Decidualization Attenuates the Contractility of Eutopic and Ectopic Endometrial Stromal Cells: Implications for Hormone Therapy of Endometriosis

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Context: Decidualization of the endometrium involves the morphological and biochemical reprogramming of the estrogen-primed proliferative endometrial stromal compartment under the continuing influence of progesterone.

Objectives: The aim of this study was to evaluate the involvement of the extracellular matrix contractility of eutopic and ectopic endometrial stromal cells during the tissue remodeling processes associated with decidualization.

Design: The effect of decidualization on the contractile profile of the endometriotic cyst stromal cells and eutopic endometrial stromal cells with or without endometriosis in the three-dimensional collagen gel culture was investigated using laser scanning microscopy, collagen gel contraction assays, and Western blot analysis.

Results: Decidualized ectopic and eutopic endometrial stromal cells in the three-dimensional collagen gel culture mimicked the morphology of decidual tissue in vivo. In vitro decidualization inhibited the contractility of these eutopic and ectopic endometrial stromal cells. Down-regulation of integrin α1β1 and α2β1 expression, suppression of Ras homology A (Rho A), Rho-associated coiled-coil-forming protein kinase (ROCK)-I and ROCK-II expression, inhibition of the differentiation into the myofibroblastic phenotype, and induction of differentiation into epithelioid decidual phenotype were observed in these cells during decidualization.

Conclusions: It is suggested that the attenuation of eutopic endometrial stromal cell-mediated contractility by decidualization is a novel and integral mechanism of the physiological endometrial tissue remodeling process during menstrual cycles. Although ectopic endometrial stromal cells have enhanced contractile profile, decidualization can attenuate the contractility of these cells. These findings may be one of the action mechanisms by which oral contraceptives and progestins ameliorate endometriosis. (J Clin Endocrinol Metab 94: 2516–2523, 2009)

Decidualization of the human endometrium is an essential preparative event for the successful establishment and maintenance of pregnancy (1). In humans, decidualization is initiated in the midsecretory phase of the menstrual cycle and is triggered mainly by ovarian sex steroid hormones, independent of pregnancy (2, 3). After embryo implantation, decidualization persists and extends throughout the endometrium, leading to the formation of the pregnancy decidua. A more restricted definition is that the decidual process consists of the morphological and biochemical reprogramming of the estrogen-primed proliferative endometrial stromal compartment under the continuing influence of progesterone (4). By this definition, the decidual process is characterized by the dramatic morphological and biochemical transformation of the endometrial stroma in which the stromal fibroblasts differentiate to become rounded, relatively large epithelioid-like or polygonal, se-

Abbreviations: 3-D, Three-dimensional; db-CAMP, dibutyryl-cAMP; FBS, fetal bovine serum; GAPDH, glyceraldehyde-3-phosphate-dehydrogenase; IGFBP-1, IGF binding protein-1; MPA, medroxyprogesterone acetate; OCs, oral contraceptives; PR, progesterone receptor; PRL, prolactin; Rho, Ras homology; ROCK, Rho-associated coiled-coil-forming protein kinase; SMA, α-smooth muscle actin.
creatory decidual cells (5–7). The morphology of the decidua is loose or sponge-like (8), the distance between the individual cells is relatively great, and there are very few collagen fibrils in the intercellular space. The decidual cells are round to oval with a giant cell volume (a nuclear volume constituting approximately 6–10% of the cell volume).

Progesterone, alone or in combination with estradiol, induces decidualization of human endometrial stromal cells in vitro (9, 10). In vitro decidualization induces morphological changes of the endometrial stromal cells, such as multilayering, the increased size of Golgi complexes, dilatation of rough endoplasmic reticulum, and formation of gap junctions, which mimic in vivo decidual transformation (9, 11). In contrast, functional modulation of the decidualized stromal cells is characterized by the secretion of prolactin (PRL) (3, 11–13) and IGF binding protein (IGFBP)-1 (3, 14); the increased expression of tissue factor (15), plasminogen activator inhibitor-1 (16), vascular endothelial growth factor (13), and growth-regulated oncogene α (17); the reduced expression of α-smooth muscle actin (SMA) (18) and matrix metalloproteinase-3 (19, 20); and the reduced activity of urinary plasminogen activator and tissue-type plasminogen activator (16, 21). The production levels of two of these affected molecules, PRL and IGFBP-1, by endometrial stromal cells are generally used as biochemical decidualization markers of progestin-induced endometrial stromal cell differentiation (14).

