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# Effects of a Levonorgestrel-Releasing Intrauterine System on the Expression of Steroid Receptor Coregulators in Adenomyosis

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**Abstract**

Although the pathophysiology of adenomyosis has not been clarified, it is thought to be related to ectopic endometrium, which depends on hormonal regulation. The levonorgestrel-releasing intrauterine system (LNG-IUS) is effective for the medical treatment of adenomyosis. However, the underlying molecular mechanisms by which LNG-IUS ameliorates adenomyosis pathology remain unclear. This study was designed to compare the expression levels of steroid receptor coregulators in human endometrium of control and participants with adenomyosis and to determine whether LNG-IUS modulated their expression. Immunohistochemistry with *H*-scores was performed. Steroid receptor coactivators were shown to have significantly decreased expressions at the secretory phase in the LNG-IUS group when compared to the other groups. Expression of transcriptional intermediary factor 2 was lower in the LNG-IUS group than in both the control group ($P = .015$) and the untreated adenomyosis group ($P = .019$) during the secretory phase. Amplified in breast cancer 1 expression was higher in the stromal cells of the untreated adenomyosis group than in those of the controls ($P = .017$) during the secretory phase; however, levels were lower in the LNG-IUS group ($P = .005$). Nuclear receptor corepressor expression increased during the proliferative phase and decreased during the secretory phase in untreated adenomyosis; this pattern was reversed in the control and LNG-IUS groups. Thus, an altered expression of steroid receptor coregulators may play a role in adenomyosis development and treatment.

**Keywords**

LNG-IUS, coregulator-related disease, steroid receptor coregulator

## Introduction

Adenomyosis is a common benign gynecologic disease that is characterized by the presence of endometrial glands and stroma located deep within the myometrium in an abnormal pattern. The precise pathogenesis of adenomyosis is unknown; however, the most widely held opinion is that it develops as a result of downgrowth and invagination of the stratum basalis into the myometrium.[1] These adenomyotic lesions, referred to as ''ectopic endometrium,'' induce a diffusely enlarged uterus and are responsible for clinical symptoms including heavy menstrual bleeding, dysmenorrhea, and pelvic pain in about two-thirds of women with the disease.[2] Due to its poorly defined margin, an adenomyotic lesion cannot be completely enucleated, and the only definite therapeutic modality of adenomyosis until recently has been hysterectomy.

Recently, the levonorgestrel-releasing intrauterine system (LNG-IUS) was demonstrated to be a novel effective medical treatment of adenomyosis, as it reduces uterine bleeding, dysmenorrhea, and uterine volume.[3-6] In addition to providing symptom relief, levonorgestrel has a direct shrinking effect on the adenomyotic deposits, which improves uterine contractility and decreases uterine size.[7] Expression of the progesterone receptor (PR) is significantly higher in adenomyosis uteri than

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in nonaffected uteri.[8] However, following LNG-IUS insertion, significant downregulation of $PR_A$ and $PR_B$ in the endometrium was observed.[9] Moreover, progestin and endogenous progesterone induced significantly higher levels of apoptosis in human adenomyotic stromal cells, suggesting that certain effects occur directly via PR in adenomyosis.[10] The molecular mechanism that underpins the effects of LNG-IUS on the endometrial glands and stromal cells in patients with adenomyosis remains unclear, though a number of mechanisms for progestin-induced growth suppression of endometrial cells have been suggested, such as downregulation of estrogen receptor (ER)[11,12] and alteration in the levels of steroid metabolizing enzymes,[13] growth factors, or cytokines.[14]

Given that the endometrium undergoes cycles of proliferation and differentiation due to the effects of estrogen and progesterone, the activity of steroid receptors in adenomyosis has been a key focus.[15] The actions of steroid receptors are influenced by specific coregulators, which are classified into 2 subclasses of coactivators and corepressors.[16-20] In response to signal transduction pathways activated by steroid hormones, these molecules stimulate or suppress the transcription of target genes in a tissue-specific manner. Several steroid receptor coregulators have been associated with normal and pathological functions in human endometrium,[21,22] endometriosis,[23-25] endometrial hyperplasia,[26,27] and breast and ovarian cancer cells.[17,28,29] Indeed, the concept of coregulator-related disease has been suggested based on links between specific coreceptors and a spectrum of diseases, including hypothyroidism, androgen insensitivity syndrome, and hormone-dependent cancers.[30] An association between hormone-dependent disorders and qualitative or quantitative abnormalities of coregulators was proposed as part of this concept. Although a number of studies on the expression of these coregulators in endometriosis have been performed, there are very few corresponding systematic studies focusing on adenomyosis. To begin to examine the steroid-receptor coregulator expression in adenomyosis, we selected 2 representative coactivators, transcriptional intermediary factor 2 (TIF-2) and amplified in breast cancer 1 (AIB-1), and a corepressor, nuclear receptor corepressor (NCoR), which is implicated in progestin-induced growth suppression.[31] The aim of the study was to determine whether the expression patterns of TIF-2, AIB-1, and NCoR differed between control and adenomyosis groups and whether LNG-IUS treatment was associated with changes in their expressions.

