

Molecular Background of Estrogen Receptor Gene Expression in Endometriotic Cells

Reproductive Sciences
2016, Vol. 23(7) 871-876
© The Author(s) 2015
Reprints and permission:
sagepub.com/journalsPermissions.nav
DOI: 10.1177/1933719115623642
rs.sagepub.com


Masao Izawa, PhD¹, Fuminori Taniguchi, MD, PhD¹,
and Tasuku Harada, MD, PhD¹

Abstract

The molecular background of estrogen receptor (ER) expression is important to understand the pathophysiology of the high estrogen environment in endometriosis. However, the molecular details have not been fully understood. The objective of this study is to evaluate the molecular background of ER α and ER β messenger RNA (mRNA) expression in endometriotic cells. The following summarizes our observations: (1) ER α mRNA expression in endometriotic cells was estimated to be approximately one-tenth of that in endometrial cells. (2) Three mRNAs, which include 3 different 5'-untranslated exons tagged to an open reading frame of wild-type ER α , were detected. (3) Expression of ER β mRNA depends mostly on 0N promoter and includes 2 open reading frames: one for a wild-type ER β 1 and another for a splice variant ER β 2. (4) Expression of ER β 1 mRNA was approximately 40-fold higher than that in endometrial cells. (5) Expression of ER β 2 mRNA was almost at a comparable level of the ER β 1. (6) ER α and ER β mRNAs are equivalently expressed in endometriotic cells. These observations show the molecular background of ER mRNA expression in endometriotic cells and provide a clue to further understanding the estrogen-dependent pathophysiology leading to clinical application in endometriosis.

Keywords

estrogen receptor α , estrogen receptor β , mRNA expression, endometriosis

Introduction

Endometriosis is an estrogen-dependent, inflammatory disease characterized by the presence of endometrium-like tissue primarily on the pelvic peritoneum and ovaries. The role of estrogen is obvious because the symptoms associated with endometriosis often disappear after menopause, and the administration of GnRH agonists or progestin relieves the pelvic lesions and associated pain. A marked upregulation of aromatase gene associated with aberrant DNA demethylation^{1,2} leads to a high estrogen environment in endometriotic tissues. Its inhibition by aromatase inhibitor is effective to reduce endometriosis-associated symptoms.³ One of the important issues to be clarified is a further understanding of the role of estrogen-dependent growth mechanism in endometriotic tissues. To explain the pathophysiology leading to clinical applications, distinct profiles of estrogen receptor (ER) expression have been documented: a higher ER β and a lower ER α expression in endometriotic tissues, which is in the inverse relationship in endometrium.⁴⁻⁷ Estrogen receptor α is the dominant receptor in the uterus and mediates a number of estrogen actions, including the stimulation of proliferation and the induction of progesterone receptor expression. In contrast, ER β antagonizes the inflammatory and proliferative actions of ER α .^{8,9} Therefore, the ER expression profile has been proposed as a major background of estrogen action in

endometriosis. However, the molecular details of ER expression in endometriotic tissues have not been fully understood so far.

Recently, several splice variants of ER β showing distinct transcriptional activities have been identified, and their roles along with the classical wild type have been extensively re-evaluated.¹⁰⁻¹³ These findings prompted us to re-evaluate the molecular background of ER expression in endometriosis. In this study, we focused on the mRNA expression in endometriotic cells.

We show the molecular background of ER mRNA expression in endometriotic cells. We observed the expression of a splice variant ER β 2 mRNA along with the wild-type ER β 1 in endometriotic cells. Importantly, these ER β transcripts were expressed almost at a comparable level of the ER α in endometriotic cells.

¹ Department of Obstetrics and Gynecology, Tottori University Faculty of Medicine, Yonago, Japan

Corresponding Author:

Masao Izawa, Department of Obstetrics and Gynecology, Tottori University Faculty of Medicine, 36-1 Nishi-cho, Yonago 683-8504, Japan.
Email: Imizawa@grape.med.tottori-u.ac.jp

Materials and Methods

Patients

The institutional review boards of Tottori University Faculty of Medicine approved this project. We obtained informed consent from all patients. Endometrial and endometriotic tissues were obtained from patients as described previously.^{1,2,14-16} Endometrial tissues were obtained from the uteri of (n = 19) cycling premenopausal women who underwent hysterectomy for uterine leiomyoma during the proliferative or secretory phase. The chocolate cyst lining of the ovaries of (n = 21) patients with endometriosis was the source of endometriotic tissue collected during the follicular or luteal phase. These patients had received no hormonal treatment before surgery. The menstrual cycle phase was determined by measuring serum estradiol and progesterone levels as well as by histological examination.