Endometriosis, a benign estrogen-dependent disease affecting 3–10% of women of reproductive age, is characterized by the ectopic growth of endometrial tissue and is associated with pelvic pain, dysmenorrhea, and infertility (22). Histologically, this disease is characterized by endometrial glands and stroma surrounded by dense fibrous tissue (23, 24). During the development of endometriotic lesions, excess fibrosis may lead to scarring and to alteration of tissue function (25). It has been suggested that type I collagen is a major contributor to endometriosis-associated fibrosis (25, 26). One approach to understanding the pathogenesis of endometriosis is to investigate the mechanisms underlying the fibrogenesis associated with this disease.

The major physiological goal of hormonal therapy for endometriosis has been the creation of a hypoestrogenic acyclic hormone environment to induce endometrial atrophy and prevent shedding of endometriotic implants, as well as to inhibit reseeding of the viable endometrium. Among the therapeutic options are antiestrogens (e.g., danazol) and regimens that induce either a medical menopause (e.g., GnRH agonists) or a pseudopregnant state [e.g., continuous combined oral contraceptives (OCs) or progestins] (27, 28). Progestins either alone or combined with estrogens are now used as the first choice of hormonal therapy on a long-term basis (29, 30). It has been reported that administration of combined OCs or progestins alone induces decidualization of both the endometriotic lesions and the eutopic endometrium (31–33).

As in vitro models for studying tissue remodeling in the eutopic endometrium and fibrosis formation in endometriosis, we have established a three-dimensional (3-D) collagen culture system with human endometrial and endometriotic stromal cells (34–36). Endometrial and endometriotic stromal cells were cultured in floating collagen lattices to reorganize and compact the collagen fibers, resulting in contraction of the collagen gels. This culture system provided a model for mechanically relaxed tissue with low tensile strength comparable to the physiological tissue remodeling of the normal cyclic endometrium and the early stages of endometriotic lesions. Contractility of the endometrial and endometriotic stromal cells was mediated by myofibroblast differentiation and by activation of the Ras homology (Rho)-Rho-associated coiled-coil-forming protein kinase (ROCK)-mediated signaling pathway (36, 37). We have also demonstrated that endometriotic stromal cells have an enhanced contractile profile compared with normal endometrial stromal cells (36).

In the present study, we investigated the role of decidualization in the physiological tissue remodeling of the cyclic endometrium and in the hormonal treatment of endometriosis with progestins.

### Patients and Methods

#### Isolation procedure and cell culture conditions of endometriotic cyst stromal cells, eutopic endometrial stromal cells with endometriosis, and normal endometrial stromal cells

Endometriotic cyst stromal cells were obtained from premenopausal patients who had undergone salpingo-oophorectomies or cystectomies for ovarian endometriotic cysts (n = 12, aged 22–35 yr). Eutopic endometrial stromal cells with endometriosis were obtained from premenopausal patients who had undergone hysterectomies for endometriosis (n = 8, aged 30–39 yr). Normal endometrial stromal cells were obtained from premenopausal patients who had undergone hysterectomies for leiomyoma and had no evidence of endometriosis (n = 9, aged 36–43 yr). The ages of the patients were statistically similar among the three groups. All patients were free of any hormonal treatments before the operation. All the specimens were diagnosed as being in the mid to late secretory phase using a standard histological examination of endometrial tissues. This study was approved by the Institutional Review Board of the Faculty of Medicine, Oita University (Oita, Japan).

