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Endometrial expression of relaxin and relaxin receptor in endometriosis

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Narrative Abstract

Our studies demonstrate significantly lower expression of relaxin and its receptor in ectopic endometriotic tissues than their expression in eutopic endometrium and in endometrium from normal controls. These data suggest that in normal and eutopic endometrium, relaxin may exert a protective effect against endometriosis.

The etiology of endometriosis, defined as the presence of endometrial stromal and glandular tissue growing at an extrauterine site, is poorly understood. The Sampson theory (1) does not sufficiently explain the etiology of this disease, since almost all women of reproductive age exhibit some degree of retrograde menstruation, but only 10–15% develop endometriosis (2). Additional factors must contribute to the development and progression of endometriosis.

Relaxin is locally synthesized by human endometrium (3) and has potent effects in the endometrium, including decidualization (4). In human and rhesus monkey endometrium, relaxin is a powerful inhibitor of matrix metalloproteinases (MMPs) (3,5,6), which play an important role in the invasive process of endometriosis (7,8). Relaxin's action in target tissues is mediated by binding to its specific receptor, leucine-rich G protein-coupled receptor 7 (LGR7). Both relaxin and its LGR7 receptor are expressed in human endometrium (3,9,10) and human decidua (11,12).

The current studies were performed to determine whether relaxin and/or its LGR7 receptor are expressed in human endometriotic tissues and whether their expression differ from that in endometrium from normal controls. We compared expression of relaxin mRNA and LGR7 mRNA in human endometriotic tissues to their expression in endometrium from normal controls.

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A total of 40 patients signed informed consent for inclusion in the study and underwent laparoscopic surgery between October 2, 2006 and May 25, 2007 at the University of Siena for the indication of an ovarian cyst identified on preoperative ultrasound. Patient age ranged between 24 and 37 years. Patients underwent laparoscopy during the proliferative (n=11) or secretory (n=29) phase of the menstrual cycle, assessed according to the last day of menstruation and confirmed by both transvaginal ultrasonography (13) and by the histologic criteria of Noyes et al. (14).

Of the 40 patients, 21 were diagnosed with ovarian endometriosis on the basis of surgical pathology. These patients were classified by laparoscopy as having stage III (n = 18) or IV (n = 3) endometriosis using the revised American Society for Reproductive Medicine classification of endometriosis (15). Patients with laparoscopic evidence and histological confirmation of extra-ovarian endometriosis were not included in the study. Of the 21 endometriosis patients, 7 had only an endometrial biopsy available (eutopic endometrium), 8 had only ectopic endometrial tissue available, and 6 had both ectopic and eutopic endometrial tissue available for this study, providing a total of 13 eutopic and 14 ectopic endometrial samples.

The remaining 19 patients were found upon laparoscopy to have non-endometriotic ovarian cysts and thus served as controls for this study. Absence of endometriosis was confirmed after laparoscopic examination of the peritoneal cavity. Surgical pathology revealed 6 serous cystadenomas, 8 mucinous cystadenomas, and 5 dermoid cysts. Intraoperative endometrial biopsies were obtained from all 19 controls.

The study protocol was approved by the local Human Investigation Committee and informed consent was obtained from all subjects prior to inclusion in the study.

Total RNAs from ectopic endometriotic lesions (n=14), as well as from eutopic endometrium from patients with (n=13) and without endometriosis (n=19), were extracted by the guanidinium-thiocyanate procedure (16). One µg of total RNA from each tissue sample was reverse transcribed into cDNA using SuperScript™ III enzyme mix (Invitrogen) and the resulting cDNAs were subjected to real-time PCR.

We used the following primers: for detection of relaxin mRNA, 5'-CCAGTGGCAGAAATTGTG-3' (forward) and 5'-CTAAGGTCAGAAGAGAACTTC-3' (reverse); for detection of LGR7 mRNA, 5'-GTGGAGACAACAATGGATGG-3' (forward) and 5'-AAGAAACCGATGGAACAGC-3' (reverse); and for detection of β-actin mRNA, 5'-ACTCTTCCAGCCTTCCTTC-3' (forward) and 5'-ATCTCCTTCTGCATCCTGTC-3' (reverse). All primer pairs span at least one exon-intron boundary to avoid amplification of genomic DNA.

