Effects of Keratinocyte Growth Factor on the Uterine Endometrial Epithelial Cells in Pigs

Hakhyun Ka* and Fuller W. Bazer[1]

Department of Biological Resources and Technology, Yonsei University, Wonju, 220-710, Korea

ABSTRACT : Keratinocyte growth factor (KGF) functions in epithelial growth and differentiation in many tissues and organs. KGF is expressed in the uterine endometrial epithelial cells during the estrous cycle and pregnancy in pigs, and receptors for KGF (KGFR) are expressed by conceptus trophectoderm and endometrial epithelia. KGF has been shown to stimulate the proliferation and differentiation of conceptus trophectoderm. However, the role of KGF on the endometrial epithelial cells has not been determined. Therefore, this study determined the effect of KGF on proliferation and differentiation of endometrial epithelial cells *in vitro* and *in vivo* using an immortalized porcine luminal epithelial (pLE) cell line and KGF infusion into the uterine lumen of pigs between Days 9 and 12 of estrous cycle. Results showed that KGF did not stimulate proliferation of uterine endometrial epithelial cells *in vitro* and *in vivo* determined by the [^{3}H]thymidine incorporation assay and the proliferating cell nuclear antigen staining, respectively. Effects of KGF on expression of several markers for epithelial cell differentiation, including integrin receptor subunits α4, α5 and β1, plasmin/trypsin inhibitor, uteroferrin and retinol-binding protein were determined by RT-PCR, Northern and slot blot analyses, and immunohistochemisty, and KGF did not affect epithelial cell differentiation *in vitro* and *in vivo*. These results show that KGF does not induce epithelial cell proliferation and differentiation, suggesting that KGF produced by endometrial epithelial cells acts on conceptus trophectoderm in a paracrine manner rather than on endometrial epithelial cells in an autocrine manner. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 12 : 1708-1714)***

Key Words : Keratinocyte Growth Factor, Porcine, Uterus, Endometrium, Epithelia

INTRODUCTION

The uterus provides an environment that supports embryonic development by synthesizing and secreting a variety of products, termed histotroph (Roberts and Bazer, 1988). Uterine secretory activity is regulated by the ovarian steroids, estrogen and progesterone, and also influenced by local growth factors and epithelial-mesenchymal interactions (Cooke et al., 1998). We have shown that KGF is a component of porcine histotroph (Ka et al., 2000; 2001). KGF is expressed in the uterine endometrial epithelia in the pig. In particular, KGF expression was higher on Day 12 of pregnancy, which coincides with maternal recognition of pregnancy in the pig, than on Day 12 of the estrous cycle (Ka et al., 2000). Expression of KGF in the porcine uterine endometrium is up-regulated by estrogens (E2), which is the maternal recognition signal in pigs (Bazer et al., 1989), and KGF functions in proliferation and differentiation of conceptus trophectoderm (Ka et al., 2001). These findings suggested that in the pig, which is the only species possessing a true epitheliochorial type of placentation, KGF plays a crucial role in epithelial-epithelial interactions between conceptus and uterus during early pregnancy. In addition to E2 of conceptus origin, other factors may induce KGF expression in the endometrial epithelial cells, because KGF gene expression is up-regulated by various factors, including steroid hormones, cytokines and other growth factors (Rubin et al., 1995).

KGF is known to function in proliferation, differentiation, migration, anti-apoptosis and angiogenesis (Werner, 1998; Szebenyi and Fallon, 1999). In the previous studies (Ka et al., 2000; 2001), we have shown that KGFR mRNA expression is localized in uterine endometrial epithelia and conceptus trophectoderm, and that KGF functions in conceptus trophectoderm proliferation and differentiation. Since uterine endometrial epithelial cells do not undergo significant proliferation during early pregnancy (Ka et al., 2001), it is not expected that KGF affects endometrial epithelial cell proliferation, but it may affect uterine epithelial cell differentiation. During early pregnancy, uterine endometrial epithelial cells undergo differentiation to increase uterine receptivity to implanting blastocysts (Burghardt et al., 1998). The uterine endometrium synthesizes and secretes a variety of proteins to nourish the early conceptus during this period (Roberts and Bazer, 1988). The increased secretory activity in the porcine uterine epithelial cells during early pregnancy includes secretion of uteroferrin, retinol-binding protein and plasmin/trypsin inhibitor (Roberts and Bazer, 1988). The expression of cell surface molecules including Muc-1, integrin receptor subunits such as α4, α5 and β1, and extracellular matrix components, changes during the

* Corresponding Author: Hakhyun Ka. Tel: +82-33-760-2369, Fax: +82-33-760-2186, E-mail: hka@dragon.yonsei.ac.kr

[1] Center for Animal Biotechnology and Genomics, Department of Animal Science, Texas A&M University, College Station, TX 77843-2471, USA.