Endometriotic cyst stromal cells were isolated from ovarian endometriotic tissues by enzymatic digestion as previously described (38). Eutopic endometrial stromal cells with endometriosis and normal endometrial stromal cells were also isolated by digesting the endometrial tissue fragments with 0.5% collagenase as previously described (38). Isolated endometriotic cyst stromal cells, eutopic endometrial stromal cells with endometriosis, and normal endometrial stromal cells were cultured in DMEM supplemented with 100 IU/ml of penicillin (Life Technologies, Inc.-BRL, Gaithersburg, MD), 50 mg/ml of streptomycin (Life Technologies, Inc.-BRL), and 10% charcoal-stripped heat-inactivated fetal bovine serum (FBS) (Life Technologies, Inc.-BRL) at 37°C in 5% CO₂ in air.

Endometriotic cyst stromal cells, eutopic endometrial stromal cells with endometriosis, and normal endometrial stromal cells in monolayer culture after the third passage were more than 99% pure as analyzed by immunocytochemical staining with antibodies to vimentin (V9; Dako, Copenhagen, Denmark), CD10 (SS2/36; Dako), cytokeratin (Dako), factor VIII (Dako), and leukocyte common antigen (2B11/H11005; Dako) and were used for the following experiments (38). The reason for using the cells after the third passage for the experiments was to obtain sufficient numbers of cells to perform the gel contraction assays and the cellular protein extraction from 3-D culture. Cells isolated from each individual patient were used for one experiment at a time. Each experiment was performed in triplicate and conducted at least five times.
In vitro decidualization of endometriotic cyst stromal cells, eutopic endometrial stromal cells with endometriosis, and normal endometrial stromal cells

Decidualization of cultured endometriotic cyst stromal cells, eutopic endometrial stromal cells with endometriosis, and normal endometrial stromal cells was induced by long-term (12 d) culture with a combination of 0.5 mM of dibutyryl-cAMP (db-cAMP; Sigma-Aldrich, St. Louis, MO) and 100 mM of medroxyprogesterone acetate (MPA; Sigma-Aldrich) or 100 mM of dienogest (17α-cyanomethyl-17β-hydroxy-4,9-estradien-3-one) (a gift from Mochida Pharmaceutical Co., Ltd., Tokyo, Japan) in the presence of 10% charcoal-stripped heat-inactivated FBS, as previously described (13, 17). Dienogest represents a new type of 19-norprogestins having a 17α-ethyl group instead of a 17α-ethyl group typical of the common 19-nortestosterone derivatives like levonorgestrel, desogestrel, and norgestimate (39). Dienogest is a synthetic steroid used as a progestin in OC pills, and its clinical application in the treatment of endometriosis is currently started.

The cells were also incubated with 0.5 mM db-cAMP, or 100 mM MPA, 100 mM dienogest alone. The culture medium was replaced every 3 d. After 12 d of treatment under these conditions, the medium was replaced with fresh culture medium containing the same amounts of db-cAMP and progestins, and the cells were further cultured for 24 h. The medium was collected and stored at −70°C for PRL and IGFBP-1 assays.

PRL and IGFBP-1 assays

The levels of production of PRL and IGFBP-1 by endometriotic cyst stromal cells, eutopic endometrial stromal cells with endometriosis, and normal endometrial stromal cells were used as markers of decidualization. The levels of PRL in the supernatants of endometriotic cyst stromal cells, eutopic endometrial stromal cells with endometriosis, and normal endometrial stromal cells were determined with a commercially available chemiluminescence immunoassay (Architect prolactin; Abbott Laboratories, North Chicago, IL). The sensitivity of the assay for prolactin was 0.8 ng/ml. The levels of IGFBP-1 in the supernatants of eutopic endometrial stromal cells with endometriosis, and normal endometrial stromal cells were also determined with a commercially available ELISA (RayBiotech, Inc., Norcross, GA). The sensitivity of the ELISA for IGFBP-1 was 5.0 ng/ml.

Collagen gel contraction assay

Cellular collagen gel contraction assays were performed as previously described (34–36). A sterile solution of acid-soluble collagen type I purified from porcine tendons (Cellmatrix type I-A; Nitta Gelatin Inc., Osaka, Japan) was prepared according to the manufacturer’s instructions. Decidualized endometriotic cyst stromal cells, eutopic endometrial stromal cells with endometriosis, and normal endometrial stromal cells were embedded in collagen gel and cultured three-dimensionally. Briefly, the cells were suspended in the collagen solution (3.0 × 10^5 cells/ml). The collagen/cell mixture (2 ml/plate) was dispensed onto 35-mm culture plates (Corning, New York, NY) coated with 0.2% BSA (Sigma-Aldrich); the mixture was allowed to polymerize at 37°C for 30 min. Immediately after polymerization, 1 ml of culture medium was added to each plate. FBS was added in the culture media during the treatment period because it is required for fibroblastic cells to contract the surrounding collagen gels to construct a tissue-equivalent environment.

