Progesterone-dependent release of transforming growth factor-beta1 from epithelial cells enhances the endometrial decidualization by turning on the Smad signalling in stromal cells

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Endometrial decidualization results from the differentiation of stromal cells in an ovarian steroid-sensitive manner. Human endometrial tissues obtained from fertile women at various stages of the menstrual cycle were subjected to immunohistochemistry to localize the components of the transforming growth factor-beta (TGF- β) system. TGF- β receptor-I and -II expression was higher in stromal cells than in epithelial cells during the secretory phase while no such variation was observed during the proliferative phase. The expression of phosphorylated Smad3 (pSmad2/3), an activated form of a component of the TGF- β signalling pathway, and translocation of pSmad2/3 from the cytoplasm to the nucleus were more pronounced in secretory endometrium. In coculture of human endometrial epithelial with stromal cells, each isolated from the proliferative endometrium, administration of progesterone stimulated decidualization as well as TGF- β signalling activation in stromal cells. Progesterone also significantly elevated the concentration of TGF- β1 in the coculture medium. Careful manipulation of the coculture, i.e. selective addition and omission of the cellular components, showed that this progesterone-induced increase in secretion of TGF- β1 come mainly from epithelial cells. Moreover, administration of TGF- β1 (10 ng/ml) directly to cultured stromal cells enhanced the expression of prolactin as well as pSmad2/3 even without progesterone. Taken together, our present data support the notion that progesterone induces stromal decidualization indirectly, i.e. by enhancing the expression and secretion of TGF- β1 from epithelial cells. The secreted, epithelial-derived TGF- β1 then acts on adjacent stromal cells, at least in part, to turn on Smad signalling that could lead to stromal decidualization.

Key words: coculture of human endometrial epithelial and stromal cells/endometrial decidualization/paracrine mediator/Smad signalling/TGF- β1

Introduction

The human uterine endometrium is a dynamic organ that undergoes remarkable periodic growth, remodelling and breakdown under hormonal control. The decidualization of human endometrial stromal cells, which develops in the late secretory stage of the menstrual cycle, is characterized by morphological and functional differentiation. For example, predecidual and particularly gestational decidual cells produce prolactin identical to pituitary prolactin and relaxin (Maslar and Roddick, 1979; Irwin et al., 1989). The most obvious features of decidualization are the stromal cell transformation. It is characterized by the transformation of the elongated fibroblast-like phenotype of endometrial stromal cell to the larger, rounder phenotype of the decidual cell (Verma, 1983; Tang et al., 1994a; Giudice and Ferenczy, 1996). This differentiation process can be induced in vitro by progesterone in estradiol-treated cultures (Huang et al., 1987; Clasen-Linke et al., 1998), by ligands which are coupled to the cAMP pathway such as PGE2 (Frank et al., 1994), gonadotropins (Frank and Gurpide, 1993) and by cAMP alone (Tang et al., 1993). However, the molecular mechanism that leads to triggering the onset of decidualization is poorly understood. Nonetheless, many studies have supported that the pre-decidualization of human endometrial stromal cells as well as complete decidualization of the stroma involves processes reminiscent of the actions of transforming growth factor-β (TGF-β) (Rizzino, 1988; Sporn and Roberts, 1990; Tang et al., 1994b).

