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Thecal Cell-Granulosa Cell Interactions Involve a Positive Feedback Loop among Keratinocyte Growth Factor, Hepatocyte Growth Factor, and Kit Ligand during Ovarian Follicular Development*

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ABSTRACT

Interactions between mesenchymal-derived thecal cells and epithelial-derived granulosa cells are essential for follicular development in the ovary. These mesenchymal-epithelial cell interactions are in part mediated by keratinocyte growth factor (KGF), hepatocyte growth factor (HGF), and Kit ligand (KL). This study investigates the hypothesis that thecal cell-derived growth factors (*e.g.* KGF and HGF) regulate granulosa cell function, and granulosa cell-derived growth factors (*e.g.* KL) regulate thecal cell function. Gonadotropin regulation of this cell-cell interaction is also examined. Sensitive quantitative RT-PCR assays were used to analyze gene expression of KGF, HGF, and KL in the ovary. Thecal cell-derived KGF and HGF stimulated KL expression in bovine granulosa cells. Granulosa cell-derived KL stimulated KGF and HGF expression in bovine thecal cells. These results suggest that thecal and granulosa cells interact in a positive feedback loop mediated by KGF, HGF, and KL. Previous studies have suggested that gonadotropins (*i.e.* FSH and LH) regulate locally produced growth factor expression in the ovary. Treatment of bovine granulosa cells with FSH and hCG (a LH agonist) directly stimulated KL expression. The LH agonist hCG was also found to stimulate both KGF and HGF expression in thecal cells. The actions of gonadotropins on follicular development may in part be indirectly regulated by KL, KGF, and HGF expression. A novel positive feedback loop was identified between thecal cells and granulosa cells that is mediated by KL, KGF, and HGF. Thecal cell-derived KGF and HGF can stimulate granulosa cell-derived KL expression, and KL, in turn, can stimulate thecal cell-derived KGF and HGF expression. Combined observations support the hypothesis that mesenchymal-epithelial cell interactions between thecal and granulosa cells can play a significant role during ovarian follicular development and mediate gonadotropin actions. (*Endocrinology* **139:** 2240–2245, 1998)

MESENCHYMAL-EPITHELIAL cell interactions are present in essentially every organ and are one of the most common cell-cell interactions between different cell types. It has long been recognized that growth and differentiation of epithelial cells are directed by adjacent mesenchymal cells during embryonic development and are optimally maintained by adjacent stroma in adult tissues. The ability of epithelial cells to feedback and communicate with mesenchymal cells is equally important. Both mesenchymal cells and adjacent epithelial cells produce factors that act in a paracrine manner to regulate cellular functions. Identification and characterization of these factors that mediate mesenchymal-epithelial cell interactions are necessary to understand the mechanisms of how cells communicate in an organ. The ovarian follicle is a useful model system to study these cell-cell interactions.

Cell-cell interactions between thecal and granulosa cells are essential for follicular development in the ovary. These mesenchymal-epithelial cell interactions are in part mediated by keratinocyte growth factor (KGF), hepatocyte growth factor (HGF), and Kit ligand (KL). Mesenchymal-derived thecal cells express the KGF and HGF genes and secrete the proteins (1). KGF and HGF can regulate granulosa cell granulosa cell function and growth (1) Epithelial-derived granulosa cells express the KL gene and secrete the protein (2). KL can regulate thecal cell function and growth (2). The expression of all of these factors increases to the highest levels in large antral follicles (2–4). Therefore, the actions of KGF, HGF, and KL are postulated to be involved in folliculogenesis, particularly during later stages of follicular development. The potential interactions among these growth factors has not been investigated and is the focus of the current study.

KGF and HGF are mesenchymal-derived growth factors that act on adjacent epithelial cells in a number of tissues (1, 5–8). KGF is a member of the fibroblast growth factor family (FGF7), and HGF is the ligand for the c-*met* receptor. KL is a multipotent growth factor that is important for ovarian follicular development, germ cell migration, melanocytes, and hematopoietic cells (9–22). Although the expression and actions of KGF, HGF, and KL have been examined in many tissues, no information is available on the potential paracrine interactions of these factors among each other. In the ovary, KGF and HGF are produced by mesenchyme-derived thecal cells and regulate the growth and steroidogenesis of epithelial-derived granulosa cells (1, 3, 4). KL is expressed by granulosa cells and has effects on oocytes, thecal cells, and ovarian stromal cells (2, 15, 23–25). The hypothesis has been developed that thecal cell-derived KGF and HGF and gran-

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ulosa cell-derived KL mediate a positive feedback loop between thecal cells and granulosa cells. This novel feedback loop is proposed to be important for ovarian follicular development and hormone (*i.e.* gonadotropin) action.