After incubation for 48 h in the presence of 10% charcoal-stripped heat-inactivated FBS, the collagen gels were photographed, and the area of the gel surface was measured with the public domain Image program 1.61 developed at the National Institutes of Health (Bethesda, MD). The incubation time was determined as previously described (34–36).

Assessment of the morphology and cell density of the endometriotic cyst stromal cells, eutopic endometrial stromal cells with endometriosis, and normal endometrial stromal cells in 3-D collagen gel culture

At the cessation of the 3-D culture, the contracted collagen gels were fixed with 4% paraformaldehyde. The morphological features and the density of the cells were observed by laser scanning microscopy (LSM5 Pascal, version 4.2; Carl Zeiss, Oberkochen, Germany).

Assessment of the expression of the receptors for collagen I in endometriotic cyst stromal cells, eutopic endometrial stromal cells with endometriosis, and normal endometrial stromal cells

Integrins α1β1 and α2β1 are the main receptors for collagen I (40, 41). The effects of decidualization on the expression of integrin α1, α2, and β1 subunits in endometriotic cyst stromal cells, eutopic endometrial stromal cells with endometriosis, and normal endometrial stromal cells were investigated by Western blotting analysis as previously described (38). The contracted collagen gels were minced and incubated in 0.02% collagenase type I (Sigma-Aldrich) in PBS for 40 min at 37°C. Decidualized or nondecidualized endometriotic cyst stromal cells, eutopic endometrial stromal cells with endometriosis, and normal endometrial stromal cells were isolated from collagen gels by centrifugation, and whole cell extracts were prepared by lysing the cells in lysis buffer (50 mM Tris-HCl, 125 mM NaCl, 0.1% Nonidet P-40, 5 mM ethylenediamine tetraacetic acid, 50 mM NaF, and 0.1% phenylmethylsulfonylfluoride). The suspension was centrifuged at 15,000 rpm for 15 min at 4°C, and the supernatant was collected. The total protein concentration was quantified using the Coomassie protein assay reagent (Pierce, Rockford, IL). The whole cell protein extract was resolved with SDS-PAGE using a 10% polyacrylamide gel under reduced conditions. After transfer to Immobilon-P transfer membrane (Millipore, Bedford, MA), the protein was stained with Ponceau S (Sigma-Aldrich) to verify uniform loading and transfer. Membranes were blocked with 5% skim milk (Becton Dickinson, Sunnyvale, CA) in Tris-buffered saline with Tween 20 (TBS-T; 50 mM Tris-HCl, 150 mM NaCl, 0.1% Tween 20, pH 7.4) overnight and subsequently incubated with primary antibodies [integrin α1 subunit (FB12; Millipore), integrin α2 subunit (2/CD49b; BD Biosciences, San Jose, CA), integrin β1 subunit (18/CD29; BD Biosciences), and glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) (Ambion, Austin, TX)] at appropriate dilutions for 1 h at room temperature. The membrane was washed three times with TBS-T and incubated with the appropriate horseradish peroxidase-conjugated secondary antibody for 1 h at room temperature. Subsequently, the membrane was washed three times with TBS-T and analyzed by enhanced chemiluminescence (Ampersham Pharmacia Biotech, Chicago, IL). The relative expression of integrin α1, α2, and β1 subunits and GAPDH protein was analyzed using the public domain Image program 1.61 developed at the National Institutes of Health.

In the experiments with cellular protein extraction, we did not use the collagen gels without cells as controls. That is because we cannot obtain cell pellet for protein extraction if the cells were not added to the collagen gel. In addition, according to the manufacturer’s instructions, collagen I used in the present study was highly purified and did not contain other cellular proteins, such as integrins and contraction-associated proteins.

