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NOTE

Gene Expressions of Keratinocyte Growth Factor and Its Receptor in the Human Endometrium/Decidua and Chorionic Villi

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Abstract. Keratinocyte growth factor (KGF) is secreted from mesenchymal fibroblasts and has a mitogenic specificity for epithelial cells in a paracrine fashion. In order to clarify the biological significance of KGF in the human endometrium which undergoes dynamic changes under the influence of sex steroid hormone, we investigated the gene expressions of KGF and its receptor (KGF-R) in the human endometrium in various sex steroid hormone milieus and chorionic villi, by RT-PCR and Northern blot hybridization. The secretory phase endometrium had a KGF mRNA level 10-fold greater than that of the proliferative phase endometrium. Similarly abundant KGF mRNA was found in decidua and pseudopregnant endometrium compared with proliferative phase endometrium. The KGF-R mRNA was detected by RT-PCR in chorionic villi from early pregnancy. These results indicate that the gene for KGF expressed in the human endometrium is mainly regulated by progesterone and that KGF might have a role in the interaction between decidua and chorion in early pregnancy in man.

Key words: Keratinocyte growth factor (KGF), Endometrium, KGF-receptor, Gene expression
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THE endometrial tissue, which consists of epithelium and stroma, undergoes dynamic changes anatomically and functionally during the menstrual cycle as well as during pregnancy under the influence of sex steroid hormones. It has been thought that the actions of sex steroid hormones in the endometrium are partly mediated through several kinds of growth factors which are locally produced in the site where they have a biological effect. Autocrine/paracrine regulation of endometrial growth by growth factor as a mediator is proposed for epidermal growth factor (EGF), transforming growth factor-α (TGF-α) and insulin-like growth factor-I [1–3]. We have previously shown that the gene expression of EGF increases during decidualization in the human endometrium, suggesting that production of EGF in the human endometrial stromal cells is mainly under the control of progesterone [4]. Extensive evidence exists that stromally derived mediators can promote epithelial proliferation and differentiation in steroid hormone responsive tissues [5].

Keratinocyte growth factor (KGF), also called FGF-7, was originally purified from human embryonic fibroblasts and detected in some stroma cell lines [6, 7]. Although other growth factors such as EGF and TGF-α induce cell proliferation and cytodifferentiation not only in epithelial cells but also in fibroblasts, KGF secreted from mesenchymal fibroblasts plays a very unique role, acting on only epithelial cells in a paracrine fashion so that the assessment of the involvement of KGF in the regulation of endometrial function, especially epithelial-mesenchymal interaction is important for a better understanding of endometrial physiology.

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Although Koji *et al.* [5] reported the KGF mRNA in stromal cells of the monkey endometrium, there are few reports on the gene expression of KGF and its change during the menstrual cycle or during pregnancy in the human endometrium. In addition, little is known about the function of KGF in the human endometrium. In this article, by reverse transcriptase-polymerase chain reaction (RT-PCR) and Northern blot hybridization, we investigated the KGF mRNA in the human endometrium and its quantitative change in different environments of sex steroid hormone such as the proliferative phase, secretory phase, pseudopregnancy and early pregnancy. Furthermore, we analyzed the gene expression of KGF-receptor (KGF-R) in chorionic villi which are in close contact with endometrium/decidua in the early stage of pregnancy.

Materials and Methods

Sample collection

Fresh endometrial tissues in the proliferative phase (day 5–15 in the menstrual cycle) and secretory phase (day 20–25) were collected from 8 endocrinologically normal patients with leiomyoma who underwent hysterectomy. Pseudopregnant endometria were also obtained from 3 hypermenorrheic patients with leiomyoma who underwent hysterectomy after treatment with 0.05 mg mestranol and 5 mg norethisterone for several weeks to stop genital bleeding before the operation. Decidua and chorionic villi of 5–9 weeks gestation were obtained from 4 artificially induced abortions of normal pregnancy, taking special care to avoid contamination of any tissue by others. All patients consented to this study. These specimens were immediately frozen in liquid nitrogen and stored at −80 °C until processing.

