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HOXB4 Immunoreactivity in Endometrial Tissues From Women With or Without Endometriosis

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Abstract

Endometriosis is a common disease characterized by the presence of ectopic endometrial tissue. Although the pathogenesis of endometriosis remains unclear, several factors have been implicated, including the dysregulation of homeobox (*HOX*) genes. Our objective was to investigate the localization and immunoreactivity of HOXB4 in endometrial tissues from women with or without endometriosis. We studied samples of eutopic endometrium (EE), endometriomas (Eoma), superficial endometriosis (SE), and deep infiltrating endometriosis (DIE) from 34 women with endometriosis, as well as eutopic endometrium from 38 women without endometriosis (EC). HOXB4 localization and immunoreactivity was assessed using immunohistochemistry and histoscore analysis. Data were analyzed with and without stratification by menstrual cycle phase. HOXB4 protein was present in the nuclei of endometrial glandular epithelial cells but not in stromal cells. HOXB4 immunoreactivity was reduced in DIE samples compared to all other groups. A smaller reduction in HOXB4 immunoreactivity was observed in SE samples compared to EC samples. HOXB4 immunoreactivity was significantly greater in proliferative compared to secretory phase samples in the EC group but not in EE, Eoma, or DIE groups. Among only proliferative phase samples, HOXB4 immunoreactivity was reduced in EE, Eoma, and DIE groups compared to EC. Based on these data, we suggest that an impaired capacity of eutopic and ectopic endometrial tissue to upregulate levels of HOXB4 during the proliferative phase may play a role in the pathogenesis of endometriosis and that further downregulation of HOXB4 may enhance ectopic implant invasiveness.

Keywords

endometriosis, HOX genes, HOXB4, immunohistochemistry

Introduction

Endometriosis affects 5% to 10% of reproductive-aged women.[1,2] Among those affected, ~80% experience dysmenorrhea, ~70% complain of pelvic pain, and ~40% have infertility.[3] Endometriosis is characterized by the presence of endometrial glands and stroma at extrauterine (ectopic) sites, most commonly in the pelvic peritoneum and ovaries.[2] A commonly accepted theory is that the ectopic implants arise from endometrial tissue that enters the peritoneal cavity through retrograde menstruation.[4] As retrograde menstruation occurs in most women but only a subset of women develop endometriosis, the establishment of ectopic implants may depend on a combination of reduced clearance by the immune system and distinct properties of certain eutopic endometrial cells that predispose them to ectopic implantation.[2,5] Eutopic endometrial cells in women with endometriosis compared to those in women without endometriosis have shown differences in the production of cytokines and metalloproteinases[6-8] as well as aberrant activation of oncogenic signaling pathways.[9,10] Alternative hypotheses propose that (1) cells are carried to ectopic sites through the blood and lymphatic systems,[11,12] (2) the

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ectopic implants arise from stem cells carried from the basalis layer of the endometrium during menstruation,[13] or (3) mesothelial cells preexisting in the peritoneum differentiate into endometrium-like tissue.[2,14]

Recent studies of the pathogenesis of endometriosis have distinguished 3 categories of implants based on anatomical locations: superficial endometriosis (SE), deep infiltrating endometriosis (DIE; defined as >5 mm beneath the peritoneum), and ovarian endometriomas (Eoma), which are cystic ovarian masses containing endometrial tissue.[15,16] Molecular differences have been identified between these categories,[17-19] prompting discussion of how the pathogenesis of these lesions may differ.[15,20]

Among the genes implicated in the pathogenesis of endometriosis are homeobox (*HOX*) genes. The *HOX* genes encode a family of transcription factors with roles in both embryonic and adult tissues.[21,22] In eutopic endometrium, *HOXC10*, *HOXC11*, *HOXD10,* and *HOXD11* are expressed most strongly in the proliferative phase and likely mediate endometrial proliferation,[23] whereas an increase in *HOXA10* and *HOXA11* expression in the mid-secretory phase is required for receptivity to implantation.[22,24,25] In ovarian endometriomas compared to eutopic endometrium from women without endometriosis, *HOXC* genes had increased messenger RNA (mRNA) expression.[26] In this study, differential methylation was identified in the regulatory regions of *HOXA*, *HOXC,* and *HOXD* genes. A separate study found reduced *HOXA11* expression in eutopic endometrium of women with endometriosis compared to women without endometriosis.[27] Consistent with these reports, a study of ectopic versus eutopic endometrial tissue from patients with stage IV endometriosis found a tendency for downregulation of *HOXA* and *HOXB* genes and upregulation of *HOXC* and *HOXD* genes.[28]

Our study focuses on *HOXB4*, a *HOX* gene whose expression in primary human endometrial glandular epithelial cells is estradiol and progestin-dependent.[29] The HOXB4 may thus be a downstream effector of dysregulated estrogen and progesterone signaling in endometriotic tissue.[2] The *HOXB4* mRNA levels were reduced in a gene expression profiling study comparing 10 ovarian endometriomas with matched eutopic endometrium (Gene Expression Omnibus Dataset ID 2835),[30,31] suggesting that decreased *HOXB4* expression may play a role in the pathogenesis of endometriosis.

The immunohistochemical localization and immunoreactivity of HOXB4 has not previously been studied in endometrial tissue from women with or without endometriosis. In the present study, we used immunohistochemistry to assess the levels of HOXB4 in eutopic endometrium (EE) and ectopic implants (Eoma, SE, and DIE) from women with endometriosis, compared to endometrial tissues from women without endometriosis (EC).

