AOGS
ACTA Obstetricia et Gynecologica Scandinavica

AOGS SHORT RESEARCH REPORT

Hypermethylation of *HOXA10* gene in mid-luteal endometrium from women with ovarian endometriomas

MASSIMILIANO FAMBRINI[1], FLAVIA SORBI[1], CECILIA BUSSANI[1], RICCARDO CIONI[1], GIOVANNI SISTI[1] & KARIN L. ANDERSSON[2]

[1]*Department of Biomedical, Experimental and Clinical Sciences, Gynecology and Obstetrics Unit, University of Florence, Florence, and* [2]*Cancer Prevention and Research Institute (ISPO), Florence, Italy*

Key words
Endometriosis, epigenetic, *HOXA10* gene, infertility, methylation, ovarian cyst

Correspondence
Massimiliano Fambrini, Department of Biomedical, Experimental and Clinical Sciences, University of Florence, Viale Morgagni, 85, 50134, Florence, Italy.
E-mail: maxfambrini@libero.it

Conflict of interest
The authors have stated explicitly that there are no conflicts of interest in connection with this article.

Please cite this article as: Fambrini M, Sorbi F, Bussani C, Cioni R, Sisti G, Andersson KL. Hypermethylation of *HOXA10* gene in mid-luteal endometrium from women with ovarian endometriomas, Acta Obstet Gynecol Scand 2013; 92:1331–1334.

Received: 28 January 2013
Accepted: 12 August 2013

DOI: 10.1111/aogs.12236

Abstract

A decrease in *HOXA10* gene expression in eutopic mid-secretory endometrium has been found in women with endometriosis-associated infertility. Promoter hypermethylation of *HOXA10* is thought to be the leading mechanism for epigenetic gene regulation in patients with endometriosis. In our series we documented significantly higher *HOXA10* promoter methylation levels in women with ovarian endometriomas than in healthy controls during the mid-luteal phase. Development of epigenetic-based strategies for non-surgical treatment of infertility related to ovarian endometriomas could be an attractive field of research in the coming years.

Introduction

Endometriosis is one of the most common benign gynecological conditions causing pain and infertility (1). Ovarian endometriomas are a frequent manifestation of the disease and surgical removal is usually offered when associated with subfertility (2). However, most of the available literature supports the conclusion that the ovarian reserve is injured by surgical stripping of ovarian endometriomas (3).

Many studies in the last few years demonstrated that the abnormal expression of some genes may contribute to endometriosis-related infertility by altering the endometrial milieu, which then becomes unfavorable for embryo implantation due to toxicity, immune dysfunction, inflammatory or apoptotic responses (4–7). *HOX* genes are developmentally regulated transcription factors that belong to a multigene family (8). These genes contain a conserved sequence element of 183 bp, known as the homeobox (8). *HOXA10* has been found to be expressed in the adult human endometrium and demonstrates a dynamic temporal pattern of expression through the menstrual cycle (9). It has been reported that expression of HOXA10 protein is maximal during the window of implantation in the human endometrium (luteal phase) (10). Preclinical and clinical studies demonstrated that *HOXA10* expression is required for endometrial receptivity. Reduced *HOXA10* expression has been observed in endometrial tissue of infertile women with endometriosis (11).

DNA methylation plays an important role in epigenetic gene regulation. However, it is still unclear whether the observed decreased expression of the *HOXA10* gene in infertile patients is related to its promoter aberrant methylation. Wu et al. (12), analyzing a few women with advanced peritoneal endometriosis, showed that aberrant methylation of the DNA regulatory sequence was associated with reduced *HOXA10* gene expression in the endometrium. However, patients were selected without considering the phase of the menstrual cycle. Szczepanska et al. (13), for the first time in luteal phase, documented the hypermethylation of DNA as a mechanism responsible for silencing *HOXA10* gene expression in women with mild peritoneal endometriosis. In this prospective case-control study, we compared the methylation status of the *HOXA10* gene promoter in the secretory phase eutopic endometrium of women with ovarian endometriomas with healthy women.

Material and methods

After the approval of the internal Ethics Committee (ref. 0028/09), women were recruited and written informed consent was obtained. Endometrial samples were collected at the time of laparoscopy in 11 women with surgically and histologically proven cystic ovarian endometriosis but no gross peritoneal or deep infiltrating endometriosis (Group A). Samples obtained from 11 healthy fertile women with at least one previous pregnancy and no present or past history of endometriosis were used as a control group (Group B).