Endometrial and Endometriotic Stromal Cells

Stromal cells were collected from endometrial and endometriotic tissues as described.^{1,2,14-16} In brief, the endometrial and endometriotic tissues were minced and digested with 0.5% collagenase. The dispersed cells were filtered through a 70 μ m nylon mesh to remove the undigested tissue pieces containing the glandular epithelium. The filtered fraction was separated further from epithelial cell clumps by differential sedimentation at unit gravity. The medium containing stromal cells was filtered through 40 μ m nylon mesh. Final purification was achieved by allowing stromal cells, which attach rapidly to plates, to adhere selectively to culture dishes within 30 minutes at 37°C in 5% CO₂ in air. To confirm the purity of the isolated stromal cells, immunocytochemical analysis was performed using cytokeratin (DAKO Corp, Kyoto, Japan) as a marker of epithelial cells, vimentin (DAKO Corp) as a marker of stromal cells, CD14 (Nichirei, Tokyo, Japan) as a marker of activated macrophages, and factor VIII F8/86 (DAKO Corp) as a marker of endothelial cells. The results showed that the purity of the stromal cells was more than 98%. Unless otherwise indicated, cells were cultured in DMEM/F12 supplemented with 10% fetal calf serum at 37°C in 5% CO₂. At the first passage, endometrial and endometriotic stromal cells were plated at a density of 5×10^5 cells/60 mm dish and incubated to reach a confluence of 80% to 90% for RNA preparation.

Analysis of ER mRNA Expression

Total cellular RNA was prepared using the RNeasy Plus kit (QIAGEN, Tokyo, Japan) according to the manufacturer's protocol. Using 2 μ g of total RNA, single-stranded complementary DNAs (cDNAs) were prepared in 20 μ L of reaction mixture containing 1 mmol/L each of deoxynucleotide, 20 units of PrimeScript RTase (Takara, Kyoto, Japan), 20 units of RNase inhibitor (Takara, Kyoto, Japan), and 20 μ mol/L of oligo-dT₁₆ primer at 42°C for 30 minutes. Then, 1 μ L of the cDNA reaction mixture was subjected to polymerase chain reaction (PCR) amplification in a 25 μ L Multiplex PCR Mixture

(Takara, Kyoto, Japan) containing 50 pmol each of the respective forward and reverse primers. Using ER α -F (5'-CAGGGTGGCAGAGAAA-GATTGG-3' in exon 2) and ER α -R (5'-GGTCTGGTAGGATCATACTCGG-3' in exon 4), a sequence of 532 bp in human ER α cDNA (Accession No: M12674) was amplified. Using ER β -F (5'-CCTGCTGTGATGAATTACAG-3' in exon 1) and ER β -R (5'-TTCTCTGTCTCCGCACAAG-3' in exon 4), a sequence of 550 bp in human ER β cDNA (Accession No: NM001437) was amplified. The conditions for PCR were 30 seconds at 94°C, 15 seconds at 58°C (ER α) or 55°C (ER β), and 30 seconds at 72°C for 38 cycles. At the end of the PCR cycles, 10 μ L of the reaction mixture was removed and subjected to electrophoresis on a 1.8% agarose gel in Tris-Borate-EDTA buffer at a constant voltage of 100 V. Separated DNA sequence stained with ethidium bromide was visualized under UV light. As an internal control, β -tubulin mRNA was assayed in parallel.

Analysis of the Alternative 5'-Untranslated Exon

Using the cDNAs as described earlier, PCR was performed. Forward primers¹⁷ in alternative 5'-untranslated exons of ER α are as follows: 1A-F: 5'-GGAGCTGGCGGGGGCGTTG-3'; 1B-F: 5'-CGCGTTTATTTAAGCCCAG-3'; 1C-F: 5'-CGGCCTTGACTTCTACAAG-3'; 1D-F: 5'-CTTCTTCACCTGAGAGAGCC-3'; 1E-F: 5'-CAGAGAAATAATCGCAGAGC-3'; 1F-F: 5'-CCAAAAGTAAAAT-GCAGGC-3'. The reverse primer, located in exon 2, was common to all reactions: 2R: 5'-CCTTGACCCCTCACAGGAC-3'. The primers¹⁸ in alternative 5'-untranslated exons of ER β are as follows: exon 0 N, 5'-AGATTTTTTAAATTTGAGATTGGGGTTG-3' and 5'-CTTACCTTACAATAAACACACC-3'; and exon 0 K, 5'-GTTGGGGTTATTTYGGGGTTGTT-3' and 5'-CCTCCAACAAACACATTC-3'. The PCR condition was 1 minute at 94°C, 1 minute at 62°C, and 1 minute at 72°C for 38 cycles. At the end of the PCR cycles, 10 μ L of the reaction mixture was removed and subjected to electrophoresis on a 1.8% agarose gel. The separated DNA sequence was stained with ethidium bromide and visualized under UV light.