Received May 3, 2005; Accepted July 28, 2005

blastocyst adhesion and implantation process (Bowen et al., 1996; Burghardt et al., 1998). In pigs, expression of integrin receptor subunits α4, α5 and β1 on the endometrial epithelial cells increases during early pregnancy, while Muc-1 expression decreases. Based on these findings in epithelial cells during early pregnancy, it is hypothesized that KGF may contribute to differentiation of uterine endometrial epithelial cells. In the present study, we determined the effect of KGF on proliferation and differentiation of porcine uterine luminal epithelial cells *in vitro* and *in vivo*.

MATERIALS AND METHODS

Animals and tissue collection

Experimental and surgical procedures involving animals were approved by the Institutional Agricultural Animal Care and Use Committee of Texas A and M University (Animal Use Protocol 2000-120). To examine the effects of KGF on proliferation and differentiation of endometrial epithelium *in vivo*, sexually mature gilts (n = 5) were catheterized on day 5 of the estrous cycle, with the catheter about 1 cm below the tubo-uterine junction of each uterine horn as described previously (Harney and Bazer, 1989). Each uterine horn was double ligated just above the uterine body so that one uterine horn could be treated and the other uterine horn could serve as the control. Intrauterine injections of recombinant KGF (rKGF; 100 ng/treatment uterine horn) or saline (1 ml/control uterine horn) were given twice a day from Days 9 to 12 after onset of estrus and the gilts were hysterectomized on Day 13. rKGF was provided by Dr. Wallace L. McKeehan (Texas A & M University). Tissue samples were taken at approximately 20 cm below the utero-tubal junction of each uterine horn. Tissue samples for paraffin sections and RNA extraction were prepared as described previously (Ka et al., 2000).

Porcine luminal epithelial cells

Porcine luminal epithelial (pLE) cell line was developed by immortalizing primary cultures by transduction with the replication-defective retrovirus (SV40) vector PLXSN-16E6E7 expressing E6/E7 proteins of human papilloma virus (HPV) type 16 as described previously (Wang et al., 2000). To assess the effect of KGF on differentiation, pLE cells were treated with rKGF (0, 1, 10 or 100 ng/ml) in serum-free DMEM/F-12 for 24 h at 37°C, and RNA and protein were extracted as described previously (Ka et al., 2001).

[^{3}H]Thymidine incorporation assay

The effect of KGF on proliferation of pLE cells was determined as described previously (Ka et al., 2001). Briefly, pLE cells were plated at 20,000 cells/cm^2 in DMEM/F-12 containing 5% FBS, then serum-starved for 24 h in serum-free DMEM/F-12, containing 2 mM glutamine and 0.1% BSA. Cells were then treated with rKGF (rKGF; 0, 1, 10, or 100 ng/ml) for 24 h at 37°C in serum-free DMEM/F-12 containing 5 μCi/ml [^{3}H] thymidine. Cells were then precipitated in 10% trichloroacetic acid for 30 min on ice, and fixed in cold methanol. The fixed cells were solubilized in 0.6 ml of 0.05% trypsin/0.1% SDS for 30 min at 37°C. [^{3}H]thymidine incorporation was counted using a Beckman LS 3801 liquid scintillation counter (Beckman Instruments, Palo Alto, CA). Total DNA content was determined using Picogreen (Molecular Probes, Eugene, OR) as described by the manufacturer. Data are expressed as dpm/μg total DNA.

RT-PCR

To characterize pLE cells and to assess effects of KGF on functional differentiation of pLE cells, expression of KGFR, integrin receptor subunits, αv, α4, α5, β1, and β3, and plasmin/trypsin inhibitor (PTI) was examined by RT-PCR. Five micrograms of total RNA from pLE cells was reverse transcribed to obtain cDNAs using Superscript II reverse transcriptase (Invitrogen, Carlsbad, CA). Newly synthesized cDNA was acid-ethanol precipitated, resuspended in 20 μl water, and stored at -20°C. The cDNAs were diluted (1:10) with sterile water prior to use in PCR reactions. The cDNA templates were amplified by PCR using AmpliTaq DNA polymerase (Perkin Elmer, Foster City, CA) and specific primers as listed in Table 1. As a loading control for differences in amounts of cDNA used for each PCR reaction for integrin expression, β-actin was also amplified. PCR products were separated on 2% agarose gel and visualized by ethidium bromide staining. The identity of each amplified PCR product was verified by sequence analysis after cloning into pCRII vector (Invitrogen).

