Published in final edited form as:

Fertil Steril. 2011 March 1; 95(3): 1133–1136. doi:10.1016/j.fertnstert.2010.09.060.

HOXA10 expression is decreased in endometrium of women with adenomyosis

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Abstract

HOXA10 gene expression is decreased in secretory phase endometrium of women with adenomyosis. Diminished expression of HOXA10 is a potential mechanism explaining decreased implantation observed in women with adenomyosis.

Keywords

Adenomyosis; HOXA10; infertility; endometrial receptivity; endometrium

Adenomyosis affects up to 19.5% of the female reproductive age population (1,2,3). Adenomyosis is defined as the presence of heterotopic endometrial glands and stroma in the myometrium. The most common presenting symptoms are dysmenorrhea, abnormal uterine bleeding, and an enlarged tender uterus. Approximately, 35% of women with adenomyosis are asymptomatic (4).

The etiology of adenomyosis is poorly understood; however, traditionally it is thought that adenomyosis results from the abnormal growth of basal endometrium into subendometrial myometrium (5). The cause of this in-growth may be mechanical disruption during surgery or pregnancy (6). Alternatively, it may result from dislocation of basal endometrium into myometrium due to dysfunctional uterine hyperperistalsis and/or dysfunctional contractility of the subendometrial myometrium (7).

A connection between adenomyosis and subfertility has not been fully established. Until relatively recently, adenomyosis was thought to account for a minor fraction of infertility; however, as women delay first pregnancies until their thirties and forties, adenomyosis is a more common diagnosis (8). Reproductive outcomes improve after successful treatment of adenomyosis with multiple modalities, including hormonal therapy and conservative surgical therapy (9,10). Thus far there is little molecular data to explain the mechanism behind subfertility in adenomyosis. Moreover, little is known about the effect of adenomyosis on known markers of endometrial receptivity, such as HOXA10.

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HOXA10 is a homeobox-containing transcription factor that is essential for embryonic uterine development and is necessary for proper adult endometrial development during each menstrual cycle (11–15). Its expression is necessary for endometrial receptivity (13,16–19). Implantation failure occurs in mice with targeted mutation of Hoxa10. Interestingly, these mice produce viable embryos, which can implant and develop normally in a wild type surrogate. In contrast, wild-type embryos fail to implant in Hoxa10 deficient mice (20). This phenotype is likely related to both the absence of Hoxa10 during embryonic uterine development and lack of adult maternal Hoxa10 expression during cyclic endometrial development. Reduction of maternal Hoxa10 results in diminished implantation proportional to the level of Hoxa10 expression, indicating that altered levels of this protein regulate the degree of the endometrial receptivity (18).

During the implantation window in the mid-secretory phase, HOXA10 messenger RNA (mRNA) expression is up-regulated in both the endometrial glandular and stromal cells (14,21,22,23). HOXA10 is up-regulated in response to estrogen and progesterone and its levels increase dramatically during the mid-secretory phase in the glands (14,21). HOXA10 has diverse effects on several aspects of adult endometrial development, such as stromal decidualization, leukocyte infiltration, and pinopod development (17,23). Furthermore, HOXA10 regulates downstream target genes that are also involved in implantation, such as β 3 integrin, empty spiracles homeobox 2 (EMX2), insulin-like growth factor-binding protein 1 (IGFBP-1), and Kruppel-like factor 9 (KLF9) (24–27). Defective endometrial HOXA10 expression has been described in association with endometriosis, polycystic ovary syndrome (PCOS), submucosal uterine leiomyomas, and hydrosalpinges, all conditions associated with abnormal implantation (, 22,28–31). Here we explore the possibility that endometrial HOXA10 expression is altered during the mid-secretory phase in women with adenomyosis.

This case-control study included 31 subjects: 19 with adenomyosis and 12 age matched fertile controls. The ages of the women ranged from 25 to 52 years. Tissue samples of endometrium were collected from nineteen women with adenomyosis undergoing hysterectomy. The initial indications for surgery were hypermenorrhea or adnexal mass. Twelve samples of normal endometrium were collected from fertile controls at hysterectomy performed for pelvic organ prolapse or benign adnexal mass. The absence of adenomyosis was confirmed histologically. Menstrual cycle dating was established from the women's menstrual history and was confirmed by endometrial histology to be in the mid-secretory phase. Adenomyosis was confirmed by histopathologic examination of the hysterectomy specimens; The average depth of myometrial invasion was 5.1 mm.

Tissue was embedded in paraffin, cut into 5 μ m sections, and mounted onto slides. Immunohistochemical analysis of HOXA10 expression was performed as previously described (32). Briefly, slides were deparaffinized and dehydrated through a series of xylene and ethanol washes, followed by permeabilization in 95% cold ethanol. After a 5-min rinse in distilled water, slides were steamed in 0.01M sodium citrate buffer for 20 min and cooled for 20 min. Slides were rinsed for 5 min in PBS with 0.1% Tween 20 (PBST), and sections were circumscribed with a hydrophobic pen. Endogenous peroxidase was quenched with 3% hydrogen peroxide for 5 min followed by a 5-min PBST wash. Nonspecific binding was blocked with 1.5% normal horse serum in PBST for 1 hour at room temperature. The primary antibody used was HOXA10 (sc-17158) (1:300) (Santa Cruz, Santa Cruz, CA). Slides were incubated with the primary antibody overnight at 4°C. Normal goat IgG (Santa Cruz, Santa Cruz, CA) was used as a negative control. Horse α -goat biotinylated secondary antibody was purchased from Vector Laboratories (Burlingame, CA) and applied for 1 h at 4°C. The sections were then incubated with biotinylated goat anti-rabbit secondary antiserum for 60 min, avidin and biotinylated peroxidase (Vectastain) for 45 min and

diaminobenzidine (400 mg/ml) for 5 min. A 16-sec exposure to hematoxylin was used as a counterstain. Slides were rehydrated through ethanol and xylene and mounted with Permount.