TGF- β is a multifunctional cytokine that regulates many biological functions ranging from growth/differentiation to apoptosis of various cell types (Sporn and Roberts, 1990). Due to their powerful effects on the cellular and molecular processes, in particular, on those associated with cellular proliferation and differentiation, angiogenesis, extracellular matrix modification and immunomodulation, TGF- βs have been identified as potential modulators of many endometrial functions. Indeed, the actions of steroid hormones are known to be mediated by locally expressed TGF- βs (Bruner et al., 1995; Godkin and Doré, 1998; Ingman and Robertson, 2002). Fluctuation in TGF- β1, TGF- β2 and TGF- β3 as well as their receptors in the endometrium has been noted (Tang et al., 1994b; Arici et al., 1996; Doré et al., 1996; Piestrzyniewicz-Ulanska et al., 2002). In human endometrium, TGF- β1 expression is up-regulated during times of increasing plasma progesterone concentrations and
down-regulated during progesterone withdrawal (Casslÿn et al., 1998). In addition, TGF-β3 expression is markedly increased during the secretory phase compared with proliferative tissues, and this increase is affected by progesterone treatment (Chegini et al., 1994; Reis et al., 2002). The secretory phase of the endometrial cycle is a critical branch point in the development and differentiation of the endometrium. This is the time of biochemical transition when full decidualization commences. It has since been suggested that TGF-β acts to define the functional destiny of the endometrium at this critical branch point of development, including progesterone-induced endometrial decidualization (Casey and McDonald, 1996; Reis et al., 2002). In support of this view, TGF-βs and their receptors are markedly expressed in decidua and villous tissues (Clark et al., 1990; Graham et al., 1992; Manova et al., 1992; Roelen et al., 1994; Schilling and Yeh, 2000). Moreover, the human endometrium expresses the necessary components of the Smad signalling pathway, whose expression and induction are regulated by TGF-βs (Luo et al., 2003), providing evidence to support the notion that TGF-βs play some specific roles in support of decidualization and placentation in an autocrine and/or paracrine manner (Lennard et al., 1995; Ando et al., 1998).

In vitro studies using monolayer culture of endometrial stromal or epithelial cells have attempted to characterize the molecular expression associated with decidualization. However, information obtained from monolayer culture did not appear to reflect the in vivo endometrium in many cases (Silleh et al., 1997; Simon et al., 1997; Kasahara et al., 2001). In addition, interaction between the endometrial components, i.e. endometrial epithelial and stromal cells, is known to be an important factor for endometrial differentiation (Osteen et al., 1994; Pierro et al., 2001). Therefore, we have utilized a well defined three-dimensional (3-D) endometrial cell culture system, which consists of human endometrial epithelial and stromal cells in a defined extracellular matrices (Park et al., 2003a), to investigate the role of TGF-β1 in the progesterone-induced endometrial decidualization, in particular, to identify molecular entity(s) that mediates endometrial decidualization. Part of these results has been published in abstract form (Park et al., 2003b).

Materials and methods

Endometrial cell preparation and reconstruction

Human endometrial tissues were obtained by curettage of hysterectomy specimens from patients with conditions other than endometrial diseases (myoma or adenomyosis), who had given informed consent and approval before operation at the Ajou University Hospital. Tissue specimens from patients of ages 30–45, who were estimated to be in the mid- or late proliferative phase of the menstrual cycle, were taken for the experiment. The protocol for cell isolation and reconstruction was identical to what we have reported previously (Park et al., 2001, 2003a). Briefly, the sampled endometrial tissues in a conical tube (Falcon, Becton Dickinson, NJ, USA) containing phosphate-buffered saline (PBS) were transported to the laboratory, where the tissue was again washed several times with PBS to remove any residual blood clots. Then the tissues were minced in 2–3 ml of Dulbecco’s modified Eagle’s medium (DMEM, Gibco Life Technologies, Grand Island, NY, USA). The tissue minces were centrifuged at 85 × g in a conical tube and to the pellet, 5 ml of 1000 units/ml collagenase (Sigma, St. Louis, MO, USA) was added and incubated in a shaker at 37°C. After 1 h of incubation, 5 ml of DMEM supplemented with 1% penicillin-streptomycin (Gibco Life Technologies) and 10% heat-inactivated fetal bovine serum (FBS, Gibco Life Technologies) was added to stop the enzyme reaction. Then 5 ml of each upper stromal portion and lower epithelial portion was seeded on a 60 mm culture dish (Falcon) in DMEM plus 10% FBS and cultured for 24 h. Dissociation and separation of the lumps of cultured cells into single cells was performed by the treatment of trypsin/EDTA for 1 h at 37°C. The stromal cells, after vigorous separation into single cells, were mixed with collagen (BD Sciences, San Diego, CA, USA) at a concentration of 5 × 10⁶ cells/ml, plated in a 24-well plate (Nunc A/S, Roskilde, Denmark) and cultured at 37°C for 1 h before collagen polymerization. The separated epithelial cells were mixed with collagen at a concentration of 1 × 10⁶ cells/ml, and 400 μl of the mixture was seeded on a cell culture insert (pore size 0.4 μm, Millipore, Billerica, MA, USA), which had been previously coated with Matrigel (BD Sciences). To induce stromal decidualization, cells were cultured for 48 h in serum-free DMEM/F-12 medium (Gibco Life Technologies) with the following hormonal combinations, whose physiological relevance was previously described (Kasahara et al., 2001; Park et al., 2003b): control, no ovarian steroid; progesterone-dominant condition, 100 nM progesterone and 1 nM estrogen added; estrogen-dominant condition, 1 nM progesterone and 100 nM estrogen added; estrogen-dominant condition, 1 nM progesterone and 100 nM estrogen added. TGF-β1 (Sigma) was treated at a concentration of 10 ng/ml when necessary. Steroids were dissolved in ethanol, with the final concentration of ethanol in the culture medium less than 0.1% (v/v). TGF-β1 was dissolved in culture medium. The overall scheme of the 3-D coculture is illustrated in Figure 1.