RNA extraction and RT-PCR

Total RNA was extracted from these specimens by means of RNeasy™ (Qiagen, CA) according to the manufacturer's protocol. Each RNA was reverse transcribed into cDNAs by AMV reverse transcriptase (TAKARA, Japan). One pair of oligonucleotides primer, corresponding to the human KGF mRNA, was synthesized in a DNA synthesizer (Applied Biosystems, Japan): nucleotide number 449–470 (5′-CACAAATGGATACTGACA-TGGA-3′) for the upstream side and nucleotide number 623–644 (5′-TCACTCTTATATCCCCTC-CTTC-3′) for the downstream side [6]. Similarly, the primers for the human KGF-R mRNA were synthesized: 5′-CTCAAGGTTCTCAAGCACTC-3′ for the upstream side and 5′-TTCCAGGCGCTT-GCTGTTTT-3′ for the downstream side, with a 4.0 kbp-long intron on genomic sequence between two primers [8, 9].

Using these primers, Taq polymerase (TAKARA, Japan), and PCR buffer (provided by the manufacturer), each cDNAs was amplified by PCR reaction for 35 cycles (1 cycle=94 °C for 1 min, 54 °C for 1 min, and 72 °C for 2 min). Then the PCR products were electrophorased in 3:1 Seakem ME: Nusieve agarose gel (TAKARA, Japan) and analyzed through visualization with ethidium bromide staining. Using ABI 373A DNA sequencer (Applied Biosystems, Japan), we confirmed that the nucleotides sequencing of these amplified DNAs completely matched to 196 bps of KGF mRNA and 172 bps of KGF-R mRNA after eluting the DNAs from agarose gel.

Northern blot hybridization

After transferring each electrophorased total RNA samples from denatured agarose gel to nylon membrane Hybond™-N+ (Amersham International, England), hybridization was performed with Rapid hybridization buffer™ (Amersham International, England) according to the manufacturer's recommendation. Eluted and sequenced 50 ng cDNA was utilized on each hybridization after labeling of random priming with the rediprime™ DNA labeling system (Amersham International, England) with [α-^{32}P]dCTP. These radioactive levels of the hybridized probe were counted in a BAS2000 Bioimage analyzer (Fuji film, Japan), and exposed on X-ray film in order to define the KGF or KGF-R mRNA length.

Results

RT-PCR

As shown representatively in Fig. 1, by 35 cycle amplification of RT-PCR (denaturation for 1 min at 94 °C, annealing for 1 min at 54 °C, and extension for 2 min at 72 °C per 1 cycle), we detected an anticipated 196 bp-long DNA band for human KGF in all samples examined, demonstrating that KGF mRNA exists in the human endometria from the proliferative phase, secretory phase, pseudopregnancy and early pregnancy. As illustrated representatively in Fig. 2, an anticipated 172 bp-long DNA band for human KGF-R was demonstrated in two human chorionic villi samples from 6 (lane A) and 7 (lane B) weeks gestation. Similar results were obtained in other RT-PCR for KGF and KGF-R using different samples of endometrium/decidua or chorionic villi.

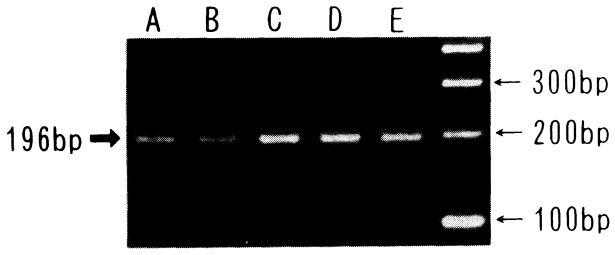

Fig. 1. Amplification of KGF mRNA by RT-PCR in human endometrium. 1 μg of total RNAs from endometria in secretory phase (lane A), proliferative phase (lane B), decidua of 7 weeks gestation (lane C), decidua of 9 weeks gestation (lane D) and pseudopregnancy (lane E) were reverse transcribed and amplified for 35 cycles by PCR with specific human KGF primers. The PCR products were separated by agarose gel electrophoresis and visualized with ethidium bromide. The marker lane contains a 100-bp DNA ladder. The KGF transcripts of 196 bp, as predicted by the primers, were detected in all samples examined.