Materials and Methods

Tissue Procurement

Samples of endometrium from 44 reproductive-aged women without endometriosis were obtained from Case Western Reserve, Cleveland, Ohio (approved by the institutional review board; IRB# 12-10-28) and Sohag University, Sohag, Egypt (transferred according to Material Transfer Agreement # M15-00189). These control women underwent laparoscopy for benign gynecological conditions other than endometriosis (eg, tubal ligation and reanastomosis). Chart review revealed no history of endometriosis in control women, and there was no evidence of endometriosis during laparoscopy. Samples from women with endometriosis were obtained from 51 women who underwent laparoscopy to diagnose or manage endometriosis at the BC Women's Centre for Pelvic Pain and Endometriosis (Vancouver, Canada). Endometriosis was diagnosed by tissue sample pathology, the gold standard for diagnosis. Surgical samples from these women with endometriosis were obtained either through the UBC Gynaecological Cancer Tissue Bank (approved by the University of British Columbia Clinical Research Ethics Board; REB numbers H05-60119 and H11-00536) or from our center's pathology archives (approved under REB numbers H14-03040 and H13-02563). Some patients provided tissue from more than 1 ectopic lesion (Table 1). Ectopic lesions from only 1 category (ie, DIE, SPE, or Eoma) were obtained from each patient, with the exception of 3 patients who provided both Eoma and DIE lesions. Whenever possible, eutopic endometrial tissue was obtained from the women who provided ectopic endometrial tissue. Eutopic endometrial tissue was obtained from all women who provided

Table 1. Sample Sizes for H-Score Analysis.[a]

Endometriosis	Sample Group	Menstrual Cycle Phase	# of Patients	# of Tissue Samples	Age, years
No	EC	Proliferative	20	20	
No	EC	Secretory	18	18	
		Total EC	38	38	39.6, SD 8.9
Yes	EE	Proliferative	18	18	
Yes	EE	Secretory	11	11	
		Total EE	29	29	39.5, SD 6.4
Yes	Eoma	Proliferative	8	10	
Yes	Eoma	Secretory	4	5	
		Total Eoma	12	15	40, SD 4.9
Yes	SE	Unable to determine as no matched EE was available	5	10	
		Total SE	5	10	40.1, SD 2.8
Yes	DIE	Proliferative	6	10	
Yes	DIE	Secretory	7	12	
		Total DIE	13	22	38.4, SD 7.6
		Total with endometriosis	34	76	

Abbreviations: DIE, deep infiltrating endometriosis; EC, eutopic endometrium from women without endometriosis; EE, eutopic endometrium from women with endometriosis; Eoma, ovarian endometriomas; SD, standard deviation; SE, superficial endometriosis.

[a]Some patients provided ectopic tissue from more than 1 lesion. Each Eoma or DIE sample represents a distinct lesion. Eoma and DIE samples were obtained from a subset of the women who provided EE samples. No EE was obtained from patients providing SE samples.

Eoma or DIE samples. No eutopic endometrial tissue could be obtained from the women who provided SE samples. No hormonal therapy was received for at least 3 months before the laparoscopy. Patients with known adenomyosis were excluded. The deep infiltrating lesions were located in zone 2 (rectovaginal and vaginal area, cul-de-sac, uterosacral ligament, parasacral ligament, bladder, appendix, and bowel serosa) or zone 3 (side walls, fallopian tubes, and peri-ureteric area).[32]

Immunohistochemistry

Only formalin-fixed and paraffin-embedded tissues were used for immunohistochemistry. Sections (4 μm) were deparaffinized and rehydrated before wet heat–induced antigen retrieval in a steamer with preheated antigen retrieval solution (#S2367; Dako, Santa Clara, CA). Endogenous peroxidase activity was quenched using Dual Endogenous Enzyme Block (#S2003; Dako) prior to incubation of the sections overnight at 4°C with a previously validated HOXB4 antibody (1:800 dilution; #2096-1; Epitomics, Burlingame, CA)[33] or non-immune rabbit immunoglobulin (1:800 dilution; sc-2027; Santa Cruz Biotechnology, Dallas, Texas). The HOXB4 immunoreactivity was detected with EnVision+ Dual Link System-HRP (#K4061; Dako) and Liquid DAB Chromogen System (#K3468; Dako) for 10 minutes. Slides were counterstained with Harris hematoxylin (Sigma, St Louis, MO), mounted, and analyzed using either an Olympus BX 41 microscope (Olympus Corporation, Shinjulu, Japan) or a Leica DM4000 B microscope (Leica Microsystems, Wetzlar, Germany).

Histoscore (H-score) Analysis

Samples were excluded from analysis for the following reasons: poor preservation of tissue, cauterized glands, absence of endometrial glands, presence of histopathological changes suggestive of exogenous progesterone treatment, or the sample was representative of another abnormality (eg, adenomyosis, endocervix, or mature cystic teratoma). We also excluded all samples from patients who had inactive eutopic endometrium. The number of included samples in each group is listed in Table 1.

Menstrual cycle phase was determined from matched eutopic endometrium according to the Noyes criteria,[34] since clinical data regarding menstrual cycle phase was not available for all patients. To assess HOXB4 immunoreactivity, 3 different reviewers who were blinded to the menstrual cycle phase of ectopic lesions and to the diagnosis associated with eutopic samples independently scored the intensity (I) of nuclear HOXB4 staining in glandular epithelial cells on a scale of 0 to 3 (0 = none; 1 = weak; 2 = moderate; 3 = strong). Reviewers estimated the proportion (P) of glandular cells with each level of intensity of HOXB4 staining. An H-score was calculated for each slide using the following formula: H-score = $\Sigma P(I+1)$[35,36] and the mean of the 3 reviewers' H-scores was used in statistical analyses.

Statistical Analysis

The Mann-Whitney rank sum test was used for unpaired 2-sample comparisons. The Wilcoxon test was used for paired statistical analyses. The Kruskal-Wallis nonparametric test was used for comparisons of more than 2 groups. Statistical tests were performed using GraphPad Prism 6 (GraphPad Software) and P values of <.05 were considered statistically significant.

Results

Patient Characteristics

There was no significant difference ($P = .91$) in the mean age of patients with analyzed lesions in the endometriosis group (39.4 [5.4] years) compared to those without endometriosis (39.6 [8.9] years). Mean age for patients providing each type of ectopic lesion is shown in Table 1. Superficial endometriotic lesions were all located in the cul-de-sac or uterosacral areas. Among the deep infiltrating lesions, 6 were uterosacral, 5 were paratubal, 3 were on unspecified peritoneum, and 2 were in the cul-de-sac. The remaining 6 deep infiltrating lesions were each from a different location (ie, pelvic side wall, parasacral region, rectovaginal region, vagina, bladder, or bowel serosa).