Transvaginal ultrasound and/or pelvic magnetic resonance imaging were used to exclude adenomyosis or intramural fibroids in all patients and ovarian cystic findings in Group B. Diagnostic hysteroscopy was performed before endometrial sampling in all patients to exclude submucous fibroids, endometrial polyps or malformations. Endometrial specimens were obtained using the Endoram device (RI-MOS, Modena, Italy) or Novak curette on days 19–23 of the menstrual cycle. Samples were split, with a portion sent for histological evaluation.

Genomic DNA was extracted from endometrial tissue samples using an automated system (BioRobot EZ1, QIAGEN, Hilden, Germany). For each sample, 1 μg of genomic DNA was modified with sodium bisulfite conversion. DNA amplification of a CpG-rich fragment within the *HOXA10* gene promoter in the 5' region upstream of the exon 1 (F1) was used, as previously described (3). The amplified region was analyzed using pyrosequencing technology, a real-time DNA-sequencing technique. A sequence primer, identifying 11 CpG sites, was used. Methylation profile was expressed as percentage of methylated sites in amplified region.

Data were compared using Student's *t*-test. Two-tailed *p*-values less than 0.05 were considered significant. The statistical package SPSS 13.0 (SPCC Inc., Chicago, IL, USA) was used throughout.

Results

The mean age of women was 34.4 ± 5.0 years. There was no significant difference in mean age between Group A (34.2 ± 5.9 years) and Group B (34.6 ± 4.2 years). Mean body mass index was 22.3 ± 1.3 in Group A and 22.5 ± 2.1 in Group B (nonsignificant). In Group A, there were 3/11 smokers (27.3%) and in Group B, 4/11 smokers (36.3%) (nonsignificant). All women experienced spontaneous regular cycles of 26–30 days during the 12 months before sample collection. Histological analysis of endometrial samples revealed normal secretive

Table 1. Characteristics of patients in Group A (endometriomas) and Group B (healthy controls).

Group A				Group B			
Patient No.	Age	Day[a]	Methylation (%)[b]	Patient No.	Age	Day[a]	Methylation (%)[b]
1	32	23	11.0	1	30	20	5.5
2	36	20	16.0	2	34	19	8.0
3	26	23	13.0	3	26	23	11.0
4	42	22	11.0	4	28	21	11.0
5	38	23	16.0	5	28	23	9.0
6	34	23	14.0	6	35	19	10.0
7	36	22	17.0	7	38	21	16.0
8	30	19	15.0	8	35	23	12.0
9	35	19	16.0	9	46	19	7.0
10	34	20	15.0	10	38	23	7.0
11	38	21	9.0	11	39	22	11.0

[a]Day of the menstrual cycle in which the sample was obtained.
[b]Percentage of methylation of *HOXA10* promoter.

endometrium in all cases. The mean diameter of endometriomas in Group A patients was 6 cm (range: 4–9). The women's age, day of the menstrual cycle in which the sample was obtained, and detailed methylation status are shown in Table 1. The mean methylation level of *HOXA10* gene promoter was 13.9 ± 2.5 in Group A and 9.7 ± 2.9 in Group B. Methylation levels were significantly higher in Group A than in Group B ($p = 0.002$).

Discussion

To our knowledge, this is the second report that demonstrates hypermethylation in the promoter region of *HOXA10* in eutopic secretive endometrium of patients with endometriosis. In our series we documented a significant higher *HOXA10* promoter methylation level in women with ovarian endometriomas than in healthy controls during the mid-luteal phase. The aberrant methylation of *HOXA10* during the secretive phase observed in our study and in the one by Szczepanska et al. (13) could be one of the mechanisms leading to the well known abnormal expression of *HOXA10* during the window of implantation in women with endometriosis. On this basis, endometriosis-related infertility could be considered a multifactorial disorder with an epigenetic component. Future studies should take into account other epigenetic alterations reported to date in endometriosis, including the aberrant methylation of progesterone receptor-B, estrogen receptor-β, steroidogenic factor-1, E-cadherin and aromatase genes (5). Patients should also be selected taking into consideration that several factors such as lifestyle and environmental factors (including diet, nutrition and smoking) can affect DNA methylation (14).

