GENETICS

Gene Expression of Transforming Growth Factor- α in Human Endometrium During Decidualization

MICHIYOSHI TAGA,^{1,2} HIDEYA SAKAKIBARA,¹ KUMIKO SUYAMA,¹ MARIO IKEDA,¹ and HIROSHI MINAGUCHI¹

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Purpose: We previously reported the gene expression of epidermal growth factor (EGF) in the process of decidualization in the human endometrium. This study was undertaken to investigate the biological significance of transforming growth factor- α (TGF- α), which shares a significant sequence homology with EGF, in the regulation of decidualization.

Methods: The gene encoding TGF- α was analyzed by Northern blot hybridization in nonpregnant human endometria and decidua from 6 to 8 weeks of gestation as well as in cultured stromal cells with or without medroxyprogesterone acetate.

Results: No transcript was detected in proliferative and secretory endometrium, whereas a transcript of 4.8 kb, which was in agreement with the size of human prepro-TGF- α mRNA previously reported, was clearly detected in decidua. Transcript of 4.8 kb was also detected in vitro in medroxyprogesterone acetate (MPA)-induced decidual cells, while no TGF- α transcript was found in endometrial stromal cells cultured without MPA.

Conclusions: These results suggest that the gene expression of TGF- α in uterine stromal cells is enhanced by stimulation from sex steroids and that TGF- α , like EGF, functions as one of the regulatory factors for decidualization in the human uterus.

KEY WORDS: transforming growth factor- α ; decidualization; gene expression; sex steroid.

INTRODUCTION

Decidualization is an important process for implantation of embryos as well as maintenance of pregnancy. Proliferation and differentiation of human endometrium throughout the menstrual cycle and subsequent decidual formation in pregnancy are subject to various hormonal signals. Although the factors that regulate steroid hormone action in the uterus are still poorly understood, there is a growing body of data implicating roles for specific growth factors in the control of endometrial function, such as epidermal growth factor (EGF), insulin-like growth factor (IGF)-I, transforming growth factor (TGF)- α (1–3), and their respective receptors (4–6). Previously we have shown the importance of EGF in the regulation of decidual formation because prepro-EGF mRNA was expressed during the decidualization process in human endometrium in both in vivo and in vitro systems (7).

TGF- α , a 50-amino acid polypeptide which shares a significant sequence homology with EGF, can bind to the EGF receptor with an affinity equal to that of EGF. Horowitz *et al.* (8) reported the immunohistochemical localization of TGF- α in human endometrium, decidua, and trophoblasts. The expression of TGF- α mRNA has been reported in rat decidua by Northern blot analysis (9,10). In the human, Haining *et al.* (11) detected mRNA for EGF and TGF- α in endometrium and decidua by polymerase chain reaction (PCR) but did not study discrete changes in mRNA levels. Therefore, there has been no definite report of studies on the change in TGF- α mRNA in the process of decidualization in human endometrium. In this study, to clarify mRNA levels at which TGF- α , like

¹ Department of Obstetrics and Gynecology, Yokohama City University School of Medicine, 3-9 Fukuura, Kanazawa-ku, Yokohama 236, Japan.

² To whom correspondence should be addressed.

EGF, is produced during the decidualization process, we examined TGF- α mRNA in human endometria/decidua by Northern blot hybridization.

MATERIALS AND METHODS

Tissue Preparation

Midproliferative- and midsecretory-phase human endometria were obtained from five hysterectomized uteri of endocrinologically normal women who had undergone hysterectomy for leiomyoma. Decidual tissues were also obtained from four patients with induced abortions of normal pregnancies of 6–8 weeks' gestation. All patients consented to participate in this study. Tissues were immediately frozen by liquid nitrogen and stored at -80°C until the time of the experiment.

In Vitro Decidualization of Endometrial Cells

In vitro decidualized cells were obtained by adding medroxyprogesterone acetate (MPA) to the medium in the primary culture of stromal cells from luteal-phase endometrium of three patients who had undergone hysterectomy for leiomyomas. After endometrial tissues were minced and digested in a Ham F-10 medium containing 0.25% collagenase and 0.05% DNase at 37°C for 3 hr, they were filtrated through 300- μm nylon mesh. After allowing stromal cells to attach to Falcon culture flasks, the culture media were aspirated for removal of unattached cells. Attached cells were further cultured in RPMI 1640 medium supplemented with 2% fetal bovine serum (FBS) with or without 1 μM MPA for 23 days. The prolactin (PRL) concentrations in the culture media were measured by radioimmunoassay to confirm that cells cultured with MPA underwent decidualization.