Immunohistochemical analysis of TGF-β receptor and Smad expression

Human endometrial tissues were taken from patients at various stages of the menstrual cycle (proliferative or secretory phase), paraffin-embedded and sectioned 3–5 μm thick according to a standard paraffin block method (Lambregt and Rothstein, 1978). Paraffin sections were treated with 4% H₂O₂ for 5 min to remove the residual peroxidases. After washing with distilled water three times, the paraffin sections were incubated with either 1/400-diluted anti-TGF-β receptor type I antibodies (anti-human rabbit polyclonal, Santa Cruz Biotech, CA, USA), 1/400-diluted anti-TGF-β receptor type II antibodies (anti-human rabbit polyclonal, Santa Cruz Biotech) or 1/400-diluted anti-pSmad2/3 antibodies (anti-human goat polyclonal, Santa Cruz Biotech) in a humidifier chamber at room temperature for 1 h. The sections were then reacted with secondary antibodies included in the LSAB-kit (DAKO A/S, Glostrup, Denmark) for 15 min according to the manufacturer’s instruction. After washing with distilled water three times, chromagen development with diaminobenzidine (DAB) (DAKO A/S) was performed, followed by counter-staining with hematoxylin. The sections were then dehydrated, cleared, mounted, and observed under a fluorescence microscope (Axioskope, Carl Zeiss, Germany). Kit solutions used for antibody dilution and subsequent processing contained carrier proteins such as 1% bovine serum albumin (BSA) to reduce the non-specific binding. To quantify the staining intensity and localization, images were analyzed by using...
image analysis software (Image Pro Plus 4.5, Media Cybernetics, San Diego, CA, USA).

**RT–PCR**
Total RNA was extracted from cultured epithelial or stromal cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s instruction. Only high purity RNA, whose absorbance ratio of 260 to 280 nm is higher than 1.9, was used for cDNA synthesis. Four to five micrograms of total RNA were subjected to cDNA synthesis using M-MLV reverse transcriptase kit (Bioneer, Daejon, Korea). For further amplification in PCR, primers were designed by a primer designing software Primer3 (available at http://frodo.wi.mit.edu/cgi-bin/prime3/primer3_www.cgi). The sequences of the primers used in these experiments were forward prolactin, 5′-GCCCTGCTGTGCTGGCTCC-3′; reverse prolactin, 5′-AGAAAGCCTTTGGTGTGTTGCTCC-3′; forward integrin β3, 5′-CGGCTCAGCTGATGTGTGTT-3′; reverse integrin β3, 5′-CATCTCCACCTAGTCCAA-3′; forward β-actin, 5′-CTTCTCGCTCCTTCCCT-3′; reverse β-actin, 5′-CTGGTCATACTCCTGCTGGCT-3′. All the primers were custom-synthesized and purified (Bioneer), and the PCR amplification was conducted using Accupower™ PCR premix kit (Bioneer) according to the manufacturer’s recommendation with 1 cycle of 3 min at 94°C, followed by 30 cycles of 10 sec at 94°C, 10 sec at 57°C and 30 sec at 72°C and 1 cycle of 2 min at 72°C. After PCR amplification, 10 μl aliquots were electrophoresed in 3% agarose gel, followed by photographic recording of the gels after staining with ethidium bromide.