Immunohistochemistry results were quantified by two evaluators blinded to the diagnosis. Each evaluator inspected four high power fields per slide to determine H-scores of glandular cells and stromal cells. The H-score was calculated using the following equation: $H\text{-score} = \sum Pi (i+1)$. Intensity of HOXA10 nuclear staining is indicated by a value of 0, 1, 2, or 3 (none, weak, moderate, strong), and Pi is the percentage of stained nuclei for each intensity (33,34).

Statistical analyses were performed using Sigma Stat 10.0. The nonparametric Mann-Whitney U test was applied for independent two-group comparisons for both glandular and stromal cells.

Endometrial tissue from 19 women with adenomyosis and 12 fertile controls were immunostained with anti-human HOXA10 as described above. All samples underwent histological evaluation and were found to be normal mid- secretory endometrium.

Immunohistochemical evaluation of HOXA10 protein expression was conducted and representative photomicrographs are shown. (Figure 1) Weak immunoreactivity was observed in stromal cells of endometrial tissue from women with adenomyosis with an H-Score of 1.4. Conversely, strong immunoreactivity for HOXA10 was found in stroma of fertile endometrium with an H-Score of 2.1. HOXA10 expression was significantly decreased in the endometrial stroma of women with adenomyosis compared to controls with a $P < 0.001$. (Figure 2)

Similar immunoreactivity was observed in glandular cells from both subjects with adenomyosis as well as controls.

Adenomyosis is increasingly associated with subfertility in the reproductive age population. There have been few studies that examine endometrial defects as a potential mechanism to explain the poor implantation rates observed clinically. In this study, we found that adenomyosis is associated with decreased HOXA10 expression, likely resulting in reduced levels of implantation.

Decreased expression of HOXA10 during the mid-secretory phase of the menstrual cycle is seen in other conditions associated with impaired implantation, such as polycystic ovary syndrome (PCOS), submucosal uterine leiomyomas, hydrosalpinx, and endometriosis (28,29,30,31,35). Women with PCOS have lower pregnancy rates and increased miscarriage rates (36,37). Furthermore, endometrial biopsies from women with PCOS show decreased expression of HOXA10 mRNA (28). Women with submucosal uterine leiomyomas have decreased expression of HOXA10 mRNA and a global decrease in HOXA10 protein is observed by immunohistochemistry, indicating a general reduction in HOXA10 not simply a change localized over the leiomyoma (30). In the endometrium of women with hydrosalpinges, HOXA10 mRNA was significantly reduced compared to matched fertile controls (29,38). Additionally, hydrosalpinges are associated with decreased in vitro fertilization (IVF) pregnancy rates; pregnancy rates improve after salpingectomy (39–41). Women with endometriosis have lower implantation rates (42). In endometriosis, a condition commonly seen with adenomyosis, HOXA10 expression does not rise normally during the mid-secretory phase (15).

HOXA10 expression is necessary for implantation and endometrial receptivity (14,16–20). Here we demonstrate that in women with adenomyosis, HOXA10 is significantly lower

during the mid-secretory phase compared with fertile controls. Another known marker of endometrial receptivity, leukemia inhibitory factor (LIF), also is associated with endometrial receptivity (43–47). LIF expression has recently been demonstrated to be decreased in women with adenomyosis during the mid-secretory phase (48). In adenomyosis, the decreased expression of both HOXA10 and LIF asserts that a change in the endometrial molecular environment plays a role in decreased implantation and receptivity.

Understanding the mechanism by which HOXA10 expression is reduced will be instrumental in finding potential options for correction. Of the conditions with impaired implantation and decreased HOXA10 expression, the potential molecular mechanism has only been ascertained for endometriosis. In endometriosis we and others have recently described an epigenetic change mediated through methylation of the HOXA10 promoter (32,49,35). Further investigation into a potential mechanism behind decreased HOXA10 levels in adenomyosis is warranted.

A potential mechanism to correct molecular defects that result in suboptimal receptivity may be targeted gene therapy. Murine litter size can be increased with the administration of HOXA10 containing DNA constructs, which increase the amount of HOXA10 in the endometrium (50). Furthermore, gene transfection of the human uterus has been successfully performed, thus we hypothesize that in the future targeted intrauterine gene therapy may be a technique to improve suboptimal implantation in women with adenomyosis (51).

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Figure 1.

HOXA10 immunohistochemistry. Immunohistochemistry identified HOXA10 protein expression in endometrial stroma and glands. Shown are representative photomicrographs demonstrating HOXA10 expression in (A) normal endometrium, (B) endometrium associated with adenomyosis, and (C) negative control omitting primary antibody. H-Scores were determined separately for glands (D) and stroma (E). Mean H score is shown for HOXA10 immunostaining in normal endometrium and adenomyosis associated endometrium in both stromal cells and glandular cells.