## Materials and Methods

### Participants and Tissue Samples

From May 2007 to May 2012, a total of 38 patients who underwent hysterectomy at Gangnam Severance Hospital, Yonsei University College of Medicine, were enrolled retrospectively in the study based on their final diagnosis and divided into adenomyosis and control groups. The final diagnosis of adenomyosis was made based on histopathologic confirmation after hysterectomy, by expert pathologists. Of the 38 women with adenomyosis, 17 were treated with an LNG-IUS (Mirena; Bayer Schering Pharma, Berlin, Germany; mean duration 16.8 months; range 4-47 months). Preoperative diagnosis for the participants was made using a 2-dimensional transvaginal ultrasound. We used the following sonographic diagnostic criteria for adenomyosis according to previous studies: myometrial cyst, distorted and heterogeneous myometrial echotexture, poorly defined focus of abnormal myometrial echotexture, and a globular and/or asymmetric uterus.[32,33] Women diagnosed with carcinoma in situ following the final pathology report were included in the study as the control group. Study participants reported that they had regular menstrual cycles of between 28 and 30 days duration. None of the participants received any hormonal treatment for at least 3 months preceding the surgery, with the exception of the LNG-IUS group, in which women who had regular menstrual cycles of between 28 and 35 days were enrolled. Menstruation cycles of the participants were recalled, and the menstrual day was determined based on the last menstruation start date. Histologic dating of the endometrium was assigned using the criteria of Noyes et al,[34] determined by expert pathologists.

Although women with the LNG-IUS experienced an improvement in symptoms (manifested as a reduction in pain or menstrual bleeding) after insertion, they underwent hysterectomies due to irregular vaginal spotting or residual symptoms that caused considerable patient discomfort. The LNG-IUS was removed shortly before the hysterectomy at the operation field. This study was carried out in accordance with the ethical standards of the Helsinki declaration and was approved by the Institutional Review Board of Gangnam Severance Hospital.

### Immunohistochemistry

Immunohistochemical staining was performed on paraffin-embedded sections by the avidin–biotin–peroxidase complex method using a Vectastain Elite ABC kit (Vector Laboratories, Burlingame, California). Antibodies for TIF-2, AIB-1, and NCoR were purchased from Bethyl Laboratories (Montgomery, Texas), Atlas Antibodies AB (Stockholm, Sweden), and Abcam (Cambridge, United Kingdom), respectively. Tissues were fixed in 10% formalin for 8 to 12 hours, dehydrated, embedded in paraffin blocks, and cut into 5-μm sections. After routine deparaffinization and rehydration, sections were first incubated with 1.5% goat serum/phosphate-buffered saline (PBS) as a blocking step for 30 minutes at room temperature. After the blocking of endogenous peroxidase activity, the sections were incubated with specific primary antibodies (TIF-2, diluted 1:450 with PBS/bovine serum albumin [BSA]; AIB-1, diluted 1:600 with PBS/BSA; NCoR, diluted 1:250 with PBS/BSA) or control nonimmunized rabbit serum at 4°C overnight. After washing with PBS, biotinylated antirabbit immunoglobulin G (IgG) was applied for 30 minutes at room temperature. For negative control of the secondary antibody, biotinylated antirabbit IgG was applied for rabbit primary antibodies. After washing with PBS, peroxidase-conjugated avidin solution was applied for 30 minutes and visualized using 0.05% 3,3′-diaminobenzidine. Light counterstaining was performed with hematoxylin.

Positive controls for TIF-2, AIB-1, and NCoR were human breast tissue with a proven expression of the corresponding epitopes. Negative controls were performed by replacing the primary antibodies with normal rabbit or mouse serum at the same concentrations as those of the corresponding primary antibodies.

### Interpretation of Immunohistochemical Staining

The immunoreactivity (IR) was semiquantitatively estimated in stained areas of the endometrial glandular cells and the stromal compartment using an immunohistochemical histological score (*H*-score).[35] The *H*-score was calculated by the formula [*H*-score = $\sum$(Pi × *i*)/100], where Pi stands for the percentage of positive cells for each intensity and *i* stands for the range of staining intensity. Pi was assigned as follows: 0 for <5%, 1 for 5% to 25%, 2 for 25% to 50%, 3 for 50% to 75%, and 4 for >75%. The staining intensity (*i*) was assigned using a 4-point scale as follows: 0 = negative, 1 = weak, 2 = moderate, and 3 = strong. Glandular cells (including the nucleus and cytoplasm) and stromal cells were classified separately for each specimen. An area was considered strong when the staining intensity was similar to the corresponding positive control. At least 3 randomly chosen areas from each sample were evaluated and averaged for each compartment. The scoring procedure was carried out by 3 different, blinded reviewers without any information about the clinical background. The interobserver discrepancies were adjusted using κ statistics.

### Statistical Analysis

The data are reported as median and interquartile range (IQR). Comparison of the continuous variables among the 3 groups was performed with the Kruskal-Wallis test, with the use of Dunn's procedure for multiple comparisons. A Mann-Whitney *U* test was used to compare the *H*-scores between the proliferative and secretory phases in each group. κ Statistics were performed to examine observer variation for the *H*-scores. For all analyses, a $P < .05$ was considered to be statistically significant. All statistical analyses were conducted using SPSS software version 18.0 (SPSS Inc, Chicago, Illinois).

## Results

The general characteristics of participants are presented in Table 1. For each group, the number of participants, mean age, mean BMI, mean gravidity and parity, and menstrual phase of endometrium classified by histologic diagnosis are shown.