Assessment of the expression of contraction-associated proteins in endometriotic cyst stromal cells, eutopic endometrial stromal cells with endometriosis, and normal endometrial stromal cells

The expression of α-SMA, Rho A, ROCK-I, and ROCK-II in endometriotic cyst stromal cells, eutopic endometrial stromal cells with endometriosis, and normal endometrial stromal cells in the above-mentioned 3-D culture were investigated by Western blotting analysis as previously described (36). SDS-PAGE and Western blotting were per-
formed as described above with primary antibodies [α-SMA (1A4; R&D Systems, Minneapolis, MN), RhoA, ROCK-I (Sigma-Aldrich), ROCK-II (Santa Cruz Biotechnology, Santa Cruz, CA), and GAPDH]. The relative expression of contraction-associated proteins and GAPDH protein was analyzed using the public domain Image program 1.61 developed at the National Institutes of Health.

**Statistical analysis**

Data are presented as the means ± s.d of representative experiments and were appropriately analyzed by the Bonferroni/Dunn test and Mann-Whitney U test with StatView 4.5 (Abacus Concepts, Berkeley, CA). Values of *P* < 0.05 were considered to indicate statistical significance.

**Results**

**Quantification of PRL and IGFBP-1 production by endometriotic cyst stromal cells, eutopic endometrial stromal cells with endometriosis, and normal endometrial stromal cells after long-term stimulation with db-cAMP and progestins**

The concentrations of PRL in the culture medium without cells were below the level of detection (<0.8 ng/ml). As shown in Fig. 1A, no PRL production was detected in unstimulated endometriotic cyst stromal cells, eutopic endometrial stromal cells with endometriosis, and normal endometrial stromal cells. Stimulation of endometriotic cyst stromal cells, eutopic endometrial stromal cells with endometriosis, and normal endometrial stromal cells with db-cAMP and MPA/dienogest combination induced a significant increase in PRL production compared with the control. Although the effect was weak, db-cAMP alone also induced PRL production by these three cell types. The PRL levels in decidualized endometriotic cyst stromal cells were lower than those in eutopic endometrial stromal cells with endometriosis or normal endometrial stromal cells. These observations were consistent with a previous report (42). Treatment with either MPA or dienogest alone had no effect on the production of PRL in these cells (data not shown).

**Collagen gel contractility of endometriotic cyst stromal cells, eutopic endometrial stromal cells with endometriosis, and normal endometrial stromal cells**

The collagen gel contractility of endometriotic cyst stromal cells, eutopic endometrial stromal cells with endometriosis, and normal endometrial stromal cells was evaluated using a collagen gel contraction assay. As shown in Fig. 3, in the presence of 10% FBS, untreated endometriotic cyst stromal cells, eutopic endometrial stromal cells with endometriosis, and normal endometrial stromal cells showed significant collagen gel contractility (89.1 ± 0.9%, 61.1 ± 1.0%, and 38.3 ± 0.4% decrease in surface area after 48 h vs. 0 h controls, respectively). Untreated
Endometriotic cyst stromal cells had significantly higher contractility than untreated eutopic endometrial stromal cells with endometriosis and untreated normal endometrial stromal cells (P < 0.0001, Bonferroni/Dunn test). Untreated eutopic endometrial stromal cells with endometriosis had significantly higher contractility than untreated normal endometrial stromal cells (P < 0.0001, Bonferroni/Dunn test). As shown in Fig. 2, the decrease in the areas of the gel surface of endometriotic cyst stromal cells, eutopic endometrial stromal cells with endometriosis, and normal endometrial stromal cells were significantly inhibited after long-term stimulation with db-cAMP+MPA and db-cAMP+dienogest. Long-term stimulation with db-cAMP alone weakly attenuated the contractility of endometriotic cyst stromal cells, eutopic endometrial stromal cells with endometriosis, and normal endometrial stromal cells. In contrast, short-term (<24 h) stimulation with db-cAMP+MPA and db-cAMP+dienogest did not affect the contractility of endometriotic cyst stromal cells, eutopic endometrial stromal cells with endometriosis, and normal endometrial stromal cells (data not shown). Long-term stimulation with MPA or dienogest alone had no effect on the contractility of these three cell types (data not shown).