RNA Extraction

Total RNA from frozen tissues of nonpregnant endometria and decidua were prepared by homogenizing tissue in buffer containing 4 M guanidine isothiocyanate, followed by centrifugation with lithium chloride. After phenol extraction and ethanol precipitation, poly(A⁺) RNA was selected by an oligo(dT) cellulose column. RNA extraction from cultured cells was also carried out by the same procedure after cells were scraped from culture plates with a rubber policeman in the homogenizing buffer.

Hybridization Probe

Human TGF- α cDNA was kindly provided by Dr. Kurachi (Osaka University, Japan). This cDNA clone, a fragment of about 2 kb, was inserted into the *EcoRI* site of the pBR327 vector.

Northern Blot Hybridization

Northern blot hybridization for nonpregnant endometrium and decidua were performed according to the methods of Maniatis *et al.* (12). Poly(A⁺) RNA from each tissue or cultured cells was resolved by electrophoresis through 1% agarose gels containing formaldehyde, followed by transfer to a nylon membrane. Blots were hybridized with 2×10^5 cpm/ml multiprimed human prepro-TGF- α cDNA. Hybridization was performed at 42°C overnight in 50% formamide, $4 \times \text{SSC}$ ($1 \times \text{SSC}$: 0.15 M NaCl and 0.015 M sodium citrate), 20 mM Tris-HCl, pH 7.0, 0.1 $\times$ Denhart reagent (20 $\times$ Denhart reagent: 0.4% each bovine serum albumin, Ficoll, and polyvinylpyrrolidone), 10% SDS, and 100 $\mu\text{g}/\text{ml}$ salmon sperm DNA. Washes were performed in $2 \times \text{SSC}$ and 0.1% SDS at room temperature for 60 min and at 50°C for 20 min. Blots were then baked at 80°C in a vacuum oven for 2 hr and exposed to X-ray film (XAR-5; Kodak Co.) with an intensifying screen at -80°C .

RESULTS

To detect TGF- α mRNA in human endometrium and decidua, endometrial and decidual poly(A⁺) RNA were analyzed by Northern blotting. As shown in Fig. 1 representatively, no transcript was detected in proliferative and secretory endometrium, whereas a transcript of 4.8 kb, which was in agreement with the size of human prepro-TGF- α mRNA reported previously, was clearly detected in decidua at 6–8 weeks of gestation. We confirmed the same finding in other endometrium/decidua samples. A transcript of 4.8 kb was also detected in MPA-induced decidual cells in vitro, while no transcript was found in endometrial cells cultured without MPA, which is illustrated representatively in Fig. 2. We obtained consistent results in other cultured cells with or without MPA. The increase in PRL concentration in the culture medium with MPA confirmed that MPA transformed the endometrial cells to decidual cells in vitro during a 23-day culture (Fig. 3).

Fig. 1. Northern blot hybridization of poly (A⁺) RNA (10 µg per lane) from proliferative- and secretory-phase human endometrium and human decidua of 8 weeks' gestation. The arrow indicates the migration of the 4.8-kb TGF-α mRNA.

DISCUSSION

Recent studies suggest that the mitogenic effects of sex steroids in the uterus are partly mediated by locally

Fig. 2. Northern blot hybridization of poly (A⁺) RNA (10 µg per lane) from in vitro decidualized or undecidualized human endometrium. The arrow indicates the migration of the 4.8-kb TGF-α mRNA.

produced peptide growth factors that act in an autocrine or paracrine fashion (13). Although it has been postulated that steroidal modulation is required for the transformation of uterine stromal cells into decidual cells, the molecular mechanisms underlying steroidal action in the process of decidualization are not fully understood.