Materials and Methods

Tissue isolation and serum-free cell culture

Bovine ovaries were obtained from young nonpregnant cycling heifers less than 10 min after slaughter. Ovaries were delivered fresh on ice by Golden Genes (Fresno, CA). Follicular phase developing follicles were isolated, and a mixture of follicles between 5–20 mm was used. Granulosa cells were isolated by microdissection from fresh tissue as previously described (26). Theca interna layers were then microdissected away from the follicle wall and enzymatically dispersed with 2 mg/ml collagenase (Sigma Chemical Co., St. Louis, MO) in Ca^{2+}/Mg^{2+}-free buffer. Cells were immediately plated in serum-free Ham's F-12 medium containing 0.1% BSA (Sigma) and were maintained at 37 C in a 5% CO_2 atmosphere. Thecal cells were treated with no factor (control) or with recombinant human KL (50 ng/ml; R&D Systems, Minneapolis, MN). Granulosa cells were treated with no factor (control) or with KGF (50 ng/ml; Life Technologies, Gaithersburg, MD), HGF (50 ng/ml; R&D Systems), FSH (100 ng/ml; National Pituitary Agency, Baltimore, MD), or hCG (100 ng/ml; 4010 IU/mg; Calbiochem, La Jolla, CA). Cell preparations obtained by this procedure have been characterized cytochemically to contain less than 3% contamination (*e.g.* endothelial cells).

Preparation of RNA

Thecal and granulosa cells were cultured as confluent monolayers in six-well plates for 72 h. Medium was removed, and total RNA was prepared by either of two methods. First, cultured thecal and granulosa cell total RNA was extracted using guanidium isothiocyanate, followed by centrifugation through a cesium chloride gradient (27). Alternatively, total RNA was prepared using the RNA-Stat 60 kit (Tel-Test, Friendswood, TX). In either procedure, denaturing extraction buffer was added directly to the culture plate to prevent RNA degradation. RNA was stored at −70 C until use.