Localization of HOXB4 in Endometrial Tissue

We first assessed the immunohistochemical localization of HOXB4 in the endometrium of women without endometriosis. HOXB4 was detectable in the nuclei of glandular but not stromal cells (Figure 1A-D), consistent with a prior report that *HOXB4* mRNA was predominantly expressed in glandular cells.[29] Positive and negative controls for HOXB4 staining (fallopian tube epithelium and ovarian epithelium, respectively) stained as expected (Figure 1E-H).[33] The localization of HOXB4 in samples from women with endometriosis was the same as that in samples from women without endometriosis (Figures 1I, 1J and 2).

Immunoreactivity of HOXB4 in Ectopic Versus Eutopic Endometrial Tissue

We next assessed HOXB4 immunoreactivity using an H-score system published previously.[35,36] Scoring of our samples had high interobserver reliability (intraclass correlation between different observers = .953). We then proceeded to compare HOXB4 immunoreactivity between groups. There was no significant difference in HOXB4 immunoreactivity between EC, EE, and Eoma groups ($P = .094$; Figure 3A). SE lesions had significantly decreased HOXB4 immunoreactivity when compared to EC samples ($P = .008$), but not when compared to EE or Eoma samples ($P = .051$ and .060, respectively; Figure 3A). DIE lesions had the lowest HOXB4 immunoreactivity, a difference that was statistically significant compared to all other groups (EC $P < .0001$, EE $P < .0001$, Eoma $P = .0002$, and SE $P = .027$; Figure 3A). HOXB4 immunoreactivity remained significantly reduced in DIE compared to EE samples in a

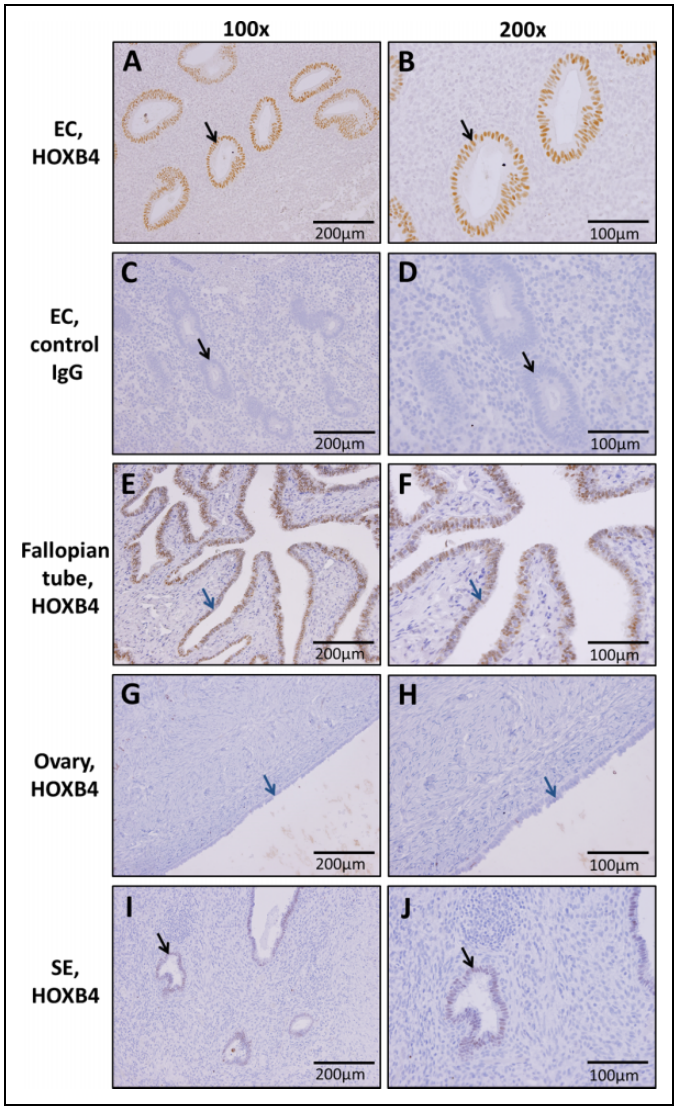

Figure 1. Immunolocalization of homeobox B4 (HOXB4) in eutopic endometrium and control tissues. A and B, Representative images of HOXB4 localizing to the nuclei of glandular epithelial cells in eutopic endometrial tissue from women without endometriosis (EC, n = 38). C and D, No staining was observed in EC tissue incubated with normal rabbit control IgG. HOXB4 immunoreactivity was detectable in (E and F) positive control fallopian tube epithelium but not in (G and H) negative control ovarian epithelium. I and J, Representative immunolocalization of HOXB4 to the nuclei of glandular epithelial cells in superficial endometriosis (SE, n = 10). Black arrows indicate endometrial glandular epithelium. Blue arrows indicate fallopian or ovarian epithelium.

paired statistical analysis comparing DIE lesions to EE samples from the same patient ($P = .040$). Indeed, when the difference in H-score was calculated for each patient, 9 of 13 patients had lower HOXB4 immunoreactivity in their DIE lesion(s) than in their EE sample (Figure 3B). Similar paired analyses comparing Eoma to EE samples from the same patient showed no significant change in HOXB4 immunoreactivity ($P = .557$; Figure 3B), consistent with the unpaired analysis.

Immunoreactivity of HOXB4 in Proliferative Versus Secretory Endometrium

As the different hormonal environments at different stages of the menstrual cycle may alter HOXB4 expression, we compared HOXB4 immunoreactivity between proliferative and secretory phase endometrium. Starting with endometrial samples from women without endometriosis, we found that HOXB4 immunoreactivity was significantly greater in the proliferative than in the secretory phase ($P = .0019$; Figures 2A-D and 3C). The statistical significance of this difference between phases was lost among samples from women with endometriosis (EE $P = .050$, Eoma $P = .971$, and DIE $P = .116$; Figures 2E-P and 3C). SE samples could not be included in this analysis as these samples had no matched EE tissue and thus menstrual cycle phase could not be determined.