**Table 1.** Characteristics of Participants.[a]

| | Control | Untreated Adenomyosis | LNG-IUS-Treated Adenomyosis |
|---|---|---|---|
| Participants, n | 23 | 21 | 17 |
| Age, years | 39.96 ± 1.61 | 38.76 ± 0.70 | 42.81 ± 1.46 |
| BMI, kg/m$^2$ | 22.16 ± 0.66 | 22.34 ± 0.65 | 23.70 ± 0.91 |
| Gravidity, n | 2.39 ± 0.39 | 3.29 ± 0.31 | 3.00 ± 0.46 |
| Parity, n | 1.61 ± 0.23 | 1.48 ± 0.15 | 1.56 ± 0.24 |
| Endometrial menstrual phase | | | |
| Proliferative, n | 15 (65.2%) | 12 (57.1%) | 9 (52.9%) |
| Secretory, n | 8 (34.8%) | 9 (42.9%) | 8 (47.1%) |

Abbreviations: BMI, body mass index; LNG-IUS, levonorgestrel-releasing intrauterine system; SD, standard deviation.
[a]Data are presented as mean ± SD.

### Immunohistochemical Staining of TIF-2 and AIB-1 in the Endometrium of Control and Participants With Adenomyosis

There was no cyclic difference in the expression of TIF-2 in endometrial tissue of the control group throughout the menstrual cycle (epithelial cell nucleus, $P = .189$; epithelial cell cytoplasm, $P = .764$; and stromal cell, $P = .436$). There was significantly decreased expression of AIB-1 in the stromal cells of the control group at the secretory phase compared to the proliferative phase ($P = .01$). However, in the untreated and LNG-IUS adenomyosis groups, the levels of AIB-1 expression were comparable throughout the menstrual cycle (epithelial cell nucleus, $P = .132$; epithelial cell cytoplasm, $P = .809$; and stromal cell, $P = .109$).

Expression of TIF-2 was detected in the cytoplasm of glandular and stromal cells (Table 2 and Figure 1). When compared to the controls, there was significantly decreased expression of TIF-2 in the LNG-IUS-treated group in stromal cells during the proliferative phase (Table 2; $P = .003$). Similarly, TIF-2 was significantly less expressed in the stromal cells of the LNG-IUS-treated group in the secretory phase than in those of the controls ($P = .015$) and untreated participants ($P = .019$). The interobserver reproducibility κ values were .394 ($P < .0001$) and .382 ($P < .0001$) for the nuclei and cytoplasm of glandular cells and .351 ($P < .0001$) for stromal cells.

The AIB-1 expression was markedly increased in stromal cells compared to glandular cells in all 3 groups (Table 2 and Figure 2). Regardless of menstrual phase, stromal cell AIB-1 expression was significantly decreased in the LNG-IUS group compared to the untreated adenomyosis group (Table 2; proliferative phase, $P = .002$; secretory phase, $P = .005$). Moreover, AIB-1 expression in stromal cells was significantly decreased in the secretory phase compared to the proliferative phase in the controls ($P = .011$), although no significant changes were noted in both untreated and treated adenomyosis groups ($P = .243$ and $P = .107$, respectively). In addition to the decreased immunohistochemistry score of the patients with adenomyosis treated with LNG-IUS, this group also exhibited atrophied glands and stroma upon microscopic observation (Figures 3 and 5). The interobserver reproducibility κ values were .410 ($P < .0001$) and .433 ($P < .0001$) for the nuclei and cytoplasm of glandular cells and .386 ($P < .0001$) for stromal cells, respectively.

We found no significant differences in the levels of coactivators between the control and untreated adenomyosis groups, with the exception of AIB-1 expression in stromal cells, which

**Table 2.** Scoring of TIF-2, AIB-1, and NCoR Immunoreactivity (*H*-Scores) in Control and Adenomyosis Endometrium.[a]

| Menstrual Phase | Control (n = 23) | Untreated Adenomyosis (n = 21) | LNG-IUS-Treated Adenomyosis (n = 17) | *P* Value |
|---|---|---|---|---|
| TIF-2 | | | | |
| Glandular cell nucleus | | | | |
| Proliferative | 15 (0-35) | 0 (0-0) | 0 (0-0) | 0.047 |
| Secretory | 55 (0-120) | 3 (0-5) | 6 (0-7.5) | 0.278 |
| Glandular cell cytoplasm | | | | |
| Proliferative | 165 (150-200) | 180 (100-200) | 90 (17.5-117.5) | 0.066 |
| Secretory | 140 (80-200) | 200 (170-240)[b] | 95 (45-145)[b] | 0.024 (0.019[b]) |
| Stromal cell | | | | |
| Proliferative | 120 (95-155)[b] | 60 (20-150) | 10 (0-30)[b] | 0.004 (0.003[b]) |
| Secretory | 90 (50-160)[c] | 80 (60-100)[b] | 0 (0-0)[b,c] | 0.008 (0.015[c],0.019[b]) |
| AIB-1 | | | | |
| Glandular cell nucleus | | | | |
| Proliferative | 30 (10-50) | 15 (0-22.5) | 5 (2.5-10) | 0.278 |
| Secretory | 25 (0-43.75) | 22.5 (10-30) | 10 (10-15) | 0.387 |
| Glandular cell cytoplasm | | | | |
| Proliferative | 5 (0-25) | 17 (0-22.5) | 0 (0-2.5) | 0.275 |
| Secretory | 7 (0-21.25) | 15 (3.75-45) | 0 (0-10) | 0.240 |
| Stromal cell | | | | |
| Proliferative | 120 (110-150) | 225 (172.5-240)[b] | 75 (40-105)[b] | 0.002 (0.002[b]) |
| Secretory | 30 (27.5-72.5)[c] | 180 (12.375-225)[b,c] | 30 (17.5-55)[b] | 0.002 (0.017[c],0.005[b]) |
| NCoR | | | | |
| Glandular cell nucleus | | | | |
| Proliferative | 210 (110-250)[c] | 5 (0-15)[b,c] | 140 (130-150)[b] | <0.0001 (<0.0001[c],0.021[b]) |
| Secretory | 45 (32.5-85) | 29 (0-110) | 2.5 (0-11.25) | 0.144 |
| Glandular cell cytoplasm | | | | |
| Proliferative | 140 (125-220)[b] | 10 (0-15)[b] | 90 (75-90) | <0.0001 (<0.0001[b]) |
| Secretory | 50 (38.75-93.75) | 60 (15-80) | 2.5 (0-8.75) | 0.063 |
| Stromal cell | | | | |
| Proliferative | 110 (60-145)[b] | 0 (0-11.25) [b] | 50 (25-80) | 0.001 (0.001[b]) |
| Secretory | 17.5 (0-85) | 30 (0-50) | 0 (0-7.5) | 0.349 |