Quantitative RT-PCR assays

Steady state levels of KGF, HGF, KL, and cyclophilin (*i.e.* IB15) messenger RNAs (mRNAs) were analyzed using a specific quantitative RT-PCR assay for each gene. These quantitative RT-PCR assays have previously been described in detail (2–4). The primers used in this quantitative analysis of KGF, HGF, KL, and 1B15 were: KGF, 5′-ATA CTG ACA TGG ATC CTG CCA AGT TTG CTC TAC AGA TCA TGC TTC-3′ (5′-primer; 45-mer) and 5′-TCC AAC TGC CAC GGT CCT GAT-3′ (3′-primer; 21-mer), which generated a specific 306-bp KGF PCR product; HGF, 5′-ACA GCT TTT TGC CTT CGA GCT ATC GGG GTA AAG ACC TAC AGG-3′ (5′-primer; 42-mer) and 5′-CAT CAA AGC CCT TGT CGG GAT A-3′ (3′-primer; 22-mer), which generated a specific 292-bp HGF PCR product; KL, 5′-GGA CAA GTT TTC GAA TAT TTC TGA AGG CTT GAG TAA TTA TTG-3′ (5′-primer; 42-mer) and 5′-AGG CCC CAA AAG CAA ACC CGA TCA CAA GAG-3′ (3′-primer; 30-mer), which generated a specific 452-bp KL PCR product [this soluble form of KL (*i.e.* KL1) is the primary form expressed by bovine granulosa cells (2)]; and 1B15, 5′-ACA CGC CAT AAT GGC ACT GGT GGC AAG TCC ATC-3′ (5′-primer; 33-mer) and 5′-ATT TGC CAT GGA CAA GAT GCC AGG ACC TGT ATG-3′ (3′-primer; 33-mer), which generated a specific 105-bp product from all cell types, demonstrating the integrity of the RNA samples. Before RT, tubes containing total RNA and specific 3′-primers were heated to 65 C for 10 min to facilitate denaturing and were cooled to room temperature to facilitate annealing. Total RNA (1 μg) was reverse transcribed for 1 h at 37 C using the following conditions: 1 μg total RNA, 1 μM specific 3′-primers of interest (up to four different primers, including 1B15), 0.1 mM deoxy (d)-NTPs, 10 mM dithiothreitol, 40 U ribonuclease inhibitor (Promega, Madison, WI), and 200 U Moloney murine leukemia virus reverse transcriptase (Life Technologies, Gaithersburg, MD) in 40 μl RT buffer (50 mM Tris-HCl, pH 8.3; 75 mM KCl; and 3 mM $MgCl_2$). After 1 h, samples were heated to 95 C for 5 min to inactivate the reverse transcriptase enzyme. Samples were immediately diluted 2.5-fold, and carrier DNA (Bluescript plasmid, Stratagene) was added to a final concentration of 10 ng/μl. This concentration of Bluescript carrier DNA (10 ng/μl) was included in all subsequent dilutions of samples and standards. Immediately before amplification, each unknown sample was further diluted 1:10 to improve the fidelity of the PCR reaction. Plasmid DNAs (*i.e.* Bluescript) containing bovine KGF, HGF, KL, or 1B15 subclones were used to generate standard curves from 1 attogram/μl (10^{-15} g/μl) to 10 pg/μl (10×10^{-9} g/μl), each containing 10 ng/μl Bluescript carrier DNA. Identical 10-μl aliquots of each sample and standard were pipetted in duplicate into a 96-well reaction plate (Marsh Biomedical Products, Rochester, NY) and sealed with adhesive film (Marsh Biomedical Products) for PCR amplification. By this design it was possible to simultaneously assay 5 known standard concentrations and 40 unknown samples for each gene. Amplification was performed in a Perkin-Elmer 9600 (Perkin-Elmer, Norwalk, CT) equipped with a heated lid using the following conditions: 0.4 μM of each primer, 16 μM dNTPs, and 1.25 U AmpliTaq polymerase in 50 μl GeneAmp PCR buffer (containing 1.5 mM $MgCl_2$; Perkin-Elmer). Each PCR amplification consisted of an initial denaturing reaction (5 min, 95 C); 25–31 cycles of denaturing (30 sec, 95 C), annealing (1 min, 60 C), and elongation (2 min, 72 C) reactions; and a final elongation reaction (10 min, 72 C). At least 0.25 μCi ^{32}P-labeled dCTP (Redivue, Amersham Life Sciences, Arlington Heights, IL) was included in each sample during amplification for detection purposes. Specific PCR products were quantitated by electrophoresing all samples on 4–5% polyacrylamide gels, simultaneously exposing the gels to a phosphor screen for 8–24 h, followed by quantitating the specific bands on a Storm PhosphorImager (Molecular Dynamics, Sunnyvale, CA). Each gene was assayed in separate PCR reactions from the same RT samples. Equivalent steady state mRNA levels for each gene were determined by comparing each sample to the appropriate standard curve. All KGF, HGF, and KL data were normalized for 1B15.

The optimal cycle number for amplification was determined for each assay to achieve maximum sensitivity while maintaining linearity (*i.e.* logarithmic phase of PCR reactions). KGF quantitative PCR products were amplified for 28 cycles, HGF and KL PCR products were amplified for 31 cycles, and 1B15 PCR products were amplified for 25 cycles. The sensitivity of each quantitative PCR assay was below 1 fg, which corresponds to less than 125 fg target mRNA/μg total RNA. For each assay, all samples were simultaneously measured in duplicate, resulting in intraassay variabilities of 11.3% (KGF), 13.6% (HGF), 8.9% (KL), and 6.5% (1B15).

Statistical analysis

All data were analyzed by a JMP 3.1 statistical analysis program (SAS Institute, Cary, NC). Effects of growth factors or gonadotropins on KGF, HGF, and KL mRNA levels were analyzed by one-way ANOVA. Observed significance probabilities of 0.05 ($P > F$) or less were considered evidence that an ANOVA model fits the data. Significant differences between treated cells and control (untreated) cells were determined using Dunnett's test, which guards against the high α (type I) error rate across the hypothesis tests (28). This multiple comparison test is recommended for multiple comparisons with controls (Dunnett's) (29, 30).