**Measurement of TGF-β concentration in the conditioned media**
To study the secretion of TGF-β1 from cells in culture, a pure population of isolated human endometrial epithelial and stromal cells were cultured separately or cocultured as described above. After 48 h in culture, cells were removed by centrifugation, and the cell-free media were collected and stored at −80°C until use. After thawing at room temperature, the medium was assayed for immunoreactive TGF-β1 by enzyme-linked immunosorbent assay (ELISA) assay with anti-TGF-β1 antibodies (Quantikine human TGF-β1 immunoassay kit, R&D systems, Minneapolis, MN, USA) according to the manufacturer’s specifications with absorbance at 450 nm in a plate reader (SpectraMax 190, Molecular Device, Sunnyvale, CA, USA). The immunoassay kit used in this experiment offered a dynamic range starting in the low pg/ml and covered three logarithms. Experiments were repeated at least three times with different media to minimize the intra- and interassay variation.

**Western blot analysis of TGF-β1 and pSmad2/3 in cultured stromal cells**
Western blot analysis was performed using standard methodology (Towbin et al., 1979). In brief, total proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS–PAGE) and transferred to nitrocellulose blotting membrane (Sartorius AG, Götingen, Germany). The membranes were blocked with 5% non-fat dry milk in Tris-buffered saline and incubated with primary antibodies for 1 h at room temperature. Blots were developed by peroxidase-conjugated secondary antibody, and proteins were visualized by enhanced chemiluminescence (ECL) procedure (Santa Cruz Biotechnology) according to the manufacturer’s recommendations.

**Statistical analysis**
Student’s t-test was used for the statistical analysis of concentrations of TGF-β1 in the culture media. P < 0.05 was considered to be statistically significant. Mann–Whitney test was used for the statistical analysis of immunohistochemistry. Unless otherwise indicated, P < 0.05 was considered to be statistically significant.

**Results**
**Expression and distribution of TGF receptor-I, -II and phosphorylated Smad3 (pSmad2/3) in normal endometrial tissues**
Human endometrial tissues were examined immunohistochemically for the expression of TGF-β receptor-I and -II as well as a signaling transducer protein pSmad2/3, and representative results are shown in Figure 2. Results demonstrated that the normal endometrial tissue throughout the estrous cycle (proliferative or secretory) expressed TGF-β receptor-I, -II and pSmad2/3, an activated component of TGF-β signalling pathway, with immunoreactivities being localized to the...
endometrial surface epithelium, glandular epithelium and stroma. However, the intensity of staining in epithelia and stroma was significantly increased in the secretory phase relative to the proliferative stage endometrium. In particular, the glandular epithelium in the secretory endometrium exhibited intense accumulation of receptor-I and -II and pSmad2/3. To quantify the level of protein expression more precisely, immuno-staining intensity was arbitrarily scored by using Image Pro Plus software. Results are summarized in Table I. The results revealed a significant increase in the level of both TGF-β receptor-I and -II protein expressions in the secretory endometrium compared to the proliferative endometrium ($P < 0.05$ using Mann–Whitney test). Similarly, the stromal expression of TGF-β receptor-I and -II protein was significantly increased compared to the respective epithelial expression in the secretory endometrium ($P < 0.05$ using Mann–Whitney test). The expression pattern of pSmad2/3 protein resembled those of TGF-β receptors in that protein expression was up-regulated in the secretory endometrium. In addition, pSmad2/3 translocation from the cytoplasm to the nucleus was greatly enhanced in the secretory endometrium ($P < 0.05$ using Mann–Whitney test) while no such translocation was noted in the proliferative endometrium. Collectively, these results suggest that in the secretory endometrium, the protein expression level of TGF-β receptor was up-regulated concomitant with activation of the TGF-β signalling.