Abbreviations: LNG-IUS, levonorgestrel-releasing intrauterine system; EM, endometrium; IQR, interquartile range; TIF, transcriptional intermediary factor.
[a]Data are presented as median and IQR.
[b,c]Significance by post hoc analysis.

was markedly increased in the untreated adenomyosis group during the secretory phase ($P = .017$). Stromal cells of the LNG-IUS-treated adenomyosis group had significantly lower levels of coactivators TIF-1 and AIB-2 than those of the untreated adenomyosis group.

### *Immunohistochemical Staining of NCoR in Endometrium of Normal and Participants With Adenomyosis*

Expression of NCoR was diffuse in glandular and stromal cells of normal endometrium (Table 2, Figures 4 and 5) and exhibited significant changes between the proliferative and secretory phases (epithelial cell nucleus, $P = .018$; epithelial cell cytoplasm, $P = .032$; and stromal cell, $P = .042$). Specifically, the expression of NCoR was significantly reduced in the secretory phase. In the LNG-IUS-treated adenomyosis group, a pattern of cyclic change similar to that observed in the control group was also observed (Table 2; glandular cell nucleus, $P = .014$; glandular cell cytoplasm, $P = .014$; and stromal cell, $P = .096$). In contrast to the tendency in the control and LNG-IUS-treated adenomyosis groups, the situation was different in the untreated adenomyosis group. Specifically, the NCoR expression pattern was reversed between the proliferative and the secretory phases in the endometrial glands and stroma, although with variable levels of statistical significance (glandular cell nucleus, $P = .093$; glandular cell cytoplasm, $P = .055$; and stromal cell, $P = .102$). The interobserver reproducibility $\kappa$ values were .389 ($P < .0001$) and .443 ($P < .0001$) for the nuclei and cytoplasm of glandular cells and .429 ($P < .0001$), respectively.

## Discussion

We observed increased expression of the TIF-2 coactivator in the cytoplasm of endometrial glandular cells and stromal cells. In stromal cells during the secretory phase, a significant decrease in TIF-2 expression was observed in the LNG-IUS-treated adenomyosis group compared with the other 2 groups. Another coactivator, AIB-1, was mainly distributed in endometrial stromal cells, and its levels were significantly increased in the untreated adenomyosis group compared to the LNG-IUS-treated group throughout the menstrual cycle. Compared to the

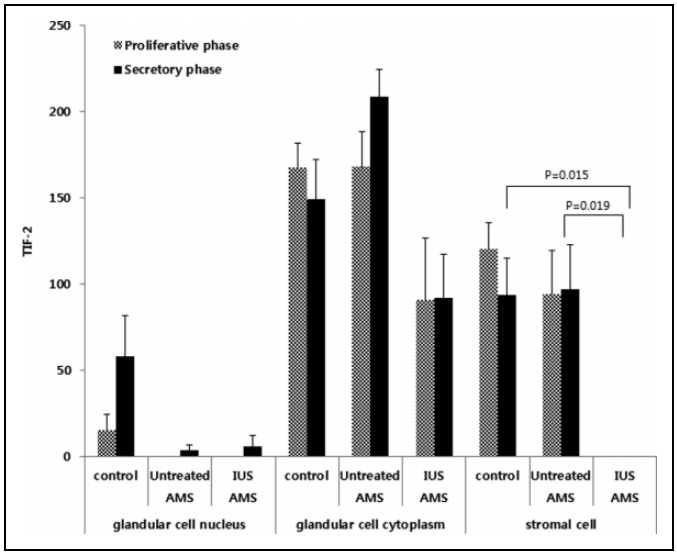


**Figure 1.** *H*-Scores of transcriptional intermediary factor 2 (TIF-2) comparison between the proliferative and the secretory phases in glandular and stromal cells of endometrium, assessed among control, untreated adenomyosis, and LNG-IUS-treated adenomyosis groups. *H*-Scores are presented as mean ± standard deviation (SD). Intergroup comparisons were performed using the Kruskal-Wallis test with Dunn's procedure. Interphase comparisons were performed using the Mann-Whitney *U* test. AMS indicates adenomyosis; IUS AMS, LNG-IUS-treated adenomyosis; LNG-IUS, levonorgestrel-releasing intrauterine system.