Northern blot hybridization

In Northern blot analysis, 2.4 kb and about 5.0 kb KGF transcripts were detected on each sample (Fig. 3-A). As illustrated representatively in Fig. 3-A, the density of the 2.4 kb KGF mRNA in secretory phase endometrium was 10-fold greater than that in proliferative phase endometrium. Similarly, decidua of 7 weeks and 9 weeks gestation and pseudopregnant endometrium treated with 0.05 mg mestranol and 5 mg norethisterone for 4 weeks had 5–10-fold and 4-fold greater KGF mRNA, respectively, than proliferative phase endometrium. These data are quantitatively illustrated in Fig. 3-B. The densities of the less abundant mRNA of about 5.0 kb were almost the same in all these samples. Similar results were obtained in other Northern blottings with different samples. KGF-R mRNA was not detected in Northern blot hybridization in chorionic villi from early pregnancy.

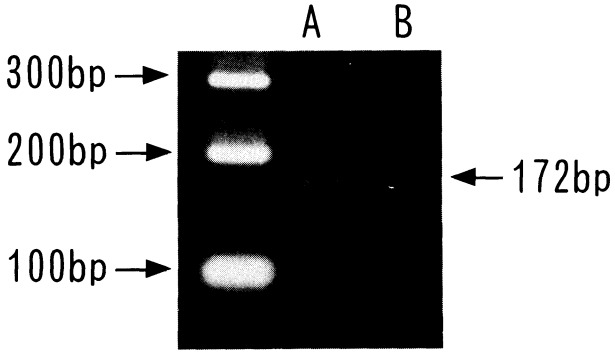

Fig. 2. Amplification of KGF-R mRNA by RT-PCR in human chorionic villi. 1 μg of total RNAs from chorion from 6 (lane A) and 7 (lane B) weeks gestation were reverse transcribed and amplified for 35 cycles by PCR with specific human KGF-R primers. The PCR products were separated by agarose gel electrophoresis and visualized with ethidium bromide. The marker lane contains a 100-bp DNA ladder. The KGF-R transcripts of 172 bp, as predicted by the primers, were detected in both samples examined.

Discussion

The uterus is comprised of heterogeneous cell populations that respond uniquely to estrogen and progesterone; the proliferation of luminal and glandular epithelia occurs in response to estrogen stimulation, whereas stromal cell proliferation is dependent upon progesterone and estrogen [10]. Sex steroid hormones cause transformation of stromal cells into decidual cells and their further proliferation. In our current experiment, we demonstrated the KGF mRNA in the human