Analysis of ER Isoform Expression

The single-stranded cDNAs prepared from endometrial and endometriotic cells were used. For the analysis of ER α isoform expression, PCR with the forward primer H1 (5'-ACGGACCATGACCATGACCCT-3' in exon 1) and the reverse primer HR5 (5'-GGTCTGGTAGGATCATACTCGG-3' in exon 5) or HR8 (5'-TCAGACCGTGGCAGGGAAA-3' in exon 8) was performed. For the analysis of ER β isoform expression, PCR with primer sets of exon 7 and the variant-specific exon 8 was performed.¹⁰ The forward primer, 5'-AGTATGTACCCTCTGGT-CACAGCG-3' in exon 7, is shared by all the ER β isoforms. The isoform-specific reverse primers were as follows: ER β 1: 5'-CCAAATGAGGGACCA CACAGCAG-3' and ER β 3: 5'-GCAGTCAAGGTGTCGACAAAGGCTGC-3'. The reverse

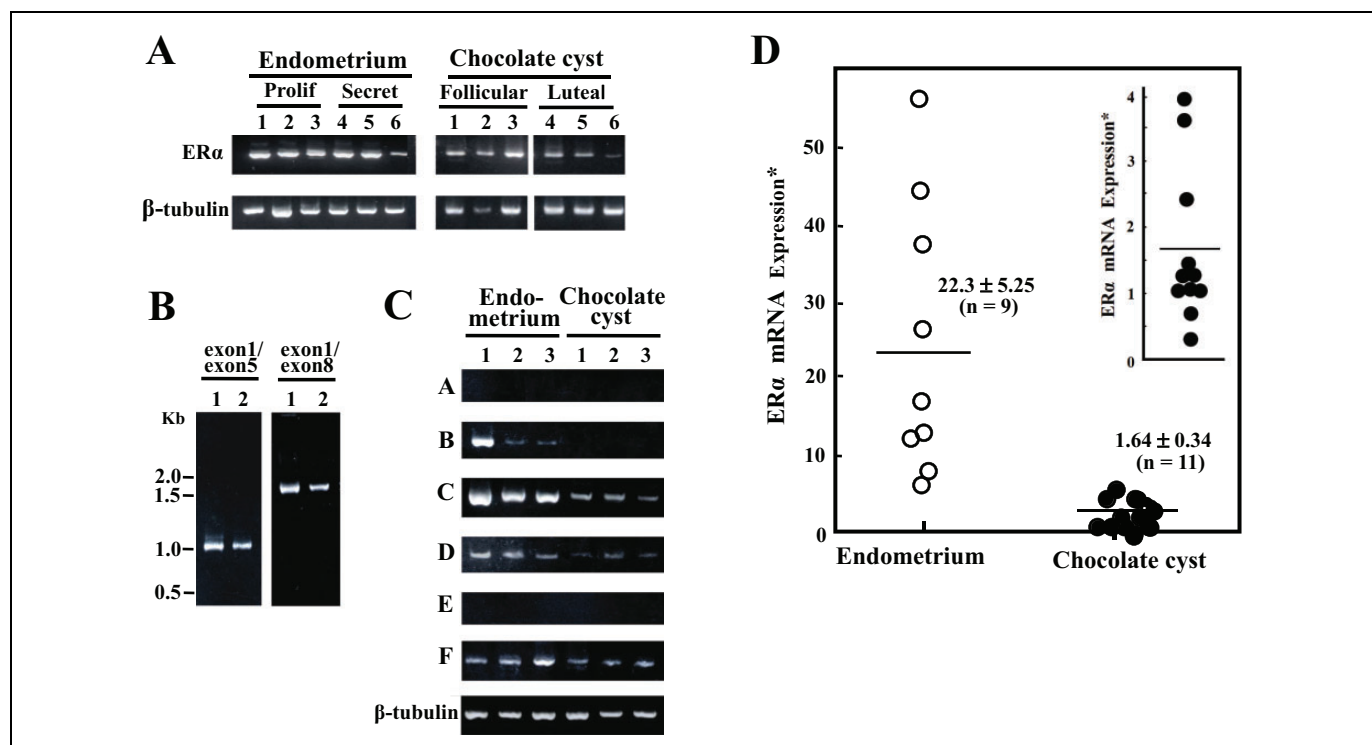


Figure 1. Estrogen receptor α (ER α) messenger RNA (mRNA) expression in endometrial and endometriotic cells. **A**, Analysis of ER α mRNA expression. Stromal cells were collected from 6 endometrial (3 from proliferative phase and 3 from secretory phase) and 6 endometriotic tissues (3 from follicular phase and 3 from luteal phase). Single-stranded complementary DNA (cDNA) was prepared from total cellular RNA and subjected to polymerase chain reaction (PCR). A sequence from exon 2 to exon 4 (532 bp in length) of ER α cDNA was amplified and subjected to a 1.8% agarose gel electrophoresis. As an internal control, β -tubulin mRNA was assayed in parallel. Amplified signals were visualized using ethidium bromide staining under UV light. **B**, Analysis of alternative splicing. Single-stranded cDNAs were prepared from endometrial (lane 1, proliferative phase) and endometriotic cells (lane 2, follicular phase). Following PCR using primer sets from exon 1 to exon 5 (1014 bp) and from exon 1 to exon 8 (1795 bp), amplified sequences were subjected to a 1.8% agarose gel electrophoresis and visualized. **C**, Analysis of untranscribed first exons in ER α mRNA. Single-stranded cDNA was prepared from 3 endometrial (lanes 1 and 2 from proliferative phase and lane 3 from secretory phase) and 3 endometriotic cells (lanes 1 and 2 from follicular phase, and lane 3 from luteal phase). Following PCR using respective 6 forward primers in the untranslated exons, A, B, C, D, E, and F, and the reverse primer in