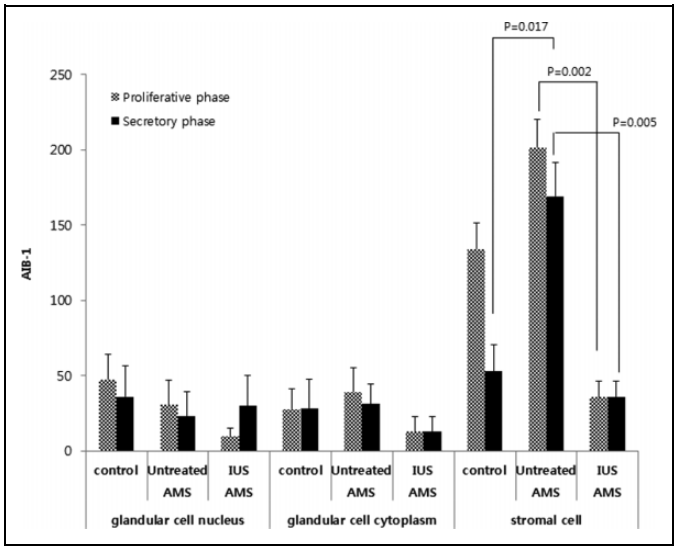


**Figure 2.** *H*-Scores of AIB-1 comparison between the proliferative and secretory phases in glandular and stromal cells of endometrium, assessed between control, untreated adenomyosis, and LNG-IUS-treated adenomyosis groups. *H*-Scores are presented as mean ± standard deviation (SD). Intergroup comparisons were performed using the Kruskal-Wallis test with Dunn's procedure. Interphase comparisons were performed using the Mann-Whitney *U* test. AMS indicates adenomyosis; IUS AMS, LNG-IUS-treated adenomyosis; LNG-IUS, levonorgestrel-releasing intrauterine system.

controls and the treated group, expression of AIB-1 was significantly increased in the untreated adenomyosis group only at the secretory phase. Expression of the NCoR corepressor, which tended to increase at the proliferative phase and decrease at the secretory phase in both the control and LNG-IUS groups, was significantly different in the untreated adenomyosis group; indeed, the expression pattern was reversed in the latter case.

Endometrial stromal and epithelial cells respond to steroid hormones (estrogen and progesterone) during the menstrual cycle. Progesterone has antiestrogenic effects due to its regulation of paracrine factors and enzymes,[36] which act mainly in the secretory phase. Paracrine gene regulation by the PR occurs in the endometrium, likely due to the activity of progesterone on the stromal PR, which stimulates paracrine factors and signal transduction; in turn, these mediators act on neighboring epithelial cells.[37] Thus, there has been intense focus on steroid receptors and related signal transduction pathways in both eutopic and ectopic endometrial pathologies.[25,38-41] Additionally, progesterone resistance is another key mechanism that has gained credence over the past decade. Since adenomyosis is also thought to be arisen from dislocation of the basal endometrium, this key pathophysiological feature is also observed.[42,43] Ectopic endometrium in adenomyosis shares the features such as altered apoptosis[44,45] and proliferation of endometrium,[46] progesterone resistance,[47,48] antioxidant, and enzymes.[49,50]

Members of the P160 steroid coactivator family interact with steroid receptors and specific transcription factors. They also recruit enzymes (including chromatin remodeling factors) that modulate transcription in order to facilitate the assembly of transcriptional activator complexes.[29] Transcriptional intermediary factor 2 is suggested to have potential roles in PR-mediated physiologic reproductive function.[51,52] However, in the pathologic state, TIF-2 seems to operate via different mechanisms. For example, the expression levels of TIF-2 and AIB-1 are significantly increased in the endometrium of patients with polycystic ovary syndrome compared to healthy controls, which suggests that these factors may increase estrogen sensitivity.[21] In addition, increased expression levels of *TIF-2* and *AIB-1* mRNAs[27,53] have been reported in endometrial carcinoma. In vitro experiments show that reduced expression of TIF-2 is associated with reduced estrogen-induced cell proliferation and downregulation of estrogen target-gene expression in breast cancer cells.[54] Furthermore, depletion or downregulation of AIB-1 leads to reduced proliferation, attenuated estrogen-dependent colony formation, and reduced survival in breast cancer cells.[28,55] Based on these previous observations and our current results, we suggest that the endometrium of patients with adenomyosis may be more exposed to estrogen with dysfunctional steroid receptors and that LNG-IUS treatment may allow the modification of signal transduction via PR by changing the expressions of coregulators. In our study, AIB-1 was expressed predominantly in stromal cells, and its level varied between groups during the secretory phase. In contrast, TIF-2 was detected in the cytoplasm of glandular cells and in stromal cells, and significant changes (compared to the LNG-treated group) were found only in stromal cells in the secretory phase. The stroma of the endometrium is the