<table>
<thead>
<tr>
<th>TGF-β receptor-I</th>
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<td>3.69 ± 1.21</td>
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<td>2.27 ± 1.30</td>
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Immuno-staining intensities of cells or organelles for transforming growth factor-beta (TGF-β) receptor-I, -II and pSmad2/3 were scored arbitrarily by using image analysis software Image Pro Plus and statistically analysed using Mann–Whitney test. Results are means ± SD from triplicate trials of each of 10 patients with normal endometrial tissues.

*P < 0.05 compared to respective proliferative endometrium.

†P < 0.05 compared to cytoplasm.

‡P < 0.05 compared to respective epithelial cells.

Expression of integrin $\beta_3$ and prolactin mRNA in human endometrial epithelial and stromal cells in coculture

Messenger RNA for integrin $\beta_3$, a cellular differentiation marker, and for prolactin, an endometrial decidualization marker, was measured by RT–PCR. In coculture, expression of both mRNAs was greatly enhanced in stromal cells (Figure 3B), but not in epithelial cells (Figure 3A), in response to progesterone treatment while the level of β-actin mRNA expression was almost constant throughout the treatment. In contrast, neither marker was detected in stromal cells if cells were cultured separately (Figure 3C), suggesting that no decidualization occurred. By comparison, expression of prolactin was not detected in epithelial cells if they were cultured separately under any hormonal combination (data not shown).

Measurement of TGF-β1 concentration in the culture medium

The amount of TGF-β1 that was secreted into the culture media from epithelial and/or stromal cells was determined to ascertain which cells secrete TGF-β1 in a progesterone-dependent manner. The level of TGF-β1 present in the culture medium was assayed by ELISA, using epithelial cell and/or stromal cell conditioned medium after 48 h in culture. As evident in Figure 4, TGF-β1 secretion by epithelial cells was enhanced by about 2.3-fold over the control with the addition of

Figure 3. RT–PCR analysis of integrin $\beta_3$ and prolactin mRNA expression in human endometrial epithelial and stromal cells in culture. Human endometrial epithelial and stromal cells were cocultured or cultured separately for 48 h under three different hormonal conditions as described in Methods (Cntr, no ovarian steroid; E, estrogen-dominant condition and P, progesterone-dominant condition). Total RNAs were isolated from epithelial cells cocultured (A), stromal cells cocultured (B) and stromal cells cultured separately (C), and equal amount of total RNA (4–5 μg) were subjected to cDNA synthesis before amplification in PCR was carried out. PCR products were analysed on an agarose gel. The β-actin mRNA is the control. Results are representatives of three experiments.
100 nM progesterone while estrogen (100 nM) had no such enhancing effect (Figure 4A). A similar progesterone-enhanced secretion of TGF-β1 was observed with the conditioned medium of epithelial/stromal cells coculture whose magnitude was comparable to that obtained from epithelial cells (Figure 4B). Also noted in Figure 4 was that 100 nM estrogen abolished the TGF-β1 secretion from epithelial and/or stromal cells in culture. Likewise, no progesterone-induced secretion of TGF-β1 from stromal cells was observed if stromal cells cultured alone (data not shown). These results clearly suggest that epithelial cells, but not stromal cells, are the cells that secret TGF-β1 in response to progesterone at least in vitro.

Expression and distribution of prolactin and pSmad2/3 protein in stromal cells in coculture under progesterone-dominant condition

Immunohistochemical observation was made to confirm the expression of prolactin and pSmad2/3 proteins in stromal cells in coculture in response to progesterone. Endometrial stromal cells were cocultured with epithelial cells for 48 h under progesterone-dominant conditions and subjected to immunostaining for prolactin and pSmad2/3. Most prolactin-positive cells were in round shape (arrows), and more than 70% of the stromal cells were prolactin positive (Figure 5A), indicating that the progesterone-induced stromal decidualization occurs in stromal cells in coculture. Under the same hormonal conditions, many stromal cells also revealed immunoreactivity against pSmad2/3 (arrows in Figure 5B) and translocation of pSmad2/3 into the nuclei, in particular, was clearly noted (an inset in Figure 5B). These results confirm and extend the data shown in Figure 3 in that the present coculture of human endometrial epithelial with stromal cells is physiologically functional similar to the human endometrium in vivo, particularly, exhibiting the progesterone-induced decidualization concomitant with activation of TGF-β signalling.