Immunoreactivity of HOXB4 in Ectopic Versus Eutopic Endometrial Tissue Stratified by Menstrual Cycle Phase

Because HOXB4 immunoreactivity varied with menstrual cycle phase, we suspected that there may be disease-specific differences in HOXB4 immunoreactivity detectable only in certain phases of the menstrual cycle. In search of such differences, we repeated our comparison between EC, EE, Eoma, and DIE groups using samples from matched menstrual cycle phases. Comparison of samples in the secretory phase yielded the same results as when phases were undistinguished: the only group with significantly reduced HOXB4 immunoreactivity compared to the EC group was the DIE group ($P = .033$; Figures 2 and 3C). In contrast, analysis of samples in the proliferative phase found that EE, Eoma, and DIE samples all had significantly decreased HOXB4 immunoreactivity compared to EC samples (EE $P = .004$, Eoma $P = .0009$, and DIE $P < .0001$; Figures 2 and 3C).

Discussion

To our knowledge, this is the first study to examine the immunohistochemical localization and immunoreactivity of HOXB4 in endometrial tissues. We demonstrated that HOXB4 localizes to the nuclei of glandular epithelial cells in eutopic and ectopic endometrial tissue. The HOXB4 immunoreactivity was significantly reduced in DIE when compared to other types of ectopic implant or eutopic endometrium from women with or without endometriosis. A smaller reduction in HOXB4 immunoreactivity was observed in SE samples compared to endometrium from women without endometriosis. When only proliferative phase tissues were studied, a decrease in HOXB4 immunoreactivity was found in both eutopic and ectopic endometrial tissues of women with endometriosis, compared to endometrium from women without endometriosis.

Strengths of this study include the use of multiple blinded observers to score immunoreactivity and the high interobserver reliability of the resulting scores. Including eutopic endometrium from women with endometriosis allowed us to assess for changes in eutopic endometrium that may predispose to endometriosis.