Results

Both thecal and granulosa cells were isolated from freshly prepared bovine ovarian follicular phase follicles of mixed sizes. Cells were plated under serum-free conditions and cultured for 72 h. A time course demonstrated that optimal stimulation was obtained at 72 h (data not shown). Quantitative RT-PCR assays were used to examine the gene expression of KL in granulosa cells as well as KGF and HGF in thecal cells. These quantitative RT-PCR assays for KL, KGF, and HGF are sensitive (<1 fg) and have a broad linear range (0.1–1000 fg), as previously described (2–4). These quantitative PCR assays have previously been validated, as described in *Materials and Methods*, with linear and parallel displacement curves and normalization with a constitutively

expressed gene cyclophilin (data not shown). As thecal cells express the KGF and HGF genes and secrete the proteins that can regulate granulosa cell growth and steroidogenesis, the effects of KGF and HGF on granulosa cell KL gene expression were examined. In addition, the regulation of KL gene expression by gonadotropins (*e.g.* FSH and LH) was examined. Finally, as granulosa cell-derived KL has been shown to regulate thecal cell growth and differentiation, the ability of KL to feedback on thecal cells to regulate KGF and HGF gene expression was also examined.

Steady state levels of KL mRNA in granulosa cells were stimulated by treatment with KGF (Fig. 1). Similarly, levels of granulosa cell KL mRNA were also stimulated by HGF (Fig. 1). Similar observations were made with KL concentrations of 25 and 50 ng/ml (data not shown). These results demonstrate that KGF and HGF regulate the gene expression of granulosa-derived growth factors such as KL. Therefore, KGF and HGF can influence granulosa cell growth (1) and growth factor (*i.e.* KL) expression.

Both cAMP analogs and gonadotropins have been shown to regulate KL gene expression in mouse and human granulosa cells. However, conflicting results have been observed in different studies (15, 31). In this study, the effects of gonadotropins (*e.g.* FSH and LH) on bovine granulosa cell KL expression were examined. Dose-response curves with the hormones indicated that optimal concentrations were used in the current study (data not shown). Both FSH and hCG (a LH agonist) stimulated KL mRNA levels in bovine granulosa cells (Fig. 2). The combined effects of FSH and hCG on KL gene expression were not synergistic, suggesting that KL mRNA levels were maximally stimulated in granulosa cells.

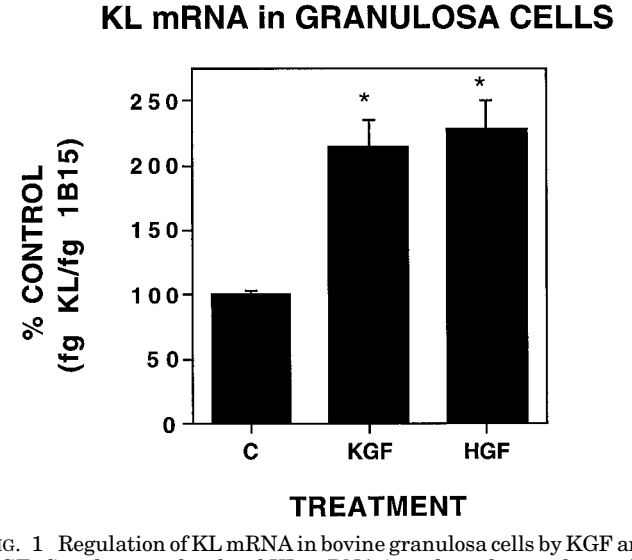

FIG. 1 Regulation of KL mRNA in bovine granulosa cells by KGF and HGF. Steady state levels of KL mRNA in cultured granulosa cells were determined using quantitative RT-PCR. Granulosa cells were placed in serum-free culture and treated with no factor (control) or with 50 ng/ml KGF or HGF. After 72 h in culture, cells were harvested, and total RNA was prepared. Levels of KL mRNA were determined and normalized to levels of cyclophilin (1B15) mRNA (femtograms of KL mRNA per fg 1B15 mRNA). Data are presented as the mean percentage of the control value ± SEM of duplicate determinations from six different sets of granulosa cell RNA. An ANOVA was performed, and significant differences from the control value were determined using Dunnett's test. *, $P < 0.05$ *vs.* control.