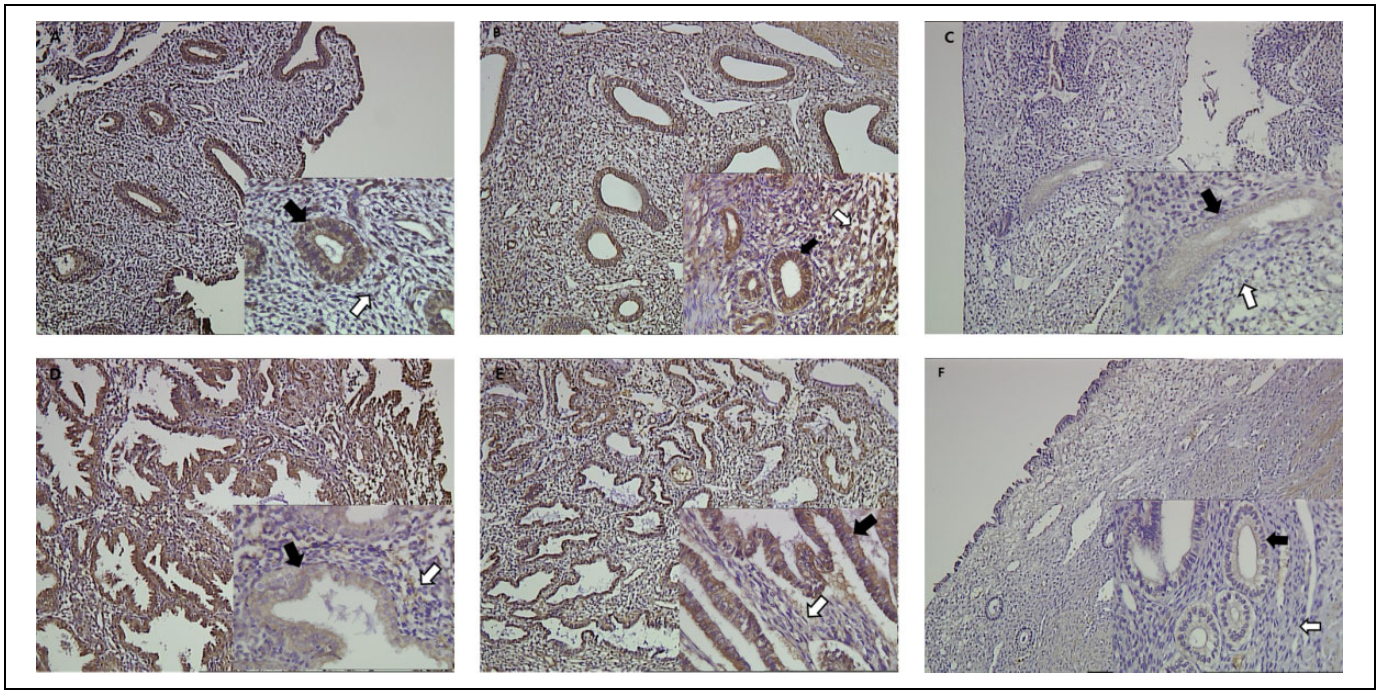

**Figure 3.** Expression of TIF-2 in endometrium from control, untreated adenomyosis, and LNG-IUS-treated adenomyosis groups in 2 phases of the menstrual cycle. A, Proliferative phase, control group, endometrium, magnification ×100 (background) and ×400 (bottom right). B, Proliferative phase, AMS, endometrium, magnification ×100 (background) and ×400 (bottom right). C, Proliferative phase, IUS AMS, endometrium, magnification ×100 (background) and ×400 (bottom right). D, Secretory phase, control group, endometrium, magnification ×100 (background) and ×400 (bottom right). E, Secretory phase, AMS, endometrium, magnification ×(background) and ×400 (bottom right). F, Secretory phase, IUS AMS, endometrium, magnification ×100 (background) and ×400 (bottom right). Black arrow: gland, white arrow: stroma. AMS, adenomyosis; IUS AMS, LNG-IUS-treated adenomyosis. (The color version of this figure is available in the online version at http://rs.sagepub.com/.)

main site of PR-dependent paracrine signaling to epithelial ER. Estrogen-dependent epithelial proliferation can be elicited by PR signals.[37] The abnormalities we observed in adenomyosis may be due to the aberrant expressions of coactivators or their disease-specific functions. For example, TIF-2 expression may confer increased ER sensitivity, whereas AIB-1 appears to be associated more with PR signaling.

The expression of NCoR in the endometrium and its relationship with progesterone have been extensively explored.[56,57] Nuclear receptor corepressor is able to form a complex with PR[57] and is recruited to a PR-responsive element in the presence of progestins,[58] thus making it likely that NCoR is involved in the downregulation of genes targeted by progesterone. Nuclear receptor corepressor is also upregulated following progestin treatment in endometrial hyperplasia.[26,31] A recent study suggested that progesterone responsiveness was modulated in the endometriotic epithelium via impaired progesterone signaling, which resulted in reduced differentiation and apoptosis.[59] In the present study, NCoR expression increased in the proliferative phase of the menstrual cycle and decreased in the secretory phase, in accordance with previous reports.[22,60] The pattern was the same in the LNG-IUS-treated adenomyosis group yet was reversed in the untreated adenomyosis group both in glandular and stromal cells. In the endometrium, the level of PR progressively increases during the proliferative phase, reaches a peak immediately before ovulation, and declines during the secretory phase.[22,40] Decreased