Northern and slot blot hybridization analyses

Effect of KGF infusion on expression of integrin receptor subunits, α4, α5 and β1 in uterine endometrium was determined by Northern blot hybridization analysis using 20 μg total cellular RNA from endometrium and [^{32}P]-radiolabeled antisense cRNA probes generated against a linearized 440 bp, 299 bp or 313 bp porcine integrin receptor subunits α4, α5 or β1, respectively. As a loading control for differences in amounts of RNA, ethidium bromide gel photographs of 18S rRNA was shown. Autoradiographs were prepared using Kodak X-OMAT X-ray film.

Masson's trichome staining

Effect of KGF on the uterine histoarchitectural changes

Table 1. Summary of PCR primer sequences, annealing temperature and expected product size

Prime	Sequence of forward and reverse primers (5'→3')	Annealing temp (°C)	Product size (bp)	GenBank accession number
Integrin α4	CAGATGGGATCTCGTCAACC TCTGCTGGACACCTGTATGC	59	440	L12002
Integrin α5	GAGCCTGTGGAGTACAAGTCC CCTTGCCAGAAATAGCTTCC	59	299	NM_002205
Integrin αv	CTGGTCTTCGTTTCAGTGTGC GCCTTGCTGAATGAACTTGG	59	295	NM_002210
Integrin β1	GACCTGCCTTGGTGTCTGTGC AGCAACCACACCAGCTACAAT	55	313	X07979
Integrin β3	AGATTGGAGACACGGTGAGC GTACTTGCCCGTGATCTTGC	59	392	AF170527
PTI	TTCATGGATGAGAAGGAATGC GAAATGCCATTGTGTTCACC	59	252	L14282
β-actin	CATCCTGACCCTCAAGTACCC GTGGTGGTGAAGCTGTAGCC	55	420	U39357

by KGF infusion was determined by Masson's Trichome staining. Briefly, uterine tissue sections were deparaffinized, rehydrated, and fixed in Bouin' solution (15 ml picric acid, 5 ml 40% formaldehyde and 1 ml glacial acetic acid) for 1 h at 56°C at room temperature. Tissue sections were then stained in Weigert's iron hematoxylin (1 part of solution A composed of 1% (w/v) of hematoxylin in 95% EtOH and 1 part of solution B composed of 8 ml of 29% ferric chloride and 2 ml of concentrated HCl in 190 ml water) for 5-6 min, in Biebrich scarlet-acid fuchsin solution (90 ml 1% Briebrich scarlet, 10 ml 1% acid fuchsin and 1 ml glacial acetic acid) for 5 min, in phosphomolybdic-phosphotungstic acid solution (5 g phosphomolybdic acid and 5 g phosphotunctic acid in 200 ml water) for 5-10 min, and in aniline blue solution (5 g aniline blue and 4 ml glacial acetic acid in 200 ml water) for 5 min, and then washed in 1% acetic acid for 4-5 min. Tissue sections were rinsed with water between each solution. The staining procedures were conducted in which nuclei were stained black, cytoplasm, keratin, and muscular and intercellular fibers red, and collagen and mucus blue.

Immunohistochemistry

To determine the effect of KGF on the uterine endometrial epithelial cell proliferation *in vivo*, expression of immunoreactive proliferating cell nuclear antigen (PCNA) was detected in uterine tissue cross-sections (5 μm) using monoclonal antibody to PCNA (Santa Cruz Biotechnology, Santa Cruz, CA) and a Super ABC Mouse IgG Kit (Biomeda, Foster City, CA) as described (Ka et al., 2001). Immunoreactive uteroferrin and retinol-binding protein was detected in uterine tissue cross-sections (5-7 μm) using specific antibodies and a Super ABC Rabbit Immunoglobulin G (IgG) Kit (Biomeda, Foster City, CA) to determine the effect of KGF on the uterine endometrial epithelial cell differentiation. Rabbit polyclonal antibody to porcine uteroferrin was produced by Dr. Jamie C. Laurenz (Texas A & M University), and rabbit polyclonal antibody to human retinol-binding protein was purchased from Dako (Carpinteria, CA). The final working antibody concentration was 2 μg/ml for uteroferrin and 5 μg/ml for retinol-binding protein. Negative controls were performed in which the primary antibody was substituted with the same concentration of purified normal rabbit IgG from Sigma.