the exon 2, amplified sequences were subjected to a 1.8% agarose gel electrophoresis and visualized. As an internal control, β -tubulin mRNA was assayed in parallel. **D**, Quantitative analysis of ER α mRNA expression. Single-stranded cDNAs from 9 endometrial (4 from proliferative phase and 5 from secretory phase) and 11 endometriotic cells (6 from follicular and 5 from luteal phase), which were randomly picked up from cells, were subjected to the TaqMan real-time PCR in triplicate. The specific probes (ER α mRNA, ESRI #69, and β -tubulin #43) were selected on the website of the Universal Probe Library System. ABI PRISM 7900HT Sequence Detection System was used. Relative ER α mRNA expression was estimated using the β -tubulin mRNA expression as an internal control. *Values (mean \pm SEM) are expressed as an arbitrary unit. Endometrium versus chocolate cyst, $P < .001$ inset, relative ER α mRNA expression in endometriotic cells is shown using a larger scale.

primer, 5'-CTTTAGGCC ACCGAGTTGATTAGA-G-3', was shared by ER β 2, ER β 4, and ER β 5.

Quantitative Real-Time PCR

Expression of ER mRNA was assessed using real-time PCR. As an internal control, β -tubulin mRNA expression was estimated in parallel. One μ L of each cDNA samples prepared from endometrial and endometriotic stromal cells (\sim 50 ng) as indicated above was subjected to the TaqMan real-time PCR in triplicate. The real-time PCR was performed using the Universal Probe Library system (Roche Diagnostics, Tokyo, Japan). The specific probe (ER α #69, ER β 1 #3, and β -tubulin #43) was selected on the website of Universal Probe Library

Assay Design Center (<https://www.roche-appliedscience.com/sis/rtPCR/upl/adc.jsp>). The FastStart TaqMan Probe Master (Roche) was used for the reaction solution. Relative expression of ER β 1, ER β 2, and ER β 5 mRNA was estimated using SYBR Premix Ex TaqII kit (Takara, Kyoto, Japan) using the respective primer sets of exon 7 and exon 8 as follows: ER β 1: 5'-CCGGCTAACCTCCTGATGC-3' and 5'-CCAAATGAGGGACCACACAGCAG-3', ER β 2: 5'-AGTATGTACCCTCTGGTCACAGCG-3' and 5'-AGGCCTTTTC TGCCCTCGATGC-3', and ER β 5: 5'-ATGGGATTATGT GCTCCACGGA-3' and 5'-CTT TAGG CCACCGAGTTGAT TAGAG-3'. ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Tokyo, Japan) was used for the real-time PCR.