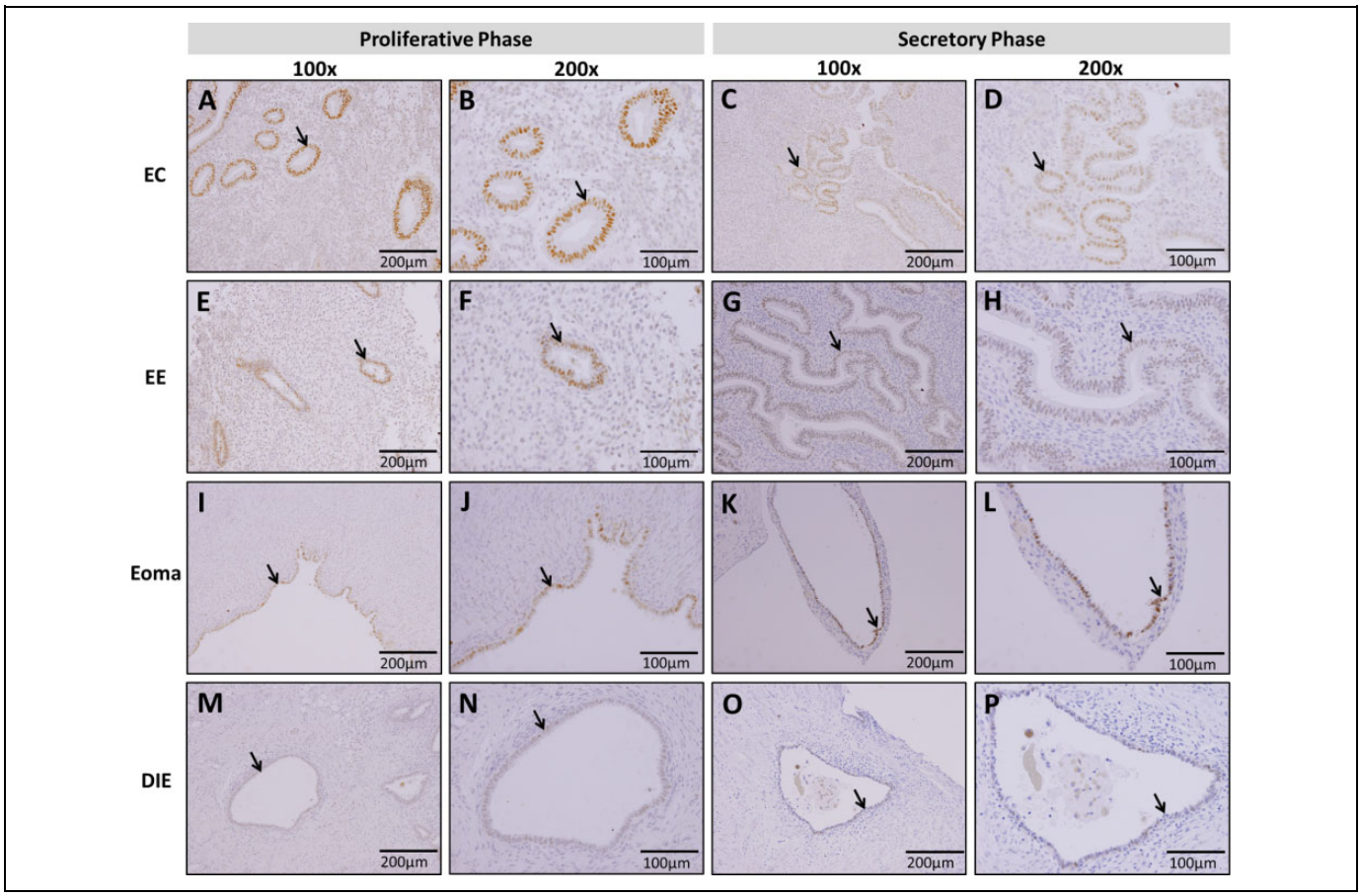

Figure 2. Immunoreactivity of homeobox B4 (HOXB4) in proliferative and secretory phase endometrial tissues from women with and without endometriosis. Shown are representative images of (A-D) eutopic endometrium from women without endometriosis (EC; proliferative phase n = 20; secretory phase n = 18), (E-H) eutopic endometrium from women with endometriosis (EE, proliferative phase n = 18; secretory phase n = 11), (I-L) ovarian endometrioma tissue (Eoma, proliferative phase n = 10; secretory phase n = 5), and (M-P) deep infiltrating endometriosis (DIE, proliferative phase n = 10; secretory phase n = 12). Arrows indicate endometrial glandular epithelium. Menstrual cycle phases of ectopic lesions were determined from matched eutopic endometrium.

Our ability to compare eutopic and ectopic lesions from the same patient helped control for interpatient variability. Stratification of samples according to type of lesion and cycle phase allowed us to identify differences that would otherwise not have appeared significant. Although stratification reduced sample size per group, this study would have an 80% power to detect a 50% reduction in HOXB4 levels compared to control samples as long as 10 or more samples were present per group (assuming H-score standard deviation of 1.5 in each group). As all groups except for secretory phase Eoma (n = 5) had 10 or more samples, we consider our study sufficiently powered.

Limitations of this study include the lack of EE samples from the patients who provided SE samples, resulting in the menstrual cycle phase of SE samples being unknown. Our conclusions regarding menstrual cycle phase-specific patterns of HOXB4 immunoreactivity are thus limited to the other sample groups. All deep infiltrating lesions available for study were from zones 2 and 3 (defined previously).[32] Other zones of the pelvis, which include the bladder dome and regions lateral to the fallopian tube, were not represented. Adolescent patients, in whom aspects of endometriosis pathophysiology may differ,[37] were also not represented in our study. We excluded the presence of adenomyosis (characterized by ectopic endometrial tissue in the uterine myometrium rather than at extrauterine sites) in study patients using high-resolution ultrasonography and magnetic resonance imaging where appropriate according to the standards.[38] However, adenomyosis can only be excluded with certainty by histopathological examination of a hysterectomy specimen. As elements of adenomyosis pathophysiology are similar to that of endometriosis,[39] investigation of HOXB4 levels in adenomyosis may be of interest for future studies. Localization of HOXB4 to glandular but not stromal cells is in contrast to the predominantly stromal expression of *HOXC10*, *HOXC11*, *HOXD10,* and *HOXD11*,[23] and is in contrast to the expression of *HOXA10*[25] and *HOXA11*[24] in both glands and stroma. The difference in HOXB4 immunoreactivity between menstrual cycle phases is not surprising, given the estradiol and progestin responsiveness of *HOXB4* expression[29], and the menstrual cycle phase-dependent expression of other *HOX* genes. HOXB4 immunoreactivity was greater in proliferative than secretory phase, similar to the increased expression of *HOXC10*, *HOXC11*, *HOXD10,* and *HOXD11* in proliferative versus secretory endometrium.[23] In contrast, *HOXA10* and *HOXA11* have peak expression in the mid-secretory phase.[22,24,25]