### Morphology and cell density of endometriotic cyst stromal cells, eutopic endometrial stromal cells with endometriosis, and normal endometrial stromal cells in contracted collagen gels

When nondecidualized endometriotic cyst stromal cells, eutopic endometrial stromal cells with endometriosis, and normal endometrial stromal cells were cultured in 3-D collagen gels, the cells contracted the initially loose network to a dense tissue-like structure (Fig. 3A). Their morphology was dendritic to stellate. In contrast, the morphology of the decidualized endometriotic cyst stromal cells, eutopic endometrial stromal cells with endometriosis, and normal endometrial stromal cells after long-term treatment with db-cAMP+MPA/dienogest remained round to polygonal in comparison to the nondecidualized cells, suggesting that the decidualized cells did not adhere to the collagen fibers. The morphologies of these eutopic and ectopic endometrial stromal cells treated with MPA/dienogest alone were similar to those of the untreated cells (data not shown).

In addition to the morphological changes, long-term stimulation with db-cAMP+MPA/dienogest decreased the cell density of endometriotic cyst stromal cells, eutopic endometrial stromal cells with endometriosis, and normal endometrial stromal cells (Fig. 3B). Long-term stimulation with db-cAMP alone weakly decreased the cell density of these three cell types. Long-term stimulation with MPA or dienogest alone had no effect on the cell density of these three cell types (data not shown).

### Expression of the receptors for collagen I in endometriotic cyst stromal cells, eutopic endometrial stromal cells with endometriosis, and normal endometrial stromal cells

To analyze the mechanisms underlying the above findings, we evaluated the expression of the integrin α1, α2, and β1 subunits in these cells. As shown in Fig. 4, the protein expressions of the integrin α1, α2, and β1 subunits in endometriotic cyst stromal cells, eutopic endometrial stromal cells with endometriosis, and normal endometrial stromal cells were significantly reduced after long-term stimulation with db-cAMP+MPA and db-cAMP+dienogest. Long-term stimulation with db-cAMP alone
were significantly reduced after long-term stimulation with db-cAMP and/or MPA/dienogest. The expression of these integrins was investigated by Western blotting analysis (Supplemental Fig. 2). The expression of GAPDH was evaluated as an internal control. The ratio of the levels of integrin subunits to the corresponding GAPDH levels in untreated conditions was defined as 100%. Data are presented as means ± SD of five repeated experiments. *, P < 0.05 vs. untreated controls (Mann-Whitney U test).

Expression of contractility-associated proteins in endometriotic cyst stromal cells, eutopic endometrial stromal cells with endometriosis, and normal endometrial stromal cells

To clarify the mechanisms underlying the attenuated contraction of collagen gels, we evaluated the expression of α-SMA, Rho A, ROCK-I, and ROCK-II proteins in endometriotic cyst stromal cells, eutopic endometrial stromal cells with endometriosis, and normal endometrial stromal cells. As shown in Fig. 5, the levels of α-SMA, Rho A, ROCK-I, and ROCK-II protein in endometriotic cyst stromal cells, eutopic endometrial stromal cells with endometriosis, and normal endometrial stromal cells were significantly reduced after long-term stimulation with db-cAMP+MPA and db-cAMP+dienogest. Long-term stimulation with db-cAMP alone weakly enhanced the expression of these proteins in these three cell types. In contrast, long-term stimulation with MPA or dienogest alone did not affect the expression of these contractility-associated proteins in these cells (data not shown). GAPDH protein was detected in all samples at almost equal quantities.