Real-time PCR was performed using the Rotor-Gene 3000 real-time quantitative PCR system (Corbett Research). Reactions were performed in duplicate using Platinum SYBR Green qPCR SuperMix (Invitrogen). Melt curve analyses and gel electrophoresis were performed to assess the purity and molecular size of each PCR product.

All reactions included RNAs from positive and negative tissue controls for the detection of relaxin and LGR7 mRNA. For detection of relaxin mRNA, total RNA extracted from human corpus luteum of pregnancy was used as a positive control and total RNA extracted from human lower uterine segment fibroblasts was used as a negative control. For detection of LGR7 mRNA, total RNA extracted from human lower uterine segment fibroblasts was used as a positive control and total RNA extracted from human skeletal muscle (Clontech) was used as a negative control.

LGR7 mRNA was detected in all samples and all reactions provided a C_t value which could be used for calculation of relative LGR7 mRNA expression (normalized to β -actin mRNA expression) via the comparative C_t method ($2^{-(\Delta\Delta C_t)}$ method) (17). However, since not all tissues showed detectable relaxin mRNA, we compared differences in proportion of tissues expressing relaxin.

Differences in the proportion of tissues expressing relaxin were compared using Fisher's Exact Test. For comparison of relative LGR7 relaxin receptor expression between tissue sample categories, mean ΔC_t values were calculated and compared using the Mann-Whitney test. P-values <0.05 were considered significant. The number of patients for which both ectopic and eutopic samples were available (n=6) was insufficient to allow for a paired analysis of relaxin and LGR7 mRNA expression.

Relaxin mRNA expression in samples from all patient categories is shown in Table 1. In normal endometrium, relaxin mRNA was detectable in 14 of 19 (73.7%) control samples [8 of 12 (66.7%) secretory phase samples and 6 of 7 (85.7%) proliferative phase samples]. In patients with endometriosis, relaxin mRNA was detectable in a significantly lower proportion of samples [11 of 27 (40.7%)] than in endometrium from normal controls [14 of 19 (73.7%)] ($p=0.0376$). Among patients with endometriosis, relaxin mRNA was detectable in a significantly lower proportion of ectopic samples [2 of 14 (14.3%)] than eutopic samples [9 of 13 (69.2%)] ($p=0.0063$). In secretory phase samples from endometriosis patients, relaxin mRNA expression was significantly lower in ectopic samples (detectable in 9.1% [1/11]) than in eutopic samples (detectable in 66.7% [6/9]), $p=0.016$.

LGR7 relaxin receptor mRNA was detectable in all samples. In normal control samples, LGR7 mRNA levels were 5.9-fold higher in the secretory phase (n=12) than in the proliferative phase (n=7) ($p=0.0031$). In patients with endometriosis, LGR7 mRNA expression was significantly lower (12.7-fold) in ectopic (n=14) than in eutopic samples (n=13) ($p=0.0003$), and significantly lower (9.7-fold) in ectopic than in normal control samples (n=19) ($p<0.0001$). Among samples from the secretory phase only, as shown in Table 1, LGR7 mRNA levels were 19.6-fold lower in ectopic than in eutopic samples ($p=0.0042$), and 15.4-fold lower in ectopic than in normal control samples ($p=0.0004$). LGR7 mRNA expression in eutopic tissue from endometriosis patients was similar to expression in endometrium from normal controls throughout the cycle.

The present study demonstrated that relaxin and LGR7 mRNA expression are significantly lower in endometriosis than in endometrium from normal controls. In patients with endometriosis, the expression of both relaxin mRNA and LGR7 relaxin receptor mRNA are significantly lower in ectopic samples than in eutopic samples and these differences were more pronounced in samples taken during the secretory phase.