TGF-β1-induced prolactin and pSmad2/3 expression in stromal cells

To evaluate and further confirm the specificity of TGF-β1 effects on stromal decidualization as a mediator for progesterone, stromal cells were grown without epithelial cells in a serum-free medium, treated with TGF-β1 at a concentration of 10 ng/ml for 48 h. Then, expression of prolactin mRNA was analyzed by RT–PCR (Figure 6).

Results revealed no prolactin mRNA expression under any hormonal condition unless TGF-β1 was added (Figure 6). In contrast, adding TGF-β1 greatly enhanced the expression of prolactin mRNA, and this enhancement was steroid-independent (Figure 6). Furthermore,
we also examined the protein expression of prolactin and pSmad2/3 by western blot analysis as shown in Figure 7. Western blotting revealed the expression of prolactin and pSmad2/3 proteins in stromal cells in response to TGF-β1 when the stromal cells were treated with TGF-β1 at 10 ng/ml for 48 h, and the enhancing effect exerted by TGF-β1 was abolished by TGF-β1-specific antibodies. Collectively, these results demonstrate that TGF-β1 is capable of inducing the stromal decidualization per se, indicating that progesterone-induced stromal decidualization is likely to be mediated by TGF-β1 in a paracrine fashion.

Discussion

Endometrial decidualization is known to be one of the differentiation processes essential for blastocyst implantation and maintenance of pregnancy. This differentiation process is marked by the onset of decidual production of prolactin (Telgmann and Gellersen, 1998) and integrin β3 (Lessey et al., 2000). The cellular mechanisms by which steroids influence decidualization are extremely important to gain insight into the normal physiology of reproduction as well as the pathophysiology of reproduction-related diseases. TGF-βs are expressed in the human endometrium and their expression are fluctuated throughout the menstrual cycle in a steroid-sensitive manner, indicating that TGF-βs may mediate steroid actions in endometrium (Sachdeva et al., 2001).

In this study, by using the 3-D coculture of human endometrial epithelial with stromal cells, we demonstrated that progesterone treatment can induce the expression of prolactin and integrin β3 mRNA in stromal cells providing evidence for in vitro endometrial decidualization consistent with in vivo decidualization. Previously, many studies demonstrated in vitro decidualization of endometrial stromal cells by using isolated stromal cells in monolayer culture in response to physiological doses of progesterone or progesterone/estrogen combination (Irwin et al., 1989; Kubota et al., 1997; Celikkakat et al., 2000; Kasahara et al., 2001; Sakai et al., 2003). Unlike the monolayered single cell culture, the present epithelial-stromal cell coculture provides a cellular environment more closely resembling in vivo endometrium thus whereby a paracrine interaction between epithelia and stroma may be allowed, including the soluble factor(s) that could mediate the steroid-induced stromal decidualization. Consistently, we show here that the onset of stromal decidualization, as evidenced by the stromal prolactin expression, is completed as soon as 48 h after steroid treatment, which is much quicker, thus more physiologically relevant than the longer treatment of steroids, ranging 7–14 days, required to detect similar results by other studies (Irwin et al., 1989; Kasahara et al., 2001). This discrepancy in duration of steroid treatment may reflect differences in prolactin expression in cultured stromal cells. Indeed, in our studies, prolactin mRNA was undetectable in stromal cells when stromal cells were exposed to 100 nM progesterone for 2 days.