Discussion

In an effort to clarify physiological and pathological tissue remodeling in the endometrium and endometriosis, our laboratory has been conducting an ongoing investigation into the contractile profiles of ectopic and eutopic endometrial stromal cells. In the present study, the following points were demonstrated: 1) decidualization inhibits the contractility of ectopic and eutopic endometrial stromal cells in vitro through down-regulation of collagen I receptor expression and suppression of Rho-ROCK-mediated signaling pathways; 2) decidualization inhibits the differentiation of ectopic and eutopic endometrial stromal cells into myofibroblastic phenotype and induces the differentiation of these cells into epithelioid decidual phenotypes; 3) decidualized ectopic and eutopic endometrial stromal cells in 3-D collagen gel culture mimic the morphology of decidual tissue in vivo; 4) although the decidualizing capacity as assessed by PRL and IGFBP-1 production is suppressed in endometriotic stromal cells, decidualization significantly down-regulates the contractility of endometriotic stromal cells as well as the eutopic endometrial stromal cells. These findings reveal the underlying mechanisms of both the physiological endometrial tissue remodeling process during decidualization and the action of OCs and progestins during the treatment for endometriosis. Alternatively, our findings also provide an alternative mechanism as to why endometriotic lesions regress during pregnancy. It is also hypothesized that ectopic and eutopic endometrial stromal cells can differentiate into two morphologically and functionally distinct cell types, a myofibroblastic phenotype and epithelioid decidual phenotype. Decidualization is suggested to be a regulatory factor of these differentiation's.

In vitro decidualization induces morphological changes of the endometrial stromal cells, which mimic in vivo decidual transformation (9, 11). It has been demonstrated that the expression of α-SMA was induced spontaneously in human endometrial stromal cells in culture (18, 36). Decidualization down-regulates the expression of α-SMA in the endometrial stromal cells in vitro (18, 43). Decrease of α-SMA expression in decidualizing stromal fibroblasts has also been reported in vivo (44). In other studies, α-SMA-positive fibroblastic cells were frequently detected in the fibrotic areas associated with endometriosis of the peritoneum, ovary, rectovaginal septum, and uterosacral ligaments (23, 24, 45). Immunohistochemical analysis led Anaf et al. (45) to suggest that endometriotic stromal cells can differentiate to α-SMA-positive myofibroblasts. We have previously demonstrated that endometriotic cyst stromal cells in 3-D collagen gel culture spontaneously differentiate into the α-SMA-positive myofibroblastic phenotype (36). α-SMA is considered the most reliable marker of myofibroblastic differentiation (46), and a correlation between the expression of α-SMA and the contractile activity of fibroblastic cells has been suggested (47). The results of our present study indicated that the reduction of contractility of the eutopic and ectopic endometrial stromal cells was associated with the inhibition of the expression of α-SMA in these cells during decidualization. We also considered that decidualization attenuated the contractility of eutopic and ectopic endometrial stromal
cells to construct the characteristic morphology of the decidua by suppressing the differentiation of these cells into the myofibroblastic phenotype and inducing the differentiation into epithelioid decidual phenotype.

In addition to the morphological changes, decidualization bestows some unique functional properties on human endometrial stromal cells (7, 10). The levels of production of PRL and IGFBP-1 by endometrial stromal cells are generally used as biochemical markers of endometrial stromal cell decidualization (14). In the present study, we demonstrated that the endometriotic cyst stromal cells retained the same capacity for decidualization as did the eutopic endometrial stromal cells with endometriosis and normal endometrial stromal cells. It is considered that our data on the endometriotic cyst stromal cell-mediated contractility do not support the progesterone resistance theory. The combination of OCs and progestins is known to be effective to control the disease progression and the painful symptoms associated with endometriosis (30). Our findings suggest an additional molecular mechanism that may underlie these therapeutic effects.

In conclusion, we demonstrated that decidualized ectopic and eutopic endometrial stromal cells in 3-D collagen gel culture mimicked the morphology of decidual tissue in vivo and that the decidualization inhibited the contractility of ectopic and eutopic endometrial stromal cells in vitro. It is suggested that the attenuation of eutopic endometrial stromal cell-mediated contractility by decidualization is a novel and integral mechanism of the physiological endometrial tissue remodeling process during decidualization. It was also suggested that the attenuation of ectopic endometrial stromal cell-mediated contractility by decidualization is one of the mechanisms by which OCs or progestins as well as the conception ameliorate endometriosis. In addition, our results indicate that research on eutopic and ectopic endometrial stromal cell biology in 3-D collagen gel culture offers new opportunities to understand the mechanisms of decidualization from the view of endometrial stromal cell-mediated contractility.

Acknowledgments

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