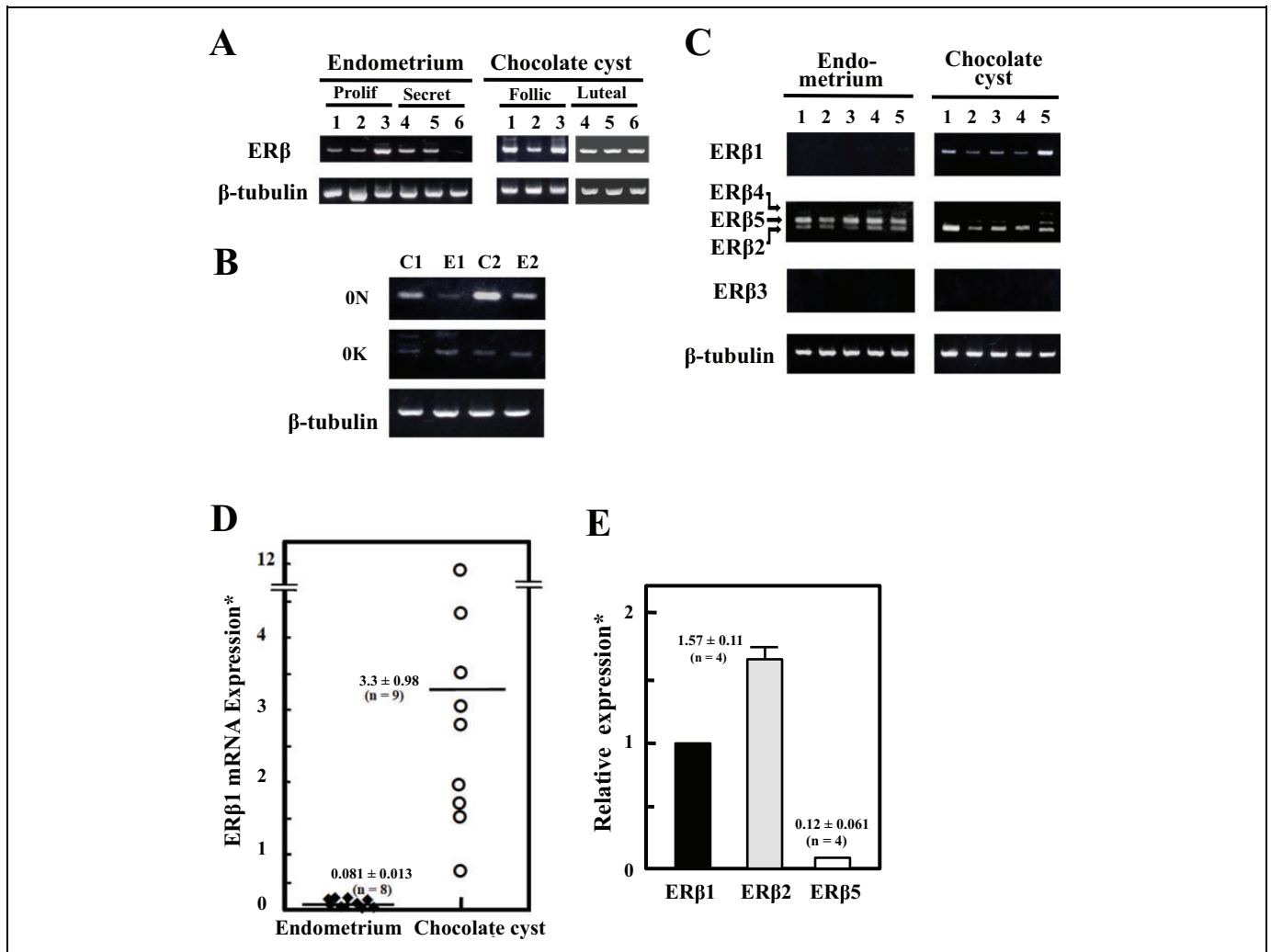


Figure 2. Estrogen receptor β (ER β) messenger RNA (mRNA) expression in endometrial and endometriotic cells. **A**, ER β mRNA expression. Stromal cells were collected from 6 endometrial (3 from proliferative phase and 3 from secretory phase) and 6 endometriotic tissues (3 from follicular phase and 3 from luteal phase). Single-stranded complementary DNA (cDNA) was prepared from total cellular RNA and subjected to polymerase chain reaction (PCR). An ER β cDNA sequence from exon 1 to exon 4 (550 bp in length) was amplified and subjected to 1.8% agarose gel electrophoresis. As an internal control, β -tubulin mRNA was assayed in parallel. **B**, Analysis of the untranslated first exons. Single-stranded cDNA was prepared from endometrial (lanes E1 and E2) and endometriotic cells (lanes C1 and C2). PCR using 2 forward primers, 0 N and 0 K, and a reverse primer located in exon 2 was performed. As an internal control, β -tubulin mRNA was assayed in parallel. **C**, Analysis of alternative splicing. Single-stranded cDNAs were prepared from 5 endometrial and 5 endometriotic cells. Following PCR with primer sets of the variant-specific exon 7 and exon 8, amplified sequences were visualized on a 1.8% agarose gel electrophoresis. As an internal control, β -tubulin mRNA expression was assayed in parallel. Arrows indicate the location of amplified sequences corresponding to ER β 2, ER β 4 and ER β 5 mRNAs. **D**: Quantitative analysis of ER β 1 mRNA expression. Single-stranded cDNAs from 8 endometrial (4 from proliferative phase and 4 from secretory phase) and 9 endometriotic cells (6 from follicular phase and 3 from luteal phase) were prepared. The specific probes (ESR2 #3 for ER β 1 and β -tubulin #43) were selected using the Universal Probe Library System. ABI PRISM 7900HT System was used. Relative ER β 1 mRNA expression was estimated using the β -tubulin mRNA expression as an internal control. *Values are expressed as arbitrary unit (mean \pm SEM). Endometrium versus chocolate cyst, $P < .006$. **E**, Relative expression of ER β 1, ER β 2, and ER β 5 mRNA in endometriotic cells. Using SYBR system with primer sets of variant-specific exon 7 and exon 8, the expression of ER β 1, ER β 2, and ER β 5 mRNAs was assayed. The expression was estimated using the β -tubulin mRNA expression as an internal control. Relative expression of ER β 2 and ER β 5 mRNA was normalized against ER β 1 mRNA expression. Values are the means \pm SEM. ER β 1 versus ER β 2, $P < .002$, ER β 2 versus ER β 5, $P < .006$.