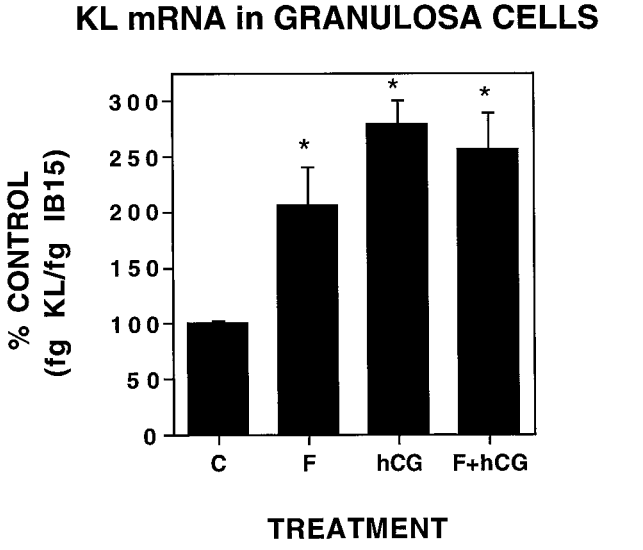

FIG. 2. Gonadotropin regulation of KL mRNA in bovine granulosa cells. Steady state levels of KL mRNA in cultured granulosa cells were determined using quantitative RT-PCR. Granulosa cells were placed in serum-free culture and treated with no factor (control C) or with 100 ng/ml FSH (F), 100 ng/ml hCG, or 100 ng/ml each of FSH and hCG (F+hCG). After 72 h in culture, cells were harvested, and total RNA was prepared. Levels of KL mRNA were determined and normalized to levels of cyclophilin (1B15) mRNA (femtograms of KL mRNA per fg 1B15 mRNA). Data are presented as the mean percentage of the control value ± SEM of duplicate determinations from six different sets of thecal cell RNA. An ANOVA was performed, and significant differences from the control value were determined using Dunnett's test. *, $P < 0.05$ *vs.* control.

The level of stimulation obtained with KGF and HGF was similar to that obtained with gonadotropins (Figs. 1 and 2). The effects of gonadotropins on bovine granulosa cell KL expression are consistent with the effects of $(Bu)_2$cAMP on mouse granulosa cells (15). These results suggest that granulosa cell-derived KL in part mediates the stimulatory actions of gonadotropins on folliculogenesis.

The ability of granulosa cell-derived KL to feedback on thecal cells was examined by analyzing KGF and HGF mRNA levels in thecal cells. Steady state levels of both KGF and HGF mRNAs were stimulated by treatment with KL (Fig. 3). Similar results were obtained with 25 and 50 ng/ml concentrations of these growth factors (data not shown). The ability of KL to stimulate KGF and HGF expression in thecal cells suggests that granulosa cells feed back on thecal cells to promote follicular growth. The ability of gonadotropin (*i.e.* hCG) to stimulate KGF and HGF mRNA levels is shown in Fig. 4. The LH agonist hCG stimulated both KGF and HGF mRNA levels in a manner similar to that of KL (Figs. 3 and 4). The ability of these growth factors to act in a paracrine manner to stimulate thecal and granulosa cell gene expression establishes a novel feedback loop that may be important for folliculogenesis (Fig. 5). The ability of gonadotropins to regulate the expression of these growth factors also suggests that the hormonal regulation of follicular development may in part be mediated by the feedback loop involving these growth factors.