Our current data show that LGR7 mRNA levels in endometrium from normal controls vary with cycle phase, with significantly (5.9-fold) higher expression in the secretory phase than in the proliferative phase, confirming previous data (9). In concert with the vast amount of data from various laboratories which demonstrate that relaxin is a potent decidualizing agent (4), these data support an important role for relaxin in human endometrial function.

Our findings that ectopic endometriotic samples exhibit decreased relaxin mRNA and LGR7 mRNA expression suggest that relaxin may be protective against endometriosis. In normal human and non-human primate endometrium, relaxin inhibits MMPs (3,5). Decreased relaxin action in ectopic endometrium may result in a compromise of relaxin-mediated inhibition of MMPs. This, in turn, may result in the increased MMP activity seen in endometriosis.

In contrast to the decreased expression of relaxin and its receptor in ectopic endometrium, eutopic endometrium from patients with endometriosis express similar levels of relaxin and its receptor to those detected in endometrium from normal controls. Similarly, previous studies have shown that MMP-1 protein expression in eutopic endometrium of endometriosis patients is comparable to those in endometrium from normal controls, whereas MMP-1 levels in ectopic endometrium are increased (18). These findings imply that the ectopic endometrium of endometriosis has unique properties, such as decreased relaxin and LGR7 receptor expression and increased MMP activity, which may confer the ability to invade surrounding tissues.

In summary, significantly decreased expression of relaxin mRNA and LGR7 relaxin receptor mRNA in ectopic endometriotic tissues, relative to their expression in eutopic endometrium and in endometrium from normal controls, suggest that relaxin may exert a protective effect against endometriosis, which may be mediated by relaxin's potent inhibition of endometrial MMPs.

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Table 1**Endometrial Relaxin and LGR7 mRNA Expression****A. RELAXIN EXPRESSION: ALL CATEGORIES**

	N ^a	%
Control (normal endometrium)	14/19	73.7
Proliferative phase	6/7	85.7
Secretory phase	8/12	66.7
Endometriosis	11/27	40.7
Ectopic endometrium	2/14	14.3
Proliferative phase	1/3	33.3
Secretory phase	1/11	9.1
Eutopic endometrium	9/13	69.2
Proliferative phase	3/4	75
Secretory phase	6/9	66.7

B. RELAXIN EXPRESSION: SPECIFIC SAMPLE CATEGORIES

	N ^b (%)		N ^b (%)	P value ^d
Control	14/19 (73.7)	Endometriosis	11/27 (40.7)	0.0376
Control	14/19 (73.7)	Eutopic endometrium	9/13 (69.2)	1.0000
Ectopic endometrium	2/14 (14.3)	Eutopic endometrium	9/13 (69.2)	0.0063
Ectopic endometrium (SP) ^c	1/11 (9.1)	Eutopic endometrium (SP) ^c	6/9 (66.7)	0.016

C. RELAXIN RECEPTOR LGR7: RELATIVE mRNA EXPRESSION

	N	Relative Expression
Proliferative phase		
Control	7	1 ^e
Eutopic endometrium	4	0.95
Ectopic endometrium	3	0.15
Secretory phase		
Control	12	1 ^e
Eutopic endometrium	9	1.27
Ectopic endometrium	11	0.064 ^f

^a N = number of samples with detectable relaxin mRNA levels/total number of samples in the category

^b N = number of samples with detectable relaxin mRNA levels/total number of samples in the category

^c SP = secretory phase

^d P values obtained using Fisher's Exact Test.

^e Expression levels (calculated as described in the text) of LGR7 mRNA in proliferative and secretory phase samples from normal controls were set to a value of 1 and expression levels in samples from endometriosis patients were computed relative to controls.

^f In the secretory phase, LGR7 mRNA levels were significantly lower (19.6-fold) in ectopic than in eutopic samples (p=0.0042), and significantly lower (15.4-fold) in ectopic than in normal control samples (p=0.0004).