Statistical Analysis

Results were analyzed using one-way analysis of variance followed by Fisher protected least significant difference test. Values were expressed as means \pm standard error of mean. $P < .05$ was considered statistically significant.

Results

Expression of ER α mRNA

Expression of ER α mRNA was observed in both endometriotic and endometrial cells (Figure 1A). We then searched for the

splice variant mRNA using reverse transcription-PCR with primer sets to amplify the open reading frame (ORF) of wild-type ER α (from exon 1 to exon 8 in ESR1). As shown in Figure 1B, only a single cDNA sequence, which includes an ORF predicting a wild-type ER α , was always amplified from cDNAs prepared from endometriotic and endometrial cells. The 5'-untranslated exons, C, D, and F, were tagged to exon 1 in endometriotic cells, while in endometrial cells, the B, C, D, and F were tagged to exon 1 (Figure 1C). Among these ER α mRNAs, the exon C-tagged mRNA was the most abundant. Relative expression of ER α mRNA in endometriotic cells was estimated to be approximately one-tenth of that in endometrial cells (Figure 1D).

Expression of ER β mRNA

Expression of ER β mRNA was observed in both endometriotic and endometrial cells (Figure 2A). In endometriotic cells, the expression depends mostly on 0N promoter (Figure 2B) and includes 2 ORFs: one for a wild-type ER β 1 and another for a splice variant ER β 2 (Figure 2C). Expressions of ER β 2 and ER β 5 mRNA were detectable in endometrial cells (Figure 2C). The expression of ER β 1 mRNA in endometriotic cells was approximately 40-fold higher than that in endometrial cells (Figure 2D). The ER β 2 mRNA expression was almost at a comparable level of ER β 1 in endometriotic cells, while the ER β 5 mRNA expression was at a marginal level (Figure 2E).

Discussion

The present study was undertaken to evaluate the molecular background of ER expression in endometriotic cells. So far, ER expression profile, a higher ER β , and a lower ER α expression in endometriotic tissues, which is in the inverse relationship in endometrium,^{4,7} has been proposed as a major background of estrogen action in endometriosis. Although the ER mRNA expression is low in endometriotic cells (Figure 1D, 2D, and 2E), the expression of ER α and ER β mRNAs is almost at a comparable level. It is interesting to note that ER β 2 mRNA^{10,11,18} was found to be coexpressed along with ER α and ER β 1 mRNAs in endometriotic cells. These observations suggest a pathophysiological role of the ER expression profile in endometriosis.