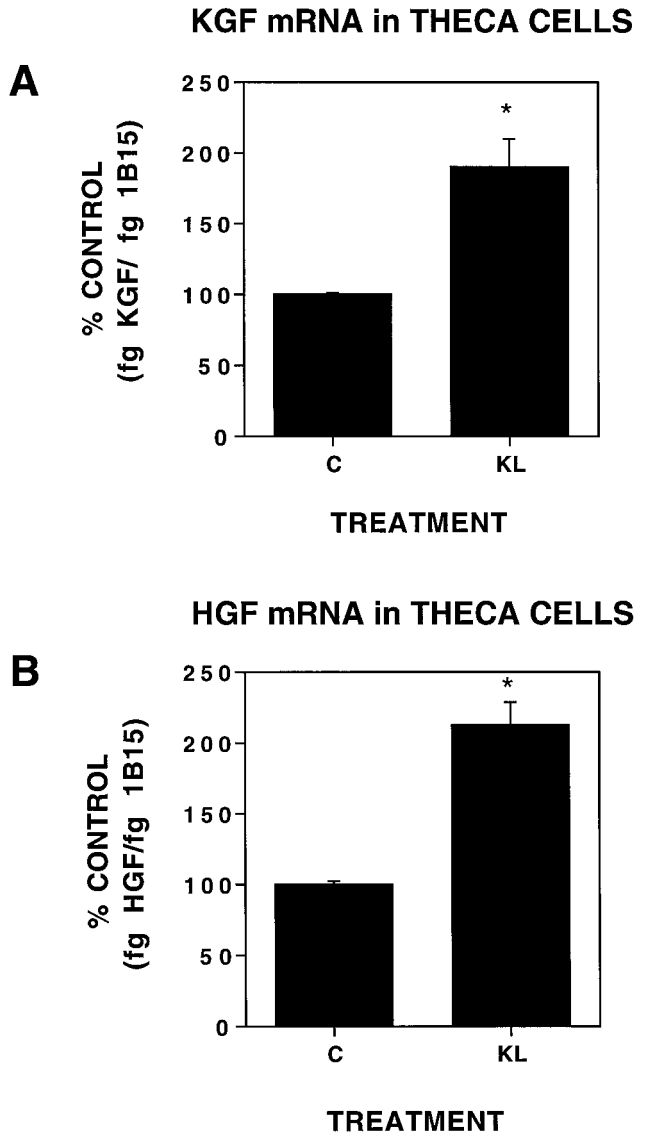

FIG. 3. Regulation of KGF (A) and HGF (B) mRNA levels in bovine thecal cells by KL. Steady state levels of KGF and HGF mRNA in cultured thecal cells were determined using quantitative RT-PCR. Thecal cells were placed in serum-free culture and treated with no factor [control (C)] or 50 ng/ml KL. After 72 h in culture, cells were harvested, and total RNA was prepared. Levels of KGF and HGF mRNA were determined and normalized to levels of cyclophilin (1B15) mRNA (femtograms of KGF or HGF mRNA per fg 1B15 mRNA). Data are presented as the mean percentage of the control value ± SEM of duplicate determinations from six different sets of granulosa cell RNA. An ANOVA was performed, and significant differences from the control value were determined using Dunnett's test. *, $P < 0.05$ *vs.* control.

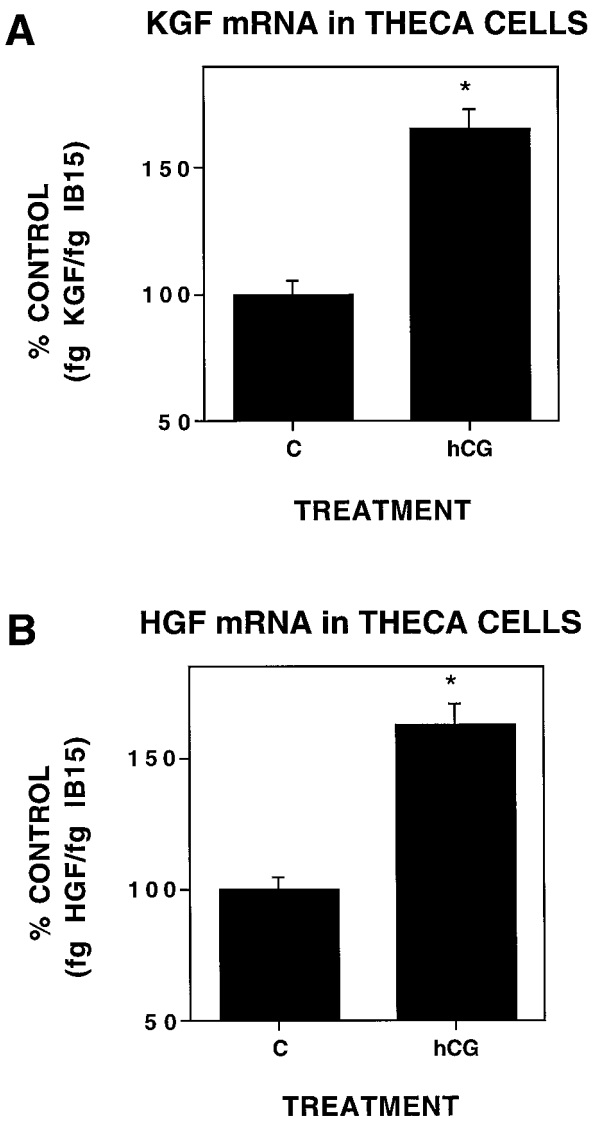

FIG. 4. Gonadotropin regulation of KGF and HGF mRNA in bovine granulosa cells. Steady state levels of KGF and HGF mRNA in cultured granulosa cells were determined using quantitative RT-PCR. Granulosa cells were placed in serum-free culture and treated with no factor [controls (C)] or 100 ng/ml hCG. After 72 h in culture, cells were harvested, and total RNA was prepared. Levels of KGF and HGF mRNA were determined and normalized to levels of cyclophilin (1B15) mRNA (femtograms of KL mRNA per fg 1B15 mRNA). Data are presented as the mean percentage of the control value ± SEM of duplicate determinations from six different sets of thecal cell RNA. An ANOVA was performed, and significant differences from the control value were determined using Dunnett's test. *, $P < 0.05$ *vs.* control.