Immunofluorescence

Immunofluorescence was conducted, as described previously (Johnson et al., 1999), to determine effect of KGF infusion on expression of integrin receptor subunits α 4, α5 and β1 proteins in the uterine endometrial epithelium. Frozen uterine tissue sections (10 μm) were fixed with methanol for 10 min at -20°C, air dried, blocked in 5% normal goat serum, and incubated in primary antibody overnight at 4°C. Monoclonal antibodies to rabbit anti-α4 (#AB1924), α5 (#AB1928) and β1 (#AB1952) were from Chemicon (Temecula, CA). Slides were then washed and incubated with fluorescein-labeled goat anti-rabbit fluorescence-conjugated IgG (1:200 dilution; Sigma) for 1 h at room temperature. Following rinsing, all slides were overlaid with coverslips and Prolong antifade mounting reagent (Molecular Probes, Eugene, OR). Fluorescence images of cells for each antibody were recorded using a Zeiss Axioplan 2 microscope fitted with a Hamamatsu C-5810 chilled 3-color CCD camera (Carl Zeiss, Thornwood, NY) with Adobe Photoshop 6.0 (Adobe Systems, Seattle, WA) image capture software.

Statistical analysis

Data from [^{3}H]thymidine incorporation assay were subjected to least-squares ANOVA using the general linear models (GLM) procedures of the Statistical Analysis System (SAS, Cary, NC). Data from Northern blot analysis were subjected to Student's *t*-test to determine the difference

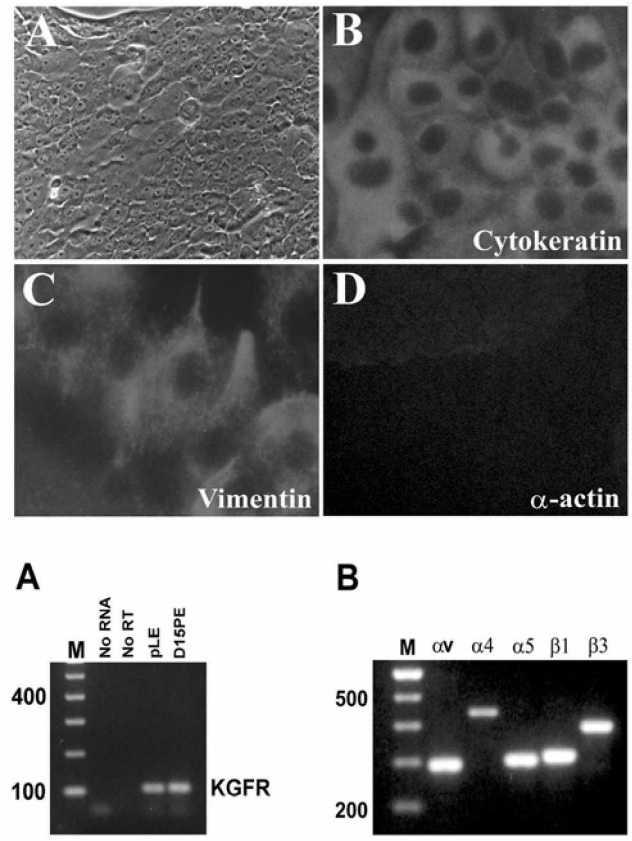

Figure 1. Characterization of pLE cells. Top: Phase contrast microscopy of pLE cells (A). Immunofluorescence analysis of expression of cytokeratin (B), vimentin (C), and the negative control for immunofluorescence (D). Bottom: RT-PCR analysis of KGFR (A) and αv, α4, α5, β1 and β3 (B) expression by pLE cells is shown when primers specific for KGFR and integrin receptors were used for PCR amplification. Note that pLE cells express KGFR and integrin receptors. D15PE, Day 15 pregnant endometrium; No RNA, no total RNA, No RT, no reverse transcriptase.

of integrin receptor subunit mRNA levels between KGF- and saline-infused uterine endometrium. The test of statistical significance was performed using the appropriate error terms according to the expectation of mean squares. Data are presented as least-squares means (LSM) with standard errors (SE). Results were considered to be significant when p was <0.05.

RESULTS

Characterization of pLE cells

The epithelial phenotype of pLE cells (Figure 1, Top) was confirmed based on a cobblestone morphology (A) and by positive cytokeratin (B), vimentin (C) and negative α-actin (D) immunostaining. KGFR, expressed in porcine trophectoderm and endometrial epithelial cells (Ka et al., 2001), and integrin receptors, αv, α4, α5, β1 and β3, expressed by porcine endometrial epithelial cells (Bowen et al., 1996), were detected in pLE cells by RT-PCR (Figure 1, Bottom).