The results in this study clearly demonstrate in both in vivo and in vitro systems that the expression of the

Fig. 3. Change in PRL concentrations in the culture media during the culture of stromal cells with or without MPA for 23 days.

gene encoding TGF- α increases during the process of decidualization in human endometrium because, using cloned human TGF- α cDNA as a hybridization probe, we have shown by Northern blot hybridization that TGF- α mRNA, which was not detected in nonpregnant human endometrium or undecidualized stromal cells cultured without MPA, was expressed in decidua from the pregnant uterus of 6–8 weeks' gestation or in vitro MPA-induced decidualized cells. Horowitz *et al.* (8) already demonstrated the immunohistochemical localization of TGF- α in human decidua, although they detected TGF- α in endometrium as well. The finding that the TGF- α transcript is expressed at a relatively high level during decidualization but not in a nonpregnant state is very similar to that of our previous report on the change in EGF mRNA during decidualization (7), suggesting that TGF- α , like EGF, functions as one of the regulatory factors for decidualization and that the gene expressions of both EGF and TGF- α in the human endometrium are enhanced during decidualization under the influence of sex steroidal stimulation in the same fashion. Therefore, the previous implication that an active autocrine/paracrine role for EGF and TGF- α and their common receptor in the mediation of mitogenic action of sex steroids in the uterine epithelium can be applicable to uterine stroma.

Although Haining *et al.* (11) reported the presence of mRNA for EGF and TGF- α at a low copy number in human endometrium and decidua using RT-PCR, they did not study discrete changes in mRNA levels because of the requirement of quantitative PCR for such analyses. Since mRNA for TGF- α may be present in very low amounts in nonpregnant human endometrium, it may not be abundant enough to be detected by Northern blot hybridization. In the rat, Northern

blot hybridization failed to demonstrate the presence of mRNA for TGF- α in nonpregnant uteri, probably because of the very low levels of message, whereas the developmentally regulated expression of high levels of TGF- α mRNA was observed in rat decidua (9). Tamada *et al.* also reported that decidualizing stromal cells synthesized TGF- α in the mouse uterus (14). Therefore, these observations of TGF- α mRNA in the rodent uterus can be extended to that of the human.

The evidence that cultured endometrial stromal cells acquired TGF- α mRNA during MPA-induced in vitro decidualization indicates that progesterone has a primary role in the regulation of TGF- α production in the decidualization process, although the possibility that estrogen is also involved in the expression of TGF- α mRNA is not completely ruled out because decidual tissue from 6–8 weeks of gestation is formed in vivo under the influence of these two hormones. The in vitro decidualization model also provides important evidence that the induction of the expression of TGF- α mRNA in decidual cells is not dependent on a direct signal unique to the conceptus but is purely regulated by a hormonal environment. This is consistent with the results of Bonvissuto *et al.* (10) that the absence of the conceptus did not eliminate the expression of mRNA and the appearance of the TGF- α peptide in the rat. Prolactin is a good marker for the decidualization process because decidua produces PRL and PRL production increases in accordance with the stimulation of sex steroids (15).

There are two possible biological roles of TGF- α in human decidua. Since human decidual tissue contains EGF/TGF- α receptor (16) and TGF- α has a stimulatory effect on the proliferation of decidual cells (17), it seems most likely that TGF- α acts to induce or amplify the cell proliferation in the process of decidual formation and, thereby, through a paracrine/autocrine mechanism, enables the decidual cells to exert the rapid and massive cell growth which is required for the extension of decidualization. In addition to its postulated role in decidual cell proliferation, it is also possible that TGF- α produced in the decidua acts upon the trophoblast, which has been reported to contain significant levels of EGF/TGF- α receptor (18). Recently we demonstrated that TGF- α stimulated trophoblast outgrowth in vitro in the mouse (19).

In conclusion, the observation that TGF- α transcript is expressed at relatively high levels during decidualization but not in the nonpregnant endometrium, together with the presence of EGF/TGF- α receptor in this tissue, suggests that TGF- α could be an important molecule which regulates the propagation of decidual

reaction by stimulating cell proliferation and differentiation of neighboring stromal cells locally through an autocrine/paracrine mechanism and plays a role in promoting the extensive vascularization required for the decidualization reaction because TGF- α has a more potent angiogenic activity than EGF (20).

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