Receptor dimerization in response to ligand binding is one of the crucial steps in ER-dependent transactivation.¹⁹ Homodimerization of ER α accelerates cellular proliferation.¹⁹ On the other hand, ER β is thought to counteract the stimulatory effects of ER α through the formation of functional heterodimer with ER α .^{20,21} Experimental evidence suggests that heterodimer-induced activation of target genes is markedly different from that induced by homodimers.^{22,23} However, these studies have been conducted based on the ligand-dependent dimerization of wild-type ERs.

Recently, multiple ER isoforms have been identified in human tissues and cells. The ER α variants have been identified in a number of human cell types. More recently, ER β variants with distinct biological functions have been identified.¹¹⁻¹³

Markedly higher ER β expression has been documented as one of the distinct gene expressions in endometriotic cells^{4,7}; however, these observations were restricted to the expression of wild-type ER β 1, and the expression had been evaluated in comparison with that in endometrial cells.

We observed the expression of splice variant ER β 2 mRNA along with the wild-type ER β 1 in endometriotic cells (Figure 2C). More importantly, these ER β transcripts were expressed almost at a comparable level of the ER α in endometriotic cells (Figure 1D, 2D and 2E). The observation suggests that the heterodimerization probably occurs in the cells. ER β /ER β and ER α /ER β dimers are known to exhibit antiproliferative effects,^{20,21} and ER α /ER β is reported to be relatively more stable than the ER β /ER β homodimer.¹³ Although ER β 2 exhibits no estrogen binding, ER β 1/ER β 2 heterodimer shows higher estrogen-induced transactivation than ER β 1/ER β 1 homodimer in a ligand-dependent manner.¹¹ To further understand the role of the ER expression profile may lead to the estrogen-dependent pathophysiology in endometriosis.

In conclusion, expression of ER isoforms, ER α and ER β , is the major circumstance for estrogen action in endometriosis. Our results demonstrated that multiple ER isoforms, ER α , ER β 1, and ER β 2, are expressed simultaneously within endometriotic cells. However, little is known regarding the preference for formation of ER homodimer versus heterodimer and how this is affected by ER expression level and ligand occupancy. On therapeutic perspectives, promoting heterodimerization of ER isoforms using selective ER modulators may become an important strategy to design therapeutics for endometriosis.²⁴ To address these issues, clarifying the molecular basis of ER expression profile and the downstream ER-dependent gene expression may lead us to an avenue of further understanding the estrogen-dependent pathophysiology in endometriosis. It is interesting to note that ER β and prostaglandin E2 integrate at Ras-like, estrogen-regulated, growth inhibitor, leading to the increase in endometriotic cell proliferation.²⁵

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported in part by a Grant-in-Aid for Challenging Exploratory Research from Japan Society for the Promotion of Science (#24659731).

References

1. Izawa M, Harada T, Taniguchi F, Ohama Y, Takenaka Y, Terakawa N. An epigenetic disorder may cause aberrant expression of aromatase gene in endometriotic stromal cells. *Fertil Steril*. 2008;89(suppl 5):1390-1396.
2. Izawa M, Taniguchi F, Uegaki T, et al. Demethylation of a non-promoter cytosine-phosphate-guanine island in the aromatase gene may cause the aberrant up-regulation in endometriotic tissues. *Fertil Steril*. 2011;95(1):33-39.