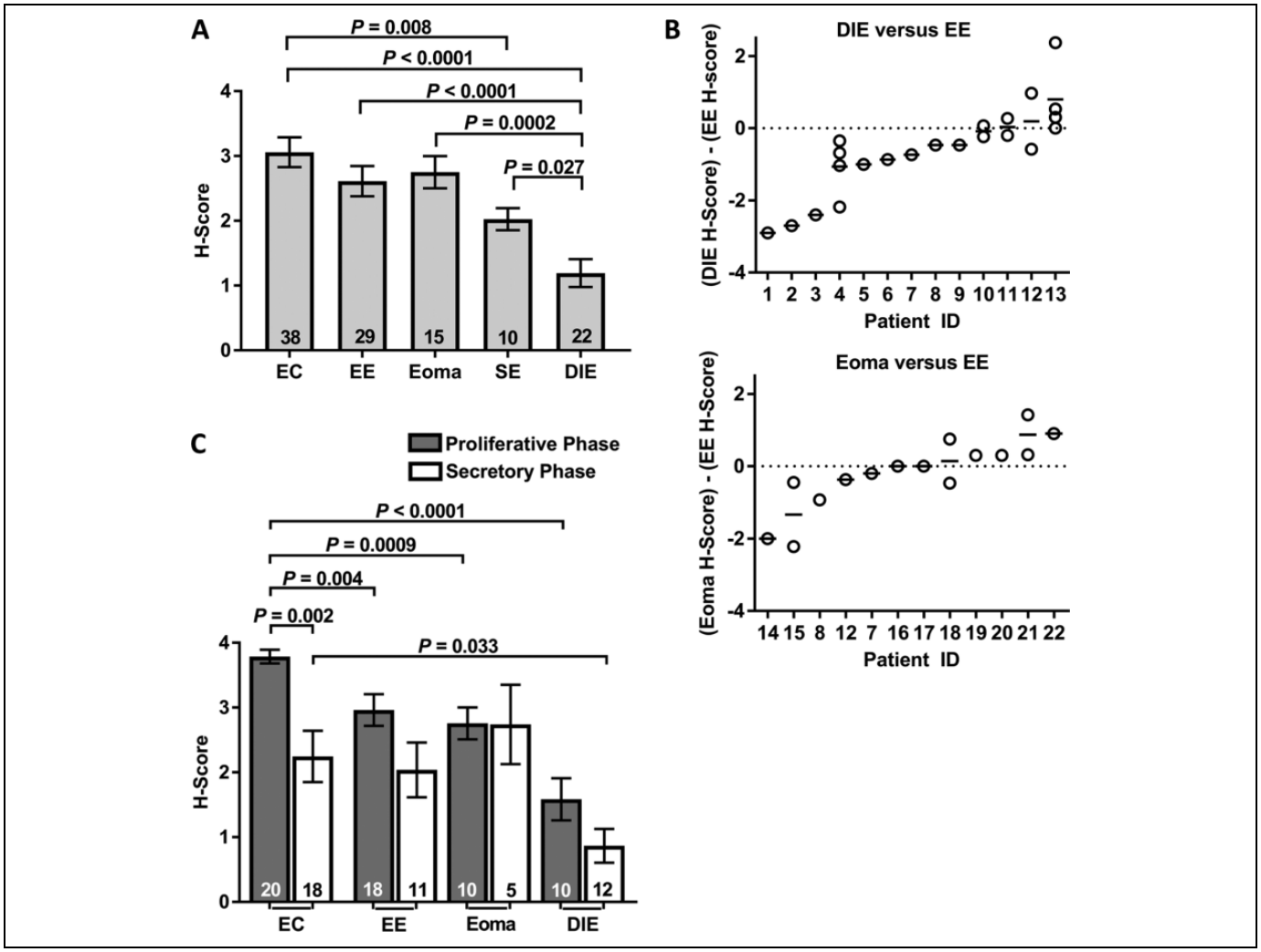

Figure 3. Quantification of homeobox B4 (HOXB4) immunoreactivity in endometrial tissues. (A) Mean HOXB4 immunoreactivity was significantly reduced in SE compared to EC samples, and in DIE samples compared to all other groups. (B) The difference in H-score between each DIE (top) or Eoma (bottom) lesion and the EE sample from the same patient. The resulting values tend to be negative for the DIE analysis but not the Eoma analysis, indicating a reduction in HOXB4 immunoreactivity in DIE but not Eoma samples compared to EE samples. Mean within each patient is indicated by a horizontal line. (C) Mean HOXB4 immunoreactivity was increased in proliferative compared to secretory phase EC samples. Among proliferative phase samples, HOXB4 immunoreactivity was reduced in EE, Eoma, and DIE samples compared to EC samples. Among secretory phase samples, HOXB4 immunoreactivity was reduced in DIE samples compared to EC samples. Menstrual cycle phases of ectopic lesions were determined from matched eutopic endometrium. For all panels error bars represent SEM. Sample size is indicated on each bar. EC indicates eutopic endometrium from women without endometriosis; EE indicates eutopic endometrium from women with endometriosis; Eoma indicates ovarian endometriomas; SE indicates superficial endometriosis; DIE indicates deep infiltrating endometriosis.

Endometrial tissue from women with endometriosis showed less of an increase in HOXB4 immunoreactivity in proliferative versus secretory phase than endometrial tissue from women without endometriosis. It's possible that the endometrial tissue of patients with endometriosis might have an impaired capacity to upregulate *HOXB4* during the proliferative phase. This dysregulation might be a factor predisposing eutopic endometrial tissue to give rise to endometriosis. The altered estrogen and progesterone responsiveness characteristic of endometriotic tissue[2] may contribute to HOXB4 dysregulation, as HOXB4 expression is estradiol and progestin-dependent.[29]

Deep infiltrating lesions had the lowest levels of HOXB4 immunoreactivity. This decrease in HOXB4 levels is consistent with the data that *HOXB* cluster genes tended to have decreased expression in ectopic versus eutopic endometrium from patients with stage IV (severe) endometriosis.[28] Our finding that deep infiltrating lesions have reduced HOXB4 immunoreactivity compared to Eoma and superficial lesions is also consistent with reports that the pathogenesis of DIE involves aspects unique from that of other endometriotic lesions.[15,17]

Deep infiltrating lesions are considered more invasive than Eoma as Eoma are typically formed by invagination of the ovarian cortex around ectopic implants rather than by direct invasion of ectopic implants.[40,41] Given our finding that HOXB4 immunoreactivity was lowest in deep infiltrating lesions, it's possible that reduced levels of HOXB4 might promote invasiveness of

endometriotic lesions. This possibility is consistent with the evidence that reduced HOXB4 enhanced the invasiveness of epithelial ovarian carcinoma cells.[33] Similarly, decreased HOXA10 is thought to increase the invasiveness of endometrial cancer cells through promotion of epithelial-to-mesenchymal transition (EMT).[42] We speculate that reduced HOXB4 might also increase invasiveness of endometriotic tissue by allowing EMT. Consistent with this notion, EMT has been implicated in the pathogenesis of endometriosis.[43-46]

An alternative interpretation of *HOX* gene expression in ectopic endometrial implants is that *HOX* gene expression drives differentiation of tissues into an endometrial-like phenotype.[47] This is based in part on the evidence that *HOXA* genes play a role in specifying uterine tissue identity during development.[47,48] Although a role for *HOXB4* in uterine tissue specification has not been reported, the lower levels of HOXB4 in deep infiltrating lesions compared to Eoma and superficial lesions might be indicative of reduced endometrial differentiation in deep infiltrating lesions.

In conclusion, we report decreased levels of HOXB4 as a feature of proliferative phase eutopic and ectopic endometrial tissues of patients with endometriosis. Among these tissues, deep infiltrating lesions had the lowest HOXB4 levels. Our findings implicate HOXB4 in the pathogenesis of endometriosis and contribute to our understanding of the heterogeneity of endometriotic lesions.

Authors' Note

This work was completed at the British Columbia Women's Hospital, Vancouver, Canada, and the BC Children's Hospital Research Institute, Vancouver, Canada.