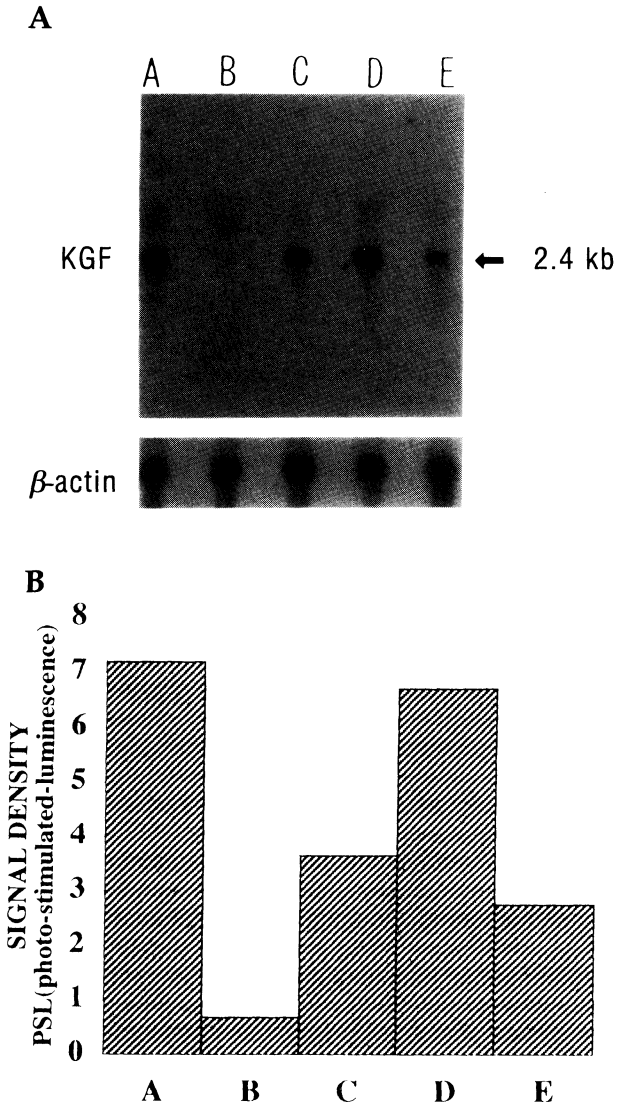

Fig. 3. Northern blot analysis of KGF mRNA in human endometria (lane A: secretory phase, lane B: proliferative phase, lane C: decidua of 7 weeks gestation, lane D: decidua of 9 weeks gestation, lane E: pseudopregnancy) with ^{32}P-labeled KGF cDNA. The mRNA of 2.4 kb and about 5 kb were detected in each sample. The dominant mRNA of 2.4 kb in secretory phase endometrium was 10-fold greater than that in proliferative phase endometrium. Similarly, decidua and pseudopregnant endometrium had 5–10-fold and 4-fold greater KGF mRNA, respectively, than proliferative phase endometrium. The densities of the less abundant 5 kb mRNA were almost the same in all these samples. The lower part of the figure shows the signal in these blots with a cDNA probe for β-actin after washing out the KGF probe, confirming equivalent quantity of RNA in all lanes. Figure 3-B shows the quantitative change in signal density of the Northern blot.

endometrium throughout the menstrual cycle because we found the KGF mRNA by RT-PCR and the dominant 2.4 kb and less abundant 5.0 kb KGF transcripts in Northern blot in this tissue. The KGF mRNA might be alternatively spliced in the human endometrium because KGF gene is reported to be transcribed as two alternative mRNAs [6]. While Siegfried *et al.* [11] reported quantitative change in KGF mRNA during the menstrual cycle caused by RT-PCR, we clarified it in the dominant KGF mRNA level by Northern blot hybridization in the human endometrium in different environments of sex steroid hormone. Our Northern blot hybridization revealed that the KGF mRNA was much more abundant in the luteal phase endometrium, pseudopregnant endometrium and decidua than in the proliferative phase endometrium, suggesting that KGF gene expression is mainly regulated by progesterone in human endometrium. Koji *et al.* [5] reported that the KGF mRNA levels were greatly increased in the endometrium, specifically during the luteal phase in naturally cycling animals or after progesterone administration to estrogen-primed spayed animals. The increase in the KGF mRNA in the luteal phase in monkeys is in agreement with our finding in man. In human endometrium, Pekonen *et al.* [12] reported KGF mRNA and KGF-R mRNA expressions in normal endometrium, myometrium, as well as in myoma and endometrial adenocarcinoma cell lines. Although Pekonen *et al.* [12] reported the presence of mRNA for KGF throughout the menstrual cycle, they lacked a detailed quantification of KGF mRNA such as a possible fluctuation in its abundance or in relation to altered ovarian steroid hormone levels. As for the localization of KGF mRNA, *in situ* hybridization revealed that a progesterone-dependent increase in KGF mRNA expression was strongest in the stromal cells in the monkey [5]. Our results showing that decidualized endometrium contained a higher level of KGF mRNA than proliferative phase endometrium indicate that KGF is produced in stromal cells and its production increases during a decidualization process under the influence of progesterone in man as well. These findings, and ours, indicate a possible role for KGF as a paracrine factor that mediates the action of progesterone.

KGF is expressed in human tissues such as kidney, gastrointestinal cells as well as in stromal

fibroblast cell lines from neonatal foreskin, adult skin and embryonic lung [6, 7]. In the rat prostate, KGF in stromal cells, which is regulated by androgen, causes stimulation of epithelial cell proliferation [13]. Pekonen *et al.* [12] reported a predominant location of the KGF-R expression in the epithelial cells with its low expression in the stromal cell population, suggesting the specificity of KGF for epithelial cell proliferation. In our current study, we did not examine the biological action of KGF in the human endometrium by separating the mesenchymal/stromal component from the epithelial cell populations.

Because endometrial epithelial cells in decidual tissue are atrophic during early pregnancy, it is unlikely that KGF produced in stromal/decidual cells exerts its cell proliferative effect on the epithelial component. We therefore assume that the epithelial component of chorionic villi, which are in close contact with decidual cells during pregnancy, is a possible target of KGF action. In our current study, we first demonstrated the gene expression of KGF-R in the human chorionic villi. This evidence indicates that KGF has a biological significance in decidua-trophoblast interaction as a paracrine mediator during early pregnancy in man.

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