Discussion

It has long been recognized that mesenchymal cells control epithelial cell functions through the production of inducer proteins that act on the adjacent epithelium. The ability of epithelial cells to influence mesenchymal cells is also important. This study expands the hypothesis and extends previous observations (1–4) that thecal cell-derived growth factors (*e.g.* KGF and HGF) regulate granulosa cell functions, whereas granulosa cell-derived growth factors (*e.g.* KL) regulate thecal cell functions. A novel positive feedback loop has been identified between thecal cells and granulosa cells that is mediated by KGF, HGF, and KL. Understanding the regulation and actions of KGF, HGF, and KL in the ovary provides insight into the mesenchymal-epithelial cell interactions that influence follicular development.

During follicular development, mesenchymal-derived thecal cells produce a number of growth factors that include KGF and HGF. Both KGF and HGF are mesenchymal-derived growth factors that act on adjacent epithelial cells in

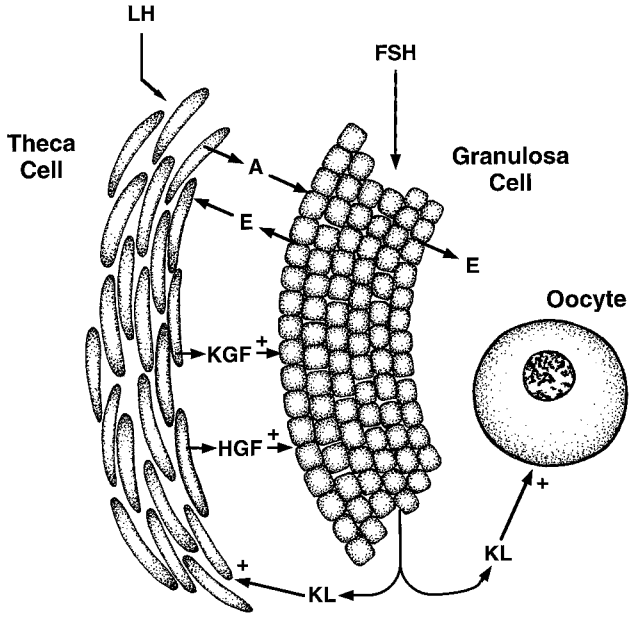

FIG. 5. Proposed schematic of thecal cell-granulosa cell interactions during follicular development. Mesenchyme-derived thecal cells produce KGF and HGF that regulate epithelial-derived granulosa cell functions. Granulosa cell expression of KL is stimulated (+) by KGF and HGF. Granulosa cell-derived KL feed back on thecal cells to regulate thecal cell growth and differentiated functions. Thecal cell expression of KGF and HGF is stimulated (+) by KL. Also illustrated is the previously established positive effect (+) of KL on oocyte development. Endocrine regulation of ovarian development is represented at the *top* with the actions of FSH and LH. Thecal cells produce androstenedione (A) under the control of LH. Androstenedione is aromatized to estradiol (E) by granulosa cells under the control of FSH. High levels of estradiol further stimulate androstenedione production by thecal cells. LH also stimulates granulosa cell function during later stages of follicular development (not shown).

a number of tissues. Gene expression of KGF and HGF is developmentally and hormonally regulated in thecal cells during follicular development (3, 4). Thecal cells have been shown to produce and secrete these growth factors (1). Granulosa cells have been shown to proliferate in response to KGF and HGF *in vitro* (1). These observations suggest that KGF and HGF may be important mediators of ovarian mesenchymal-epithelial cell interactions that promote folliculogenesis. The current study demonstrates that thecal cell-derived KGF and HGF also stimulate KL expression in granulosa cells. This is the first report of the regulation of KL by these mesenchymal growth factors. Although these results show that KGF and HGF regulate KL mRNA levels, the effects of KGF and HGF on KL protein production remain to be elucidated. The actions of KGF and HGF on granulosa cell KL expression indicate that these growth factors also alter cellular parameters other than cell growth (1). As granulosa cell-derived KL is important for oocyte maturation, thecal cells may indirectly regulate oocyte function by influencing granulosa cell production of KL.