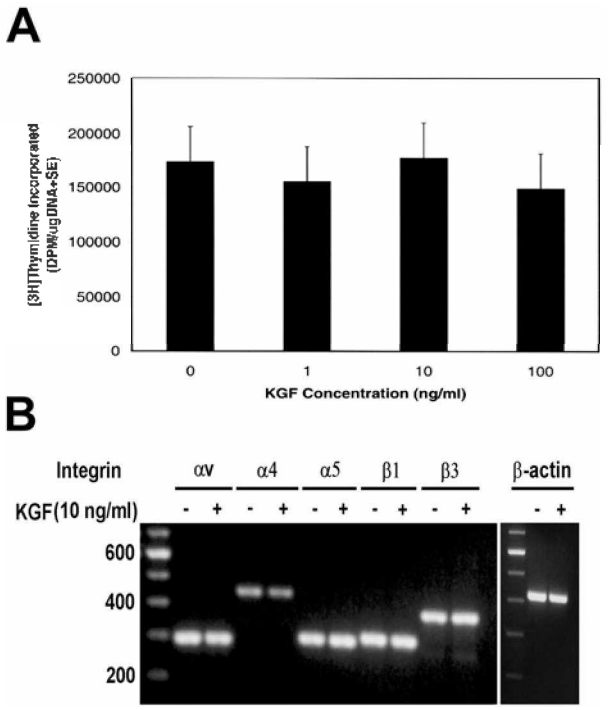

Figure 2. Effect of KGF on pLE cell proliferation (A) and differentiation (B). A: Effect of KGF on [^{3}H]thymidine incorporation in pLE cells. pLE cells were cultured in DMEM/F12 containing 5% FCS for 24 h, serum-starved for 24 h, and treated with recombinant rat KGF (0, 1, 10 or 100 ng/ml) for 24 h in serum-free DMEM/F12 containing [^{3}H]thymidine (5 µCi/ml). Nucleic acids were precipitated using trichloroacetic acid, and [^{3}H]thymidine incorporation was determined. This experiment was repeated four times and in triplicate within each replication. [^{3}H]thymidine incorporation data were subjected to least squares regression analyses of variance using DNA concentration as a covariate. rKGF did not increase [^{3}H]thymidine incorporation in pLE cells ($p>0.05$). B: Effect of KGF on expression of integrin receptors in pLE cells. pLE cells were cultures in DMEM/F12 containing 5% FCS for 24 h, serum-starved for 24 h, and treated with KGF (0 or 10 ng/ml) for 24 h in serum-free DMEM/F12. Total cellular RNA was extracted from cells and was subjected to RT-PCR analysis to detect expression of integrin receptors, αv, α4, α5, β1, and β3. As a loading control for differences in amounts of cDNA used, ethidium bromide gel photographs of β-actin amplication was used. Data indicate that expression of integrin receptors was not affected by KGF in pLE cells.

Effect of KGF on pLE cell proliferation and differentiation

DNA synthesis by pLE cells, as measured by [^{3}H]thymidine incorporation, did not change ($p>0.05$) in response to treatment with increasing doses of rKGF (Figure 2A). Expression of integrin receptor subunits, αv, α4, α5, β1 and β3, and PTI mRNAs, as markers of LE differentiation, measured by RT-PCR, was not affected by KGF treatment in pLE cells (Figure 2B; data for PTI not

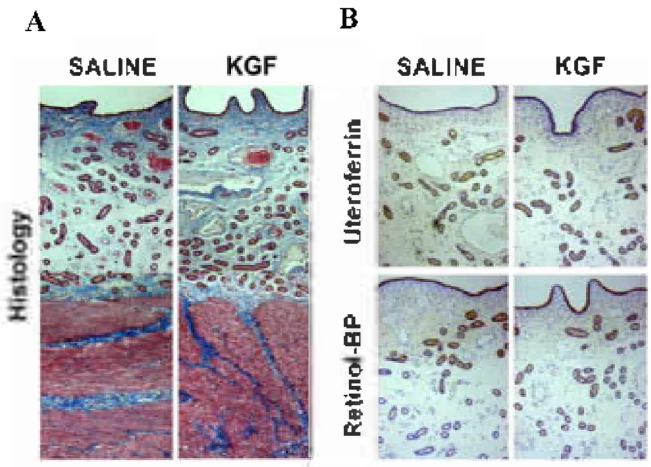

Figure 3. Effect of KGF on uterine morphology determined by Masson's Trichome staining (A) and expression of uteroferrin and retinol-binding protein (RBP) in the uterine endometrium from KGF- or saline-infused uteri determined by immunohistochemistry (B). KGF infusion did not affect any significant changes in uterine morphology and expression of uteroferrin and RBP expression.

shown).

Effect of KGF on expression of PCNA in the uterine endometrium

Immunoreactive PCNA expression was used as a marker for cell proliferation. Lack of immunoreactive PCNA protein in LE and GE, in agreement with the result shown previously (Ka et al., 2001), was observed in both saline- and KGF-infused uterine endometrium (data not shown).

