee of Tongji hospital. Steroids were dissolved in sesame oil and injected subcutaneously (0.1 ml/mouse). Control animals received oil only. Sesame oil and steroid hormones were purchased from Sigma Chemical Co. Mice were sacrificed at 12 hours following injection.

Adult female mice (age 8–10 weeks) were mated with fertile males of the same strain to induce pregnancy (day 1: vaginal plug). Mice were sacrificed in the time period between 8:30 AM and 09:00 AM on each day of pregnancy. Some uteri were fixed in 4% paraformaldehyde, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E). To perform other studies, deciduomal tissues were extracted from mouse uteri and separated from the myometrium. Tissues were rapidly frozen and kept at −72°C for subsequent analysis.

Immunohistochemistry Staining

Slides were deparaffinized and dehydrated through a serious of xylene and ethanol washes. After a 5-minute rinse in distilled water, an antigen-presenting step was performed by steaming the slides in 0.01 M sodium citrate buffer for 20 minutes, followed by removing the staining jar from the steam chamber and cooling it for 20 minutes. Slides