KL is expressed in granulosa cells of healthy developing follicles. Granulosa cell-derived KL promotes follicular development and is important for oocytes, thecal cells and stromal-interstitial cells (2, 15, 23–25). As gonadotropins directly stimulate granulosa cell functions and promote folliculogenesis, the regulation of KL expression in granulosa cells by gonadotropins was investigated. Two previous studies addressed the direct regulation of KL in granulosa cells by gonadotropins and produced conflicting results. KL expression was stimulated by $(Bu)_2$cAMP in mouse granulosa cells, suggesting that gonadotropins may stimulate KL expression (15). In human granulosa-luteal cells, KL expression was decreased in response to FSH and hCG, suggesting that gonadotropins inhibit KL expression (31). In addition, *in vivo* experiments suggest that both mouse and rat granulosa cell KL expression can be increased by LH, hCG, and PMSG (32, 33). In the current study, both FSH and hCG stimulated gene expression of KL in purified bovine granulosa cells. These results suggest that gonadotropins (*i.e.* FSH and LH) may in part promote follicular development by directly stimulating KL expression in granulosa cells. The explanation of why gonadotropins decreased KL expression in human granulosa cells is not known. Potential differences in luteinized granulosa cells need to be investigated. Analysis of the potential regulation of KL by gonadotropins in other species also remains to be elucidated.

Epithelium-derived granulosa cells are proposed to feedback on mesenchyme-derived thecal cells to regulate thecal cell functions. Previous work has shown that granulosa cell-derived KL acts on thecal cells to promote cell proliferation and differentiation (2). The possibility that KL feedback on thecal cells may also regulate KGF and HGF expression was investigated in the current study. Treatment of thecal cells with KL stimulated both KGF and HGF gene expression. The ability of KL to stimulate thecal cell KGF and HGF expression is significant because it establishes a positive feedback loop between granulosa cells and thecal cells. Thecal cell-derived KGF and HGF can stimulate granulosa cell KL expression, and granulosa cell-derived KL can stimulate thecal cell KGF and HGF expression. As the expression of KGF, HGF, and KL is greatest in large antral follicles (2–4), the positive feedback among these factors may be particularly important during later stages of follicular development. Communication between thecal cells and granulosa cells through the production and actions of KGF, HGF, and KL appears to be a mesenchymal-epithelial cell interaction that may in part promote folliculogenesis.

Hormones such as FSH and LH stimulate follicular growth *in vivo* (34–38). The actions of such hormones in the ovary are necessary for follicular development and reproductive viability. Although these hormones increase follicular growth *in vivo*, no proliferative effect is apparent on purified cells *in vitro*. As a result, the hypothesis has developed that gonadotropins and steroids indirectly stimulate follicular growth by influencing local mesenchymal-epithelial cell interactions in the ovary. The current study supports previous work suggesting that hormones can directly regulate KGF and HGF expression in thecal cells. The current study also demonstrates that FSH and hCG directly stimulate KL expression in granulosa cells. The ability of gonadotropins to influence KGF, HGF, and KL gene expression provides an indirect mechanism for gonadotropins to regulate folliculogenesis. Gonadotropins may also indirectly influence oocyte functions by stimulating granulosa cell functions such as KL expression. These observations provide an expanded mech-

anism to explain hormone-induced follicular growth in the ovary.

The current hypothesis involving mesenchymal-epithelial cell interactions and these growth factors suggested by this study is summarized in Fig. 5. This schematic of thecal and granulosa cells depicts both the endocrine regulation and the paracrine regulation of thecal and granulosa cell functions. It is well established that gonadotropins regulate thecal and granulosa cell differentiated functions (see FSH and LH, Fig. 5). Steroid-mediated thecal cell-granulosa cell interactions via androgen and estrogen are critical and are influenced by gonadotropins. Locally produced growth factors, such as KGF, HGF, and KL, may also be important for regulating thecal and granulosa cell functions. The combined endocrine and paracrine regulation of KGF, HGF, and KL expression in the ovary is postulated to be important for follicular development. The autocrine actions of these growth factors may also be a factor (not shown in Fig. 5). For example, previous observations have suggested that HGF may act on thecal cells in an autocrine manner in the rat (39). It is anticipated that a complex network of autocrine, paracrine, and endocrine cell-cell interactions involving these and other growth factors will be needed for optimal follicular development. The current study used purified cell populations to demonstrate a potentially important cell-cell interaction in the follicle. Future studies involving coculture and *in vivo* studies will be required to assess the physiological importance of this interaction to follicle development and hormone actions. The novel positive feedback loop between thecal cells and granulosa cells suggested in the current study provides insight into potential mesenchymal-epithelial cell interactions required for follicular development.

Acknowledgments

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