Effect of KGF on the uterine morphology

In Masson's Trichome staining to determine effect of KGF on the uterine morphology, no distinctive differences in uterine histoarchitecture between saline- and KGF-infused uteri were observed (Figure 3A).

Effect of KGF on expression of uteroferrin and retinol-binding protein in the uterine endometrium

Endometrial expression of uteroferrin and retinol-binding protein was determined by immunohistochemistry. No distinctive effect of KGF on expression of these proteins was observed (Figure 3B).

Effect of KGF on expression of integrin receptors in the uterine endometrium

Expression of integrin receptor subunit β1 mRNA (~ 4.2 kb) was detected in the uterine endometrium by Northern blot analysis, but was not noticeably different in both saline- and KGF-infused uterine endometrium (Figure 4A). Expression of integrin receptor subunits α4 and α5 mRNA was not detectable in the endometrium by Northern blot

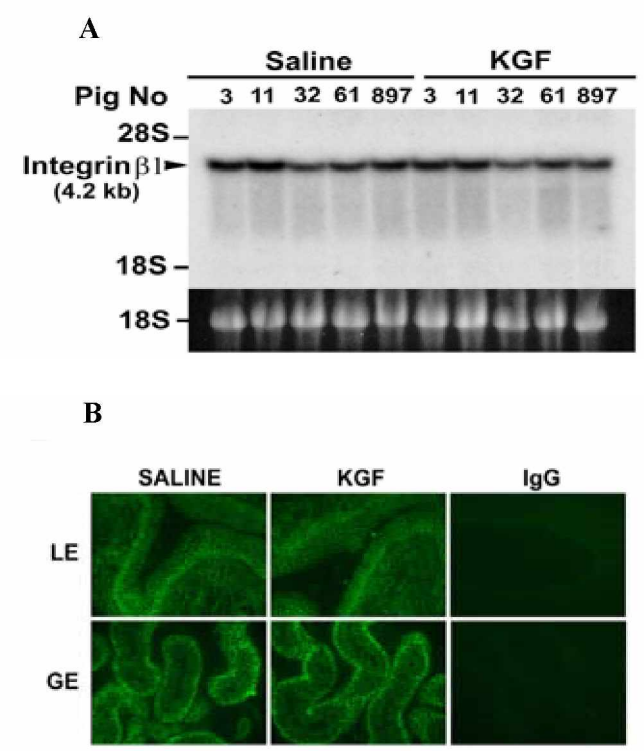

Figure 4. Effect of KGF on integrin receptor β1 expression. Northern blot analysis (A) and immunofluoroscence (B) of integrin receptor β1 expression in the uterine endometrium from KGF- or saline-infused uteri. As a loading control for differences in amounts of RNA used, ethidium bromide gel photograph of 18S rRNA was shown. KGF infusion did not increase expression of integrin receptor β1 mRNA and protein. LE: luminal epithelium, GE; glandular epithelium. Magnification = 183×.

analysis. Expression pattern of integrin receptor subunits, α4, α5, and β1 protein, determined by immunofluorescence, was in agreement with the result previously reported (Bowen et al., 1996), but no distinctive effect of KGF infusion on expression of these proteins were observed (Figure 4B; data for α4 and α5 not shown).

DISCUSSION

The uterus provides an environment for sperm transport, regulation of luteolysis for the control of cyclicity, receipt and growth of preimplantation embryos, implantation, placentation and fetal development. In pigs which form a true epitheliochorial type of placentation, uterine endometrial secretions are essential for the support of fetal development (Roberts and Bazer, 1988). Secretory products include transport proteins (Roberts and Bazer, 1988; Harney et al., 1993), enzymes (Fazleabas et al., 1983), extracellular matrix proteins (Garlow et al., 2002) and growth factors (Simmen et al., 1990; Ka et al., 2000). Among those secretory proteins KGF is produced by the uterine endometrium during pregnancy and diestrus of the estrous

cycle with a unique cell-type specific expression (Ka et al., 2000).

KGF has been shown to stimulate epithelial cell proliferation in various tissues (Werner, 1998). KGF also induces differentiation of cells by changing expression of various genes, such as uPA, aromatase, surfactant proteins-A -B and -D, syndecan-1 and Na^{-}/K^{+} ATPase in many tissues and organs (Rubin et al., 1995; Werner, 1998). The known functions of KGF in many cells and tissues and the pattern of KGF and KGFR expression in the porcine uterus (Ka et al., 2000) made us to hypothesize that KGF of endometrial origin acts on the proliferation and differentiation of conceptus trophectoderm and differentiation of uterine endometrial epithelial cells.