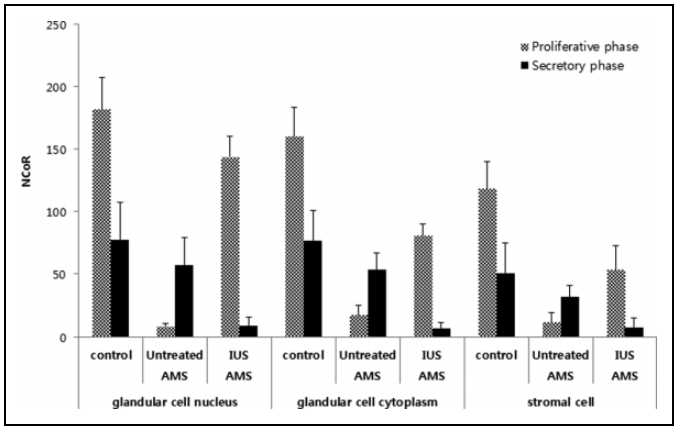


**Figure 4.** H-scores of NCoR comparison between the proliferative and secretory phases in glandular and stromal cells of endometrium, assessed between control, untreated adenomyosis, and LNG-IUS-treated adenomyosis groups. H-scores are presented as mean ± SD. AMS, adenomyosis; IUS AMS, LNG-IUS-treated adenomyosis. Intergroup comparisons were performed using the Kruskal-Wallis test with Dunn's procedure. Interphase comparisons were performed using the Mann-Whitney *U* test.

NCoR expression during the proliferative phase in adenomyosis may affect the activity of PR, thereby engendering progesterone resistance. Therefore, decreased expression of NCoR during the proliferative phase and the loss of its cyclic expression throughout

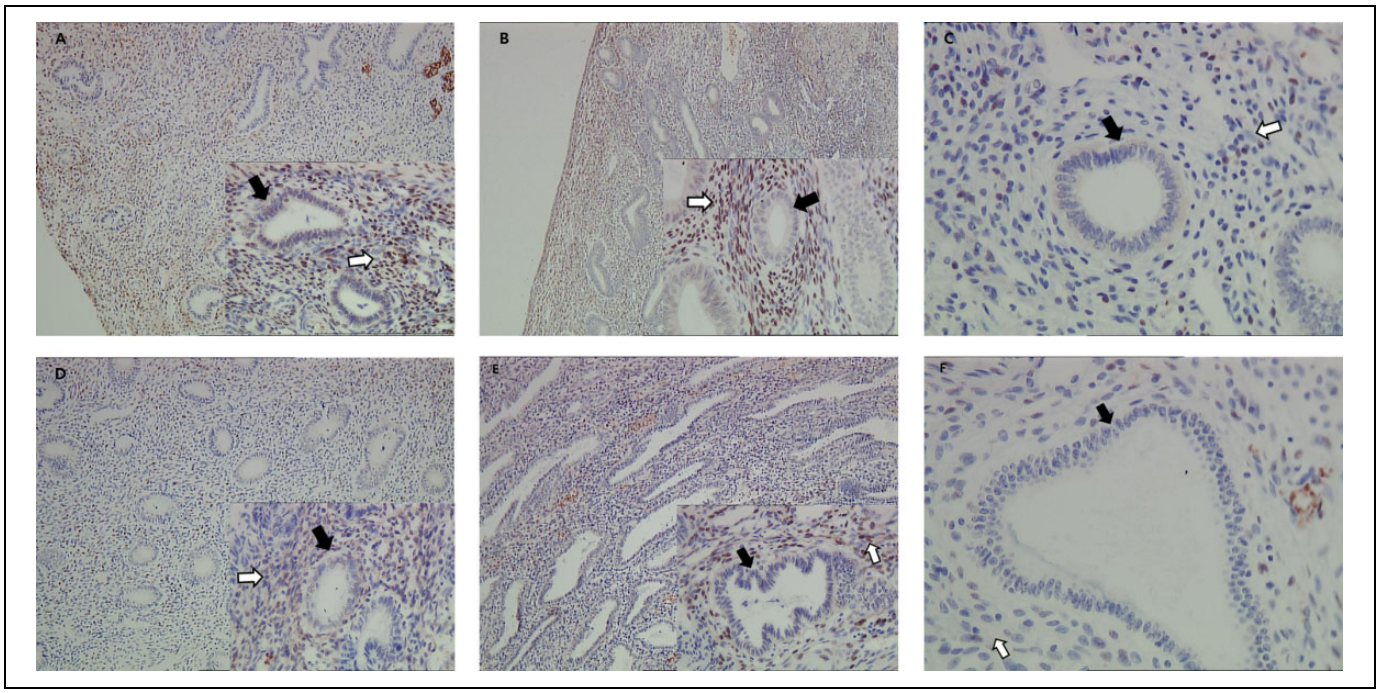

**Figure 5.** Expression of AIB-1 in endometrium from control, untreated adenomyosis, and LNG-IUS-treated adenomyosis groups in 2 phases of the menstrual cycle. A, Proliferative phase, control group, endometrium, magnification ×100 (background) and ×400 (bottom right). B, Proliferative phase, AMS, endometrium, magnification ×100 (background) and ×400 (bottom right). C, Proliferative phase, IUS AMS, endometrium, magnification ×400. D, Secretory phase, control group, endometrium, magnification ×100 (background) and ×400 (bottom right). E, Secretory phase, AMS, endometrium, magnification ×100 (background) and ×400 (bottom right). F, Secretory phase, IUS AMS, endometrium, magnification ×400. Black arrow: gland, white arrow: stroma. AMS, adenomyosis; IUS AMS, LNG-IUS-treated adenomyosis. (The color version of this figure is available in the online version at http://rs.sagepub.com/.)