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Declaration of Conflicting Interests

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References

1. Eskenazi B, Warner ML. Epidemiology of endometriosis. *Obstet Gynecol Clin North Am*. 1997;24(2):235-258.
2. Bulun SE. Endometriosis. *N Engl J Med*. 2009;360(3):268-279. doi:10.1056/NEJMra0804690.
3. Sinaii N, Plumb K, Cotton L, et al. Differences in characteristics among 1,000 women with endometriosis based on extent of disease. *Fertil Steril*. 2008;89(3):538-545. doi:10.1016/j.fertnstert.2007.03.069.
4. Sampson J. Peritoneal endometriosis due to the menstrual dissemination of endometrial tissue into the peritoneal cavity. *Am J Obstet Gynecol*. 1927;14:422-469.
5. Ahn SH, Monsanto SP, Miller C, Singh SS, Thomas R, Tayade C. Pathophysiology and immune dysfunction in endometriosis. *BioMed Res Int*. 2015;2015:795976. doi:10.1155/2015/795976.
6. Hornung D, Ryan IP, Chao VA, Vigne JL, Schriock ED, Taylor RN. Immunolocalization and regulation of the chemokine RANTES in human endometrial and endometriosis tissues and cells. *J Clin Endocrinol Metab*. 1997;82(5):1621-1628. doi:10.1210/jcem.82.5.3919.
7. Tseng JF, Ryan IP, Milam TD, et al. Interleukin-6 secretion in vitro is up-regulated in ectopic and eutopic endometrial stromal cells from women with endometriosis. *J Clin Endocrinol Metab*. 1996;81(3):1118-1122. doi:10.1210/jcem.81.3.8772585.
8. Osteen KG, Bruner KL, Sharpe-Timms KL. Steroid and growth factor regulation of matrix metalloproteinase expression and endometriosis. *Semin Reprod Endocrinol*. 1996;14(3):247-255. doi:10.1055/s-2007-1016334.
9. Kao LC, Germeyer A, Tulac S, et al. Expression profiling of endometrium from women with endometriosis reveals candidate genes for disease-based implantation failure and infertility. *Endocrinology*. 2003;144(7):2870-2881. doi:10.1210/en.2003-0043.
10. Wu Y, Kajdacsy-Balla A, Strawn E, et al. Transcriptional characterizations of differences between eutopic and ectopic endometrium. *Endocrinology*. 2006;147(1):232-246. doi:10.1210/en.2005-0426.
11. Machado DE, Abrao MS, Berardo PT, Takiya CM, Nasciutti LE. Vascular density and distribution of vascular endothelial growth factor (VEGF) and its receptor VEGFR-2 (Flk-1) are significantly higher in patients with deeply infiltrating endometriosis affecting the rectum. *Fertil Steril*. 2008;90(1):148-155. doi:10.1016/j.fertnstert.2007.05.076.
12. Abrao MS, Podgaec S, Dias JA Jr, , et al. Deeply infiltrating endometriosis affecting the rectum and lymph nodes. *Fertil Steril*. 2006;86(3):543-547. doi:10.1016/j.fertnstert.2006.02.102.
13. Hufnagel D, Li F, Cosar E, Krikun G, Taylor HS. The role of stem cells in the etiology and pathophysiology of endometriosis. *Semin Reprod Med*. 2015;33(5):333-340. doi:10.1055/s-0035-1564609.
14. Ferguson BR, Bennington JL, Haber SL. Histochemistry of mucosubstances and histology of mixed müllerian pelvic lymph node glandular inclusions. Evidence for histogenesis by müllerian metaplasia of coelomic epithelium. *Obstet Gynecol*. 1969;33(5):617-625.
15. Tosti C, Pinzauti S, Santulli P, Chapron C, Petraglia F. Pathogenetic mechanisms of deep infiltrating endometriosis. *Reprod Sci*. 2015;22(9):1053-1059. doi:10.1177/1933719115592713.
16. Koninckx PR, Meuleman C, Demeyere S, Lesaffre E, Cornillie FJ. Suggestive evidence that pelvic endometriosis is a progressive disease, whereas deeply infiltrating endometriosis is associated with pelvic pain. *Fertil Steril*. 1991;55(4):759-765.
17. Van Langendonckt A, Luyckx M, Gonzalez MD, Defrère S, Donnez J, Squifflet J. Differential expression of genes from the homeobox A cluster in deep endometriotic nodules and peritoneal lesions. *Fertil Steril*. 2010;94(6):1995-2000. doi:10.1016/j.fertnstert.2010.01.003.
18. Samartzis N, Samartzis EP, Noske A, et al. Expression of the G protein-coupled estrogen receptor (GPER) in endometriosis: a tissue microarray study. *Reprod Biol Endocrinol RBE*. 2012;10:30. doi:10.1186/1477-7827-10-30.