3. Olive DL, Pritts EA. Treatment of endometriosis. *N Engl J Med*. 2001;345(4):266-275.
4. Brandenberger AW, Lebovic DI, Tee MK, et al. Oestrogen receptor (ER)-alpha and ER-beta isoforms in normal endometrial and endometriosis-derived stromal cells. *Mol Hum Reprod*. 1999; 5(7):651-655.
5. Fujimoto J, Hirose R, Sakaguchi H, Tamaya T. Expression of oestrogen receptor-alpha and -beta in ovarian endometriomata. *Mol Hum Reprod*. 1999;5(8):742-747.
6. Matsuzaki S, Fukaya T, Uehara S, Murakami T, Sasano H, Yajima A. Characterization of messenger RNA expression of estrogen receptor-alpha and -beta in patients with ovarian endometriosis. *Fertil Steril*. 2000;73(6):1219-1225.
7. Xue Q, Lin Z, Cheng YH, et al. Promoter methylation regulates estrogen receptor 2 in human endometrium and endometriosis. *Biol Reprod*. 2007;77(4):681-687.
8. Harris HA. The unexpected science of estrogen receptor-beta selective agonists: a new class of anti-inflammatory agents? *Nucl Recept Signal*. 2006;4:e012.
9. Xing D, Feng W, Miller AP, et al. Estrogen modulates TNF-alpha-induced inflammatory responses in rat aortic smooth muscle cells through estrogen receptor-beta activation. *Am J Physiol Heart Circ Physiol*. 2007;292(6):H2607-H2612.
10. Moore JT, McKee DD, Slentz-Kesler K, et al. Cloning and characterization of human estrogen receptor beta isoforms. *Biochemical and Biophysical Research Communications*. 1998;247(1):75-78.
11. Leung YK, Mak P, Hassan S, Ho SM. Estrogen receptor (ER)-beta isoforms: a key to understanding ER-beta signaling. *PNAS*. 2006;103(35):13162-13167.
12. Paulmurugan R, Tamrazi A, Massoud TF, Katzenellenbogen JA, Gambhir SS. In vitro and in vivo molecular imaging of estrogen receptor alpha and beta homo- and heterodimerization: exploration of new modes of receptor regulation. *Mol Endocrinol*. 2011;25(12):2029-2040.
13. Chakraborty S, Willett H, Biswas PK. Insight into estrogen receptor beta-beta and alpha-beta homo- and heterodimerization: a combined molecular dynamics and sequence analysis study. *Biophys Chem*. 2012;170:42-50.
14. Iwabe T, Harada T, Tsudo T, et al. Tumor necrosis factor-alpha promotes proliferation of endometriotic stromal cells by inducing interleukin-8 gene and protein expression. *J Clin Endocrinol Metab*. 2000;85(2):824-829.
15. Sakamoto Y, Harada T, Horie S, et al. Tumor necrosis factor-alpha-induced interleukin-8 (IL-8) expression in endometriotic stromal cells, probably through nuclear factor-kappa B activation: gonadotropin-releasing hormone agonist treatment reduced IL-8 expression. *J Clin Endocrinol Metab*. 2003;88(2):730-735.
16. Izawa M, Harada T, Deura I, Taniguchi F, Iwabe T, Terakawa N. Drug-induced apoptosis was markedly attenuated in endometriotic stromal cells. *Hum Reprod*. 2006;21(3):600-604.
17. Figtree GA, McDonald D, Watkins H, Channon KM. Truncated estrogen receptor alpha 46-kDa isoform in human endothelial cells: relationship to acute activation of nitric oxide synthase. *Circulation*. 2003;107(1):120-126.
18. Suzuki F, Akahira J, Miura I, et al. Loss of estrogen receptor beta isoform expression and its correlation with aberrant DNA methylation of the 5'-untranslated region in human epithelial ovarian carcinoma. *Cancer Sci*. 2008;99(12):2365-2372.
19. Powell E, Shanle E, Brinkman A, et al. Identification of estrogen receptor dimer selective ligands reveals growth-inhibitory effects on cells that co-express ERalpha and ERbeta. *PLoS One*. 2012; 7(2):e30993.
20. Lindberg MK, Moverare S, Skrtic S, et al. Estrogen receptor (ER)-beta reduces ERalpha-regulated gene transcription, supporting a "ying yang" relationship between ERalpha and ERbeta in mice. *Mol Endocrinol*. 2003;17(2):203-208.
21. Saji S, Hirose M, Toi M. Clinical significance of estrogen receptor beta in breast cancer. *Cancer Chemother Pharmacol*. 2005; 56(suppl 1):21-26.
22. Pettersson K, Grandien K, Kuiper GG, Gustafsson JA. Mouse estrogen receptor beta forms estrogen response element-binding heterodimers with estrogen receptor alpha. *Mol Endocrinol*. 1997;11(10):1486-1496.
23. Tremblay GB, Tremblay A, Labrie F, Giguere V. Dominant activity of activation function 1 (AF-1) and differential stoichiometric requirements for AF-1 and -2 in the estrogen receptor alpha-beta heterodimeric complex. *Mol Cell Biol*. 1999;19(3):1919-1927.
24. Paterni I, Granchi C, Katzenellenbogen JA, Minutolo F. Estrogen receptors alpha (ER α) and beta (ER β): subtype-selective ligands and clinical potential. *Steroids*. 2014;90:13-29.
25. Monsivais D, Dyson MT, Yin P, et al. ER β - and prostaglandin E2-regulated pathways integrate cell proliferation via Ras-like and estrogen-regulated growth inhibitor in endometriosis. *Mol Endocrinol*. 2014;28(8):1304-1315.