Since each of the TGF-β isotypes has different spatiotemporal distribution in the endometrium (Chegini et al., 1994; Doré et al., 1996), specific TGF-β isotype(s) may serve different roles in endometrial physiology. Which isotype is implicated in endometrial decidualization? The answer is complex and controversial. However, much evidence suggests that TGF-β2 is localized primarily to stromal cells whereas TGF-β1 and TGF-β3 are to epithelial cells within the human endometrium (Bruner et al., 1995; Ando et al., 1998). Moreover, it has previously been demonstrated that TGF-β1 is implicated in epithelial–mesenchymal interaction and in regulation of trophoblast attachment, growth and differentiation (Lennard et al., 1995; Tse et al., 2002). In our studies with epithelia-stroma coculture, results coincide with many previous reports not only in that progesterone stimulates the stromal decidualization as evidenced by an increase in expression of integrin β3, and prolactin mRNA (Irwin et al., 1989; Kubota et al., 1997), but also in progesterone-enhanced release of TGF-β1 into the culture media (Polli et al., 1996). In addition, our results also demonstrate that adding TGF-β1 directly to cultured stromal cells can induce the stromal decidualization per se without progesterone. Based on this observation, we propose that TGF-β1 is the principal mediator for steroid action that leads to the stromal decidualization. Furthermore, manipulation of the coculture, i.e. adding a cell component to or omitting from the coculture, enables us to confirm that epithelial, but not stromal, secretion of TGF-β1 is mainly induced by progesterone, consistent with the idea of stromal decidualization in a paracrine fashion. Our study, to our best knowledge, provides the first clear evidence that endometrial epithelial cells secrete TGF-β1 in response to progesterone, and it remains to be an interesting task to see whether the epithelial cell-derived TGF-β1 directly acts on adjacent stromal cells, inducing stromal decidualization.

The fact that epithelial cells, but not stromal cells, are the source of TGF-β1 release in response to progesterone deserves further comments on putative roles for TGF-β1 in directing blastocyst opposition and trophoblast invasion/differentiation. A recent finding suggests that hepatocyte growth factor (HGF) expressed and secreted from the placenta stimulates invasion and motility of the trophoblast cells while TGF-β1 antagonizes the HGF-stimulated trophoblast cell invasion and motility, at least in part, through adverse effects on expression of inducible nitric oxide synthase (iNOS) (Tse et al., 2002). The anti-inflammatory effect of TGF-β1 on trophoblast invasion, which could result from progesterone-induced, epithelial-derived secretion, might be one of the possible mechanisms of TGF-β1 action in decidualization which involves the regulation of trophoblast invasion, in particular, in trophoblast-epithelium attachment as suggested by Godkin and Doré (1998) and in endometrial carcinoma–stromal cell interaction (Albright and Kaufman, 1995).

The biological availability of active TGF-βs is regulated both at transcription and at latent protein activation. TGF-βs are released as latent proforms that become active after proteolytic cleavage by plasmin (Cassil and et al., 1998). The antibodies utilized in this study (ELISA assay) did not fully differentiate between latent and active TGF-β1. Therefore, the difference in amount of TGF-β1 present in the conditioned medium as indicated in Figure 4 would be larger if only...
the active form of TGF-β1 is taken into account. Mass spectrometric analysis would clarify this issue in the near future.

Our study also demonstrated expression of pSmad2/3, an intracellular mediator of TGF-β signalling, in stromal cells concomitant with the progesterone-induced stromal decidualization in vitro. In addition, expression of both type-I and -II receptor for TGF-β was detected in human endometrial tissues, with expression of both receptors being higher in the stroma of secretory endometrium. Similarly, pSmad2/3 protein expression was enhanced in secretory endometrium relative to proliferative endometrium concomitant with more protein translocation into the nucleus. A similar result has been described elsewhere.

For example, Luo and associates (2003) reported that human endometrium, isolated endometrial epithelial and stromal cells all expressed necessary components of Smad signalling pathway, and their expression and induction were regulated by TGF-β through its receptor. In support of this line of speculation, Lin and associates (2004) reported a cyclic expression of TGF-β signalling components (Smad2 and 4) in rat endometrium during the menstrual cycle, and TGF-β signalling functioning during pre-implantation by switching the endometrium from the neutral phase to the receptive phase, suggesting that TGF-β signalling could be turned on and off depending on physiological context.

In summary, the present 3-D coculture of human endometrial epithelial cells with stromal cells allows achievement of an improved in vitro system for studying interactions between cells through soluble signals. The results obtained thereby lend support to the view that progesterone-induced stromal decidualization may be principally mediated by TGF-β1 secreted from the endometrial epithelial cells by turning on the Smad signalling in stromal cells.

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References


Transforming growth factor-β in endometrial decidualization


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