Results from our previous studies show that KGF increases conceptus trophectoderm cell proliferation and differentiation, confirming our working hypothesis and suggesting that KGF acts as a histotroph for conceptus development (Ka et al., 2001). In this study, results show that KGF does not induce proliferation and differentiation of endometrial epithelial cells *in vivo* and *in vitro*. Because there is very little cell proliferation detected in pig uterine endometrium *in vivo* during early pregnancy and diestrus of the estrous cycle determined by PCNA staining (Ka et al., 2001), it is not surprising that KGF does not induce endometrial epithelial cell proliferation. This finding is consistent with the result of Slayden et al. (2000) in primate uterus. Thus, it is not likely that KGF acts on cell proliferation in the porcine uterine endometrial epithelial cells. Rather, we expected KGF to induce endometrial epithelial cell differentiation, because endometrial epithelial cells express KGFR (Ka et al., 2000) and undergo differentiation during early pregnancy for implantation. In the primate uterus Slayden et al. (2000) also reported that KGF increased spiral artery growth and inhibited glandular apoptosis during the menstrual cycle, even though there was no effect of KGF on endometrial cell proliferation. In the present study, however, we could not detect any significant effect of KGF *in vitro* and *in vivo* on expression of markers of endometrial epithelial differentiation in the porcine endometrial epithelial cells.

Our results suggest is that the only target cell of KGF in the porcine uterus is the conceptus trophectoderm, not the uterine endometrial epithelium, for induction of proliferation and differentiation during early pregnancy. Thus, KGF mediates its function in cell proliferation and differentiation in a paracrine manner, but in an autocrine a manner. KGF is a well established paracrine mediator in many tissues and organs, especially in the epithelial-mesenchymal interactions (Cunha, 1994; Hom et al., 1998). Differential response of KGF in the trophectoderm and endometrial epithelial cells may result from various factors modulating KGF actions. These factors include salt concentrations, presence of heparan sulfate proteoglycan, which is a low-affinity receptor for FGFs, and composition of core protein of heparan sulfate proteoglycan (McKeehan et al., 1998; Berman et al., 1999).

There are other possibilities that KGF does not induce differentiation of endometrial epithelial cells. Firstly, in the *in vitro* study it is probable that pLE cells lose some of its original characteristics which are important for KGF-mediated function. It is not clear whether integrins are directly regulated by KGF, or whether dose and duration of KGF treatment is effective to induce epithelial cell responses, because there is no evidence to show the direct effect of KGF on these markers expression in other studies. KGF infused into the uterine lumen from Day 9 to 12 *in vivo* also did not have any effect on expression of markers, integrin receptors, uteroferrin and retinol-binding protein. In this study, KGF infused into the uterine lumen may be redundant for endometrial epithelial cell differentiation, because KGF is present in the uterine lumen on Day 12 of the estrous cycle and pregnancy (Ka et al., 2000). It is also possible that the amount of KGF was not enough to induce significant effects of KGF in the uterus, compared to the study of Slayden et al. (2000) which used 0.5 mg/ml of KGF daily for 4 days and observed the effects of KGF. Also, KGF action may not be associated with expression of markers chosen in this study for uterine endometrial epithelial cell differentiation but other unknown factors may be affected. More comprehensive study which includes a microarray technology may answer these possibilities.

In summary, KGF did not affect proliferation and expression of several markers of differentiation in uterine endometrial epithelial cells, suggesting that KGF may induce proliferation and differentiation of conceptus trophectoderm in a paracrine manner, but not in endometrial epithelial cells in the porcine uterus. However, a little further study remains to determine the detailed role of KGF on uterine endometrial epithelium in pigs.

REFERENCES

Bazer, F. W., J. L. Vallet, J. P. Harney, T. S. Gross and W. W. Thatcher. 1989. Comparative aspects of maternal recognition of pregnancy between sheep and pigs. J. Reprod. Fertil. Suppl. 37:85-89.

Berman, B., O. Ostrovsky, M. Shlissel, T. Lang, D. Regan, I. Vlodavsky, R. Ishai-Michaeli and D. Ron. 1999. Similarities and differences between the effects of heparin and glypican-1 on the bioactivity of acidic fibroblast growth factor and the keratinocyte growth factor. J. Biol. Chem. 274:36132-36138.

Bowen, J. A., F. W. Bazer and R. C. Burghardt. 1996. Spatial and temporal analyses of integrin and Muc-1 expression in porcine uterine epithelium and trophectoderm *in vivo*. Biol. Reprod. 55:1098-1106.