19. Santulli P, Chouzenoux S, Fiorese M, et al. Protein oxidative stress markers in peritoneal fluids of women with deep infiltrating endometriosis are increased. *Hum Reprod Oxf Engl*. 2015;30(1): 49-60. doi:10.1093/humrep/deu290.
20. Sanchez AM, Viganò P, Somigliana E, Panina-Bordignon P, Vercellini P, Candiani M. The distinguishing cellular and molecular features of the endometriotic ovarian cyst: from pathophysiology to the potential endometrioma-mediated damage to the ovary. *Hum Reprod Update*. 2014;20(2):217-230. doi:10.1093/humupd/dmt053.
21. McGinnis W, Krumlauf R. Homeobox genes and axial patterning. *Cell*. 1992;68(2):283-302.
22. Du H, Taylor HS. The role of hox genes in female reproductive tract development, adult function, and fertility. *Cold Spring Harb Perspect Med*. 2015;6(1):a023002. doi:10.1101/cshperspect.a023002.
23. Akbas GE, Taylor HS. HOXC and HOXD gene expression in human endometrium: lack of redundancy with HOXA paralogs. *Biol Reprod*. 2004;70(1):39-45. doi:10.1095/biolreprod.102.014969.
24. Taylor HS, Igarashi P, Olive DL, Arici A. Sex steroids mediate HOXA11 expression in the human peri-implantation endometrium. *J Clin Endocrinol Metab*. 1999;84(3):1129-1135. doi:10.1210/jcem.84.3.5573.
25. Taylor HS, Arici A, Olive D, Igarashi P. HOXA10 is expressed in response to sex steroids at the time of implantation in the human endometrium. *J Clin Invest*. 1998;101(7):1379-1384. doi:10.1172/JCI1057.
26. Dyson MT, Roqueiro D, Monsivais D, et al. Genome-wide DNA methylation analysis predicts an epigenetic switch for GATA factor expression in endometriosis. *PLoS Genet*. 2014;10(3): e1004158. doi:10.1371/journal.pgen.1004158.
27. Szczepańska M, Wirstlein P, Skrzypczak J, Jagodziński PP. Expression of HOXA11 in the mid-luteal endometrium from women with endometriosis-associated infertility. *Reprod Biol Endocrinol RBE*. 2012;10:1. doi:10.1186/1477-7827-10-1.
28. Borghese B, Mondon F, Noël JC, et al. Gene expression profile for ectopic versus eutopic endometrium provides new insights into endometriosis oncogenic potential. *Mol Endocrinol Baltim Md*. 2008;22(11):2557-2562. doi:10.1210/me.2008-0322.
29. Gao J, Mazella J, Tseng L. Hox proteins activate the IGFBP-1 promoter and suppress the function of hPR in human endometrial cells. *DNA Cell Biol*. 2002;21(11):819-825. doi:10.1089/104454902320908469.
30. Barrett T, Wilhite SE, Ledoux P, et al. NCBI GEO: archive for functional genomics data sets–update. *Nucleic Acids Res*. 2013; 41(Database issue): D991-D995. doi:10.1093/nar/gks1193.
31. Hever A, Roth RB, Hevezi P, et al. Human endometriosis is associated with plasma cells and overexpression of B lymphocyte stimulator. *Proc Natl Acad Sci U S A*. 2007;104(30): 12451-12456. doi:10.1073/pnas.0703451104.
32. Bedaiwy MA, Pope R, Henry D, et al. Standardization of laparoscopic pelvic examination: a proposal of a novel system. *Minim Invasive Surg*. 2013;2013:153235. doi:10.1155/2013/153235.
33. Zhang X. *The Expression and Invasion-Suppressive Function of HOXB4 in Epithelial Ovarian Cancer [Dissertation]*. Vancouver, British Columbia: University of British Columbia; 2014.
34. Noyes RW, Hertig AT, Rock J. Dating the endometrial biopsy. *Am J Obstet Gynecol*. 1975;122(2):262-263.
35. Sharpe-Timms KL, Ricke EA, Piva M, Horowitz GM. Differential expression and localization of de-novo synthesized endometriotic haptoglobin in endometrium and endometriotic lesions. *Hum Reprod Oxf Engl*. 2000;15(10):2180-2185.
36. Browne H, Taylor H. HOXA10 expression in ectopic endometrial tissue. *Fertil Steril*. 2006;85(5):1386-1390. doi:10.1016/j.fertnstert.2005.10.072.
37. Gargett CE, Schwab KE, Brosens JJ, Puttemans P, Benagiano G, Brosens I. Potential role of endometrial stem/progenitor cells in the pathogenesis of early-onset endometriosis. *Mol Hum Reprod*. 2014;20(7):591-598. doi:10.1093/molehr/gau025.
38. Struble J, Reid S, Bedaiwy MA. Adenomyosis: a clinical review of a challenging gynecologic condition. *J Minim Invasive Gynecol*. 2016;23(2):164-185. doi:10.1016/j.jmig.2015.09.018.
39. Benagiano G, Brosens I, Habiba M. Structural and molecular features of the endomyometrium in endometriosis and adenomyosis. *Hum Reprod Update*. 2014;20(3):386-402. doi:10.1093/humupd/dmt052.
40. Scurry J, Whitehead J, Healey M. Classification of ovarian endometriotic cysts. *Int J Gynecol Pathol*. 2001;20(2):147-154.
41. Brosens IA, Puttemans PJ, Deprest J. The endoscopic localization of endometrial implants in the ovarian chocolate cyst. *Fertil Steril*. 1994;61(6):1034-1038.
42. Yoshida H, Broaddus R, Cheng W, Xie S, Naora H. Deregulation of the HOXA10 homeobox gene in endometrial carcinoma: role in epithelial-mesenchymal transition. *Cancer Res*. 2006;66(2): 889-897. doi:10.1158/0008-5472.CAN-05-2828.
43. Xiong Y, Liu Y, Xiong W, et al. Hypoxia-inducible factor 1α-induced epithelial-mesenchymal transition of endometrial epithelial cells may contribute to the development of endometriosis. *Hum Reprod*. 2016;31(6):1327-1338. doi:10.1093/humrep/dew081.
44. Proestling K, Birner P, Gamperl S, et al. Enhanced epithelial to mesenchymal transition (EMT) and upregulated MYC in ectopic lesions contribute independently to endometriosis. *Reprod Biol Endocrinol*. 2015;13:75. doi:10.1186/s12958-015-0063-7.
45. Bartley J, Jülicher A, Hotz B, Mechsner S, Hotz H. Epithelial to mesenchymal transition (EMT) seems to be regulated differently in endometriosis and the endometrium. *Arch Gynecol Obstet*. 2014;289(4):871-881. doi:10.1007/s00404-013-3040-4.
46. Matsuzaki S, Darcha C. Epithelial to mesenchymal transition-like and mesenchymal to epithelial transition-like processes might be involved in the pathogenesis of pelvic endometriosis. *Hum Reprod Oxf Engl*. 2012;27(3):712-721. doi:10.1093/humrep/der442.
47. Zanatta A, Pereira RM, Rocha AM, et al. The relationship among HOXA10, estrogen receptor α, progesterone receptor, and progesterone receptor B proteins in rectosigmoid endometriosis: a tissue microarray study. *Reprod Sci Thousand Oaks Calif*. 2015;22(1): 31-37. doi:10.1177/1933719114549846.
48. Block K, Kardana A, Igarashi P, Taylor HS. In utero diethylstilbestrol (DES) exposure alters Hox gene expression in the developing müllerian system. *FASEB J Off Publ Fed Am Soc Exp Biol*. 2000;14(9):1101-1108.