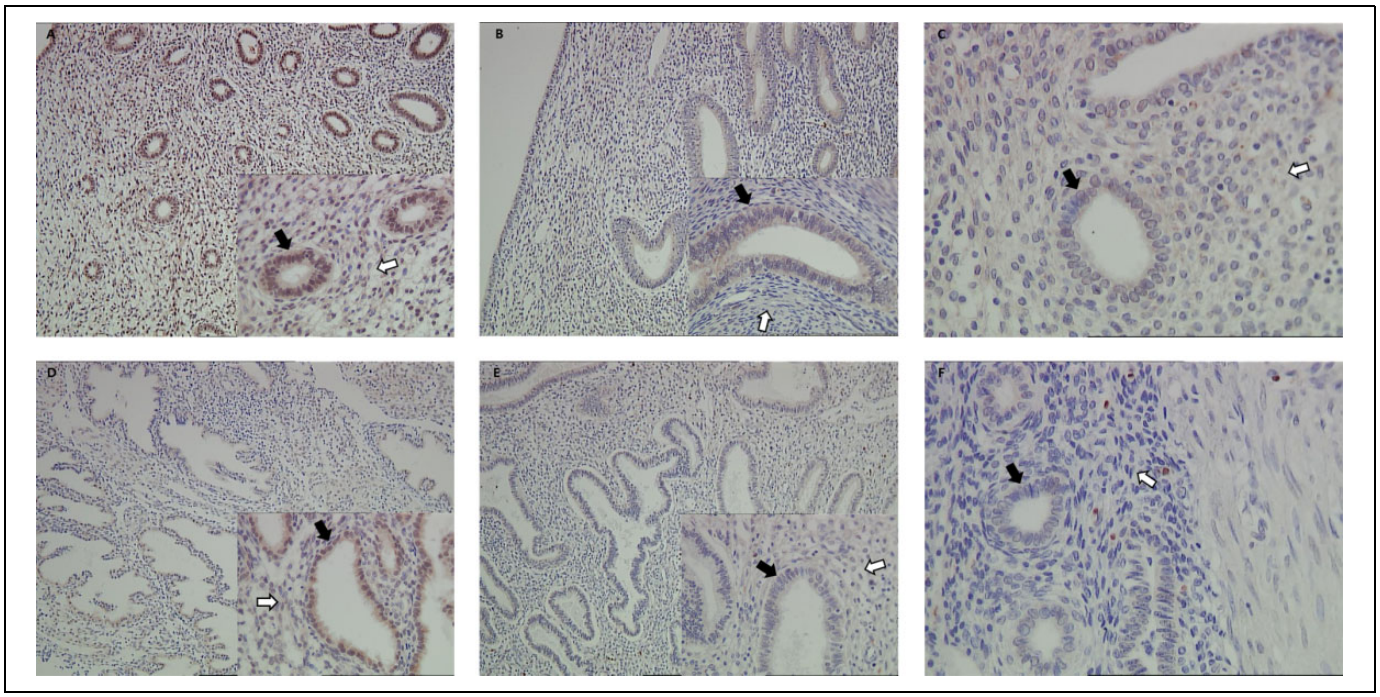

**Figure 6.** Expression of NCoR in endometrium from control, untreated adenomyosis, and LNG-IUS-treated adenomyosis groups in 2 phases of the menstrual cycle. A, Proliferative phase, control group, endometrium, magnification ×100 (background) and ×400 (bottom right). B, Proliferative phase, AMS, endometrium, magnification ×100 (background) and ×400 (bottom right). C, Proliferative phase, IUS AMS, endometrium, magnification ×400. D, Secretory phase, control group, endometrium, magnification ×100 (background) and ×400 (bottom right). E, Secretory phase, AMS, endometrium, magnification ×100 (background) and ×400 (bottom right). F, Secretory phase, IUS AMS, endometrium, magnification ×400. Black arrow: gland, white arrow: stroma. AMS, adenomyosis; IUS AMS, LNG-IUS-treated adenomyosis. (The color version of this figure is available in the online version at http://rs.sagepub.com/.)

menstruation in untreated adenomyosis may implicate NCoR in the pathogenesis of this disease. Furthermore, increased NCoR expression following LNG-IUS treatment during the proliferative phase suggests a possible beneficial role of LNG-IUS in adenomyosis via modification of gene expression.

To our knowledge, this is the first report to characterize and compare steroid receptor coregulators in adenomyosis and to subdivide adenomyosis into untreated and LNG-IUS-treated groups. The findings suggest that further study of steroid hormone signaling in adenomyosis will be helpful in clarifying the origins of its pathophysiology and in stratifying patients based on the molecular composition of their hormone-responsive signaling networks. The main weakness of our study is that the expression of coregulators was only evaluated using semiquantitative immunostaining intensity scoring. A secondary analysis using either Western or Northern blot analysis or quantitative real time polymerase chain reaction (RT-PCR) would bolster our initial observations. Another limitation was the inherent treatment bias associated with the selection of the LNG-IUS group, as they ultimately failed their treatment with LNG-IUS in proceeding with the hysterectomy.

In conclusion, altered expressions of both steroid receptor coactivators and corepressors may have a role in adenomyosis development. Moreover, LNG-IUS, which has been broadly applied to patients with adenomyosis in clinical practice, may affect the disease by adjusting the signal transduction pathways that are activated by steroid hormones.

## Authors' Note

The work reported was performed at Severance Hospital, Yonsei University College of Medicine, Seoul, Republic of Korea.

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