Burghardt, R. C., J. A. Bowen and F. W. Bazer. 1998. Endocrine

control of trophoblast-uterine epithelial cell interactions. In The Endocrinology of Pregnancy. (Ed. F. W. Bazer). Humana Press Inc., Totowa, New Jersey, pp. 199-228.

Cooke, P. S., D. L. Buchanan, T. Kurita, D. B. Lubahn and G. R. Cunha. 1998. Stromal-epithelial cell communication in the female reproductive tract. In The Endocrinology of Pregnancy. (Ed. F. W. Bazer). Humana Press Inc., Totowa, New Jersey, pp. 491-506.

Cunha, G. R. 1994. Role of mesenchymal-epithelial interactions in normal and abnormal development of the mammary gland and prostate. Cancer 74:1030-1044.

Fazleabas, A. T., R. D. Geisert, F. W. Bazer and R. M. Roberts. 1983. Relationship between release of plasminogen activator and estrogen by blastocysts and secretion of plasmin inhibitor by uterine endometrium in the pregnant pig. Biol. Reprod. 29:225-238.

Garlow, J. E., H. Ka, G. A. Johnson, R. C. Burghardt, L. A. Jaeger and F. W. Bazer. 2002. Analysis of osteopontin at the maternal-placental interface in pigs. Biol. Reprod. 66:718-25.

Harney, J. P. and F. W. Bazer. 1989. Effect of porcine conceptus secretory proteins on interestrous interval and uterine secretion of prostaglandins. Biol. Reprod. 41:277-284.

Harney, J. P., T. L. Ott, R. D. Geisert and F. W. Bazer. 1993. Retinol-binding protein gene expression in cyclic and pregnant endometrium of pigs, sheep, and cattle. Biol. Reprod. 49:1066-73.

Hom, Y. K., P. Young, A. A. Thomson and G. R. Cunha. 1998. Keratinocyte growth factor injected into female mouse neonates stimulates uterine and vaginal epithelial growth. Endocrinol. 139:3772-3779.

Johnson, G. A., R. C. Burghardt, T. E. Spencer, G. R. Newton, T. L. Ott and F. W. Bazer. 1999. Ovine osteopontin: II. Osteopontin and α_v β_3 integrin expression in the uterus and conceptus during the peri-implantation period. Biol. Reprod. 61:892-899.

Ka, H., J. A. Jaeger, G. A. Johnson, T. E. Spencer and F. W. Bazer. 2001. Keratinocyte growth factor is up-regulated by estrogen in the porcine uterine endometrium and functions in trophectoderm cell proliferation and differentiation. Endocrinol. 142:2303-2310.

Ka, H., T. E. Spencer, G. A. Johnson, F. W. Bazer. 2000. Keratinocyte growth factor: expression by endometrial epithelia of the porcine uterus. Biol. Reprod. 62:1772-1778.

McKeehan, W. L., F. Wang and M. Kan. 1998. The heparan sulfate-fibroblast growth factor family: diversity of structure and function. Prog. Nucleic Acids Res. Mol. Biol. 59:135-176.

Roberts, R. M. and F. W. Bazer. 1988. The functions of uterine secretions. J. Reprod. Fertil. 82:875-892.

Rubin, J. S., D. P. Bottaro, M. Chedid, T. Miki, D. Ron, H. G. Cheon, W. G. Taylor, E. Fortney, H. Sakata, P. W. Finch and W. J. LaRochelle. 1995. Keratinocyte growth factor. Cell Biol. International 19:399-411.

SAS Institute Inc. 1990. SAS User's Guide: Version 6. Statistical Analysis System Institute Inc., Cary, North Carolina.

Simmen, R. C., F. A. Simmen, A. Hofig, S. J. Farmer and F. W. Bazer. 1990. Hormonal regulation of insulin-like growth factor gene expression in pig uterus. Endocrinol. 127:2166-2174.

Slayden, O. D., J. S. Rubin, D. L. Lacey and R. M. Brenner. 2000. Effects of keratinocyte growth factor in the endometrium of rhesus macaques during the luteal-follicular transition. J. Clin. Endocrinol. Metab. 85:275-285.

Szebenyi, G. and J. F. Fallon. 1999. Fibroblast growth factors as multifunctional signaling factors. Int. Rev. Cytol. 185:45-106.

Wang, G., G. A. Johnson, T. E. Spencer and F. W. Bazer. 2000. Isolation, immortalization and initial characterization of uterine cell lines: an *in vitro* model system for the porcine uterus. *In vitro* Cell Dev. Biol. Anim. 36:650-656.

Werner, S. 1998. Keratinocyte growth factor: a unique player in epithelial repair processes. Cytokine Growth Factor Rev. 9:153-165.