Concerning therapeutic implications, recent studies on animal models have demonstrated that heterotopic endometrial tissue could induce aberrant methylation of *HOXA10* in the eutopic endometrium, resulting in decreased *HOXA10* expression (15). If demonstrated in humans, this finding would confirm the importance of surgical removal of peritoneal endometriosis in infertile patients.

However, concerning ovarian endometriomas, which *per se* may be detrimental to the ovarian reserve, current evidence points toward an even lower ovarian reserve after surgery (2,3). Novel non-surgical strategies to manage ovarian cystic endometriosis are advocated to prevent iatrogenic injury to the follicular reserve and adhesion formation following surgical excision. Our data, if confirmed in larger studies, indicate a new target for developing novel non-surgical ways to treat endometrioma-associated infertility. 5-Azacytidine (5-ac) is a demethylating agent widely used in gene methylation experiments, including studies on patients with endometriosis (14,15). Recently Lu et al. (16) found that *HOXA10* mRNA and protein expression were markedly upregulated in the eutopic endometrium of patients with endometriosis after treatment with 5-ac, indirectly suggesting that *HOXA10* expression is controlled by methylation of the promoter. Drugs such as demethylating agents to reverse aberrant methylation could represent an attractive field of research to develop new epigenetic-based strategies for non-surgical treatment of infertility related to ovarian endometriomas.

References

1. Giudice LC, Kao LC. Endometriosis. Lancet. 2004;364:1789–99.
2. Jadoul P, Kitajima M, Donnez O, Squifflet J, Donnez J. Surgical treatment of ovarian endometriomas: state of the art? Fertil Steril. 2012;98:556–63.
3. Raffi F, Metwally M, Amer S. The impact of excision of ovarian endometrioma on ovarian reserve: a systematic review and meta-analysis. J Clin Endocrinol Metab. 2012;97:3146–54.
4. de Ziegler D, Borghese B, Chapron C. Endometriosis and infertility: pathophysiology and management. Lancet. 2010;376:730–8.
5. Guo SW. Epigenetics of endometriosis. Mol Hum Reprod. 2009;15:587–607.
6. Kao LC, Germeyer A, Tulac S, Lobo S, Yang JP, Taylor RN, et al. Expression profiling of endometrium from women with endometriosis reveals candidate genes for disease-based implantation failure and infertility. Endocrinology. 2003;144:2870–81.
7. Altmäe S, Martínez-Conejero JA, Salumets A, Simón C, Horcajadas JA, Stavreus-Evers A. Endometrial gene expression analysis at the time of embryo implantation in women with unexplained infertility. Mol Hum Reprod. 2010;16:178–87.
8. Du H, Taylor HS. Molecular regulation of mullerian development by Hox genes. Ann N Y Acad Sci. 2004;1034:152–65.
9. Taylor HS. The role of HOX genes in human implantation. Hum Reprod Update. 2000;6:75–9.
10. Modi D, Godbole G. HOXA10 signals on the highway through pregnancy. J Reprod Immunol. 2009;83:72–8.
11. Zanatta A, Rocha AM, Carvalho FM, Pereira RM, Taylor HS, Motta E, et al. The role of the Hoxa10/HOXA10 gene in the etiology of endometriosis and its related infertility: a review. J Assist Reprod Genet. 2010;27:701–10.
12. Wu Y, Halverson G, Basir Z, Strawn E, Yan P, Guo SW. Aberrant methylation at HOXA10 may be responsible for its aberrant expression in the endometrium of patients with endometriosis. Am J Obstet Gynecol. 2005;193:371–80.

13. Szczepańska M, Wirstlein P, Luczak M, Jagodziński PP, Skrzypczak J. Reduced expression of HOXA10 in the midluteal endometrium from infertile women with minimal endometriosis. Biomed Pharmacother. 2010;64:697–705.
14. Nasu K, Kawano Y, Tsukamoto Y, Takano M, Takai N, Li H, et al. Aberrant DNA methylation status of endometriosis: epigenetics as the pathogenesis, biomarker and therapeutic target. J Obstet Gynaecol Res. 2011;37:683–95.
15. Lee B, Du H, Taylor HS. Experimental murine endometriosis induces DNA methylation and altered gene expression in eutopic endometrium. Biol Reprod. 2009;80:79–85.
16. Lu H, Yang X, Zhang Y, Lu R, Wang X. Epigenetic disorder may cause down regulation of HOXA10 in the eutopic endometrium of fertile women with endometriosis. Reprod Sci. 2013;20:78–84.